B

B Cell Activating Transcription Factor

► BATF

B Cell Activation Protein BL34

► Regulator of G-Protein Signaling 1 (RGS1)

B Cell Stimulatory Factor-2 (BSF-2)

► IL6

B Lymphocyte Antigen CD19

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Synonyms

B4; CD19 molecule; CVID3

Historical Background

CD19 was first identified as biomarker of normal and neoplastic B lymphocytes as B4 antigen with the use of anti-B4 monoclonal antibody against CD19 (Anderson et al. 1984). Anti-B4 antibody in turn was encoded from cDNA clones of human tonsillar cDNA library, by selectively hybridizing with RNA from $CD19^+$ cell lines. Both human and mouse CD19 molecules are highly homologous with a conserved cytoplasmic domain with no homology with other known proteins and an immunoglobulin-like extracellular domain, which gave CD19 molecule the status of a member of Ig superfamily (Tedder and Isaacs 1989).

Introduction

The CD19 molecule is a 95 KDa cell surface protein of B lymphocytes and follicular dendritic cells (FDC). It is considered as a biomarker of B cell because of its continued expression throughout B cell differentiation stages starting from late pro-B cell stage until terminally differentiated plasma cells (Nadler et al. 1983; Schriever et al. 1989). It is a co-receptor of B cell receptor complex having an important role in BCR-mediated signaling for B cell differentiation and activation.

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Genomic Organization and Protein Structure

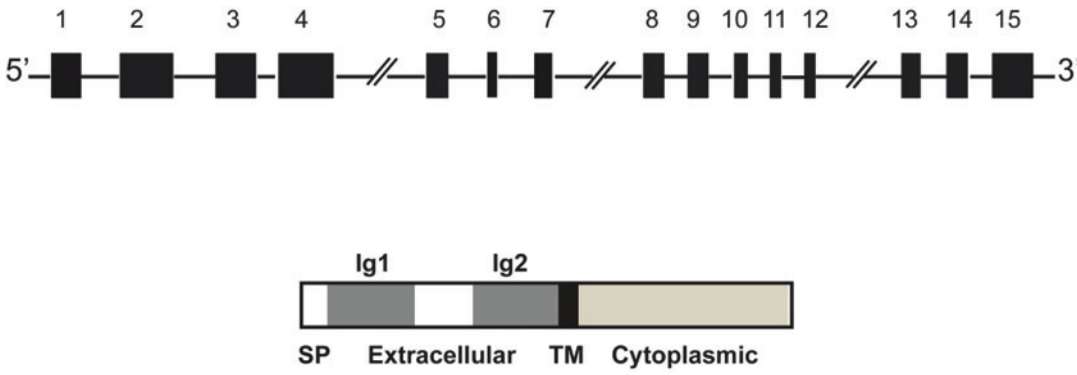
CD19 antigen is encoded by *CD19* gene located on chromosomes 16 (16p11.2) and 7 in human and mice, respectively. *CD19* gene is spanned in around 8 kb region in human and in 6 kb in mouse and consists of 15 exons. Out of 15 exons, 2 exons encode for extracellular Ig-like domain, 1 encodes for transmembrane region, 9 encode for cytoplasmic tail, and the rest of the exons encodes for 5′ and 3′ UTR, leader peptide, and poly-A region. There are specific conserved sites between human and mouse CD19 gene including all exons, exon-intron boundaries, and regions flanking 5′ and 3′ untranslated regions suggesting putative functional and evolutionary role of these regions and a high regulation of their expression (Zhou et al. 1992). There are multiple CD19 transcripts, while two predominant isoforms have been isolated in vivo. CD19 promoter region lacks TATA box and has a very small 5′ untranslated region (Fig. 1).

CD19 protein is 556 amino acids long and is classified as class I transmembrane protein. Structurally it is divided into extracellular, transmembrane, and cytoplasmic domains having specific interaction sites for various signaling molecules. Extracellular domain consists of two C2-type Ig-like domains separated by a non-Ig-like, potentially disulfide-linked domain and N-linked carbohydrate addition sites. This domain is 276 amino acids long, and through this domain, CD19 interacts with CD21 and CD81 (Bradbury et al. 1992).

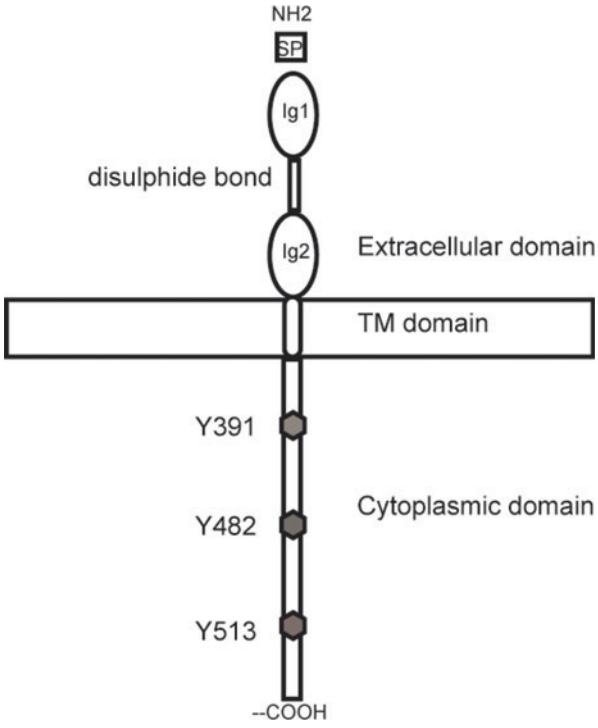
Transmembrane domain is a small 22-amino-acid-long element, consists mainly of hydrophobic amino acids, and lacks charged residues. This domain is important for intermolecular association with other components of BCR signaling complex like TAPA1 and Leu13. Cytoplasmic domain is highly conserved across species and consists of 242 amino acids in length. This domain is highly charged and has 19% acidic and around 10% basic residues with some localized regions of strong net negative charge. CD19 contains nine highly conserved cytoplasmic tyrosine residues (Fig. 2); however, three tyrosine residues Y391, Y482, and Y513 have important biological functions (Wang et al. 2002). Following CD19 cross-linking, these tyrosine residues get phosphorylated and serve as a docking site for several SH2-containing cytoplasmic signaling molecules of B lymphocytes. They mediate its interaction with signaling components PI3K, Grb2, Sos, and Vav and several protein tyrosine kinases: Lyn, Lck, and Fyn.

CD19 Expression on B Cell and Its Regulation

CD19 expression on B cell surface starts from as early as late pro-B cell stage (on D_H-J_H rearranged



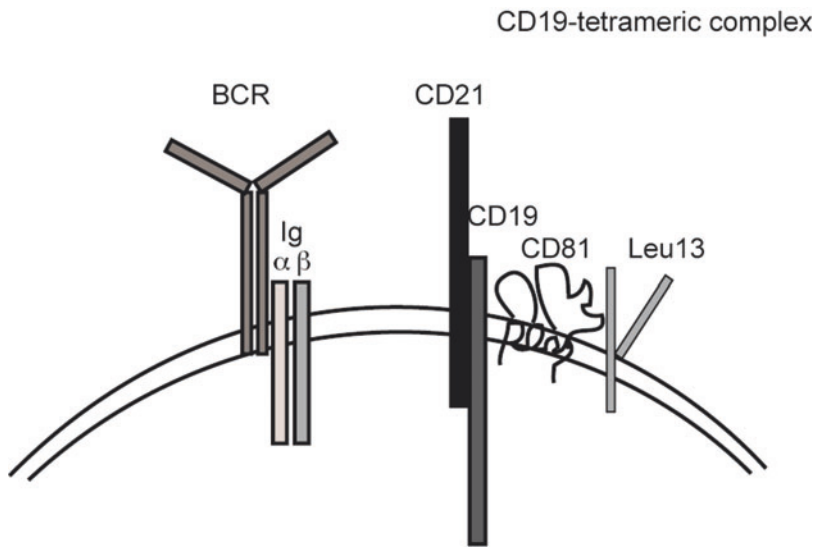
B Lymphocyte Antigen CD19, Fig. 1 Schematic representation of human *CD19* gene. The filled *boxes* represent the exon sequences and the *line* represents introns. Exons of the *CD19* gene encode different regions of the CD19 protein: extracellular, transmembrane, and cytoplasmic



B Lymphocyte Antigen CD19, Fig. 2 Schematic representation of molecular structure of CD19 protein. The two extracellular immunoglobulin-like domains are separated by a non-immunoglobulin domain possibly with disulfide bond. A small transmembrane region is followed by long cytoplasmic domain having multiple tyrosine residues. Three important tyrosine residues are shown in the picture

pro-B cells) and continues until differentiated plasmablast stage. Its continuous presence on B cell surface advocates their role in B cell development and activation. Progression through early pro-/prestage to immature B cell stage requires augmentation of pre-BCR signaling through CD19; in addition, it also helps in positive and negative selection of immature B cells. CD19 role in early B cell development was evident with impaired B cell proliferation in $CD19^{-/-}$ mice due to impairment of pre-BCR signaling (Otero et al. 2003). CD19 also has an extended role in peripheral B cell development, which starts from the egress of B cell from the bone marrow to the periphery. CD19 expression is threefold higher in mature B cell than that of immature B cell and is slightly more on B1 cells compared to B2 cells (Carter et al. 2002). B1 cell differentiation and maturation are dependent on CD19-mediated signaling as $CD19^{-/-}$ mice show diminished population of B1 cells, while overexpression of CD19 leads to extended B1 cell population. In the subpopulation of B2 cells, both marginal zone and follicular B cell compartment require survival signal from CD19 for their maintenance (Otero et al. 2003).

CD19 expression is vital for B cell development and function; hence, its density on B cell surface is tightly regulated; moreover, its expression is lineage specific, and it expresses only on B cell and follicular dendritic cells among all members of the hematopoietic system. CD19 expression level is crucial for correct B cell development as it has been shown through studies on $CD19^{-/-}$ mice and overexpressing CD19 transgenic mice that both low (absence of positive selection) and high (negative selection) expressions of CD19 lead to defect in B cell development and disturbed pro-/pre- to mature B cell ratio (Carter and Fearon 1992; Engel et al. 1995). Transcription factor PAX5 (also known as BSAP: B cell lineage-specific activator protein) expressed at all B cell developmental stages except terminally differentiated plasma cells and considered as master regulator of B cell commitment and differentiation of early lymphoid progenitors correlates with CD19 expression (Fuxa and Busslinger 2007). Cloning and characterization



B Lymphocyte Antigen CD19, Fig. 3 Schematic representation of CD19-associated signaling complex. On the cell surface of B cell, CD19 forms a tetrameric complex with CD81, CD21, and Leu13. CD81 is essential for surface expression of CD19, CD21 connects CD19 to BCR via antigen-bound complement, and CD19 has long cytoplasmic tail with multiple tyrosine residues. B cell receptor complex consists of antigen binding immunoglobulin with immunoreceptor tyrosine activation motifs (ITAMs) containing polypeptides Igα and Igβ. Co-activation of CD19 receptor complex and B cell receptor complex leads to dual activation of receptors and lowers threshold for B cell activation

of CD19 gene have reported several PAX5-binding site, and in particular a high-affinity binding site was identified in the promoter region instead of TATA box (Kozmik et al. 1992). Studies have shown that Pax5 is required for normal expression of CD19, starting from late pro-B cell stage until terminal differentiation to plasma cell. Surface expression of CD19 molecules requires the presence of tetraspanin molecule CD81, and in the absence of CD81, expression of CD19 on B cell surface is greatly reduced as CD81 regulates the transport of CD19 to plasma membrane. CD81-deficient mice show almost 50% reduction in CD19 expression compared to controls, and patients deficient in CD81 did not express CD19 on the B cell surface (Shoham et al. 2003).

Role of CD19 in B Cell Signaling

On B cell surface, CD19 associates with three different molecules (CD21, a complement receptor; CD81, a member of the tetraspanin family; and CD225 (Leu13)) and forms tetrameric co-receptor complex (Fig. 3). This complex is referred as CD19-CD21 complex and is mediated by transmembrane and adjacent extracellular portions of CD19. CD21 is a cell surface molecule with short cytoplasmic tail and an extracellular domain comprised of 15–16 consensus repeat. Extracellular domain of CD21 binds with complement C3d-bound antigens and connects CD19 to BCR to enhance BCR signaling. CD81 is a member of transmembrane 4 superfamily and is important for surface expression of CD19. It mediates association of membrane protein complex to cytoskeleton and involved in BCR regulation. The role of Leu13 (16 kDa) from this complex is not fully known. Out of this multimolecular signaling complex, only CD19 has long cytoplasmic tail, which is responsible for intracellular signaling. Phosphorylated tyrosine residues of CD19 at Y482 and Y513 allow association with PI3K and at Y391 recruits Vav. The mechanism of other tyrosine kinases Lyn and Fyn association with CD19 is still not clear. CD19 serves as a co-receptor for

BCR and by associating with components of BCR complex modulates signaling through BCR. CD19 plays a critical role in both early antigen-independent phase of B cell development and antigen-dependent activation of peripheral B cell.

CD19 in Pre-BCR and BCR Signaling

CD19 expresses on the surface of B cell at late pro-B cell stage prior to the expression of BCR and thus functions independently of BCR. It plays a role in late pro-B cell to large pre-B (proliferating) cell stage transition by modulating the proliferative signal emanating through pre-BCR. Another important role of CD19 is during positive and negative selection of immature B lymphocyte. Based on the BCR signaling threshold, a B cell can be either positively selected or eliminated. As CD19 augments the BCR signals, it may have a role in these selection events. $CD19^{-/-}$ mice show reduced number of mature B cell suggesting a defective positive selection of immature B cell (von Muenchow et al. 2014).

Naïve B cell, on their surface, expresses BCR with an antigen-recognizing domain immunoglobulins (Ig), immunoreceptor tyrosine-based activation motifs (ITAMs) containing signaling components Igα (CD79a) and Igβ (CD79b), and signal-modulating co-receptor components CD19-C21 complex. Upon antigen recognition and ligand binding, phosphorylation of ITAMs occurs and recruits tyrosine kinases Lyn (hyperlink) and Syk (hyperlink). Both tyrosine kinases phosphorylate several adaptor proteins and the co-receptor CD19. Phosphorylated Cd19 further recruits several other molecules as Vav, Bruton's tyrosine kinase (Btk), and PI3 kinase (PI3K) via its cytoplasmic domains and lowers the threshold for B cell activation. CD19 interacts with a variety of proteins through which it involves intracellular signal transduction downstream of BCR.

CD19 and Lyn

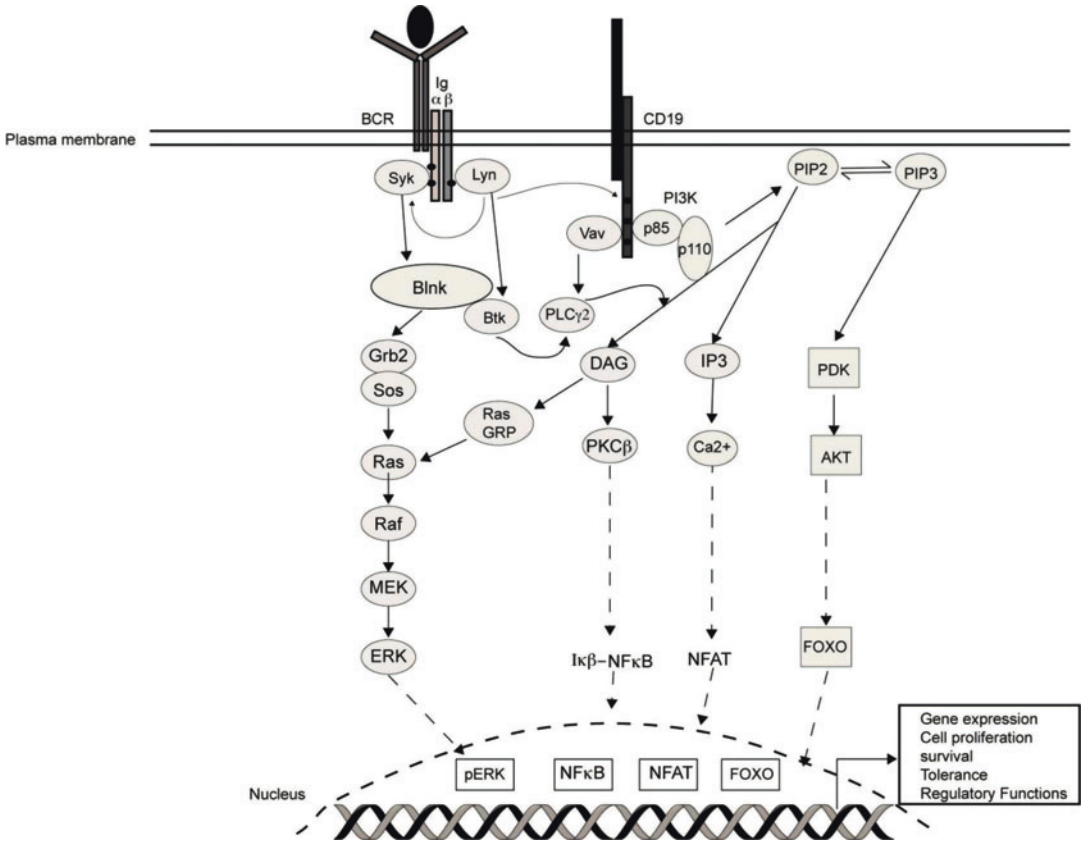
CD19 is primarily phosphorylated by Src family protein tyrosine kinase Lyn at tyrosine 513, as this phosphorylation event is absent in Lyn-deficient B cell before or after BCR ligation. However, reports suggest that CD19-deficient primary B cells have greatly compromised Lyn phosphorylation; hence, there exist an interdependence between CD19 and Lyn. In addition, data exist in support of independent Lyn and CD19 activity as well. By regulating Lyn kinase activity, CD19 regulates the B cell signaling threshold, and this interaction of Lyn with co-receptor complex may reduce the threshold of BCR activation by up to 10^4-fold (Fearon and Carroll 2000).

CD19 and Vav

Vav proteins are highly expressed in peripheral lymphocytes and spleen cells and have been shown to modulate BCR-mediated PI3K signaling. The Vav family of proteins is cytoplasmic guanosine nucleotide exchange factor for Rho family GTPases. CD19 phosphorylation recruits Vav into a signaling complex of CD19 via SH2 interaction, which facilitates its subsequent phosphorylation by activated PTK. Vav binds with PIP3 produced by PI3K and thus its activation is controlled by PI3K. On the other hand, Vav regulates PI3K through Rac1, a member of the Rho family GTPases (Bustelo 2014).

CD19 and PI3K

Phosphoinositide 3-kinases (PI3K) are a family of lipid kinase that plays an important role in B cell differentiation. It produces phosphatidylinositol (PtdIns) 3,4,5 (PIP3) from PtdIns 4,5 (PIP2). PIP3 then activates the downstream signaling molecules Akt, followed by modulation of downstream transcription factors Foxo1 and Foxo3 causing its exclusion from the nucleus and degradation which allows progression of cell cycle. PI3K activity is equally important in both early and late B cell differentiation. In B cell, PI3K activation is primarily mediated by CD19. Upon BCR cross-linking, phosphorylated CD19 binds with regulatory subunits of PI3K, p85alpha with its SH2 domain at YxxM motif present in the cytoplasmic region. However, existence of other adaptor molecules for PI3K-CD19 association is reported. One such molecule is B cell adapter for PI3K (BCAP), having four YxxM motifs for binding of SH2 domain of p85alpha. $CD19^{-/-}$ $BCAP^{-/-}$ primary B cells have been shown to have defective BCR-mediated PI3K activation



B Lymphocyte Antigen CD19, Fig. 4 Overview of BCR- and CD19-mediated signaling. Upon antigen recognition by BCR, tyrosine phosphorylation of Igα and Igβ molecule recruits several tyrosine kinases Lyn, Syk, and Btk. This engagement of BCR triggers tyrosine phosphorylation of CD19 which in turn recruits PI3K consisting of p85α and p110δ. Phosphorylated Blnk links these two pathways. Subsequent activation and integration of multiple signaling pathways lead to activation of transcription factors, culminating into gene regulation

and developmental block at B cell maturation. Any defect in CD19-mediated phosphorylation of PI3K or defect of PI3K signaling due to deletion or inactivation of its regulatory subunits p110δ and p85α reduces BCR-mediated phosphorylation of Akt, FoxO, and protein kinase D, followed by reduced Ca^{2+} flux, impaired cell cycle progression, and impaired B cell homeostasis (Baracho et al. 2011) (Fig. 4).

CD19 and Complement

The complement system is a cascade of serum-soluble protein that constitutes an important part of the innate immune system. Activation of complement is an essential component of early response against infection. Cleavage products of complement rapidly recruit effector cells and facilitate lysis and phagocytosis of microbes. In addition these cleavage products collectively called as C3d (g) bind with CR2 receptor (CD21) on B cell surface and FDC and bridge innate and adaptive arm of the immune system. Upon engagement of CD21 with C3d (g) bound with antigen, recruits CD19-CD21-CD81 complex to the lipid raft and augment B cell signaling through CD19 cytoplasmic chain. However, co-engagement of BCR is required for CD19 recruitment to raft and its signaling (Del Nagro et al. 2005).

CD19 and Toll-Like Receptors

Toll-like receptor (TLR) families play an important role in innate immune response, which serves

as a first line of defense and is T cell independent. Upon encounter with pathogenic microorganisms, innate immune system recognizes specific molecular patterns on their cell components such as lipopolysaccharide (LPS), peptidoglycan, and bacterial DNA and RNA. LPS, a component of gram-negative bacterial cell wall, is recognized by TLR, a pattern recognition receptor, expressed by many cell types. In human and mice, there are 10–13 different types of TLRs which mostly signal via adaptor protein myeloid differentiation factor 88 (MyD88) and TIR domain-containing adaptor inducing interferon-beta (TRIF). However, two different types of TLRs are expressed on B cell along with BCR, TLR4, and RP105, out of which RP105 is preferentially expressed on mature B cell. CD19 plays an important role in the regulation of TLR signaling through B cell-specific receptor RP105. Upon RP105 ligation, CD19 recruits Lyn and Vav and regulates intracellular $Ca2^{+}$ mobilization (Hua and Hou 2013).

CD19 Animal Models

Both CD19-deficient ($CD19^{-/-}$) mice (Engel et al. 1995) and human CD19 transgenic mice (hCD19TG) (Zhou et al. 1994) that overexpress CD19 show a dramatic decrease in peripheral B lymphocyte numbers. This decrease in number in case of hCD19TG is because of increased cell surface signaling through CD19 and resulted feedback signaling to inhibit the development of bone marrow precursors of B cells, while in $CD19^{-/-}$ mice B cell precursors, development is largely unaffected; instead later stages of B cell growth are affected with a special reduction in marginal zone B cell. Upon BCR cross-linking and LPS stimulation, $CD19^{-/-}$ B cell shows reduced proliferation, while this is increased in hCD19TG mice depending on gene dosage. In both cases B cells were able to proliferate clonally and secrete antibody. Antibody secretion in hCD19TG is skewed toward IgG2b, while there is an overall decrease in all isotypes in CD19−/− mice. T cell-dependent humoral response is reduced in $CD19^{-/-}$ mice with reduced proliferation and reduced germinal center formation and memory cell selection. These phenotypes of both mice suggest that CD19 acts as a regulator of cell surface receptor signaling having crucial role both at early development in the bone marrow and at later stages of clonal expansion after antigen encounter in peripheral B cell pool.

CD19 and Its Association with Human Diseases

Studies have shown that B cell regulates immune response in a variety of ways including antigen presentation, cytokine production, antibody production, and T cell help and influence on the function of other immune cells including dendritic cells and macrophages. On the surface of B cell, CD19 molecule along with its co-receptor complex members CD21, CD81, and CCL25 signals with BCR to lower the threshold of BCR-dependent signaling and regulates the immune response (Carter and Fearon 1992). CD19 is considered as positive response regulator of BCR signaling. Any defect or deficiency of CD19 affects the humoral immune response and leads to malfunction of immune system resulting into disease.

Mutation in cd19 gene in mice leads to hypogammaglobulinemia, low $CD5^{+}$/B1-B cells, impaired T cell-dependent germinal center formation, and impaired B cell memory. CD19 mutation in humans has been reported: insertion and deletion of base pair in exons 6 and 11, respectively, result in frameshift mutation and insertion of an early stop codon prior to tyrosine residues in the cytoplasmic chain which are critical for CD19-mediated signaling. Depending on the type of mutation, there could be either complete lack of CD19 surface expression or a severely reduced expression. Phenotype of disease includes a normal number of precursor mature B cell but reduced $CD5^{+}$ B cell and $CD27^{+}$ memory B lymphocytes along with decreased serum level of IgG antibodies. These patients show hypogammaglobulinemia similar to $CD19^{-/-}$ mice because of poor antigen-specific response by mature B cell, poor response to vaccination (toward rabies vaccine), and increased susceptibility to bacterial infection (van Zelm et al. 2006).

A subset of common variable immunodeficiency (CVID) is linked with mutation of *CD19* gene. Reported case of one patient with mutation in splice acceptor site of intron 5 had similar immunological and clinical phenotype as observed before with loss of CD19 surface expression and antibody deficiency (Kanegane et al. 2007).

As we have already seen, CD19 acts as a positive regulator of BCR signaling, while negative regulators for BCR signaling are CD22, CD72, and FcγRIIB that dampen BCR signals. These are collectively called as response regulators, which establish signaling threshold that controls the duration and intensity of B cell activation. Any alteration in CD19/CD22 loop activities contributes to autoimmunity both in mice and human. hCD19Tg mice which express threefold more CD19 are reported to be autoimmune prone, and they show increased proliferation in response to antigen and have anti-dsDNA serum antibodies. An autoimmune disease systemic sclerosis (SSc) is characterized by tissue fibrosis and production of disease-specific autoantibodies. B cells from SSc patients show higher expression level of CD19 by 20% and upregulated CD19 signaling pathway resulting in chronic hyperactivation of B cells, precisely $CD27^{+}$ memory B cells compared to naïve B cells (Yoshizaki and Sato 2015). Dysregulated (both high and low) CD19 expression has been reported in the case of systemic lupus erythematosus (SLE), another kind of autoimmune disease where autoantibodies and immune complex aid into the pathogenesis (Sato et al. 2000).

CD19 antigen is a specific B cell marker and is expressed by all normal and malignant B cells and is used as a marker to differentiate between B and T cell leukemia. It is expressed in acute lymphoblastic leukemias (ALL), chronic lymphocytic leukemias (CLL), and B cell lymphomas. Expression level of CD19 in different types of malignancy differs significantly and ranges from normal to high levels and is also a useful marker as diagnostic tool. Occasionally CD19 expression also associates with non-B cell malignancies such as acute myeloid leukemias (AML) and multiple myeloma (MM) and is considered as a result of aberrant regulation of CD19 by PAX5. Studies in $CD19^{-/-}$ mice have identified the role of CD19 in the stabilization of c-Myc protein, which is an established proto-oncogene in human cancers. CD19/c-Myc activation loop has been shown to have a role in malignant B cell transformation and lymphoma genesis (Wang et al. 2012).

CD19 Therapeutics

As CD19 is expressed by majority of B lymphoid malignant cells and autoreactive B cells, antibody-mediated therapy targeting CD19 is a new advancement in the field of antibody therapy. CD19 targeting has evolved through years, and considerable advancement has been made for its precise use. Initially CD19 monoclonal antibodies have been used for lymphoma therapy, and transient reduction of tumor cells was reported; later anti-CD19 monoclonal antibodies were conjugated with immunotoxins such as ricin A and saporin and evaluated successfully against human and murine malignant B cells. Combination therapy of anti-CD19 with chemotherapy also gave a successful result in experimental models. Combination of anti-CD19 with cytokine treatment induces antibody-mediated cellular toxicity mechanisms (Hammer 2012). Advancement and improvement to this antibody therapy have been done with the use of humanized anti-CD19 antibody (Medi-551), which is a fucosylated antibody and has increased affinity to FcγRIIIA. Targeted immunotherapy against CD19 has been developed in the form of bi-specific T cell engager (BiTE) antibody, a monoclonal antibody (blinatumomab) having antibody-binding site specific for both CD19 and CD3. It engages cytotoxic T cell and then guides it to CD19-expressing B cells and subsequently leads to lysis of malignant cells; however, normal B cells get lysed too. Alternative approach toward B cell targeting is chimeric antigen receptor (CAR) approach. CAR T cell immunotherapy involves patient-derived T cells, which are genetically modified to express synthetic antigen receptors having specificity toward CD19. They are composed of three

domains, an extracellular CD19 recognition domain derived from a single-chain variable fragment (scFv), which is linked via a flexible hinge region to transmembrane domain, and a cytoplasmic signaling domain that triggers the T cell activation. CARs have undergone a series of modifications; first-generation CARs utilized cytoplasmic CD2ζ region, while second- and third-generation CARs included a variety of co-stimulatory molecules for improved T cell stimulation. Clinical trials of CARs are undergoing and have shown promising results in B cell malignancies (Katz and Herishanu 2014).

Other than B cell malignancy, anti-CD19 antibodies are also considered for the treatment of autoimmune diseases. Previously anti-B cell approaches have been used for targeting B cells in non-Hodgkin lymphoma, rheumatoid arthritis, and other autoimmune diseases as SLE and ANCA-associated vasculitis using rituximab (anti-CD20) and belimumab. However, these approaches are not suitable for targeting neither pre-B cell nor antibody-secreting plasma cell where CD20 is not expressed; hence, CD19 appears to be a promising approach for specific targeting of B cells as it starts expressing before CD20 (B1 antigen) at pro-B cell and continues till plasma cell differentiation (Mei et al. 2012).

Summary

CD19 is a biomarker for B cells and function as a co-receptor for BCR on B cell surface. CD19 along with its signaling complex plays an important role in signaling cascade emanating downstream of BCR and is involved in positive regulation of activation threshold of BCR. CD19 plays an important role in B cell development, and any deregulation of CD19 results in impairment of B cell development, which is associated with either B cell deficiency or autoimmunity in patients. Hence, it maintains a balance among humoral response and tolerance induction. CD19-based immunotherapy is emerging as a promising clinical development for the management of B cell malignancies and autoimmunity. CD19 monoclonal antibodies anti-B4-br, BiTE, SAR 3419, MEDI-551, and chimeric antigen receptor toward B cells (anti-CD19-CAR) are under clinical trials in different phases and look promising for the treatment of B cell malignancies.

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B1BKR

▶ Bradykinin Receptors

B_1R

▶ Bradykinin Receptors

B220

▶ CD45 (PTPRC)

B2BKR

▶ Bradykinin Receptors

B_2R

▶ Bradykinin Receptors

B4

▶ B Lymphocyte Antigen CD19

Baculoviral IAP Repeat-Containing 5

▶ Survivin

Baculoviral IAP Repeat-Containing Protein 5

▶ Survivin

BAD (BCL-2-Associated Agonist of Cell Death), BBC6 (BCL2-Binding Component 6), BCL2L8 (BCL2-Like Protein 8)

▶ BCL-2 Family

BAF

▶ SWI/SNF Chromatin Remodeling Complex

BAFF/BLyS Family

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Synonyms

Cytokines

APRIL: A proliferation-inducing ligand
Synonyms: TNFSF13a; TALL-2; TRDL-1; CD256

BAFF/BLyS: B cell-activating factor of the TNF family/B lymphocyte stimulator
Synonyms: TNFSF13b; TALL-1; zTNF-4; THANK; CD257

Receptors

BCMA: B cell maturation antigen
Synonyms: TNFRSF13a; TNFRSF17; CD269

TACI: Transmembrane activator and calcium modulator and cyclophilin ligand interactor
Synonyms: TNFRSF13b; CD267

BAFFR/BR3: BAFF receptor/BLyS receptor 3
Synonyms: TNFRSF13c; Bcmd; CD268

Overview

The BAFF/BLyS family includes two cytokine ligands and three receptors, all of which are members of the TNF/TNF receptor superfamily. Members of the BLyS family play critical and varied regulatory roles among cells of the B lymphocyte lineage: they control the selection and survival of pre-immune B cells, govern aspects of selection and differentiation among activated B cells, and impact formation and survival of antibody-secreting plasma cells. General features of these ligands and receptors are summarized in Table 1, and receptor expression patterns on B cell subsets are summarized in Table 2 and Fig. 1. In this entry, we provide a brief history of the discovery and nomenclature of the receptors and ligands, followed by a treatment of their molecular structure, ligand-receptor interactions, and consequent signaling. We then provide an overview of physiological and pathophysiological relevance, with emphasis on their roles in the biology of B lymphocytes. Both BAFF/BAFFR and BLyS/BR3 nomenclatures are in common use for this ligand-receptor pair; herein we use the latter.

Historical Background

Ligands

Both BLyS and APRIL were discovered through genome-wide homology searches. BLyS was identified simultaneously by multiple groups and, as a result, has appeared in the literature under the different names listed above. Many of these acronyms are misnomers, because they erroneously ascribe direct costimulatory or mitogenic activity to BLyS. This arose from observations

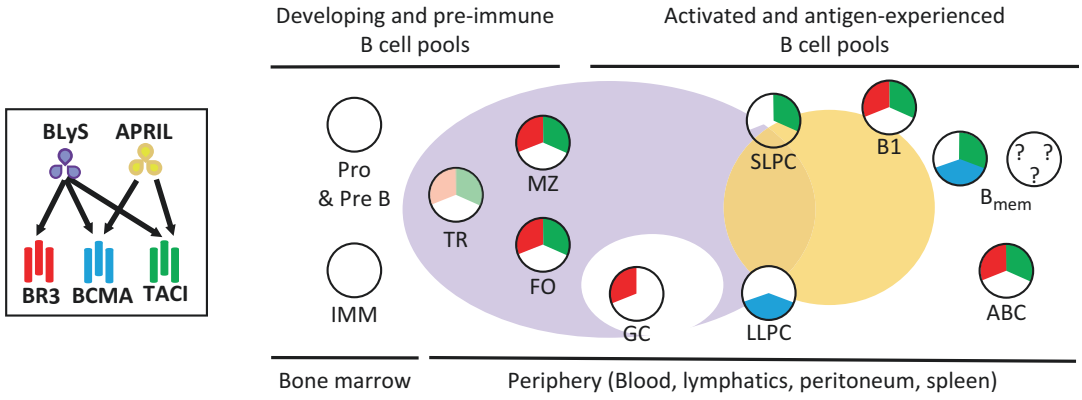
BAFF/BLyS Family, Table 1 BLyS family ligand and receptor family member characteristics (mice)

		Ligands		Receptors		
		BLyS	APRIL	BR3	TACI	BCMA
Molecular characteristics	**Molecular structure(s)**	Soluble trimer, 60-mer	Soluble trimer	Type III transmembrane proteins		
	Molecular weight (kDa)	31	31	18	27	20.4
Cellular characteristics	**Binding partner(s)**	TACI BCMA BR3	TACI BCMA	BLyS	APRIL BLyS	APRIL BLyS
	NF-kB signaling			Noncanonical (NF-kB2)	Canonical (NF-kB1)	Canonical (NF-kB1)
	B cell defects in mutants or knockouts	Reduced mature B cell numbers; impaired GC selection	Impaired TI responses	Reduced mature B cell numbers; impaired GC selection	Increased pre-immune pool; increased autoantibody production; CVID	Reduction in BM plasma cells

BAFF/BLyS Family, Table 2 BLyS receptor distribution and ligand requirements of B cell subsets (mice)

B cell subsets	Location	Subset	T ½ (days)	Production rate (10^6 / day)	Steady-state size (10^6)	Receptor(s)	Cytokine requirement
Developing and pre-immune	Bone marrow	Pro		15	5	None	None
		Pre	3.5	15	50	None	None
		IMM	3	10–15	30–40	BR3, TACI	?
	Circulation, secondary lymphoid organs	TR	2–4	1.5	~10	BR3, TACI	BLyS
		FO	90–100	0.4–0.5	30–40	BR3, TACI	BLyS
		MZ	45–156	0.5	7–10	BR3, TACI	BLyS
	Spleen, peritoneal cavity	B1	16–17 (splenic)	Variable	Variable	BR3, TACI	Partial APRIL dependence
Antigen-experienced	Spleen, lymph node	GC	15	Variable	Variable	BR3	BLyS
	Spleen, blood, bone marrow	SLPC	3–5	Variable	Variable	TACI, BCMA	?
		LLPC	≥20 weeks	Variable	Variable	BCMA	APRIL (BLyS?)
		B_{mem}	≥20 weeks	Variable	Variable	TACI, BCMA	?
		ABC	Weeks–months	Variable	Variable	BR3, TACI	BLyS independent; APRIL unknown

that BLyS caused B cell hyperplasia in vivo and augmented the abundance of proliferating cells following BCR ligation in vitro. While these findings were consistent with the notion that BLyS had mitogenic or costimulatory properties, subsequent work has definitively established that the primary action of BLyS is survival rather than activation or mitogenesis. Similarly, the initial



BAFF/BLyS Family, Fig. 1 BLyS receptor distribution defines distinct homeostatic niches. The BLyS family consists of two cytokines and three receptors (*Left box*). All are members of the TNF/TNFr superfamily. The three receptors are cell surface homotrimers, and the ligands exist primarily as soluble trimers. BLyS can bind all three receptors, whereas APRIL binds only TACI and BCMA. BLyS family receptor expression differs among developing, pre-immune, and antigen-experienced B cell subsets (*Right panel*). These varying patterns of receptor expression dictate reliance on and responsiveness to BLyS or APRIL, schematized by the violet or yellow areas, respectively. Developing B cells in the bone marrow do not express receptors for BLyS family ligands. As newly formed B cells exit to the periphery as transitional (TR) B cells, BR3 and TACI expression ensue and increase as these mature to join the follicular (FO) or marginal zone (MZ) subsets. This allows them to compete for BLyS signals via BR3, which are required for survival. Although TACI sequesters BLyS on TR and FO B cells, it is dispensable for survival of pre-immune subsets. Antigen-driven activation leads to the formation of germinal centers (GCs). GC B cells continue to express BR3 but extinguish TACI expression. Among GC B cells, competition for locally produced BLyS enables appropriate GC B cell selection. GC B cells that survive give rise to memory B cells (B_{mem}) and long-lived plasma cells (LLPC). Several memory B cell subsets have been defined, with varying combinations of BLyS receptor expression. Most memory B cells are BLyS independent. LLPC predominantly express BCMA and relocate to the bone marrow. Since BCMA can bind either BLyS or APRIL, there are likely redundant survival niches for these cells. Short-lived plasma cells (SLPC) are formed in the absence of a GC reaction and express TACI and possibly BCMA, but are also largely BLyS independent

characterization of APRIL was done in cell lines derived from malignancies, and the major focus was thus on proliferation. However, as with BLyS, it appears that APRIL acts primarily as a survival and differentiation factor, rather than a direct activator.

Receptors

BCMA, TACI, and BR3 are the three BLyS family receptors. BCMA was discovered within a chromosomal translocation in a human T cell lymphoma. TACI derives its name from the ability to interact with calcium-modulating cyclophilin ligand (CAML). BR3 (a.k.a. BAFFR) is the most recently discovered member of the receptor trio. Early research indicated that neither TACI nor BCMA deficiency substantially impacts B cell compartments, despite the profound effects of BLyS. This accordingly led to the search for a third receptor that could interact with BLyS and/or APRIL. Studies of the A/WySn mouse, which has a severe reduction in mature B cells, led to the discovery of the third receptor, BR3 (initially termed Bcmd, for B cell maturation defect).

Molecular Structure and Binding Relationships of BLyS Family Members

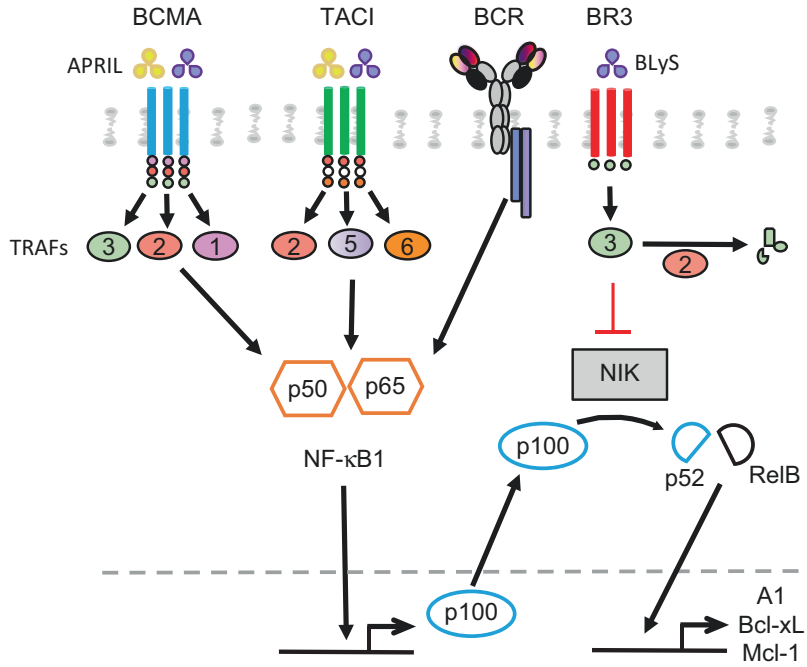
Ligands

BLyS and APRIL share about 50% structural homology with each other, but only 20–30% with other members of the TNF family. Both are produced as Type II transmembrane proteins and then undergo furin cleavage to be released as soluble proteins, though in some instances BLyS may remain membrane anchored, as can a mutated form of APRIL lacking the furin

cleavage site (Bossen and Schneider 2006; Vincent et al. 2013). Soluble BLyS forms multimers. BLyS trimers are most commonly associated with biological activity, as are the 60-mers, which may engage many receptors at a single site. Despite its structural similarity to BLyS, APRIL has not been reported to form 60-mers, although there is evidence that APRIL multimerizes by binding to sulfated proteoglycans, effectively concentrating APRIL or APRIL signaling at a local site (Sindhava et al. 2013). BLyS-APRIL heterotrimers are observed, and, although their in vivo role is not yet ascertained, they are biologically active and correlate with autoimmunity (Stohl et al. 2011).

Receptors

The BLyS family receptors are Type III transmembrane proteins (Bossen and Schneider 2006; Coquery and Erickson 2012). Structurally, the BLyS family receptors present considerable differences from other TNF receptor family members but share several motifs among themselves. For instance, all contain one (BR3, BCMA) or two (TACI) cysteine-rich domains, essential for receptor-ligand interactions. Similarly, the cytoplasmic domains of all three BLyS family receptors have one or more TNF receptor-associated factor (TRAF)-binding domains and interact with one or more TRAF proteins to transduce signals as shown in Fig. 2 (Rickert et al. 2011).



BAFF/BLyS Family, Fig. 2 Summary of BLyS family receptor signaling. All BLyS family receptors signal via TRAFs (TNF receptor-associated factors). Each of the three receptors has a distinct set of TRAF-binding motifs that enable and dictate their downstream signaling features. BR3 binds only TRAF3 and is one of the few cell signaling molecules that operates via the noncanonical NF-kB pathway exclusively. In contrast, BCMA and TACI each have three TRAF-binding sites and enable signaling via several downstream pathways, including canonical NF-kB. Further, B cell receptor (BCR) signals activate NF-kB1 (nuclear factor kappa B1, subunits p65 and p50) which translocate into the nucleus and induce p100 transcription, the substrate for NF-kB-inducing kinase (NIK). NIK is degraded by TRAF3 activity, unless TRAF3 is recruited downstream of BLyS/BR3 engagement and degraded in a TRAF2-dependent manner. This allows NIK to process p100 to p52. p52 forms heterodimers with RelB, which induce the transcription of antiapoptotic genes like Bcl-xL, A1, and Mcl-1

Binding Relationships

BCMA and TACI can bind both BLyS and APRIL, but the BR3 receptor binds only BLyS (Table 1). These differential binding capacities, coupled with distinct patterns of receptor expression in different B cell subsets (Table 2), allow considerable breadth in the targets and activities of the two ligands (Cancro 2004; Vincent et al. 2013). Crystallographic analysis of BLyS and APRIL has presented us with an electrostatic model for the basis of interaction between this subfamily's cytokines and receptors (Bossen and Schneider 2006). BR3 is heavily positively charged, whereas the putative binding sites in BLyS and APRIL have negatively versus positively charged residues, respectively, which may explain why BLyS binds BR3 strongly but APRIL does not bind to this receptor. Furthermore, the TACI and BCMA combining sites have mixed electrostatic charges, which is consistent with their abilities to interact with both BLyS and APRIL, albeit with differing affinities.

Overview of Primary B Cell Development and Characteristics

BLyS, APRIL, and their receptors are now well established as survival and homeostatic factors for cells within the B lymphocyte lineage. This is evidenced by the phenotypic features of mice and humans with mutations or deficiencies in BLyS receptors and ligands (Table 1), indicating the scope of B cell function affected by this family. Accordingly, a brief discussion of B cell development and differentiation is necessary to place BLyS family members into their biological context and to better understand their physiological roles. Table 2 summarizes key features and receptor-ligand interactions for B cell developmental and activation subsets (Cancro 2004; Srivastava et al. 2005; Anderson et al. 2006; Amanna and Slifka 2010).

B cells arise from hematopoietic stem cells in the bone marrow (BM), where they undergo immunoglobulin heavy and light chain gene rearrangements during the pro-B and pre-B cell stages, and then express a complete B cell antigen receptor (BCR) on the surface. At this point, they are termed immature (IMM) B cells. A young adult mouse produces almost 15 million immature B cells per day (Table 2), which undergo extensive negative selection, resulting in the elimination of cells with BCRs that either do not meet minimal tonic signaling thresholds or that interact strongly with self-antigens. The 10% of IMM B cells that survive this checkpoint migrate to the periphery and pass through the transitional (TR) developmental stage. Negative selection based on BCR specificity also occurs at the TR stage, such that only about 30% of TR B cells initially exiting the bone marrow survive to join the mature FO or MZ pools. Residence in all of these pools is brief; developing B cells transit the bone marrow stages in 2–3 days, and the recent bone marrow emigrés in TR pools either die or join the mature FO and MZ B cell pools within 3 days. In contrast, FO B cells have a life span of about 100 days and thus comprise 75–80% of peripheral B cell pool, whereas MZ B cells have a life span of about 1 month. The immunoglobulin isotype of BCRs on these preimmune B cell subsets is IgM and IgD.

BLyS Family Members Govern Pre-immune B Cell Homeostasis

BR3 and TACI are first expressed on TR B cells (Table 2); their levels increase through the TR stages and are highest on FO and MZ B cells. Accordingly, cells in the pro- and pre-B cell compartments have no functional reliance on either BLyS or APRIL. The primary role of the BLyS/BR3 signaling axis is readily surmised from the phenotype of BLyS or BR3 knockout mice and BR3 signaling mutants such as the A/WySn strain, which have a 90% reduction in FO B cells and virtually no MZ B cells. Likewise, humans with BR3 deficiency have very few mature naïve B cells (Darce et al. 2007). In contrast, BCMA or APRIL knockout mice have no disturbances in their peripheral pool, but TACI knockouts slowly accumulate more B cells in the FO and MZ pools, implying a regulatory role for this receptor.

Despite continuous turnover, the total number of cells within the FO and MZ B cell subsets remains relatively constant in normal adults. This steady-state situation is a function of the rate at which newly formed cells enter these mature B cell pools and their life span within the pool. Thus, stringent selection controls the number of immature B cells entering from the bone marrow to the periphery, and competition for survival factors helps to regulate life span thereafter. BLyS is the key survival factor, and systemic BLyS determines homeostatic "space" for pre-immune B cells. Radiation-resistant stromal cells – primarily among so-called fibroblastic reticular cells – are the main sources of systemic BLyS (Cremasco et al. 2014), supporting the notion that BLyS availability is linked to organism size: larger organisms produce more BLyS and therefore have a larger steady-state primary B cell pool size.

Interplay between BCR and BLyS-BR3 signals is required to pass the TR checkpoint. This checkpoint is flexible, with systemic BLyS acting as a rheostat to control the extent of BCR specificity-based selection at the TR checkpoint. Higher systemic BLyS relaxes selection by rescuing cells with BCR signal strength that falls above the threshold for negative selection or below the tonic signaling threshold for positive selection. Lower systemic BLyS makes TR selection more stringent, because only those cells with optimal tonic BCR signaling pass the checkpoint. B cells that fail to signal through either BCR or BR3 die, indicating a signaling integration to ensure survival, and Stadanlick et al. showed that the NF-kB signaling pathway may integrate signals downstream of these two receptors (reviewed in Stadanlick and Cancro (2006)). BR3 signals primarily via the noncanonical or NF-kB2 pathway and relies on availability of the p100 precursor to NF-kB. Although the BCR itself does not signal through the NF-kB2 pathway, p100 is a target of BCR signaling. Thus, BCR signaling replenishes p100, which is in turn used by BR3 signaling, which in turn induces expression of antiapoptotic genes (Fig. 2). This may in part explain why both a functional BCR and a functional BR3 are necessary for pre-immune B cell survival. More recent studies have shown that some downstream intermediates of BCR and BR3 signaling may be shared, further suggesting a complex relationship that mediates the interplay between BCR-mediated selection and BLyS-mediated survival.

In contrast to the B-2 lineage that is the focus of this encyclopedia entry, cells of the B-1 lineage are generated from fetal liver precursors, self-renew in the periphery, have a limited BCR repertoire, and are more common in coelomic cavities and mucosal interfaces than in secondary lymphoid organs. There is increasing evidence that BLyS family members play key roles in survival and homeostasis of B-1 B cells and may in fact integrate homeostasis of the two lineages (reviewed in (Sindhava et al. 2013)).

Overview of Antigen-Experienced B Cell Development and Characteristics

Antigen encounter results in the activation of FO and MZ B cells, leading to proliferation and further differentiation. Some activated B cells quickly differentiate into antibody-secreting cells termed short-lived plasma cells (SLPC), which afford rapid antibody formation early in the immune response and persist for several days (Table 2). A later and more antigen-focused antibody response results from the germinal center (GC). Some B cells activated by antigen initiate GCs in the spleen and lymph node after receiving cognate T cell help. GC B cells undergo successive rounds of somatic hypermutation (SHM) of immunoglobulin genes and selection, culminating in BCRs with greatly improved affinity for the antigen. As GCs progress and resolve, they yield memory B cells (B_{mem}) and long-lived plasma cells (LLPC), both with lifespans measured in months or years (Table 2). During immmune responses, activated B cells may undergo "class switching" from IgM and IgD to other Ig heavy chain isotypes, such as IgG or IgA. In general, SLPC display limited class switching and primarily secrete IgM antibody, whereas B_{mem} and LLPC show extensive class switching.

BLyS Family Roles among Antigen-Experienced B Cell Subsets

BLyS receptor profiles change with activation and subsequent differentiation (Table 2), yet the consequences of BLyS ligand and global receptor knockouts/mutations are generally more subtle for antigen-experienced compared to pre-immune B cell subsets (Table 1). Part of the challenge in studying BLyS family roles for antigen-experienced subsets involves the primacy of the BLyS-BR3 axis in generating and maintaining their preimmune precursors. For example, knockouts of either BLyS or BR3 yield a paucity of FO and MZ B cells and a limited BCR repertoire. Thus, sophisticated mouse models and experimental systems are needed to parse the roles of BLyS family cytokines and receptors in the differentiation, selection, and survival of antigen-experienced B cells.

Recent investigations into the role of BLyS in shaping GC dynamics used both wild-type inbred and chimeric mouse models to reveal that this cytokine is spatially segregated, and the main driver of this separation is TACI (reviewed in (Goenka et al. 2014)). Upon antigen binding and cognate interactions, a GC is seeded, and these signals along with IL-21 from T follicular helper (TFH) cells lead to proliferation and TACI down-regulation by GC B cells. In contrast, FO B cells continue to express TACI, allowing them to bind and sequester BLyS. The GC is thereby "insulated" from systemic BLyS and is a BLyS-poor microenvironment relative to the surrounding follicle. However, TFH themselves secrete BLyS, serving as a local source of the cytokine within the GC that is required for efficient selection of high-affinity GC B cell clonotypes - and thus, ultimately, for optimal generation of B_{mem} with high-affinity BCRs and LLPC that secrete antibody with high affinity for the antigen. These observations are reminiscent of the selection process that occurs at the transitional stage during primary B cell development, with the added aspect of localized BLyS production that promotes survival of B cells with appropriate affinity.

TACI-APRIL interactions are key to T-independent (TI) responses (reviewed in Oropallo et al. 2011). Whereas APRIL knockout mice show muted TI responses and reduced class switching to IgG and particularly IgA, APRIL transgenics show enhanced and more durable production of IgM, IgG, and IgA. TACI knockouts likewise have impaired antibody responses to some TI antigens, and fail to maintain wild-type levels of flu-specific plasma cells or IgM, IgG, and IgA antibody following flu infection.

As noted above, TACI, BR3, and BLyS all play key roles in effective T cell-dependent immune responses. LLPC, one product of the GC, require BCMA for generation and maintenance. Both BLyS family ligands are important for this subset: BLyS neutralization has little effect on LLPC, whereas when both BLyS and APRIL are neutralized or eliminated, there is a marked reduction in LLPC numbers (reviewed in (Cancro et al. 2009, Stohl et al. 2011)). The ability to salvage survival through either BLyS or APRIL likely offers a competitive advantage for LLPC over pre-immune subsets in anatomic locales such as bone marrow. Consistent with the idea that APRIL is the primary mediator of plasma cell persistence, SLPC and LLPC are observed in close proximity to cells that elaborate APRIL, such as myeloid cells, macrophages, and osteoclasts.

Memory B cells in mice express TACI and/or BCMA, but not BR3, and those from humans have been reported to express all three BLyS receptors; yet B_{mem} persistence appears to be largely independent of BLyS and APRIL. Nevertheless, unswitched (IgM+ IgD+) B_{mem} are somewhat sensitive to BLyS neutralization in mice, while IgG+ B_{mem} are unaffected. BLyS clearly plays roles in the development and persistence of human B cell memory (reviewed in Karnell and Ettinger (2012)), although, somewhat paradoxically, long-term BLyS depletion does not significantly affect existing memory in SLE patients (Chatham et al. 2012). Together, these observations suggest differences in key BLyS family receptor-cytokine interactions for the persistence of different B_{mem} subsets.

A recently described B cell subset termed age-associated B cells (ABCs) accumulates with age and is associated with autoimmunity in both mice and humans (reviewed in Naradikian et al. (2016)). While the ABC subset is heterogeneous, a shared characteristic is expression of and dependence upon the transcription factor T-bet. ABCs display SHM, suggesting they are antigen-experienced. Although ABCs express both BR3 and TACI (Table 2), they appear to be largely BLyS independent, similar to conventional B_{mem}.

Pathophysiological Roles for BLyS Family Members

Malignant B cells, like their normal counterparts, may also depend on BLyS or APRIL for survival. Myeloma cells are sustained by APRIL produced by osteoclasts; and often, transformed B cells themselves are a source of BLyS, thereby perpetuating their own persistence. Therapeutic antibodies targeting B cells were initially developed with a view to treating B cell malignancies; some, including anti-CD20 (rituximab) and an antibody targeting BLyS (belimumab), have proven useful in treating autoimmune disorders.

A clear link between BLyS and autoimmunity is conclusively demonstrated by studies of murine transgenics, knockouts, and mutants: BLyS overexpression leads to SLE-like disease, including autoantibody formation, immune complex deposition, and proteinuria, while BLyS reduction improves disease symptoms. Likewise, serum BLyS levels are elevated and may correlate with clinical disease in several human rheumatologic disorders including rheumatoid arthritis, Sjogren's syndrome, and inflammatory bowel disease. Intensive research on BLyS as a therapeutic target culminated in development of belimumab, an anti-BLyS antibody therapy approved in 2011 for treatment of SLE (Stohl et al. 2011).

Peripheral tolerance risks are posed by both T cell-dependent and T cell-independent antigen activation (Oropallo et al. 2011; Goenka et al. 2014). Dysregulated GC selection and/or resolution could result in autoreactive clonotypes in B_{mem} and LLPC pools, where they may persist for years. In the case of TI activation via Toll-like receptors (TLRs), signaling cross talk between the BCR and TLR pathways normally leads to an early death as the early SLPC response wanes and the affinity-matured antibody response comes into play; however, there is mounting evidence that an inappropriately prolonged SLPC response, particularly in the context of elevated BLyS, may result in the rescue and recruitment of self-reactive cells into long-lived effector pools. Given the evidence that antigen-experienced subsets such as B_{mem} and LLPC are BLyS independent and therefore not affected by belimumab (Cancro et al. 2009; Chatham et al. 2012), the need for more targeted therapies is apparent.

The ABC subset is associated with autoimmunity in both mice and humans, although causality has yet to be established (Naradikian et al. 2016). Nevertheless, there is mounting evidence in mouse models that ABCs develop in response to antigens that engage the BCR and nucleic acid sensing TLRs, within an inflammatory cytokine milieu. Hence, ABCs may be generated in response to viral antigens as well as self-antigens such as chromatin or apoptotic debris. Their accumulation with age may simply reflect appropriate immune responses to pathogens, whereas in autoimmune-prone genetic backgrounds, they may result from inadvertent or dysregulated SLPC or GC responses.

Mice overexpressing APRIL, and APRIL knockouts, do not exhibit overt pathophysiology; however, this does not preclude a potential pathogenic role for this cytokine. As noted above, APRIL is a survival factor for plasma cells and thus may help to maintain autoreactive clones, thereby contributing to pathogenic autoantibody production. Furthermore, LLPC are recalcitrant to B cell ablation therapies as they lose some of the surface markers targeted by such agents. Therefore, understanding the role of BLyS and APRIL in mediating the survival of potentially long-lived autoreactive LLPC can aid in the development of better therapies. Indeed, mouse studies point to important roles for BCMA in controlling B cell homeostasis, regulating plasma cell differentiation, and maintaining tolerance in an autoimmune-prone

context (Coquery and Erickson 2012). For example, mouse models of lupus that are BCMA deficient display exacerbated symptoms and increased SLPC and LLPC in secondary lymphoid organs. Mutations in TACI are associated with CVID (common variable immune deficiency), and some of these mutations also promote autoimmunity (Romberg et al. 2013). In summary, it is likely that mutations in any of the three BLyS family receptors could contribute to the development, heterogeneity, and penetrance of a range of human autoimmune diseases.

Summary The BLyS family of receptors and ligands plays a pivotal role in maintaining peripheral homeostasis of B cells in both mice and humans. Their influence on differentiation and maintenance of antigen-experienced subsets is also beginning to be appreciated. Apart from regulating selection and life span, the BLyS/BR3 axis is relevant in autoimmunity and is a current therapeutic target.

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BAI3

▶ ADGRB3

BAK (BCL-2 Antagonist Killer), BCL2L7 (BCL-2 Like 7), CDN1

▶ BCL-2 Family

BANF

▶ Merlin (*NF2*)

Basic FGF

▶ FGF (Fibroblast Growth Factor)

Basic Leucine-Zipper Transcription Factor, ATF-Like

▶ BATF

BATF

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Synonyms

B cell activating transcription factor; Basic leucine-zipper transcription factor, ATF-like; B-ATF; SFA-2; SFA2; SF-HT-activated gene-2

Historical Background

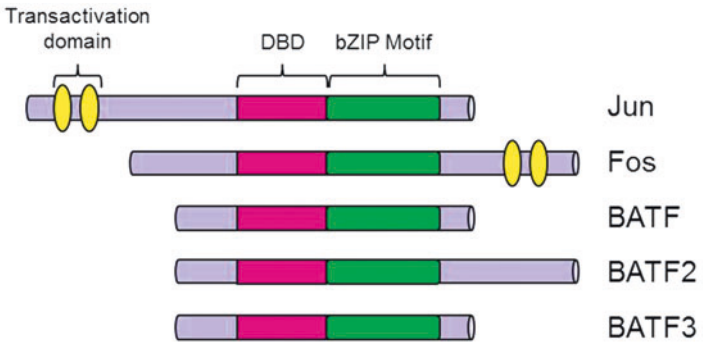
The basic leucine-zipper transcription factor, ATF-like (BATF) was first identified from a cDNA library of Epstein-Barr virus (EBV)-stimulated B cells, as a nuclear protein containing a basic leucine-zipper motif. A strong hybridization band for BATF was detected in polyadenylated mRNA from cell lines and several human tissues such as the lung and Raji Burkitt's lymphoma by Northern Blot analysis (Dorsey et al. 1995). Only a few months later, another study described a new bZIP transcription factor, which they called SF-HT-activated gene 2 (SFA-2), which was found highly expressed in T and B cells, especially when these cells were transformed by human T cell leukemia virus type I (HTLV-I) (Hasegawa et al. 1996).

Both studies showed that BATF does not form homodimers but builds heterodimers preferentially with Jun-family proteins. These dimers can then bind to AP-1-binding sites on the DNA, thereby regulating gene expression (Dorsey et al. 1995; Hasegawa et al. 1996).

Localization and Structure of BATF

In humans, the gene encoding for BATF (125 amino acids) is located on chromosome 14q24 whereas the murine *Batf* maps to chromosome 12q. Human and murine BATF have 96% homology, with only five different amino acids. Highest homology was observed in the bZIP region, as well as on serine and threonine residues in the basic region and the N-terminus, which are important for posttranslational modification of BATF via phosphorylation. In both species, the BATF gene is in close proximity to the genes encoding the bZIP transcription factor JDP2 (Jun dimerization protein 2) and fos (Williams et al. 2001; Murphy et al. 2013).

By comparing the sequences of cDNA and genomic DNA of BATF, three exons were identified. In the first exon (exon I, 304 nt), the initiator codon for translation is encoded, as well as 20 amino acids. In exon II (105 nt), 35 amino acids are encoded, which comprise the basic DNA-binding domain and the first leucine residue of the bZIP domain. The remaining part of this domain is encoded in exon III (530 nt), in addition to 44 carboxyterminal residues. A polyadenylation site is located about 20 nt away from the end of exon III (Meyer et al. 1998).



BATF, Fig. 1 Domain structures of AP-1 family proteins Jun, Fos, BATF, BATF2, and BATF3. All proteins contain a DNA-binding domain (DBD) and a leucine-zipper motif (bZIP). BATF family proteins lack the transactivation domain contained in other AP-1 proteins such as Jun. The bZIP motif facilitates dimerization, AP-1 consensus sequences are bound via the DBD of both factors (Modified from Murphy et al. 2013)

The BATF mRNA is about 0.9 kb, and the translated protein has a molecular weight of ca. 13.7 kDa (Hasegawa et al. 1996).

In contrast to other bZIP proteins, BATF, as well as the related proteins BATF2 (also known as SARI) and BATF3 (JDP-1 or p21^{SNFT}), only contains a DNA-binding domain and the leucine-zipper motif but no transactivation domain (Fig. 1).

Expression and Regulation of BATF

Further studies showed that *BATF* mRNA is expressed mainly in hematopoietic tissues and cells, with low expression in thymus and bone marrow and moderate expression in the spleen, lymph nodes, appendix, and peripheral blood mononuclear cells (PBMC). As there was no detectable expression of *BATF* mRNA in fetal liver, it was assumed that BATF is expressed at later time points in the development of myeloid and lymphoid lineages (Echlin et al. 2000).

This was confirmed later when it was shown that there was no *Batf* expression in embryonic tissue, but an upregulation occurred shortly after birth. Furthermore, differential expression of *Batf* was observed during T cell development in the thymus. Here, analysis of thymocytes revealed *Batf* mRNA expression in CD4$^-$CD8$^-$ double negative (DN) and CD4$^+$ or CD8$^+$ single positive (SP) cells, while CD4$^+$CD8$^+$ double positive (DP) cells showed no detectable expression. Besides thymic expression, *Batf* was also expressed in B cells and CD4$^+$ and CD8$^+$ cells isolated from the spleen (Williams et al. 2001).

In naïve T cells, *Batf* mRNA expression was induced by stimulation with anti-CD3 (αCD3) antibodies in vitro (Williams et al. 2001). In mouse M1 myeloid leukemia cells, it was shown that leukemia inhibitory factor (LIF) and interleukin 6 (IL-6) were able to upregulate BATF expression in a signal transducer and activator of transcription 3 (STAT3)-dependent manner (Senga et al. 2002).

In the absence of IL-6, also IL-1 could induce *Batf* mRNA expression in in vitro differentiated Th17 cells (Ikeda et al. 2014) and under Th9-favoring conditions (IL-4 and transforming growth factor beta, TGFβ), a STAT6-dependent upregulation of *Batf*-mRNA expression was observed (Jabeen et al. 2013). CD8$^+$ T cells that were stimulated with IL-12 in the presence of αCD3/αCD28 as well as B cells stimulated with IL-4 and lipopolysaccharide (LPS) also show increased expression of *Batf* (Betz et al. 2010; Kuroda et al. 2011).

BATF Expression in Different Cell Types

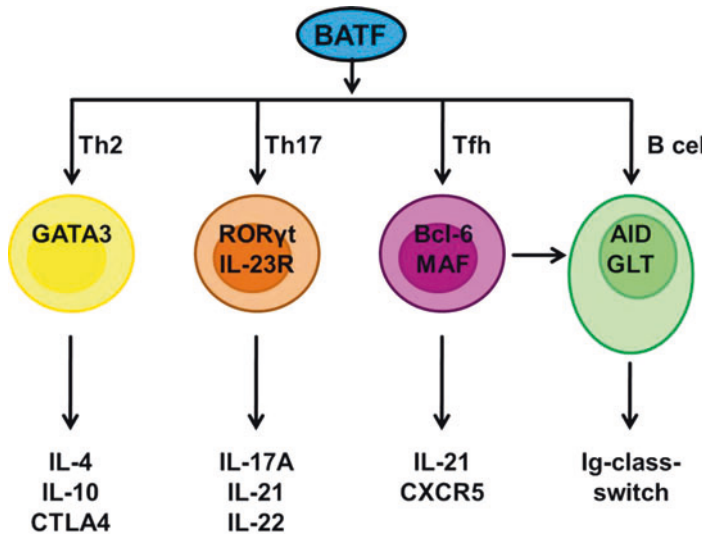
Analyzing models overexpressing or lacking BATF (reviewed in (Sopel et al. 2016)) have contributed to elucidating the role of this transcription factor in different cell types. While BATF

expression has been shown to be induced in hematopoietic stem cells (HSC) by granulocyte-colony stimulating factor (GM-CSF) via STAT3 (Wang et al. 2012), the best characterized cell types expressing BATF are lymphoid cells, especially B and T cells. Figure 2 depicts key targets of BATF in B cells and T cell subsets.

In B cells, BATF has been shown to be important for the class-switching of immunoglobulins (Ig). Here, BATF directly influenced the expression of the gene *Aicda* (encodes for activation induced deaminase, AID) via interaction with a regulatory region behind the transcriptional start sequence and additionally facilitated germline transcripts (GLT) of I-region promoters upstream of the switch-regions. Therefore, mice lacking BATF presented a deficiency in antibodies of switched isotypes, while IgM levels in the serum were comparable to wild-type mice. Furthermore, in the absence of BATF, no germinal centers were observed in the spleen (Betz et al. 2010; Ise et al. 2011).

Overexpression of BATF using different cell-specific approaches revealed a defect in natural killer T (NKT) cell development, while the global deletion of BATF did not impair NKT cell development (reviewed in (Sopel et al. 2016)). Beside NKT cells, also $CD4^+$ and $CD8^+$ T cell development in spleen, lymph nodes, and thymus was not observed to be altered in the absence of BATF (Schraml et al. 2009; Betz et al. 2010).

Regarding $CD4^+$ T cells, first studies using in vitro differentiation approaches in BATF-deficient mice revealed normal differentiation of T helper cells type 1 (Th1) and regulatory T cells (Treg), while the development of Th17 cells was profoundly impaired. Here, e.g., *Rora*, *Rorc*, *Il17*, and *Il21* were identified as target genes of BATF. In addition, $CXCR5^+$ follicular T helper (Tfh) cells were also found diminished in the absence of BATF, with *Bcl6* and *cmaf* being directly regulated by BATF (Schraml et al. 2009; Betz et al. 2010; Ise et al. 2011). Differentiation of Th2 cells in vitro provided contradictory results, which might be



BATF, Fig. 2 Impact of BATF expression on downstream targets in B cells and T cell subsets. BATF has been described to directly bind to the *Gata3* promoter in Th2 cells. Furthermore, it has been shown to influence IL-4, IL-10, and CTLA-4 expression in these cells. Regarding Th17 cells, *Rorc* (encoding for RORγt), *Il23r* (encoding for IL-23 receptor), *Il17a*, *Il21*, and *Il22* have been identified as BATF target genes. In Tfh cells, BATF targets *Bcl6* and *Cmaf* expression with influence on IL-21 and CXCR5. Ig class-switch is mediated by BATF via direct interaction with *Aicda* (encodes for AID) and germline transcription of I-region promoters upstream of switch regions. *AID* activation-induced deaminase, *GLT* germline transcripts (Modified from Murphy et al. 2013)

attributed to different genetic backgrounds; however, later studies using BATF-deficient mice in models of experimental asthma showed decreased Th2-associated cytokine secretion, e.g., IL-4, IL-5, IL-13, indicating a role for BATF in Th2 cells. This has been supported by a recent study where BATF binding to the promoter region of *Gata3*, the main transcription factor of Th2 cells, has been confirmed. Furthermore, in Th9 cell differentiation, BATF has also been revealed as a key factor, as naïve $CD4^+$ T cells in vitro hardly develop into Th9 cells under Th9-favoring conditions, when BATF is lacking (Jabeen et al. 2013; Ubel et al. 2014; Sahoo et al. 2015).

Together, the lack of antibodies with switched isotypes, Tfh and Th17 cells, as well as reduced Th2 and Th9 differentiation made BATF an interesting target to study in allergic diseases such as asthma where it has been shown that mice lacking BATF are protected from developing experimental allergic asthma in different models (Jabeen et al. 2013; Ubel et al. 2014; Sahoo et al. 2015).

BATF in $CD8^+$ T cells has been associated mainly with chronic viral infections. It has been shown that BATF was rapidly induced in a chronic infection with murine lymphocytic choriomeningitis virus (LCMV) and expression was maintained over a long period of time (Quigley et al. 2010). In accordance with this, mice lacking BATF expression and infected with LCMV display an increased viral load in spleen, lung, and liver in concomitance with reduced numbers of virus-specific $CD8^+$ T cells. Furthermore, in the absence of BATF, $CD8^+$ T cells were impaired in their activation, proliferation, and effector maturation under LCMV conditions. These results suggested that BATF, especially in $CD8^+$ T cells, is necessary to control LCMV infections (Grusdat et al. 2014; Kurachi et al. 2014).

Cooperation of BATF-Jun with Interferon-Regulatory Factors

BATF is an AP-1 family protein and as described above dimerizes with Jun proteins. It has been shown previously that these heterodimers preferentially bind to TRE (12-O-tetradecanoylphorbol-13-acetate (TPA)-response element) sequences but can also bind to CRE (cyclic AMP response element) sequences on the DNA (Echlin et al. 2000). Recently, it has been proposed that the BATF-Jun dimer can interact with interferon regulatory factor 4 (IRF4) and that this complex binds to AP-1-IRF composite elements (AICE) in Th17 cells and in $CD4^+$ T cells preactivated and stimulated with IL-21 (Li et al. 2012).

Summary

The AP-1 transcription factor BATF is predominantly expressed in hematopoietic tissues, especially in T and B cells. It is able to form dimers with Jun-family proteins and bind to AP-1 consensus sequences.

Studies using BATF-deficient mice have revealed a crucial role of BATF in the development of follicular helper cells (Tfh) and Th17 cells, as well as in B cells, where BATF facilitates class-switch recombination of immunoglobulins. Recently, it has been shown that BATF also influences Th2 and Th9 responses, especially in allergic diseases, such as experimental asthma. Furthermore, in $CD8^+$ T cells, BATF was associated with antiviral immune responses.

As BATF has been shown to be important for the differentiation of several T helper cell subsets and their cytokine expression, as well as Ig class-switch in B cells, it emerges as an interesting molecule for novel therapeutic approaches for diseases such as asthma, colitis, or other T cell-derived cytokine-driven diseases. Targeting BATF, e.g., by using a small molecule inhibitor, might reduce several effector molecules at the same time, which would be advantageous to blocking single molecules by antibodies. However, as the antiviral immune response mediated by $CD8^+$ T cells is strongly impaired in the absence of BATF, it will be challenging to inhibit BATF expression, e.g., in $CD4^+$ T cells only, as to keep side effects at a minimum.

See Also

▶ IRF4

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B-ATF

▶ BATF

BatK

► CSK-Homologous Kinase

BAX

► Apoptosis Regulator BAX

BAX (BCl2-Associated X Protein), BCL2L4 (BCL-2 Like 4)

► BCL-2 Family

BB2

► Intercellular Adhesion Molecule 1

BB2R

► Gastrin-Releasing Peptide Receptor (GRPR)

BC067047

► P-Rex

Bcl10-Interacting MAGUK Protein 3

► CARMA1

BCL-2 (B-Cell Lymphoma 2)

► BCL-2 Family

BCL-2 Family

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Synonyms

A1 (BCL2-related protein A1), BFL-1, BCL2L5 (BCL-2 like 5); BAD (BCL-2-Associated Agonist of cell Death), BBC6 (BCL2-Binding Component 6), BCL2L8 (BCL2-like protein 8); BAK (BCL-2 antagonist Killer), BCL2L7 (BCL-2 like 7), CDN1; BAX (BCl2-associated X protein), BCL2L4 (BCL-2 like 4); BCL-2 (B-cell lymphoma 2); BCL-W, BCL2L2 (BCL-2 like 2); BCL-XL, BCL2L, BCL2L1 (BCL-2 like 1); BID (BH3 Interacting domain Death agonist); BIK (BH3 interacting Killer), NBK; BIM (BCL-2 Interacting Mediator of cell death), BCL2L11 (BCL-2-like Protein 11), BOD; BMF (BCL-2-Modifying Factor); BOK (BCL-2 related Ovarian Killer), BCL2L9 (BCL-2 like 9); HRK (Harakiri BCL2 interacting protein), DP5 (Neuronal Death Protein-5); MCL-1 (Myeloid Cell Leukemia-1), BCL2L3 (BCL-2 like 3); NOXA, PMAIP (Phorbol-Myristate-Acetate-induced Protein), APR (Adult T cell leukemia-derived PMA-responsive); PUMA (p53 upregulated modulator of apoptosis), BBC3 (BCL2-Binding component 3)

Historical Background

The BCL-2 protein, the founding member of this family of proteins, was discovered in 1985. The gene *BCL2* was identified as the main protagonist in the chromosomal translocation t(14;18) in a subset of B-cell lymphomas, placing it under the control of the promoter of the immunoglobulin heavy chain genes (Cotter 2009). In contrast to previously identified oncogenes that mainly promote cell proliferation, BCL-2 was the first

oncogene shown to inhibit cell death (Vaux et al. 1988). Since then other members of the BCL-2 family have been discovered, and the family of BCL-2 proteins now consists of approximately 20 members. In mammalian organisms, BCL-2 proteins play an essential role in the control of programmed cell death, and in particular apoptosis. Recent investigations have helped to unveil some facets of their regulation and their molecular mode of action on membrane organelles and mitochondria.

Structure and Classification of BCL-2 Proteins

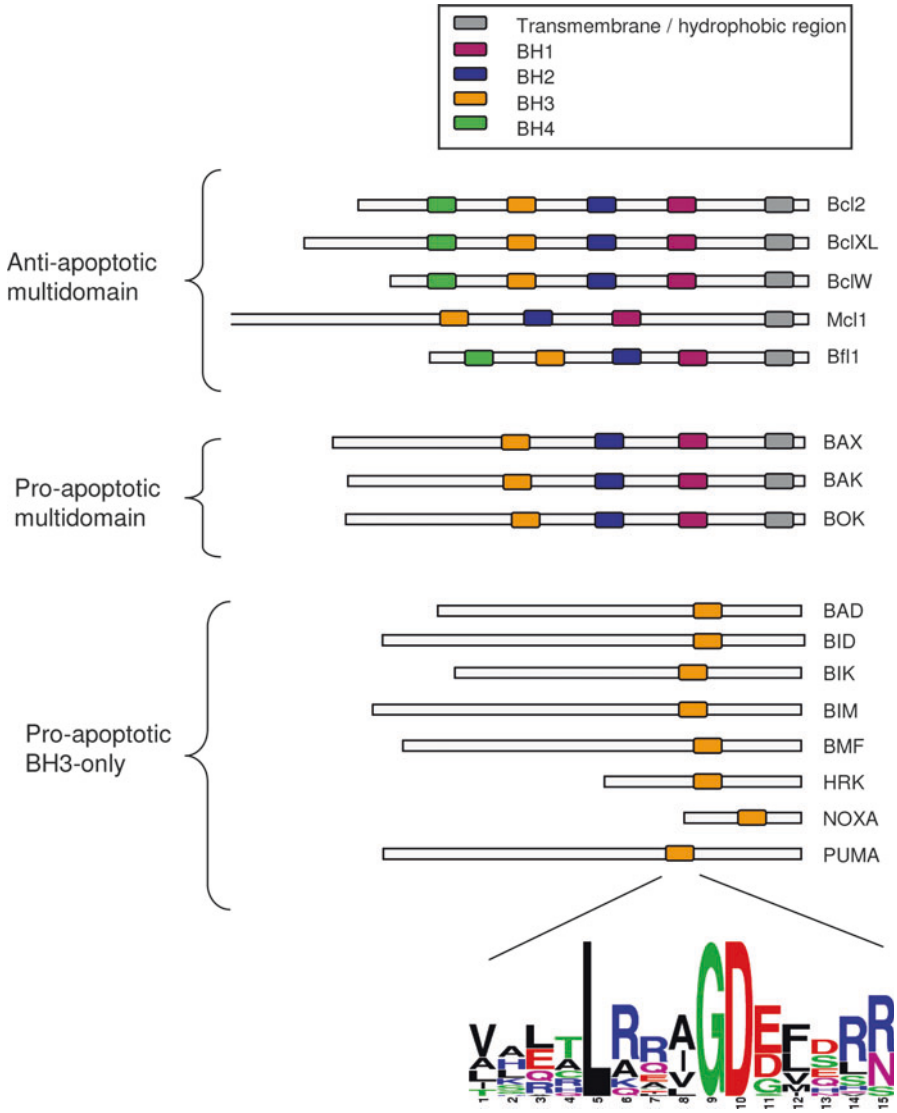
BCL-2 proteins are divided into three groups, based on functional as well as structural criteria (Chipuk et al. 2010): (1) functionally, depending on their effect on apoptosis – either pro- or anti-apoptotic; (2) structurally according to the presence of one or multiple homology domains – all members of the BCL-2 family share one or more regions of sequence homology, called the BCL-2 homology domains 1–4 (BH1 to BH4). The anti-apoptotic proteins of the BCL-2 family constitute the first group of proteins. They are multidomain proteins, usually containing BH1-BH4 domains. This group principally includes the proteins BCL-2, BCL-XL, MCL-1, and A1 (Fig. 1). The corresponding proteins functionally counteract the pro-apoptotic proteins of the BCL-2 family. The pro-apoptotic proteins are divided into two groups: (1) the multidomain pro-apoptotic proteins (BAX, BAK, BOK) of the BCL-2 family, and (2) the BH3-only proteins, containing this sole homology domain (such as BID, BIM, PUMA, BAD, NOXA, BMF, HRK, BIK) (Fig. 1). The BH3 domain is, therefore, the only conserved region of homology among the proteins of the BCL-2 family. While this domain is an essential region for the activity of the BCL-2 proteins, it is short and consists of approximately 15 amino acids organized in an amphipathic helix (Fig. 1). The BH3 domain is also present in proteins that are only loosely connected to the BCL-2 family, such as the proteins MULE and Beclin-1.

Remarkably, despite important differences in their amino-acid sequences, all multidomain proteins of the BCL-2 family possess a similar secondary structure consisting mostly of α-helices and a similar overall fold (Chipuk et al. 2010). This similarity extends to the proteins that have opposing functions, either pro- or anti-apoptotic. In BCL-XL, the spatial juxtaposition of α-helices from the BH1-BH3 regions defines a globular structure with a hydrophobic groove on the surface of the molecule. This hydrophobic groove enables BCL-XL to interact with the BH3 domain of pro-apoptotic proteins. In contrast to the multidomain proteins of the BCL-2 family, the BH3-only proteins are structurally diverse, with the exception of BID, which has an overall fold reminiscent of the multidomain proteins. Members of the BH3-only subset, such as BAD or BIM, tend to be intrinsically unfolded proteins and they probably acquire a stable fold only upon their interaction with other members of the BCL-2 family.

Mitochondrial Membrane Permeabilization by BCL-2 Proteins

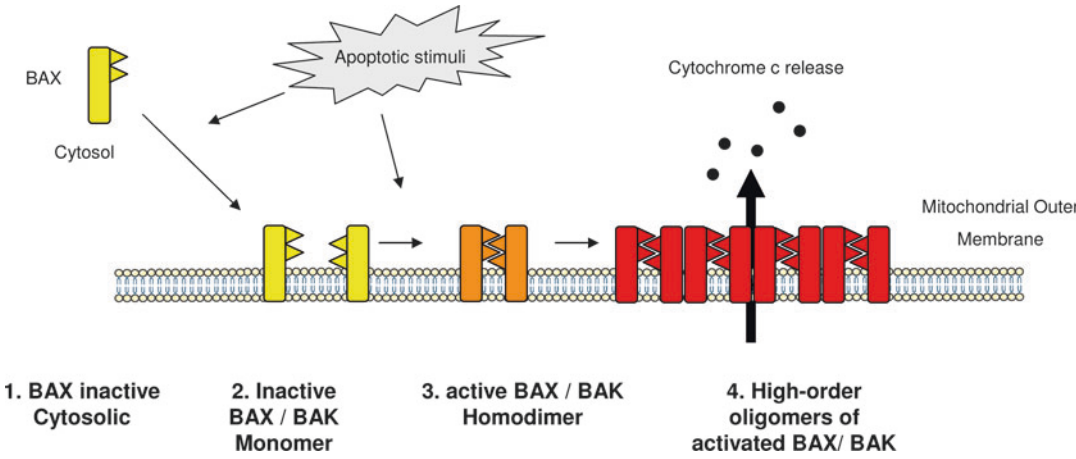
In mammalian cells, mitochondrial outer membrane permeabilization (MOMP) is an early and crucial event during the induction of apoptosis (Tait and Green 2010). The MOMP leads to the release of pro-apoptotic factors, such as cytochrome c, into the cytosol. There, cytochrome c induces a cascade of biochemical events that lead to the activation of caspases, a family of proteases involved in the execution of the death sentence.

The proteins of the BCL-2 family are key players in the MOMP (Kuwana et al. 2002). The pro-apoptotic multidomain proteins of the BCL-2 family, i.e., BAX and BAK, play an essential role in the MOMP through their ability to form membrane-inserted oligomers (Chipuk et al. 2010; Westphal et al. 2010). How BAX and BAK insert and ultimately permeabilize mitochondrial membranes is a complex question and represents the focus of intense research. According to a commonly accepted model, several steps are required for BAX/BAX



BCL-2 Family, Fig. 1 *Structural domains and organization of the BCL-2 proteins.* BCL-2 proteins can be classified according to their pro- or anti-apoptotic effects and the presence of one or multiple BCL-2-homology (BH) domains. The BH3 domain is the only domain of homology shared by all members of the family. It consists of 15 AA with a preference for the motif depicted in the sequence logo in the lower part of the figure (adapted from the server Prosite, http://expasy.org/cgi-bin/prosite/). While the membrane localization domains are indicated here for the multidomain BCL-2 proteins, several members of the BH3-only proteins also possess membrane targeting domains with an affinity for lipids

oligomerization and MOMP (Fig. 2). The first step consists of the mitochondrial recruitment of these proteins. While BAK is constitutively present at the mitochondrial level, BAX is cytosolic in healthy cells. In its cytosolic form, the C-terminal extremity of BAX is sequestered in its BH3-binding pocket and BAX is therefore locked in a monomeric, inactive form. The first step in BAX activation consists of the release of BAX from this intramolecular lock, and this step is a requisite for the insertion of BAX into the MOM. The next step is common to BAX and BAK, and consists of the direct activation of these proteins. Some proteins of the BH3-only



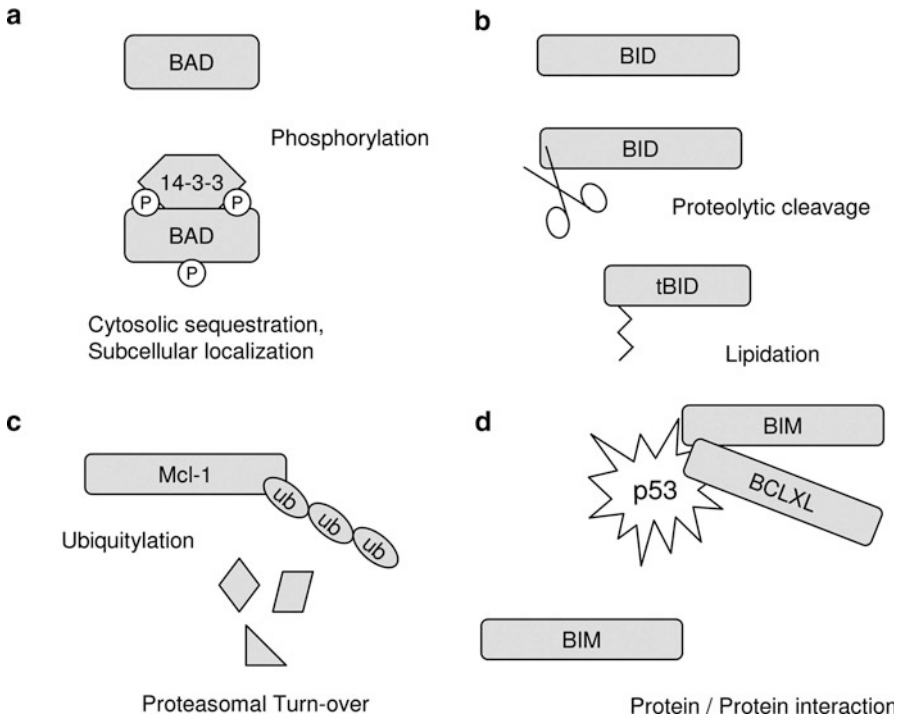
BCL-2 Family, Fig. 2 *A model for the activation of BAX/BAK and the induction of MOMP.* The activation of the pro-apoptotic multidomain proteins BAX and BAK is an essential step that leads to mitochondrial membrane permeabilization and apoptosis. While BAK is constitutively present at the mitochondrial level, BAX is normally cytosolic. The first step in the activation of BAX consists of a cytosolic to membrane translocation, possibly occurring as a consequence of the release of the carboxy-terminal tail of BAX from an inhibitory internal interaction with the hydrophobic groove of this molecule. The second step consists of the activation of BAX/BAK per se and probably results in the shaping of the BH3 domains of BAX or BAK. Reciprocal interactions and homodimer formation are rendered possible once this shaping has allowed reciprocal interactions between their BH3 domains and hydrophobic grooves. Further interactions implicating other parts of BAX/BAK lead to higher order complex- and pore-formation, resulting in MOMP and apoptosis

subset, in particular BID, BIM, or PUMA, can directly activate BAX and BAK through direct contacts (Gavathiotis et al. 2008; Gallenne et al. 2009). The multimerization of BAX and BAK also requires the release of these molecules from the inhibitory effect of anti-apoptotic proteins of the BCL-2 family, such as BCL-2, BCL-XL, or MCL-1. Anti-apoptotic proteins of the BCL-2 family negatively regulate the multimerization of BAX and BAK through two mechanisms: (1) the direct sequestration of BAX and/or BAK, and (2) indirectly, through the neutralization of the BH3-only proteins endowed with the ability to activate BAX/BAK, such as BID (Billen et al. 2008). Overall, the MOMP is a complex process that is intimately associated with the formation of complexes between proteins of the BCL-2 family. While the role of BCL-2 proteins in MOMP is well accepted, many questions about the precise mechanisms still remain, such as the exact nature of the pore formed by BAX and BAK and the contribution of accessory mitochondrial proteins to this process.

Regulation of the BCL-2 Network: Role of the BH3-Only Proteins

Cell survival is the result of a delicate balance between the activities of the pro- and anti-apoptotic proteins of the BCL-2 family. Apoptosis occurs when this balance is tipped over in favor of the pro-apoptotic signal. The BH3-only proteins play an upstream regulatory role in the BCL-2 network. While the proteins of this subset generally stimulate apoptosis, a complex picture of the mode of action of BH3-only proteins has recently emerged.

All BH3-only proteins are able to neutralize the anti-apoptotic proteins of the BCL-2 family, but the interaction of BH3-only proteins with anti-apoptotic BCL-2 proteins is characterized by its selectivity. There are large differences in the interaction spectra among BH3-only proteins (Chen et al. 2005; Certo et al. 2006). Some BH3-only proteins, such as BAD, neutralize selected anti-apoptotic proteins, such as BCL-2 and BCL-XL, while others, such as BIM and PUMA, bind all



BCL-2 Family, Fig. 3 *Posttranslational regulation of proteins of the BCL-2 family.* The BCL-2 proteins are regulated through the direct modulation of their activation status, their subcellular localization, protein stability, or their functional sequestration. Posttranslational modifications, such as phosphorylations, proteolytic cleavage, ubiquitylation, lipidation, interaction with chaperones or with specific molecules are frequently encountered. For example, the protein BAD is regulated by phosphorylation and association with proteins of the 14-3-3 family (panel **a**). BID becomes active upon engagement of death receptors: A proteolytic cleavage by Caspase-8 creates a truncated version of this protein (tBID) and unmasks a site for N-myristoylation of this protein (panel **b**). The protein MCL-1 is regulated by ubiquitylation, a posttranslational modification that controls its turnover through proteasomal degradation (panel **c**). Finally, protein interactions can also regulate the activity of BCL-2 proteins. The protein p53 is able to functionally neutralize the anti-apoptotic proteins of the BCL-2 family, such as BCL-XL or MCL-1, despite the absence of a BH3 domain; by doing so, the cytosolic accumulation of p53 might release pro-apoptotic proteins of the BCL-2 family, such as BIM, from preexisting inhibitory interactions (panel **d**)

pro-survival proteins. Some BH3-only proteins, such as BIM, BID, and PUMA, do not only neutralize the anti-apoptotic proteins of the BCL-2 family, but can also activate the pro-apoptotic proteins BAX and BAK through labile interactions (Gavathiotis et al. 2008; Gallenne et al. 2009). These differences in terms of mode of action of the BH3-only proteins translate into differences in apoptotic potency, and proteins such as BAD behave more as sensitizers toward apoptosis rather than true inducers. The study of how the effector BCL-2 proteins are regulated in living cells has until now been a difficult task. New biochemical as well as functional approaches will certainly help to track the dynamic interactions between BCL-2 proteins, and to clarify the regulation of BCL-2 proteins during the life/death decision.

An important aspect of the regulation of BH3-only proteins is that they are kept under control by specific stimuli. Apoptosis-modulating stimuli operate on each BH3-only protein, via an array of regulations ranging from transcriptional to posttranslational (Fig. 3). For example, PUMA is induced transcriptionally following severe DNA damage, essentially through the activation of the transcription factor ▶ p53 (Yu and Zhang 2008). On the other hand, the BH3-only protein

BAD is phosphorylated and thereby inactivated by pro-survival kinases, such as the kinase cascades RAF-MEK-ERK or PKB-mTOR. The survival of cells requires their constant exposure to trophic factors that activate these cascades, and BAD becomes activated by dephosphorylation in response to growth factor deprivation (Danial 2008). BID is another member of the BH3-only subset whose activity is under regulation through the engagement of a family of cell surface receptors known as death receptors. BID is activated by a proteolytic cleavage generating the truncated, active form of the molecule called tBID. Cellular studies have helped to establish the basics on the regulation of each BH3-only protein and the sentinel function of BH3-only proteins, but the regulation of BCL-2 proteins remains a complex topic, involving several protagonists with different tissue-specific expression patterns and partially redundant functions.

Various Physiological Functions

In addition to the regulation of programmed cell death, proteins of the BCL-2 family regulate several physiological processes. These processes are diverse, and range from the control of mitochondrial morphogenesis and Ca^{2+} fluxes in the endoplasmic reticulum to various aspects of cell metabolism. Cell proliferation and the integrity of the genome are also regulated by BCL-2 proteins, through interactions established with regulatory proteins of the cell cycle and DNA repair machinery. A detailed overview of these mechanisms is clearly beyond the scope of this chapter, but the regulation of autophagy and inflammatory cytokine production by BCL-2 proteins provide two well-known examples. Autophagy is a process whereby cellular macromolecules or organelles become isolated inside cellular membrane and fuse with lysosomes to promote their elimination and recycling of their components. BCL-2 and BCL-XL have been shown to interact with Beclin-1, an essential regulator of autophagy. The interaction between Beclin-1 and BCL-2 is possible because Beclin-1 possesses a BH3 motif (Maiuri et al. 2007). BCL-2 and BCL-XL also play a role in the regulation of the metabolism of inflammatory cytokines, such as Interleukin-1, through molecular interactions established with the inflammasome, an intracellular protein complex involved in the regulation of Caspase-1, the enzyme responsible for the maturation processing of this cytokine (Bruey et al. 2007). The proteins of the BCL-2 family therefore exert pleiotropic effects that extend far beyond the regulation of programmed cell death.

BCL-2 Proteins and Cancer

Reduced sensitivity to apoptosis is one of the hallmarks of cancer cells. Deregulation of BCL-2 protein expression is frequently observed and it was shown to contribute to this disease (Yip and Reed 2008; Frenzel et al. 2009). Overexpression of the anti-apoptotic proteins of the BCL-2 family was the mechanism first reported to account for apoptosis resistance in cancer cells. While it is now well accepted that most cancer cells present a reduced sensitivity to apoptosis due to modulation of the BCL-2 regulatory system, the mechanisms that lead to the altered regulation of BCL-2 proteins are complex. Alterations in the genome of cancer cells, epigenetic mechanisms, and posttranslational modifications often concur to shape the BCL-2 proteome in cancer cells (Yip and Reed 2008; Frenzel et al. 2009).

The regulation of the proteins of the BCL-2 family has attracted considerable attention as a possible approach for cancer treatment. Indeed, inducing tumor regression through the death of cancer cells is the main goal of cancer treatment, and most chemotherapeutic agents are apoptosis inducers in cancer cells (Fulda and Debatin 2006). In a growing number of situations, apoptosis of cancer cells induced by medical treatments was found to depend on the modulation of BCL-2 proteins: treatment-induced apoptosis could either be blocked by the overexpression of anti-apoptotic proteins, such as BCL-XL or MCL-1, or by the reduction of the expression of pro-apoptotic proteins of the BCL-2 family.

For example, colorectal cancer cells with a BAX knockout were found to be insensitive to the commonly used chemotherapeutic agent 5-fluorouracil (Zhang et al. 2000). More recently, specific BH3-only proteins were found to account for cell death induced by specific targeted therapies. For example, the BH3-only protein BAD mediates the apoptotic response of liver cancer cells exposed to the kinase inhibitor sorafenib, currently the only medical treatment for this tumor (Galmiche et al. 2010).

The realization that BCL-2 proteins play a pivotal role in the response of cancers to medical treatments led to intense efforts aiming to identify compounds that would directly target these proteins and could be used as a new line of targeted therapies in oncology. In recent years great advancements have been made along this line, principally with the search for BH3-mimetic compounds that bind the hydrophobic groove formed by BH1-BH3 of the anti-apoptotic BCL-2 proteins, thus favoring apoptosis. To date, the compound with the best characterized BH3-mimetic activity is ABT-737 that was developed by the Abbott laboratories (Oltersdorf et al. 2005). ABT-737 binds with high affinity to the anti-apoptotic proteins BCL-2, BCL-XL, and BCL-W, but not to MCL-1, thus demonstrating a BAD-like reactivity. ABT-737 exerts a strong anticancer activity on Small Cell Lung Carcinoma cells, which frequently overexpress BCL-2 (Oltersdorf et al. 2005). An orally active derivative, ABT-263, has been developed. ABT-263 has shown promising effects in animal models with xenografted tumors, leading to sustained regression and demonstrating the safety of the inhibition of BCL-2 proteins in the entire organism. Studies aiming to test BCL-2 inhibitors in animal models that more closely mimic human tumors are now eagerly awaited. In parallel, the identification of compounds with reactivities that differ from those of ABT-737 as well as the understanding of cancer cell addiction to anti-apoptotic proteins of the BCL-2 family are the focus of future research. BCL-2 proteins have acquired the status of potential targets in oncology, and advances in this field are expected in the coming decade.

Summary

BCL-2 proteins are pivotal regulators of apoptosis. Over the past decade, intense research efforts have helped to better understand how these proteins mutually interact and regulate the mitochondrial membrane permeabilization, a critical step in apoptosis execution. In addition to their role as important effectors, BCL-2 proteins have also emerged as key integrators for the cell signaling pathways regulating programmed cell death. Extensive work still remains to fully understand the functionality of the intricate network of BCL-2 proteins, but recent advances have demonstrated the therapeutic potential of targeting BCL-2 proteins in cancer therapy. The introduction of drugs with a new mode of action, called BH3 mimetics, holds great promise in cancer research. It is also expected to facilitate the exploration of the physiological functions and the regulation of these important signaling molecules.

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Bcl-2-Associated X Protein

► Apoptosis Regulator BAX

BCL2L4

► Apoptosis Regulator BAX

Bcl-2-Like Protein 4

► Apoptosis Regulator BAX

BCL-W, BCL2L2 (BCL-2 Like 2)

► BCL-2 Family

BCL-XL, BCL2L, BCL2L1 (BCL-2 Like 1)

► BCL-2 Family

Bcmd

► BAFF/BLyS Family

BCYM3

► Transient Receptor Potential Cation Channel Subfamily V Member 4 (TRPV4)

BDKRB1

► Bradykinin Receptors

BDKRB2

► Bradykinin Receptors

BDR2

► Hippocalcin

BERG36

► Tristetraprolin (ZFP36) and TIS11B (ZFP36-L1)

BESC

▶ ENaC

Beta Chemokine Exodus-2

▶ CCL-21

Beta Spectrin

▶ Spectrin

Beta-1 Metal-Binding Globulin

▶ Transferrin

Beta-Catenin

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Synonyms

Catenin (cadherin-associated protein), beta 1 (88kD); Catenin beta; Catnb; Ctnnb; CTNNB1

Historical Background

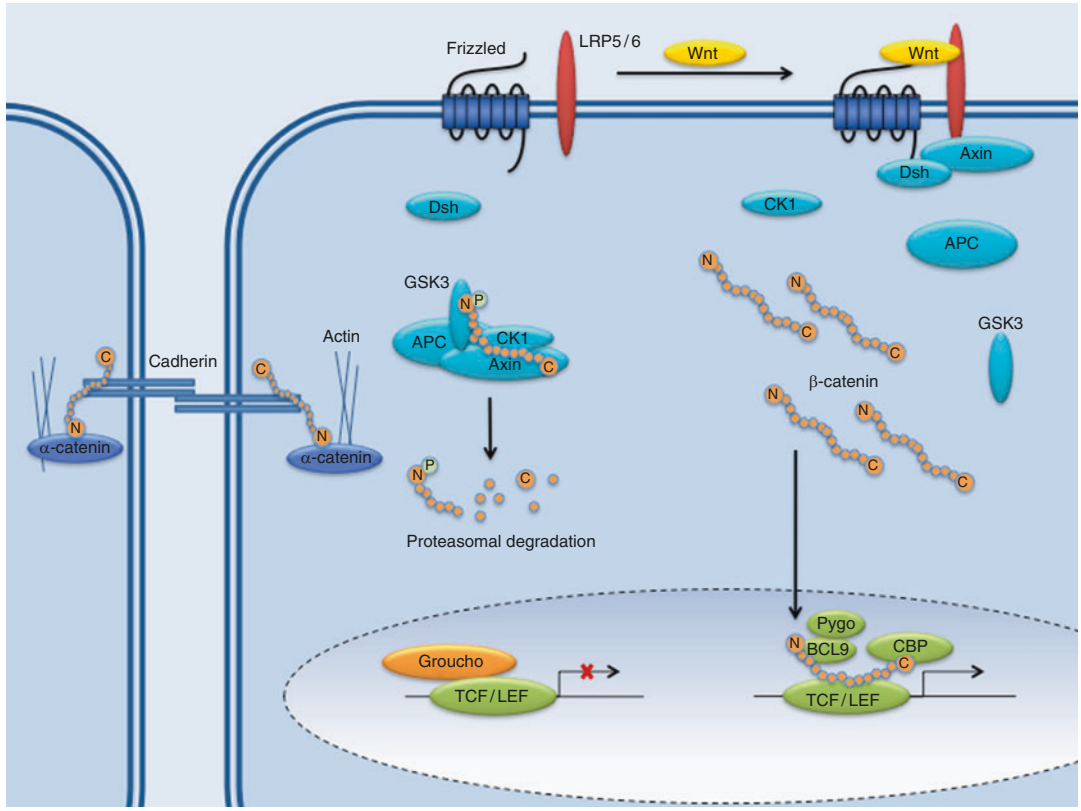
Beta-catenin (β-catenin) (Armadillo in *Drosophila*) is a multifunctional protein involved in two essential cellular events: cell–cell adhesion and the canonical Wnt signaling pathway (Takemaru 2006). β-Catenin/armadillo (Arm) was initially identified as a segment polarity protein in *Drosophila* in the early 1980s, and later recognized as a key downstream effector of the Wnt pathway. Meanwhile, β-catenin was shown to be an integral component of cadherin-mediated cell adhesion complexes. Over the past two decades, interdisciplinary research has tremendously advanced our knowledge of β-catenin function and its involvement in human disorders (Takemaru et al. 2008; Cadigan and Peifer 2009; MacDonald et al. 2009). At cell–cell adhesion junctions, β-catenin interacts with type-I cadherins and α-catenin, which in turn associates with the actin cytoskeleton. In canonical Wnt signaling, β-catenin acts as a transcriptional coactivator through its interaction with transcription factors and cofactors to stimulate expression of target genes. In recent years, aberrant activity of β-catenin signaling has been linked to various diseases, especially cancer.

Structural Features of β-Catenin

Human or mouse β-catenin consists of 781 amino acid residues, harboring a central structural core of 12 Arm repeats, flanked by unique N- and C-termini (Takemaru et al. 2008; Mosimann et al. 2009). The Arm repeat domain is highly conserved between vertebrates and other species but the terminal portions are diverged. The three-dimensional structure of the Arm repeat region has been determined, forming a twisted superhelical structure with a positively charged groove. Many β-catenin-binding partners bind to the Arm repeat domain. The precise structures of the N- and C-terminal tails remain unknown and may not form a rigid structure on their own. β-Catenin is subjected to posttranslational modifications such as ubiquitination, phosphorylation, and acetylation that control its protein stability, subcellular localization, and protein–protein interactions (Verheyen and Gottardi 2010). Plakoglobin (γ-catenin) is a close homologue of β-catenin in vertebrates and can fulfill some of the same functions (Zhurinsky et al. 2000).

β-Catenin as a Key Transcriptional Coactivator in the Canonical Wnt Pathway

β-Catenin is best known for its function as a transcriptional coactivator downstream of



Beta-Catenin, Fig. 1 A simplified current model of the Wnt/β-catenin signaling pathway. β-Catenin has a dual function, acting in both cell adhesion and canonical Wnt signaling. See text for details

canonical Wnt signaling. Wnts are secreted extracellular proteins that play diverse roles in embryonic development and tissue homeostasis, including cell proliferation, cell fate decisions, and stem cell maintenance, as well as cell movement and polarity (Angers and Moon 2009; Cadigan and Peifer 2009; MacDonald et al. 2009). Core components of the Wnt/β-pathway are highly conserved in evolution from primitive cnidarians to humans.

Our current understanding of the Wnt/β-catenin signaling pathway is summarized in Fig. 1. In the absence of a Wnt ligand (Fig. 1, left), β-catenin is captured by the multi-protein "destruction complex," composed of the tumor suppressors Axin and adenomatous polyposis coli (APC), and the protein kinases casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK3). CK1 acts as a priming kinase and phosphorylates β-catenin at serine 45, allowing subsequent phosphorylation at threonine 41, serine 37, and serine 33 by GSK3. Phosphorylated β-catenin is then recognized by the E3 ubiquitin ligase receptor β-TrCP and targeted for ubiquitin-mediated proteasomal degradation. Therefore, under unstimulated conditions, cytosolic β-catenin is maintained at low levels. In the nucleus, the DNA-binding HMG-box T-cell factor/lymphoid enhancer factor (TCF/LEF) proteins keep Wnt target genes off by recruiting transcriptional corepressors such as Groucho (TLE). Extracellularly, the activity of Wnts is regulated by several secreted antagonists including Dickkopfs (DKKs), secreted frizzled-related proteins (sFRPs), and Wnt inhibitory factors (WIFs). Upon engagement with the seven transmembrane frizzled (Fz) receptors and the low-density lipoprotein receptor-related protein (LRP) coreceptors LRP5/6 (Fig. 1, right), Wnts

trigger activation of the cytoplasmic protein disheveled (Dsh) and phosphorylation of the cytoplasmic tail of LRP5/6. This promotes recruitment of Dsh and Axin to the receptor complex at the plasma membrane, resulting in inhibition of β-catenin phosphorylation and degradation. Consequently, β-catenin accumulates in the cytoplasm and then translocates into the nucleus where it displaces Groucho and forms a complex with TCF/LEF transcription factors, leading to activation of Wnt target genes. Thus, activation of the Wnt pathway at the cell surface is ultimately translated into changes in gene expression through the TCF/β-catenin complex in the nucleus.

It is noteworthy that several negative regulators of β-catenin signaling, including APC, Axin, and Chibby (Cby), have been shown to contain both nuclear localization and nuclear export signals that enable them to shuttle between the nucleus and cytoplasm, and facilitate nuclear export of β-catenin (Willert and Jones 2006; Cadigan and Peifer 2009; MacDonald et al. 2009). In contrast, nuclear β-catenin interactors, such as TCF and BCL9/Pygopus (Pygo), appear to retain β-catenin in the nucleus. Detailed information on Wnt signaling can be found on the Wnt Homepage (http://www.stanford.edu/~rnusse/wntwindow.html).

Mechanisms of Target Gene Activation by β-Catenin

β-Catenin exerts its activation potential through assembly of coactivator and chromatin-remodeling complexes (Willert and Jones 2006; Takemaru et al. 2008; Mosimann et al. 2009). The C-terminal activation domain of β-catenin interacts with various positive cofactors such as the histone acetyltransferases CBP/p300, SWI/SNF ATPase subunit BRG1, and Parafibromin (Hyrax; a component of the RNA polymerase II-associated PAF1 complex). On the other hand, the N-terminal portion of β-catenin directly binds to the bridging molecule BCL9 (Legless), which in turn recruits the PHD-finger protein Pygo. Other β-catenin coactivators include TIP49 (Pontin), MED12, TRRAP, MLL1/2, and TBL1/TBLR1. The signaling activity of β-catenin is negatively regulated by its antagonists such as ICAT and Cby. There is also evidence that the TCF/β-catenin complex can function as a transcriptional repressor (Cadigan and Peifer 2009; MacDonald et al. 2009).

A considerable number of direct target genes of the TCF/β-catenin complex have been identified in various model systems including c-Myc, cyclinD1, Axin2, and TCF/LEF (for a comprehensive list of Wnt target genes, see the Wnt homepage). In general, cellular responses to Wnt signals vary significantly among different cell types, and many Wnt/β-catenin target genes are regulated in a cell-type specific manner. There are a number of reagents/tools available to monitor β-catenin signaling activity including cell-based reporters, transgenic reporter animals, and direct β-catenin target genes (Moon et al. 2004; Barker and Clevers 2006; Chien et al. 2009).

β-Catenin at the Crosstalk with Other Signaling Pathways

Besides the canonical Wnt pathway, β-catenin signaling activity is positively or negatively regulated by a variety of other signaling pathways including Akt (protein kinase B), Src, PTEN, p53, NF-κB, epidermal growth factor (EGF), integrin-linked kinase (ILK), insulin-like growth factor (IGF), and prostaglandin E2 (PGE2) (Moon et al. 2004; MacDonald et al. 2009).

In addition to TCF/LEF factors, β-catenin has been shown to serve as a coactivator or, in some cases, a corepressor for many DNA-binding transcription factors including members of the nuclear hormone receptor family and HMG-box-containing Sox proteins (Takemaru et al. 2008; MacDonald et al. 2009). For instance, the vitamin A, vitamin D, and androgen receptors physically interact with β-catenin in a ligand-dependent fashion to potentiate activation of their target genes, while suppressing expression of TCF/β-catenin-dependent genes. Thus, it is apparent that β-catenin, via these transcription

factors, could impact a broader range of gene expression programs.

β-Catenin in Development and Disease

The Wnt/β-catenin pathway has been studied extensively in a wide spectrum of model organisms including *C. elegans*, *Drosophila*, zebrafish, *Xenopus*, and mouse, and proven to be essential for numerous aspects of embryonic development such as segmentation, axis formation, and brain patterning (Cadigan and Nusse 1997; Chien et al. 2009). In mice, β-catenin deficiency results in embryonic lethality at the gastrulation stage (Grigoryan et al. 2008). Over the last decade, through the use of conditional mouse models, β-catenin has been activated and inactivated in various tissues in a temporal and tissue-specific manner (Grigoryan et al. 2008). These studies revealed important roles of Wnt/β-catenin signaling in development and homeostatic maintenance of many organs. In adults, Wnt/β-catenin signaling is crucial for maintaining self-renewal of pluripotent stem cells in skin, blood, intestine, and brain, and for tissue regeneration and repair following injury (Reya and Clevers 2005; Clevers 2006; Stoick-Cooper et al. 2007). Remarkably, recent studies identified the Wnt/β-catenin target and orphan receptor Lgr5 (GPR49) as a marker for stem cells in the adult intestinal epithelium and hair follicle (Barker and Clevers 2010).

More recently, dysregulation of Wnt/β-catenin signaling activity has been linked to the pathogenesis of a wide range of human diseases such as bone density defects and cancer (Logan and Nusse 2004; Clevers 2006; MacDonald et al. 2009).

Loss-of-function mutations in the Wnt coreceptor LRP5 are associated with osteoporosis-pseudoglioma syndrome (OPPG) characterized by low bone mass and loss of vision. Conversely, activating mutations in LRP5 cause increased bone density. These findings clearly demonstrate that Wnt/β-catenin signaling positively regulates bone formation.

Constitutively activated β-catenin signaling, due to loss-of-function mutations in APC or Axin or gain-of-function mutations in β-catenin itself, is associated with a variety of human malignancies including melanoma and colon and hepatocellular carcinomas (Polakis 2000; Takemaru et al. 2008). Remarkably, greater than 70% of colon cancers show aberrant Wnt/β-catenin signaling activity. Mutations in APC or Axin compromise their function within the β-catenin destruction complex, while oncogenic mutations in the N-terminal regulatory domain of β-catenin block its degradation via the ubiquitin-proteasome pathway. In addition, some tumor types show loss of expression of the secreted Wnt antagonists sFRPs and WIF1 due to epigenetic silencing by hypermethylation (Barker and Clevers 2006; Takemaru et al. 2008). All of these alterations ultimately lead to stabilization and nuclear translocation of β-catenin, followed by activation of target gene expression. Hence, β-catenin is an attractive molecular target for cancer therapeutics as well as other Wnt-related diseases. To date, small molecules that disrupt TCF/β-catenin or CBP/β-catenin interaction or stabilize Axin protein and therefore inhibit β-catenin-dependent transcription have been reported (Moon et al. 2004; Barker and Clevers 2006; Takemaru et al. 2008).

Summary

β-Catenin plays crucial roles in diverse biological processes as a pivotal component of cell–cell adhesion and Wnt signaling. It serves as a protein network hub by mediating numerous protein–protein interactions to ensure proper development and homeostasis of multiple tissues. Recent advances in genome-wide RNAi screens and proteomics approaches greatly facilitate the identification of novel β-catenin regulators (Angers and Moon 2009). The realization that β-catenin signaling is perturbed in various human diseases continues to fuel worldwide research efforts in the future. Certainly, a better understanding of β-catenin functions has broad impact on human diseases, stem cell biology, and regenerative medicine.

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Beta-Chemokine Exodus-2

▶ CCL-21

Betaine/GABA Transporter-1 (BGT-1)

▶ GABA Transporters

Beta-Nerve Growth Factor

▶ NGF

Beta-NGF

▶ NGF

Bex

▶ BEX3

BEX3

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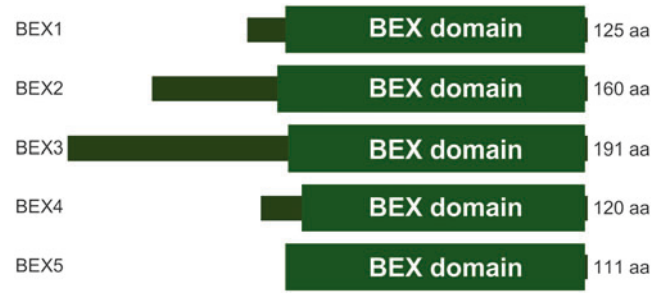
Synonyms

Bex; DXS6984E; HGR74; NADE; NGFRAP1



BEX3, Fig. 1 BEX family proteins. All BEX family proteins share a characteristic BEX domain. BEX2 and BEX3 have an uncharacterized N-terminal region

Historical Background

The Brain-Expressed X-linked (BEX) is a family of five proteins including BEX1, BEX2, BEX3, BEX4, and BEX5 (Kazi et al. 2015). BEX-family proteins are characterized by a highly conserved BEX domain (Fig. 1). Function of the BEX domain is still poorly understood. Recent studies identified a role of BEX domain containing proteins in growth control. All BEX genes cluster to the human Xq22 chromosome. BEX3 was the first BEX-family protein described and was initially named HGR74 (Rapp et al. 1990).

BEX3 Gene and Spice Variants

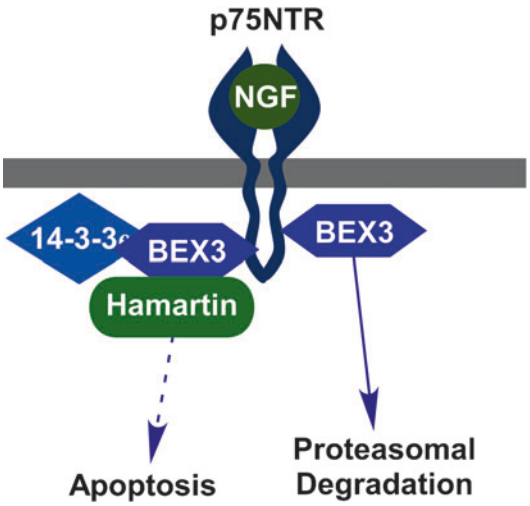
Human BEX gene encodes three splice variants known as BEX3 isoforms a, b and c (Fig 2). Although all three isoforms contain an intact BEX domain, isoform c has an N-terminal uncharacterized region with proline-rich (PXXP) motif, indicating that isoform c might associate with SRC-homology 3 (SH3) domain-containing proteins. BEX3 expression was described in various human tissues including testis, prostate, ovarian granulosa cells, and seminal vesicles (Rapp et al. 1990). The mouse homolog of BEX3 is known as Nerve Growth Factor Receptor Associated Protein 1 (NGFRAP1). Mouse BEX3 was identified as a nerve growth factor receptor (p75NTR)-associating protein and was named NADE (Mukai et al. 2000). In mouse, BEX3 expression has been described in brain, heart, lung, stomach, small intestine, and muscle tissues (Mukai et al. 2000). BEX3 expression was also described in pillar cells (Sano et al. 2001), and higher expression was detected during mouse embryonic development (Sharov et al. 2003). Mouse BEX3 has a short sequence of nuclear export signal (NES). Therefore, BEX3 is localized both to the cytosol and also to the nucleus (Mukai et al. 2000; Alvarez et al. 2005).

BEX3 in Neurotrophin Receptor Signaling

The nerve growth factor (NGF) and its receptor (p75NTR) have been well studied for their involvement in development and maintenance of the nervous system (Descamps et al. 2001). BEX3 interacts with the transmembrane receptor p75NTR in response to NGF in PC12 cells (Mukai et al. 2000) and in cortical neurons (Park et al. 2000). NGF-induction elevates BEX3 expression in oligodendrocytes (Mukai et al. 2000). The association of BEX3 with p75NTR is mediated through the death domain of p75NTR and is required for NGF-induced apoptosis (Mukai et al. 2000; Mukai et al. 2002). Furthermore, BEX3 associates with the adaptor protein 14-3-3ε (YWHAE). The 14-3-3 family proteins associate with phosphoserine and phospho-threonine containing proteins, regulating a wide range of cellular processes including apoptosis, development, proliferation, and signal transduction (Zhao et al. 2011). BEX3 and 14-3-3ε complex formation is necessary for NGF-induced p75NTR/BEX3-mediated apoptosis in oligodendrocytes and PC12nnr5 (Kimura et al. 2001). The tuberous sclerosis complex 1 (TSC1) gene product Hamartin forms complex with BEX3, and siRNA-mediated knockdown of TSC1 gene in PC12h cells was shown to protect cells from

BEX3, Fig. 2 BEX3 splice variants. BEX3 gene encodes three different splice variants. All three isoforms have a functional BEX domain. Isoform c is the longest BEX3 isoform having a N-terminal region with proline-rich (PXXP) sequence

NGF-induced apoptosis indicating that BEX3-Hamartin association is also required for NGF-induced apoptosis in PC12 cells (Yasui et al. 2007). Therefore, it is likely that BEX3 forms a multi-protein complex to induce apoptosis in PC12 cells in response to NGF. Another NGF receptor, the tropomyosin-related kinase A (TRKA) constitutively binds to BEX3 and both TRKA and BEX3 are expressed in embryonic rat dorsal root ganglia (DRG) neurons, further suggesting a role of BEX3 in neuronal development (Calvo et al. 2015). BEX3 has two boxes which contain sequences that are targets for ubiquitination. Expression of BEX3 was detected in PCNA and PC12 cells after inhibition of proteasome activity suggesting that BEX3 is a target of ubiquitination-dependent degradation (Mukai et al. 2000; Mukai et al. 2003). Furthermore, BEX3 was found to be rapidly degraded in the proteasomes (Mukai et al. 2000; Alvarez et al. 2005). Collectively, the current data suggest that BEX3 forms multi-protein complexes to induce apoptosis (Fig. 3).



BEX3, Fig. 3 BEX3 in NGF receptor signaling. Upon NGF-stimulation BEX3 binds with NGF-receptor p75NTR and form complex with 14-3-3ε and Harmartin inducing apoptosis

Role of BEX3 in Cancer

BEX proteins play differential roles in cancer. BEX1 has been shown to be a tumor suppressor while BEX2 acts as an oncogene (Kazi et al. 2015; Lindblad et al. 2015). There are a few studies describing the involvement of BEX3 in cancer. The human ovarian carcinoma cell line (PA-1) and the mouse teratocarcinoma cell line (F9) express BEX3 (Kim et al. 2004). In PA-1 and F9 cell lines, BEX3 was found to be associated with mitochondria suggesting a role in regulation of mitochondrial function. Breast cancer cell lines express the NGF receptors p75NTR and TRKA, as well as BEX3 (Descamps et al. 2001; Tong et al. 2003) indicating a possible involvement of BEX3 in breast cancer. Overexpression of BEX3 in MDA-MB-231 human breast cancer cells suppressed in vivo tumor formation suggesting that BEX3 acts as tumor suppressor in breast cancer (Tong et al. 2003). Furthermore, BEX3 associates with SMAC (Yoon et al. 2004). SMAC is a mitochondrial protein that induces cytochrome c-dependent caspase activation (Du et al. 2000). Association of BEX3 with SMAC inhibits XIAP-mediated SMAC ubiquitination but promotes

TRAIL-induced apoptosis in MCF7 breast cancer cells (Yoon et al. 2004). Thus, in breast cancer BEX3 appears to be a proapoptotic gene.

Summary

Role of BEX proteins in human pathophysiology has not been extensively studied. Current studies suggest that BEX3 plays an important role in signaling by the nerve growth factor receptors. BEX3 induces apoptosis by forming multi-protein complexes in response to NGF stimulation, which is required apoptosis. Therefore, BEX3 expression is required for maintaining basal levels of NGF signaling. Involvement of BEX3 in cancer is also evident. BEX3 acts as a tumor suppressor in breast cancer by inducing apoptosis.

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bFGF

▶ FGF (Fibroblast Growth Factor)

Bgp

▶ CEACAMs

bHLHb19

▶ Transcription Factor 4

bHLHb27

▶ Inhibitor of DNA Binding 4 (ID4)

BID (BH3 Interacting Domain Death Agonist)

▶ BCL-2 Family

BIK (BH3 Interacting Killer), NBK

▶ BCL-2 Family

BIM (BCL-2 Interacting Mediator of Cell Death), BCL2L11 (BCL-2-Like Protein 11), BOD

▶ BCL-2 Family

Bimp3

▶ CARMA1

Binary mRNA

▶ Structural mRNAs

Binding Immunoglobulin Protein

▶ HSPA5

BiP

▶ HSPA5

BIR1

▶ GIRK2

BIRC5

▶ Survivin

BIT

▶ Sirpa

Bitter Taste Receptors

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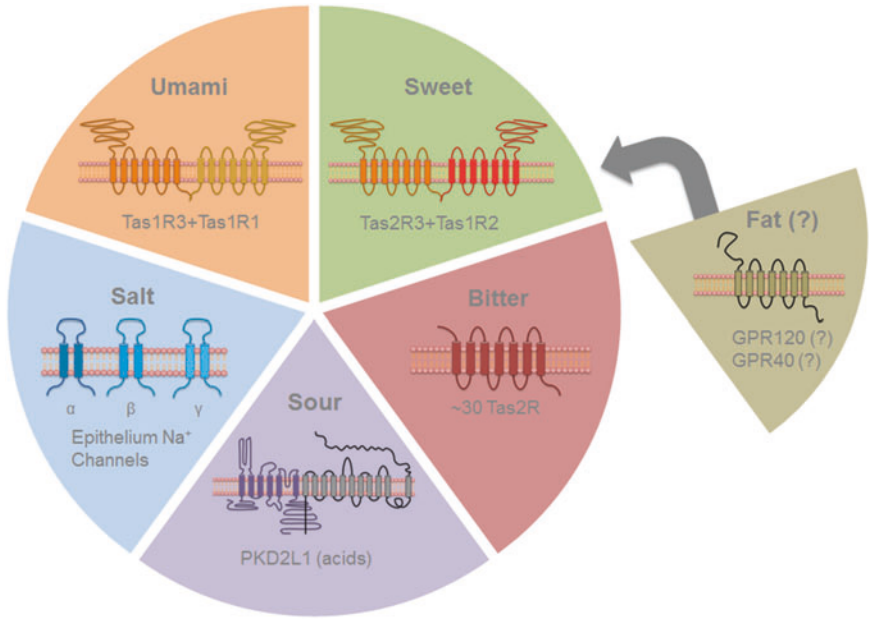
Synonyms

T2R; Tas2R; TAS2R



Bitter Taste Receptors, Fig. 1 The five taste modalities and their receptors. Umami, sweet, and bitter taste receptors are members of the GPCR family, while salt taste perception is mediated by epithelial sodium channels and sour taste perception is mediated by acidic compounds acting at the PKD2L1 receptor. The sixth proposed taste modality, fat, is pictured with the GPR120 receptor, which has been implicated in its function along with GPR40

Historical Background

Mammals are able to detect and interpret five main taste qualities: sweet, salty, sour, umami, and bitter, with a possible sixth modality (fat) having been recently identified (Liu et al. 2011). Like all sensory systems, those involved in the detection of taste are thought to have evolved as a tool to enhance survival in new environments and to increase fitness (Fig. 1).

The molecular basis of bitter taste detection is thought to have evolved for more practical purposes. Plants often produce poisonous secondary metabolites in order to protect themselves from ingestion by predators, and as such mammals, birds, and other animals have evolved the ability to detect which plants and plant material are and are not safe to consume. Bitter taste is detected in humans by ~25 members of the bitter taste receptor (Tas2R) subfamily of G-protein coupled receptors (GPCRs). Since a large proportion of poisonous compounds produced by plants are bitter in taste, the ability to sense bitter taste proved to be advantageous in avoiding harm. However, the correlation between toxicity and bitterness is complicated. Many bitter compounds (such as those found in coffee, beer, and broccoli) are not toxic at concentrations typically consumed, while others even present health benefits such as chemoprotection.

The molecular players responsible for the perception of bitter taste had not been known or understood until the early 2000s: until then it had been hypothesized that there must exist a large family of genes whose products were able to detect bitter compounds, as the chemical entities responsible for evoking bitter taste are structurally diverse (Adler et al. 2000). The first biochemical evidence to prove the existence of these molecules came from Chandrashekar et al. (2000), who used a heterologous expression system to express three candidate taste receptors, mTas2r5 and mTas2r8 from mice, and hTas2R4 from humans, in modified HEK-293

cells. They showed that cells expressing both the mTas2r5 receptor and Gα15 responded specifically to cycloheximide, a compound that is exceptionally aversive to mice, through a G-protein-coupled response resulting in the release of endogenous Ca^{2+} stores at concentrations similar to the sensitivity of cycloheximide-induced aversion in live mice. Additionally, by assaying a selection of 11 human Tas2Rs, hTas2R4 was found to respond significantly to high levels of denatonium and 6-n-propyl-2-thiouracil and was found to be 70% identical in sequence to the mouse bitter receptor mTas2r8. To determine whether mTas2r5 receptor polymorphisms had any effect on ligand binding or corresponded to the *Cyx* cycloheximide-tasting locus, three previously characterized cycloheximide taster strain and one nontaster strain mTas2r5 sequences were isolated and compared to the mTas2r5 taster and nontaster strains DBA/2J and C57BL/6 (Chandrashekar et al. 2000). It was found that all the taster strains had the same mTas2r5 alleles as the DBA/2J strain and that all the nontasters harbored the same alleles as those found in the C57BL/6 strain; additionally, the nontaster strains exhibited a change in cycloheximide sensitivity compared to the taster strains, indicating that mTas2r5 is indeed a detector of the bitter ligand cycloheximide. Coupled with the finding that mTas2r5 associates with the taste transduction G-protein gustducin, the authors demonstrated that the Tas2R family of GPCRs is essential in the transduction of bitter taste stimuli.

Evolution, Genetic Regulation, and Location

The dynamic evolution of bitter taste receptors has been documented in the past using comparative genomics and phylogeny-based methods to detect gains and losses across vertebrate, teleost fish, cetacean, and other species. Feng et al. (2014) found evidence of massive losses of Tas2R and Tas1R genes in their analysis of six toothed-whale species and five baleen species, such that all three members of the Tas1R gene family and 10 Tas2R receptor genes were pseudogenized, with the exception of Tas2R16 in three baleen whale species. Massive pseudogenization or absence of bitter taste receptor genes has also been found in teleost fish (Picone et al. 2014). These discoveries are in accordance with the belief that vertebrate bitter taste receptor gene evolution was heavily influenced by environmental factors, namely due to the changing feeding behaviors of animals (Dong et al. 2009). Herbivorous species of animals would most likely encode and express the largest number of bitter taste receptors as their diets consist of many more bitter molecule-containing foods than omnivores or carnivores. As for the major gene losses in aquatic species such as whales and fish several other reasons have been presented, among them (a) that the high concentration of sodium in the ocean would conceal any bitter tastant that could present itself to taste receptor cells in the oral cavity and (b) that engulfing food whole may have rendered their taste perceiving machinery obsolete (Feng et al. 2014). In contrast, lobe-finned fishes such as the coelacanth species *Latimeria chalumnae* have been the only fish species to date to exhibit a large collection of bitter taste receptors (58) which closer resemble those of vertebrates more than teleost fish. Interestingly, coelacanths not only have the largest repertoire of bitter taste receptors among fish but also among vertebrates, with frogs (49), mice (~36), and humans (~30) rounding out the top four (Picone et al. 2014).

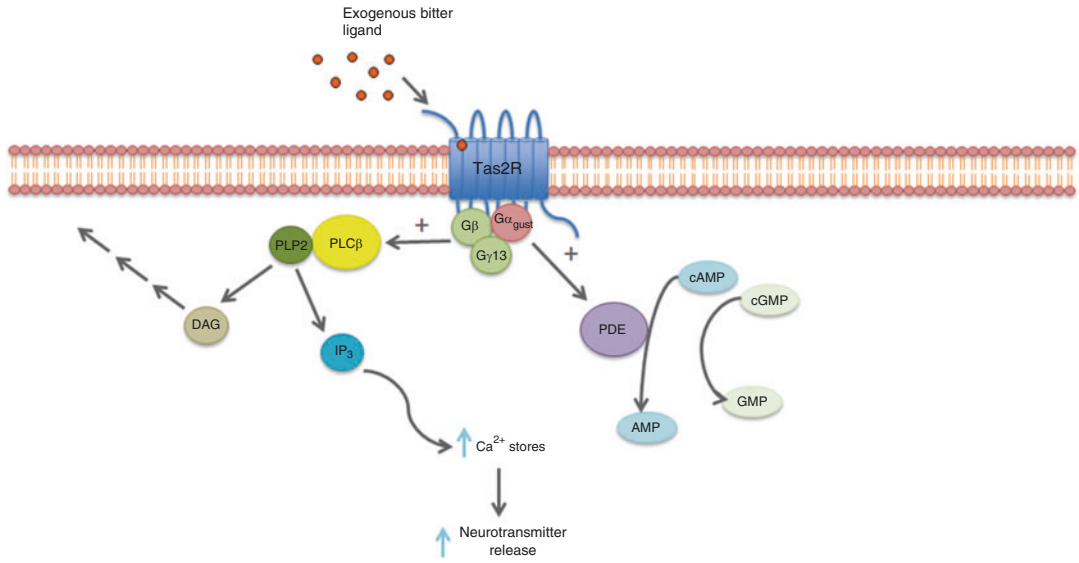
The human bitter taste receptor family consists of 43 Tas2R genes (around 40% of which are pseudogenes), the majority of which are found in two multigene clusters; 10 gene sequences on chromosome 7, and 20 on chromosome 12, while only Tas2R1 is encoded on chromosome 5 (Bachmanov and Beauchamp 2007). Interestingly, the organization of mTas2r sequences in the mouse genome very closely resembles that of humans, where two clusters of mTas2r genes of 10 and 29 sequences are encoded on chromosome 6. The conservation of these motifs has led to the suggestion that the arrangement of Tas2R gene clusters was determined prior to the divergence of primates (Andres-Barquin and Conte 2004).

The Tas2R family of receptors display a low degree of sequence similarity with Class A/rhodopsin-like GPCRs (Di Pizio et al. 2016). As such, they were classified with the frizzled family of GPCRs; however, in most studies they are reported as distant relatives of classical Class A GPCRs. In contrast to the TAS1R family, all Tas2R genes contain no spliceosomal introns. Additionally, Tas2R gene products exhibit short N-terminal extracellular domains and as such are much shorter in length than their Tas1R counterparts (300 amino acids *versus* ~800 amino acids). Tas2R genes, as with Tas1Rs and salt receptors (epithelial sodium channels or ENaCs), are highly conserved across vertebrates; mouse taste receptor genes in some cases share at least 70% sequence identity with their human counterparts (Chandrashekar et al. 2000).

Oral Bitter Taste Perception

Bitter taste receptors in the oral cavity are expressed on type II taste receptor cell (TRC) microvilli, which in turn are bundled into taste buds on the tongue (Avau and Depoortere 2016). Neurophysiological studies have lent credence to two possible modes of ubiquitous expression of Tas2Rs: (a) that Tas2Rs may be co-expressed in the same TRC and that all Tas2Rs may be expressed in any given Tas2R-positive cell or (b) that different Tas2Rs may be selectively expressed in a given TRC (Bachmanov et al. 2014). The majority of human Tas2Rs are responsive to more than one bitter molecule as the number of natural and synthetic bitter molecules far outnumber the amount of receptors present in any given mammalian species (Fig. 2).

Tas2Rs are almost without exception expressed in α-gustducin containing cells, a Gα protein implicated in the transduction of bitter taste signals (Andres-Barquin and Conte 2004). The involvement of α-gustducin in the transduction of bitter taste signals is crucial for full activation and signaling to occur, as demonstrated through the use of mouse knockout models (Wong et al. 1999). However, lacking the α-gustducin subunit does not limit the potential for GPCR activation as bitter taste potentiation may still occur with the help of other Gα protein subunits



Bitter Taste Receptors, Fig. 2 Tas2R signaling in the oral cavity. Exogenous bitter ligand induces the activation of the heterotrimeric G-protein complex and dissociation of Gα-gustducin and Gβ3 and Gγ13. Gβγ lead to the activation of PLCβ and cleavage of PIP2, increasing levels of DAG and IP_3. IP_3 induces intracellular Ca^{2+} release and neurotransmitter release. Activated Gα-gustducin leads to a decrease in cNMP levels

expressed in TRCs. This finding has raised the question of whether or not α-gustducin is simply favored due to relative abundance in TRCs, whether different Tas2Rs are selective for particular G-protein subunits to become fully activated, or other biological factors may play a role in their coupling to bitter taste receptors (Behrens and Meyerhof 2009).

Co-localization and mouse knockout studies were performed early on in the elucidation of bitter taste receptor signaling in order to determine the factors necessary for proper signal transduction of bitter taste stimuli. For signaling to occur, the formation of a heterotrimeric G-protein complex between α-gustducin and Gβ3 and Gγ13 occurs the most often, while some trimers are comprised of Gβ1 subunits (Behrens and Meyerhof 2009). Tas2R stimulation and activation of the G-protein heterotrimer leads to the activation of PLCβ2, whose induction causes an increase in cellular levels of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) via the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP2) (Behrens and Meyerhof 2009). An IP_3-dependent increase in intracellular calcium induces the activation of the TRPM5 transient receptor potential channel, whose induction generates a depolarization across the TRC cell membrane. An action potential is then reached and the resulting neurotransmitters released act on taste nerves enervating to the brain. Additionally, the α-gustducin subunit activates phosphodiesterase (PDE) resulting in a decrease in cellular cNMPs, such as cAMP and cGMP; however, the exact reason for which these changes in cNMPs occur is not well known (Behrens and Meyerhof 2009).

Tas2Rs Beyond the Oral Cavity

Although dubbed "bitter taste" receptors, the Tas2R family of GPCRs have in recent years been discovered in a multitude of tissues outside of the oral cavity including the gut, the airway, the heart, the thyroid, the brain, and breast epithelium, among a list of tissues that is rapidly expanding (see Avau and Depoortere 2016; Shaik et al. 2016), where sensing bitterness would not necessarily be associated with poison sensing.

Taste receptors have been shown to reside on the surface of gut endocrine cells and display bitter tastant-mediated contractility (Avau et al. 2015). The bitter tastant denatonium benzoate induced contractions in human gastric smooth muscle through intracellular calcium release and extracellular calcium influx, while intragastric denatonium administration caused gastric emptying delay. Additionally, healthy human volunteers who were subjected to intra-gastric denatonium benzoate administration displayed increased hunger satiation and a decrease in tolerance of nutrient volume, suggesting that Tas2Rs are involved in a protective negative feedback loop in the gut, whereby ingestion of bitter and potentially toxic compound causes a decrease in alimentary intake (Avau et al. 2015).

Tas2Rs have been documented on both solitary chemosensory cells and ciliated cells in the airway, and display interesting roles in both innate airway immunity and cell autonomous responses. PLC-dependent calcium release and trigeminal nerve stimulation was observed when a broad-acting stimulant of mTas2rs (denatonium benzoate) was applied to isolated mouse SCCs from nasal epithelium, as was a cessation of breathing upon application to anaesthetized rats (Finger et al. 2003). A different response was observed in human SCCs responsive to denatonium benzoate expressing the bitter taste receptor Tas2R47, where bitter agonist stimulation lead to a "calcium wave" which proceeded through gap junctions to other epithelial cells in the nose and stimulated release of antimicrobial peptides involved in preventing increased bacterial colonization (Lee et al. 2014).

In addition to their role in innate immunity of the upper airway, several studies have elucidated the involvement of Tas2Rs in airway smooth muscle contraction. Deshpande et al. (2010) noted that receptors expressed on airway smooth muscle were not only functional and signaled in a calcium-dependent fashion but were able to induce a higher level of bronchial relaxation than a commercially available β_2-agonist. The efficacy

of Tas2Rs in comparison to β_2-adrenergic-induced bronchodilation has been called into question by some but recognized by the majority as having a bona fide therapeutic potential, perhaps most effectively as a combination therapy with existing β_2 agonists.

The expression of Tas2Rs in cancer cells has recently been identified in both breast (Singh et al. 2014) and pancreatic cancer (Gaida et al. 2016). Tas2R4 expression was downregulated by 20–30% in the breast cancer cell lines MDA-MB-231 and MCF-7 when compared to the noncancerous cell line MCF-10A. Functional calcium assays were conducted using quinine, dextromethorphan, and phenylthiocarbamide, showing that although reduced in number, Tas2Rs are functional in breast cancer cells. In pancreatic cancer, Tas2R38 was identified on the surface of lipid droplets, and stimulation of the receptor by phenylthiourea or N-acetyl-dodecanoyl homoserine was found to induce activation of p38 and ERK1/2 while upregulating NFATC1 expression (Gaida et al. 2016). These findings are significant as it could link Tas2Rs with a broad range of disease states, making them possible targets for new cancer therapies.

Summary

Bitter taste receptor research has grown steadily since their identification in the early 2000s, and their discovery in tissues outside of the oral cavity is intriguing. Though many aspects of their biology remain a mystery, such as ligand specificity and their therapeutic potential in diseases such as in the airway, their presence in many areas outside of the mouth lend credence to a more important role than previously thought in a multitude of biological processes in humans and other mammals alike.

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BKB1R

▶ Bradykinin Receptors

BKB2R

▶ Bradykinin Receptors

BKR1

▶ Bradykinin Receptors

BKR2

▶ Bradykinin Receptors

BL34

▶ Regulator of G-Protein Signaling 1 (RGS1)

BL-AC/P26

▶ CD69

BLPI

▶ Secretory Leukocyte Protease Inhibitor (SLPI)

BM-90

▶ Fibulins

BMAL-1

▶ Plasminogen Activator Inhibitor-1

BMF (BCL-2-Modifying Factor)

▶ BCL-2 Family

BMK1

▶ MEK5/ERK5

BMYB

▶ B-Myb

B-Myb

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Synonyms

BMYB; MYB proto-oncogene like 2; Myb-like protein 2; Myb-related protein B, MYBL2; v-myb

avian myeloblastosis viral oncogene homolog-like 2; v-myb myeloblastosis viral oncogene homolog (avian)-like 2

Historical Background

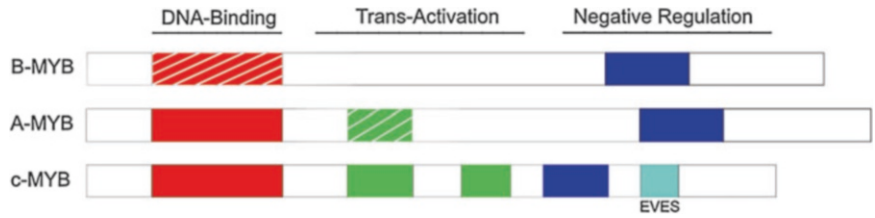
B-MYB is a member of the Myeloblastosis transcription factor (TF) family which is present in all vertebrates. The other members of the family are A-MYB and c-MYB. c-MYB was the first one to be discovered as a homologue of the v-MYB oncogene carried by two different avian leukemia viruses, Avian Myeloblastosis Virus (AMV) and E26 (Fig. 1) which cause acute myeloblastic leukemia and can also transform immature hematopoietic cells in culture.

A-MYB and B-MYB were discovered later via homology to c-MYB. A, B, and c-MYB are structurally very similar with nearly identical DNA binding domains (Fig. 2). In humans, B-MYB gene is located on chromosome 20q13.1.

Although c-MYB was the first to be discovered, B-MYB is believed to be the ancestral progenitor of the Myeloblastosis transcription factor family. Of the three isoforms of MYB, B-MYB is most closely related to the MYB found in *Caenorhabditis elegans*, *Drosophila melanogaster*, and Sea urchins. B-MYB is also suggested to be part of evolutionary conserved protein machinery as it binds to E2F and pRB along with various other proteins in a manner similar to *D.melanogaster* MYB (dMYB).

Unlike A and c-MYB whose expression is tissue specific and dependent on the stage of development, B-MYB is ubiquitous and present in all rapidly proliferating cells at all stages of mammalian development and in the adult.

B-Myb, Fig. 1 Structures of AMV v-MYB and c-MYB. This figure depicts the conserved functional domains present in c-MYB. The *boxes* represent the most conserved domains present among the mouse, chicken, and human proteins. In comparison to c-MYB, the AMV v-MYB protein is truncated at both amino and carboxy terminal and also has many point mutations, indicated by *stars*, all of which affect the transformation activity of AMV-v-MYB. The EVES domain is located near the carboxy terminus and is involved in auto inhibition of c-MYB activity



B-Myb, Fig. 2 Structure of the MYB proteins. The three vertebrate MYB proteins have nearly identical DNA-binding domains (shown as *red boxes*) located near the amino-terminus. The remainder of the proteins diverges between them at other domains. There is some sequence similarity between identity of the trans activation domains of A-MYB and c-MYB (*green boxes*). The most weakly conserved domain is the negative regulation domain indicated by *blue boxes* located near the carboxy terminus

B-MYB in Cell Cycle Progression

A large growing body of work has investigated the key role played by B-MYB in cell cycle progression. B-MYB transcription begins in late G1 phase and is highest during S-phase (Robinson et al. 1996).

There have been several studies demonstrating that B-MYB is an E2F-regulated gene and its expression is regulated by the RB family of pocket proteins acting on the B-MYB promoter. The binding of E2F at the B-MYB promoter along with the p107 and p130 pocket proteins (RB family of proteins) represses B-MYB expression and hence inhibits cell cycle progression/control (W-FLam and JWatson 1993). High levels of B-MYB in S-phase lead to the transactivation of a number of target genes required for cell cycle progression including c-myc, DNA polymerase-α, Hsp70, cdc2, DNA topoisomerase II-α, and B-MYB itself.

B-MYB expressed in S-phase is functionally activated by phosphorylation through cyclinA/CDK2 (Saville and Watson 1998). The phosphorylation of B-MYB potentially activates B-MYB by obstructing the binding of corepressors to the B-MYB promoter thereby increasing transcriptional activity. The level of B-MYB protein is regulated by ubiquitination of phosphorylated B-MYB through the Ubiquitin ligase SKP2 followed by its proteasome-mediated degradation to restrict its presence and activity to only the S-phase of cell cycle.

Another function of B-MYB relevant to cell cycle progression is the direct link to clathrin and filamin, two important components of mitotic spindle fibers. This suggests that genome instability caused due to lack of B-MYB in zebrafish, *Drosophila*, or mice can be due to improper formation of spindle fibers.

DREAM complex:

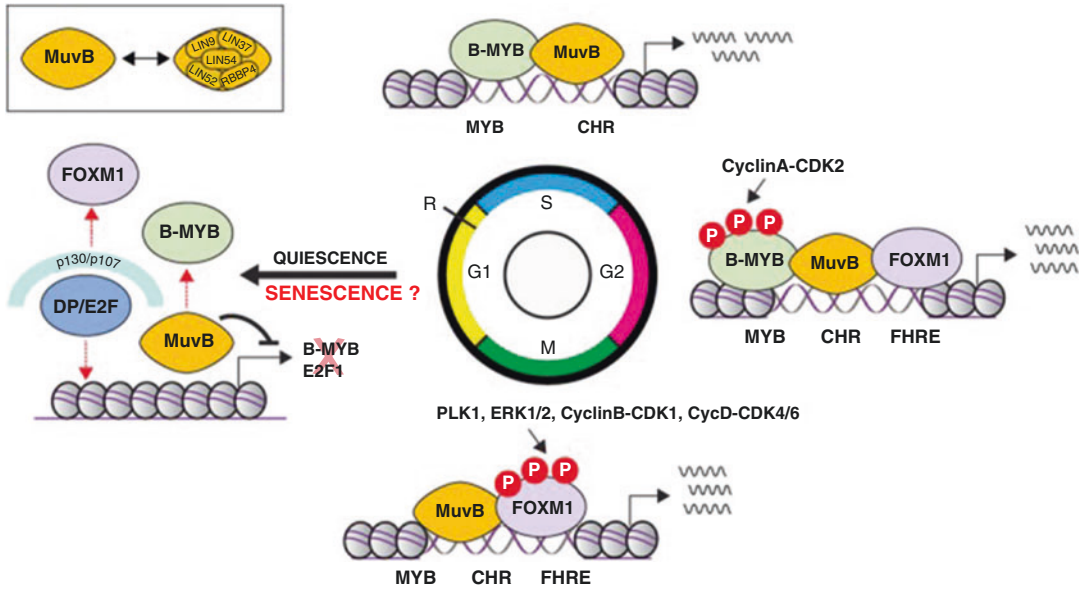
As described by Sadasivam and DeCaprio, the DREAM complex is the master coordinator of cell cycle-dependent gene expression (Sadasivam and DeCaprio 2013). DREAM is a multisubunit complex formed by the assembly of p130 and p107 (RB family of proteins) with Dimerization partner (DP), E2F, and a Multivulval class B (MuvB) core complex which represses most if not all gene expression in quiescence (Litovchick et al. 2007) (Chan et al. 2014). The MuvB core complex comprises of LIN9, LIN37, LIN 52, LIN 54, and RBBP4 (Sadasivam et al. 2012). DeCaprio and colleagues have shown that in mammalian cells, the MuvB core complex dissociates from p130 and sequentially recruits B-MYB, during S phase, and FOXM1, in G2 phase, to activate mitotic gene expression (Schmit et al. 2007) (Sadasivam et al. 2012). Even though the role of the DREAM complex in cellular senescence is not fully understood, studies have shown that disorganization of the DREAM complex leads to suppression of Ras-induced senescence (Litovchick et al. 2011). The components and functions of these complexes are highly conserved in vertebrates, flies, and worms (Sadasivam and DeCaprio 2013) (Fig. 3).

There are a number of published studies describing the involvement of B-MYB in carcinogenesis. This is not surprising as B-MYB plays a crucial role in cell cycle progression and its overexpression is associated with several different types of cancer and aggressive tumor growth. Cytogenetic analysis of many types of cancer has detected amplification of chromosome 20q13, the chromosomal location of B-MYB.

Role of B-MYB in Cellular Senescence and Aging

Cellular senescence is defined as a program of stable growth arrest which normal cells undergo after a finite number of divisions called the Hayflick limit (Hayflick and Moorhead 1961). As B-MYB is required for and promotes cell cycle progression, it indirectly suggested that B-MYB might have a role in preventing senescence. This was demonstrated by studies in which B-MYB inhibition by RNA interference was shown to induce senescence. (Johung et al. 2007).

A considerable amount of work along with previous research in our lab has shown that B-MYB is one of the most highly downregulated TFs upon senescence growth arrest and the



B-Myb, Fig. 3 Role of the DREAM complex in cell cycle regulation: Association of MuvB complex (LIN9, LIN37, LIN52, LIN54, and RBBP4) with different factors at different phases in cell cycle regulates gene expression during the cell cycle. In quiescence, when cells are arrested MuvB binds to p130/p107, E2F4, and DP to form the DREAM complex, which inhibits all cell cycle-dependent gene expression and hence arrest cell growth. When cells exit quiescence, p130 dissociates from MuvB and E2F allowing activator E2Fs to activate genes required for progression through S phase. MuvB binds to B-MYB in S phase to regulate late S phase genes. In G2 phase, MuvB-B-MYB complex recruits FOXM1 followed by proteasomal degradation of B-MYB. Active FOXM1 remains bound to MuvB and regulates the expression of genes required in G2-M transition. Figure from (Mowla et al. 2014)

downregulation was reversed when senescence was bypassed upon inactivation of the p16-pRB and p53-p21 tumor suppressor pathways, two key pathways known to have role in establishing and maintaining senescence. Ongoing research in our lab has found that ectopic expression of B-MYB bypasses senescence in the conditionally immortal human mammary fibroblasts (HMF3); these cells can be induced to undergo senescence synchronously by altering the growth conditions. This suggests that loss of B-MYB expression may have causative role in senescence.

Senescence can be triggered in response to a variety of intrinsic and extrinsic stimuli including: progressive telomere shortening, changes in telomeric structure, oxidative stress, oncogene overexpression, loss of cell contact, and DNA damage. Senescence growth arrest is induced and maintained mainly via p53-p21 and p16-pRB tumor suppressor pathways. There is evidence suggesting that B-MYB can suppress senescence by inhibiting the p16-pRB pathway (Huang et al. 2011). They showed that B-MYB is a transcriptional repressor of the cell cycle inhibitor, $p16^{INK4A}$, suggesting that inhibition of $p16^{INK4A}$ by B-MYB leads to cyclinD/CDK4,6 activation which subsequently phosphorylates and inactivates pRB leading to cell proliferation thereby overcoming cell cycle arrest.

B-MYB is repressed both during quiescence and senescent growth arrest by RB-mediated repression. In quiescence the RB family members p107 and p130 along with E2F4 bind to the E2F site on the B-MYB promoter to form the repressive DREAM complex to repress B-MYB transcription, thereby promoting cell cycle arrest. However, in senescence RB-mediated repression of B-MYB is stronger due to the destabilization of B-MYB mRNA by RB-mediated overexpression of the miR29 and miR30 family of micro RNAs (miRNAs). This suggests that the level of B-MYB might be very important and act

as a deciding factor between cell proliferation, cell senescence, and quiescence. Thus, moderately low levels of B-MYB lead to quiescence whereas extremely low levels of B-MYB, due to miRNA-mediated degradation, manifest senescence whereas high levels of B-MYB lead to cell cycle progression.

B-MYB has recently emerged as a candidate that plays a role in attenuating senescence and as a potential candidate for regulating entry into senescence. It has vital antisenescence qualities due to its role in cell proliferation and growth. Loss of B-MYB expression has an important role in causing senescence growth arrest as silencing of B-MYB expression in primary human foreskin fibroblasts induces senescence (Johung et al. 2007), and overexpression of B-MYB can rescue Ras-induced premature senescence in rodent cells (Masselink et al. 2001).

Other Key Roles of MYB

Cell Death

A number of studies have found that B-MYB plays a role in cell death and have suggested that B-MYB promotes cell cycle progression through the overexpression of antiapoptotic genes namely clusterin, survivin, and BCL2.

Development

Of the three members of the Myeloblastosis family of TFs, B-MYB is the only one found to be present in embryonic stem (ES) cells. It has a critical role in early embryonic development as mice lacking B-MYB die at a very early stage of development as a consequence of defects in formation of inner cell mass in the blastocyst (Tanaka et al. 1999). Along with maintaining the self-renewal capacity of ES cells (Zhan et al. 2012), Tarasov et al. have shown that knockdown of B-MYB in murine ES cells leads to delayed transit through G2/M, severe mitotic spindle, and centrosome defects leading to polyploidy. Loss of B-MYB also leads to a reduction in Oct4 expression which eventually leads to ES cell differentiation as differentiated cells have tight cell cycle checkpoint controls capable of identifying chromosomal abnormalities and promoting apoptosis. (Tarasov et al. 2008).

Nutrient and Metabolic Signaling

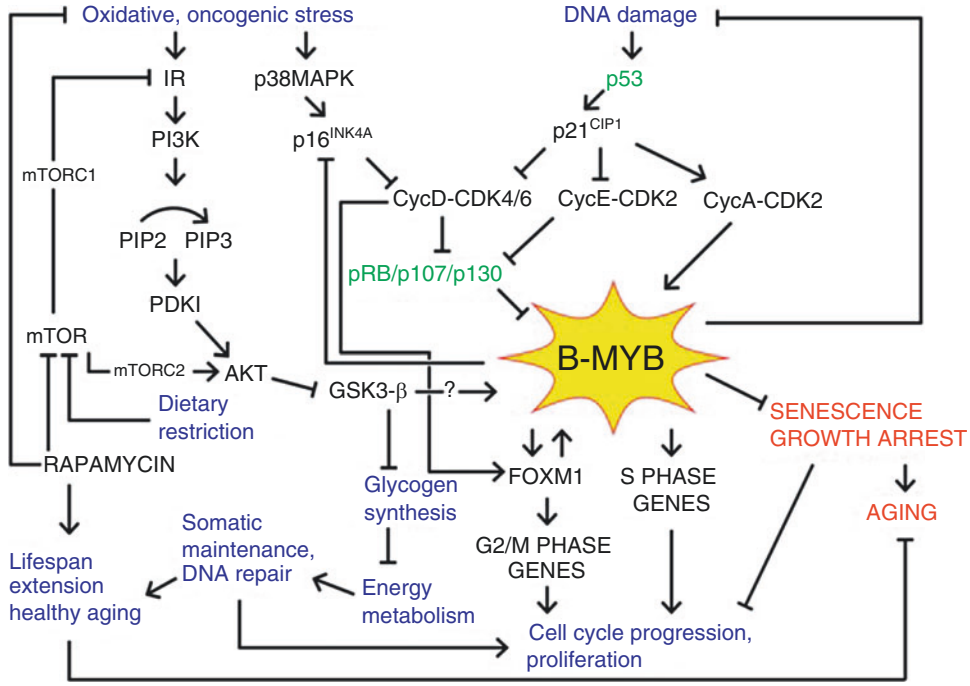
Recently, there has been increasing interest in the role of B-MYB in nutrient and metabolic signaling and linking it to antiaging signaling. Numerous studies have attempted to explain the increase in lifespan of organisms by inhibition of mechanistic target of rapamycin (mTOR) pathway by rapamycin or through dietary restriction. A connection between B-MYB and inhibition of mTOR by rapamycin can be explained by the reduction of oxidative stress and premature senescence which results in an increase in replicative life span by rapamycin thereby connecting this pathway to cell senescence and hence to B-MYB (Li et al. 2012).

There is further evidence suggesting that mTOR can affect the B-MYB pathway in Arabidopsis (Ye et al. 2012). Although until now no evidence has been discovered in mammals this suggests there may be a potential link (Fig. 4).

Summary

B-MYB plays a unique role in maintaining cell homeostasis thereby playing a critical role in a variety of biological processes. Mice lacking B-MYB die very early in development as a consequence of the impaired inner cell mass formation in the blastocyst suggesting a critical role in development. Although B-MYB plays many roles, its key role is in cell cycle progression. DeCaprio and colleagues have shown that in mammalian cells, the MuvB core complex dissociates from p130 and sequentially recruits B-MYB, during S phase, and FOXM1, in G2 phase, to activate mitotic gene expression (Schmit et al. 2007; Sadasivam et al. 2012).

Because of the oncogenic discovery of c-MYB the literature surrounding MYB family of proteins is always skewed towards their oncogenic potential because of which there are only a few studies in literature that deal with the loss of B-MYB in senescence growth arrest. A considerable body of data has shown that



B-Myb, Fig. 4 Schematic representation of the pathways which relate B-MYB to cellular senescence and aging. This shows how loss of B-MYB expression can lead to senescence growth arrest and therefore assist in aging. It also demonstrates how DNA damage, dietary restriction, and oxidative/oncogenic stress may lead to senescence growth arrest and aging through B-MYB. Figure from (Mowla et al. 2014)

repression of B-MYB expression can prevent proliferation in both normal and tumor cells. As B-MYB is suggested to have causative role in senescence, more research is needed to better understand mechanisms on how B-MYB blocks senescence and is integrated into senescence-inducing pathways.

Levels of B-MYB expression maintained and regulated by p53-p21 and p16-pRB pathways critically determine if a particular cell will undergo proliferation, quiescence, or apoptosis. This poses the further question: Can levels of B-MYB itself be used as an informative biomarker?

Studies have shown that removal of senescent cells can prevent or delay age related tissue dysfunction and extend health span (Baker et al. 2011). Although there is much work on the importance of DREAM complex in quiescence and its role in repressing cell cycle-dependent gene expression, very little research has focused on role of DREAM complex in senescence. So it is necessary to further examine the role of the DREAM complex in cellular senescence.

Evidence in the literature suggests that there may be a link between B-MYB and FOXM1. They have common downstream targets such as CCNB1, PLK1, and AURK1, which are required for progression into mitosis. Their levels are also repressed during G0, and their activities are regulated by cell cycle-dependent phosphorylation. They also undergo cell cycle-dependent ubiquitin-mediated proteasome degradation. Further research is required to establish a potential link between B-MYB and FOXM1.

It is recommended that further research be undertaken to determine the role of B-MYB in preventing senescence and identifying downstream targets, particularly those targets that may be involved in the stability of senescence arrest. These targets will represent novel, important and direct targets for developing new therapies that promote healthier aging and increase vitality of

the older population through stimulating regeneration, repair, and wound healing, while retaining the tumor suppressor properties of senescence, if possible. These targets will also be new therapeutic cancer targets, for developing small molecule inhibitors and activators aimed at inducing senescence in tumors. Even though targeting TFs is challenging, it may be possible to develop therapeutics targeting B-MYB itself because of its extensive posttranslational modifications.

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BNSP

► Osteopontin (*Spp1*)

BOK (BCL-2 Related Ovarian Killer), BCL2L9 (BCL-2 Like 9)

▶ BCL-2 Family

Bone Sialoprotein 1

▶ Osteopontin (*Spp1*)

Bp50

▶ CD40

Bpk

▶ BTK

BPTP3

▶ Tyrosine-Protein Phosphatase Nonreceptor Type 11 (PTPN11)

Bradykinin Receptors

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Synonyms

B1BKR; B_1R; B2BKR; B_2R; BDKRB1; BDKRB2; BKB1R; BKB2R; BKR1; BKR2; Kinin B_1; Kinin B_2

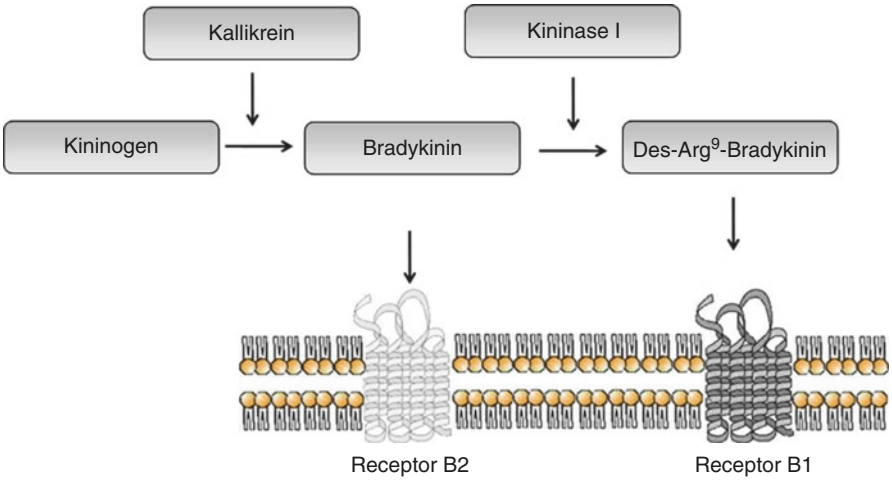
Historical Background

A primary mediator of inflammation, the nonapeptide bradykinin (BK) is a pharmacologically active peptide of the kinin group released in tissues and circulation as a consequence of coagulation cascade activation, more specifically by the kininogen cleavage by kallikrein. The enzyme kallikrein was described in 1930 by Werle and Frey. It was the first component of the kallikrein-kinin system (KKS) discovered, followed by the identification of bradykinin (BK) by Rocha e Silva and colleagues in 1949. In 1970s Regoli and coworkers characterized molecularly the two subtypes of kinin receptors B_1 and B_2, based in their pharmacological and expression profiles differences (Fig. 1). These findings enabled the subsequent development of different agonists and antagonists for these receptors (Leeb-Lundberg et al. 2005). The genes encoding these receptors were cloned in 1990s and after that, animal models for the study of this system were generated: the B_2 knockout mice (Borkowski et al. 1995), the B_1 knockout mice (Pesquero et al. 2000), and the knockout mice for both kinin receptors (Cayla et al. 2002).

Structural Aspects

The BK receptors are typical G protein coupled receptor (GPCR), consisting of a single polypeptide chain that spans the membrane seven times, with the N-terminal domain being extracellular and the C-terminal domain being intracellular. These receptors are present in different species of mammals like human, monkey, rats, mice, rabbit, and others.

In humans both receptors, B_1 and B_2, are homologues preserving 36% of identity at the amino acid level (Leeb-Lundberg et al. 2005). These receptors are encoded by three-exon genes. B_1 receptor gene is in tandem with the B_2 receptor gene, located sequentially (5′ direction) separated by only 12 kb at cromossome 14q32 in humans. This composition can vary between



Bradykinin Receptors, Fig. 1 Kallikrein-kinin system

species like the deletion of exon 2 in mice (Cayla et al. 2002).

Knockout animal models of each kinin receptor gene by homologous recombination have been done. The B_2 receptor knockout mice are fertile, apparently healthy, and when smooth muscle or neurons of these mice are stimulated with bradykinin they failed to produce response (Borkowski et al. 1995). The B_1 receptor knockout mice are healthy, fertile, normotensive, and they are analgesic in behavioral tests of chemical and thermal nociception (Pesquero et al. 2000). The generation of a knockout mouse of both receptors ($B_1B_2^{-/-}$) was also done. Due to the fact that both genes are in close chromosomal position, $B_1B_2^{-/-}$ mice could not be obtained by simple breeding of the single knockout lines. The B_1 receptor gene was inactivated in embryonic stem cells derived from B_2-deficient animals. These animals are normotensive and protected from endotoxin-induced hypotension (Cayla et al. 2007). Recently, another model of double-knockout of kinin receptors was generated by complete deletion of the gene locus (Kakoki et al. 2010).

Pharmacological Aspects

Kinins are locally released from their origin molecules, the kininogens, as a result of limited proteolysis by a class of serine proteases called kallikreins. The metabolite generated is the nonapeptide bradykinin or a decapeptide, kallidin (Lys-BK). Kinins cleavage by the kininase II also named angiotensin converting enzyme (ACE) generates inactive metabolites terminating bradykinin activity. The action of carboxipeptidases on kinins generates des-Arg^9-BK (DBK) or Lys-des-Arg^9-BK (Lys-DBK). The B_2 receptor has high affinity for the intact kinins, those generated by either plasma or tissue kallikreins, BK and Lys-BK, in all mammalian species. B_1 receptor responds to different kinin metabolites, either DBK or Lys-DBK, generated by arginine carboxypeptidases, such as carboxypeptidase N and M. In humans, plasma kallikrein forms BK, whereas tissue kallikreins form kallidin. In rodents, both plasma and tissue kallikrein generate BK. Receptor affinity for agonist ligands: B_2 receptor, BK $\approx$ Lys-BK $>>$ des-Arg^9-BK and Lys-des-Arg^9-BK; B_1 receptor, Lys-des-Arg^9-BK $>$ Lys-BK $\approx$ des-Arg^9-BK $>>$ BK (Leeb-Lundberg et al. 2005).

Peptide antagonists for the kinin B_1 receptor were the first antagonists generated based on modifications of the agonist structure, such as [Leu8] des-Arg^9-BK and Lys-[Leu8]des-Arg^9-BK. The search for antagonists showed that the spatial orientation of the C-terminal region of the peptide molecule is critical for antagonism. Many antagonists the for B_2 receptor have been generated.

The most known peptide antagonist is the icatibant or HOE-140. Non-peptide ligands for the kinin receptors have been designed and are yet a great field of study, since peptides are generally poor drugs for oral bioavailability and brain penetration (Leeb-Lundberg et al. 2005).

Signaling Pathways

In different species both kinin receptors are identified as seven transmembrane G protein coupled receptor. Various signal transduction mechanisms have been described for kinins depending on the cellular type. BK or DBK stimulates B_2 or B_1 receptors, respectively. Through the phospholipase C pathway (by Gq activation), kinin signaling leads to inositol 3-phosphate (IP_3) generation and intracellular calcium mobilization, whereas through the ▶ phospholipase A_2 pathway (activated through Gi or calcium-dependent mechanisms) it leads to arachidonic acid release, also by activating the endothelium nitric oxide synthase (eNOS) and producing nitric oxide (NO). B_2 receptor has also been found to directly interact with other eNOS in a G protein-independent manner (Leeb-Lundberg et al. 2005).

BK also transiently promotes tyrosine phosphorylation of ▶ MAP Kinases and activates a Janus-activated kinase/STAT (JAK-STAT) pathaway. This involves tyrosine phosphorylation of both the Janus-activated kinase family tyrosine kinase Tyk2 and STAT3 followed by STAT3 nuclear translocation. B_2 activates multiple transcription factors that regulate the induction of several cytokines involved in tissue injury and inflammation as well as B_1 receptor induction. Besides these classical pathways, IL-1β and ▶ TNF-α can stimulate the expression of B_1 and B_2 receptors by pathways involving activation of ▶ NF-κB and MAPKs. Although the B_1 and B_2 receptors seem to couple to similar cellular signal transduction pathways, the patterns of signaling are different (Leeb-Lundberg et al. 2005; Brechter et al. 2008).

B_1 and B_2 receptor form homodimers and these receptors were found to spontaneously heterodimerize. Heterodimerization was associated with a specific proteolytic degradation of the participating B_2 receptor and an increase in both agonist-dependent and -independent signaling of the heterologous receptor complex. The existence of a B_2 receptor and angiotensin receptor 1 (B_2/AT1) heterodimeric complex may have implications for blood pressure. The B_2/ACE interaction modulates ACE activity (Sabatini et al. 2008).

B_2 receptor function is controlled by short-term mechanisms involving fast ligand dissociation, receptor desensitization and internalization, and, after long-term stimulation, downregulation of the receptor occurs. In contrast, B_1 receptors elicit persistent responses and signaling that are subjected to very limited desensitization and receptor internalization with very slow ligand dissociation (Couture et al. 2001).

Kinins and Disease

The kallikrein-kinin system (KKS) is present in numerous pathologies and the role it plays may vary. It can maintain the danous state of disease or play a protective role, as summarized below in Table 1.

Generated during inflammation and tissue injury, bradykinin contributes to the initiation and maintenance of inflammation, to exciting and sensitizing sensory nerve fibers, thus producing pain as reviewed by Couture and coalleagues in 2001. Thus the B_2 receptor is involved in acute inflammation, including increased vascular permeability, venoconstriction, arterial dilatation, and pain through the activation of sensory nerve terminals. This receptor has a limited role in the cellular component of the inflammatory response involving leukocyte recruitment within the microcirculation. The activation of B_2 receptors in sensory neurons promotes hyperalgesia. Bradykinin can sensitize nociceptors following the release of prostaglandins, cytokines, and nitric oxide either from sensory neurones, endothelial and immune cells or fibroblasts in addition to its interaction with mast cell mediators. The blockade of B_2 receptors located on sensory neurons may be responsible for the analgesic property of B_2 receptor antagonists. The pro-inflammatory effects of

Bradykinin Receptors, Table 1 Kinin receptors' presence in various diseases

Disease	Effect
Inflammation	Pro-inflammatory[a]
Pain	Hyperalgesia[a]
Infection	BK increase:
	Vascular leakage and vasodilation[a]
	Arterial vasodilatation[a]
Imune system (autoimmune diseases)	Imune cells stimulation and regulation[b]
Bone (arthritis and periodontitis)	Stimulate bone resorption[c]
Respiratory system (asthma and rhinitis)	Increase in the expression of kinins[d]
Neurological disease	
Alzheimer	Improvement of cognitive deficits[e]
Epilepsy	Deleterious and protective effects[e]
Sclerosis	B_1R increases blood–brain barrier permeability[f]
Kidney nephrophaty	Chemokine production[g]
	Macrophage accumulation[g]
Metabolism	
Diabetes	Prevention of progression of insulin-dependent diabetes[h]
Obesity	B_2 absence enhance senescence in mice[i]
	$B_1^{-/-}$ mice are protect from diet-induced obesity[j]
Cardiovascular	Hypertrophy[k]
	Cardiopathy[k]
	Hypertension[k,l]
	Atherosclerosis[m]
Liver	Attenuates fibrosis/ hepatocellular damage[n]
Cancer	Tumor growth[l]
	Angiogenesis stimulation[l]

[a]Couture et al. (2001)
[b]Schulze-Topphoff et al. (2009)
[c]Brechter et al. (2008)
[d]Proud (1998)
[e]Lemos et al. (2010)
[f]Schulze-Topphoff et al. (2009)
[g]Klein et al. (2010)
[h]Kakoki et al. (2010)
[i]Kakoki et al. (2006)
[j]Mori et al. (2008)
[k]Sharma (2003)
[l]Leeb-Lundberg et al. (2005)
[m]Merino et al. (2009)
[n]Kouyoumdjian et al. (2005)

B_1 receptors include promotion of blood-borne leukocyte trafficking, edema and pain. B_1 receptors are primarily involved in persistent inflammatory pain and are expressed in macrophages, fibroblasts, or endothelial cells, where they may be responsible for inflammation mediators releasing (prostaglandins, cytokines, and nitric oxide) that sensitize or activate the nociceptors.

Because of its multicellular location and the mode of persistent signaling mechanism, the B_1 receptor is likely to exert a strategic role in inflammatory diseases, particularly those with an immune etiology (asthma, rheumatoid arthritis, multiple sclerosis, and diabetes). In addition to the pro-inflammatory effects of kinin receptors, B_1 receptors may exert a protective effect in brain inflammatory diseases such as multiple sclerosis by reducing T-lymphocyte infiltration into the brain (Schulze-Topphoff et al. 2009).

Kinins exert influence on multiple players of the immune system (i.e., macrophages, dendritic cells, T and B lymphocytes). BK is capable of modulating the activation, proliferation, migration, and effector functions of immune cells. Kinin receptors seem to be important in autoimmune conditions, such as rheumatoid arthritis, lupus, and myasthenia gravis (Schulze-Topphoff et al. 2008).

Kinin receptors are present in osteoblasts, osteoclasts, and fibroblasts, linking the kallikrein-kinin system with rheumatoid arthritis, periodontitis, and bone resorption. They can stimulate bone resorption through prostaglandins. Kinin B_1 and B_2 receptors synergistically potentiate IL-1β and TNF-α-induced prostaglandin biosynthesis in osteoblasts by a mechanism involving increased levels of cycloxygenase-2 (Brechter et al. 2008).

Many studies have demonstrated increased kinin generation associated with asthma, allergic rhinitis, and during viral rhinitis (Proud 1998). The first studies began with the analysis of the presence of kinins after allergen stimulation in allergic subjects and absence of them in non-allergic subjects. The inflammatory infiltration and relation between kinins and the chronic phase of the disease were then observed. Kinins are also associated with the release of the mast cell granule constituents, histamine, and tryptase,

major mediators of acute phase. The kinin concentration increase during asthma is associated with the augment in histamine and other inflammatory markers, including eicosanoids. The administration of bradykinin by nasal spray to the upper airways of normal, nonatopic subjects, or of asymptomatic atopic individuals has been shown to result in the dose-dependent induction of symptoms of nasal obstruction, modest rhinorrhea, nasal irritation, and sore throat, but not sneezing (Proud 1998).

Kinin receptors are involved with brain damage in different forms. They act in multiplus sclerosis, epilepsy, and Alzheimer's disease. Kinin B_2 receptor promotes survival and protects against brain injury by suppression of apoptosis and inflammation induced by ischemic stroke. In epilepsy, the kinin B_2 receptor also plays a neuroprotector effect and the kinin B_1 receptor plays a deleterious, pro-epileptogenic action in animal models (Leeb-Lundberg et al. 2005). Kinin receptors are involved in neurodegeneration and increase of amyloid-b concentration, associated with Alzheimer's disease (Lemos et al. 2010). More recently it was shown that during the aging process, the B_1 receptor could be involved in neurodegeneration and memory loss. Nevertheless, the B_2 receptor is apparently acting as a neuroprotective factor (Lemos et al. 2010). In inflammatory brain disease, like sclerosis, kinin B_1 receptors are important in limitating migration of lymphocytes through the central barrier and inflammation in the brain (Schulze-Topphoff et al. 2009).

Kinins receptors are present in the kidney and are involved with kidney disease, such as renal failure and nephropathy. Since kinin receptors are present in patients in end stage of renal failure, treatment with a B_1 receptor antagonist reduces both glomerular and tubular lesions and improve renal function trough the reduction of renal chemokine expression and macrophage accumulation in glomerulonephritis (Klein et al. 2010). Genetic association between B_1 receptor polymorphisms and end-stage renal failure have been reported, as the B_2 receptor polymorphism is associated with diabetic nephropathy (Leeb-Lundberg et al. 2005).

Lack of B_1 and B_2 receptors exacerbates diabetic complications, enhances the nephropathy (glomerulonephritis), neuropathy (decrease the time of nervous impulse), and bone mineral loss caused by insulin-dependent diabetes in mice (Kakoki et al. 2010). The development of diabetic retinopathy increases vascular permeability, neovascularization, inflammation and B_2 activation contributes to vascular permeability and edema, which suggests the correlations between the KKS and microvascular complications of diabetes. Studies performed in diabetic mice demonstrated that the absence of B_2 receptor in these animals increases indicators of senescence like alopecia, skin atrophy, kyphosis, osteoporosis, testicular atrophy, lipofuscin accumulation in renal proximal tubule and testicular Leydig cells, and apoptosis in the testis and intestine (Kakoki et al. 2006).

The kinin B_2 receptor agonist BK may participate in the regulation of substrate utilization by several tissues by improving blood flow and substrate delivery to the tissues and also by promoting translocation of glucose transporters. It appears to improve the release of insulin and improve insulin sensitivitiy. Furthermore, insulin may activate the kallikrein-kinin system, which consequently may increase its metabolic effects. However, in experimental diabetes mellitus, BK may participate in the inflammatory reaction leading to Langerhans islets destruction (Damas et al. 2004). Kinin B_1 receptor is involved in obesity, as shown by Mori et al. The absence of B_1 receptor in mice decreases plasma leptin levels, increases leptin sensibility, protects mice from diet-induced obesity (diet with 45% of fat), and augments energy expenditure.

The KKS has importante role in various pathological processes of the cardiovascular system, such as hypertension, cardiac failure, ischemia, left ventricular hypertrophy, and endotoxemia. There is activation of BK activity in endotoxemia. On the other hand, it seems that there is deficient activity of the KKS in hypertension, cardiac ischemia, and development of left ventricular hypertrophy. These pathological states might be due to a genetic abnormality of the KKS or down-regulation of the BK receptors (Sharma 2003). Several studies have detected a significant

association between the B_2 receptor 58 C/T polymorphism and hypertension (Leeb-Lundberg et al. 2005). Kinin B_1 receptor deficiency aggravates atherosclerosis and aortic aneurysms in mice under cholesterolemic conditions, supporting an antiatherogenic role for the kinin B_1 receptor (Merino et al. 2009).

The KKS is also present in the liver and is related to liver disease. BK can induce portal hypertensive response when injected in the liver. This hepatic hypertensive response to BK is mediated by the B_2 receptor and modulated by the L-Arg/nitric oxide pathway. There is also evidence of the participation of BK in the pathogenesis of vasodilatation and ascites formation in cirrhotic patients (Kouyoumdjian et al. 2005).

Finally, the ability of BK to stimulate vessel growth and increase vascular permeability may contribute to the biological behavior of tumors. Evidence for increased generation of kinins and kinin receptors detection in different types of cancer has been reported (Leeb-Lundberg et al. 2005).

Summary

Considering the knowledge gathered since the classical pharmacological models were established and the more recently gene target animal models, much has been changed concerning the kinin receptors function. In the beginning, the kinin receptors were first implicated with pain and inflammation. Nowadays they are still important in this area of study; however, they have been implicated with different diseases like asthma, arthritis, sepsis, kidney disease, hypertension, cardiopathy, diabetes, and cancer among others. In the last years, new implications of kinin receptors in obesity and immunology are described, as well as interaction of kinin receptors and other proteins like ACE and AT1 receptor. These implications will bring new possibilities for therapies involving kinin ligands (agonists and antagonists). Moreover, ongoing tests with new drugs affecting the KKS are on the way. The main goal is to develop more potent and tissue specific ligands, with increased disposability, central permeability, and reduced collateral effects. The field of study of these receptors is wide and promising.

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Brain Angiogenesis Inhibitor

► ADGRB3

Brca1

► BRCA1 and BRCA2

BRCA1 and BRCA2

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Synonyms

BRCA1: Brca1; BRCA1/BRCA2-containing complex, subunit 1; BRCAI; BRCC1; Breast and ovarian cancer susceptibility protein 1; Breast cancer 1; Breast cancer 1, early onset; Breast cancer type 1 susceptibility protein; Breast cancer type 1 susceptibility protein homolog; IRIS; PSCP; RING finger protein 53; RNF53

BRCA2: BRCA1/BRCA2-containing complex, subunit 2; Brca2; BRCC2; Breast and ovarian cancer susceptibility gene, early onset; Breast cancer 2; Breast cancer 2 tumor suppressor; Breast cancer 2, early onset; Breast cancer susceptibility protein BRCA2; Breast cancer type 2 susceptibility protein homolog; FACD; FAD; FAD1; FANCB; FANCD; Fancd1; Fanconi anemia group D1 protein; Fanconi anemia group D1 protein homolog; RAB163

Historical Background

Most breast and ovarian cancers (BOC) are sporadic, meaning they occur by chance with no known cause. A diagnosis of hereditary breast and ovarian cancer syndrome (HBOC) has been considered when multiple cases of breast and/or

ovarian cancer on the same side of the family. Most women who have breast or ovarian cancer do not have HBOC. HBOC is an inherited genetic condition; this means that the cancer risk is passed from generation to generation in a family.

The first evidence for the existence of a gene involved in breast cancer susceptibility was proven in 1990 by mapping predisposition to young-onset breast cancer (Hall et al. 1990). Intense efforts to isolate the gene have proceeded since it was first mapped to chromosome arm 17q in 1990. In 1994, a candidate for the gene was identified by positional cloning methods (Miki et al. 1994) and was identified as *BRCA1* gene.

A second locus, *BRCA2*, was mapped to chromosome arm 13q (Wooster et al. 1994), and it was suggested that this gene may account for a proportion of early onset breast cancer roughly equal to that resulting from *BRCA1*.

The official symbols (*BRCA*, italic for the gene, nonitalic for the protein) are the official names.

When chromosome loss is observed in breast and ovarian tumors from patients who carry *BRCA1*-predisposing alleles, the wild-type copy of *BRCA1* is invariably lost while the presumptive mutant allele is retained. This observation at this time let the investigator to propose the hypothesis that *BRCA1* is a tumor suppressor gene and that the functional BRCA1 protein is present in normal breast and ovarian epithelium tissue and is altered, reduced, or absent in some breast and ovarian tumors.

Thus, although the terms "breast cancer susceptibility gene" and "breast cancer susceptibility protein" describe an abnormal gene, *BRCA1* and *BRCA2* are normal; it is their mutated form that is abnormal.

BRCA1/2 Structure

The *BRCA1/2* genes are tumor suppressor genes. Diseases associated with a mutation in *BRCA1/2* include breast, ovarian, pancreatic, gastric, and prostate cancers, and melanoma and patients with a mutation in *BRCA2* include Fanconi Anemia. Both *BRCA1* and *BRCA2* are involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair (homologue repair, HR).

The *BRCA1* gene is in the chromosome 17q21.31 from base pair 41,196,312 to base pair 41,277,500, is composed of 24 exons, and encodes a nuclear protein of 1863 amino acids (molecular mass 207,721 Da) that plays a role in maintaining genomic stability.

The *BRCA2* gene originally was mapped to an interval of ~6 cM on human chromosome 13q12-q13 (Wooster et al. 1994). Subsequently, a gene was identified that carried independent mutations in several different families (Wooster et al. 1995). This gene was found to have 27 exons and to encode a protein of 3418 amino acids having an estimated molecular mass of 384 kDa (Tavtigian et al. 1996). One very unusual aspect of the gene structure is the presence of a large coding exon (exon 11) of ~5 kb encoding almost half of the BRCA2 protein.

The BRCA1 protein contains several important domains to achieve its function. It has a Zinc finger, C3HC4 type, and is 40–60 amino acids long and one of the main functions is the interaction with associated proteins. It has also a serine domain and by a posttranslational modification is present in the nucleus as a phosphoprotein that acts as a tumor suppressor. This protein also contains nuclear localization signal and nuclear export signal motifs. The ring domain is an important element of ubiquitin E3 ligase. The E3 ubiquitin-protein ligase activity is required for its tumor suppressor function. The encoded protein forms a large multisubunit protein complex known as the BRCA1-associated genome surveillance complex (BASC). The protein combines with other tumor suppressors, DNA damage sensors, and signal transducers and also associates with RNA polymerase II, and through the C-terminal domain, also interacts with histone deacetylase complexes. The BRCA1 protein plays a role in transcription, DNA repair of double-stranded breaks, and recombination.

The BRCA2 protein contains several copies of a 70 amino acid motif called the BRC motif, and these motifs mediate binding to the RAD51 recombinase which functions in DNA repair. BRCA2 acts by targeting RAD51 to ssDNA

over double-stranded DNA, enabling RAD51 to displace replication protein-A (RPA) from ssDNA and stabilizing RAD51–ssDNA filaments by blocking ATP hydrolysis.

BRCA1/2 are considered tumor suppressor genes, as tumors with *BRCA1/2* mutations generally exhibit loss of heterozygosity (LOH) of the wild-type allele.

BRCA1/2 Relevance and Cellular Physiology and Function

Germline mutations in one of the breast cancer susceptibility genes, *BRCA1* (MIN #113705) or *BRCA2* (MIN#600185), are the major and most widely known risk factors for breast and/or ovarian cancer (BOC) hereditary syndrome (HBOC) (Miki et al. 1994; Wooster et al. 1995). Although the mutations are present in about 40% of the patients with strong family BOC background, HBOC occurs in 5–10% of all BOC cases; in turn, individuals with such inheritance have a 50–80% risk of developing breast cancer and a 30–50% risk of ovarian cancer in their lifetime, while other malignancies such as prostate and pancreatic cancer have been less frequently observed (Robson and Offit 2007; Roy et al. 2012). Furthermore, cancers as melanoma and colon have been detected in families with *BRCA2* mutations (Easton, et al. 1997; Robson and Offit 2007; Roy et al. 2012). Moreover, *BRCA1* carriers have a 4-fold increased risk of colon cancer, whereas male carriers face a 3-fold increased risk of prostate cancer.

Characteristic features in affected families are an early age of onset of breast cancer (often before age 50), increased chance of bilateral cancers (cancer that develop in both breast, and both ovaries, independently), frequent occurrence of breast cancer among men, increased incidence of tumors of other specific organs, such as the prostate, gastric, melanoma, and pancreas and other cancers as included in the NCCN guidelines.

Since the discovery of *BRCA1* and *BRCA2* genes, thousands of genetic variants with different clinical significance have been reported, at the beginning in the Breast Cancer Information Core Database (http://research.nhgri.nih.gov/bic/) and now included in the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), with near 6000 and 4000 cases reported for *BRCA1* and *BRCA2*, respectively, including 1000 different variants in each gene classified as pathogenically responsible for HBOC. The large extension of these genes and the rare hot spot mutations are the consequence of the genetic diversity and thus, the limitation for establishing population panels useful for screening studies.

Alternative splicing plays a role in modulating the subcellular localization and physiological function of this gene. Many alternatively spliced transcript variants, some of which are disease-associated mutations, have been described for this gene, but the full-length natures of only some of these variants have been described.

The frequency of *BRCA1* and *BRCA2* mutation carriers in women with BOC depends on the population analyzed but appears to be similar across ethnicity (Kurian 2010). However, significant variation has been demonstrated in the spectrum of *BRCA1/2* mutations according to ethnic and/or geographical diversity (Neuhausen 1999; Solano et al. 2012). Racial mixture in the South American population has been reported in epidemiological and molecular studies (Wang et al. 2008). In particular, the population of Argentina (the largest analyzed for *BRCA1/2* in South America) consists of an admixture of European ancestry – mainly from Spain and Italy – and an Amerindian component in a variable degree which is observed in more than 50% of the population (Marino et al. 2006; Martinez Marignac et al. 2004; Solano et al. 2012).

As mentioned above, the utility of the panels is undoubtedly; however, attention needs to be drawn to the implementation of a mutation panel as a putative standard screening anticipating its impact in health care, as in a report of "a non-mutation detected" for this, the panel should be followed by the full sequence of *BRCA1/2*. This panel may become, however, the unique analysis in a patient's lifetime, in many countries at least, that is, he/she might never being studied for a total sequence because of the heterogeneity of some health insurance system. This secondary

effect may prove harmful and confusing for patients and doctors, who may never realize the test performed is practically useless. In addition, there is limited information available regarding population-specific risk and very systematic studies of the prevalence of genetic variants predisposing to breast cancer relevant to the population of Latin America.

Founder effects are most prominent in geographically, culturally, or religiously isolated populations that undergo rapid expansion from a limited number of ancestors, when, because of low genetic diversity, some alleles become more frequent. The term "founder" is used for those mutations where haplotype studies revealed shared polymorphic markers consistent with common ancestor. The *BRCA1/2* founder effect in Ashkenazi Jews population is very well described. The most well-characterized three founder mutations are two in *BRCA1* gene: c.68_69delAG (BIC: 185delAG) and c.5266dupC (BIC: 5382insC) and one in *BRCA2* c.5946delT (BIC: 6174delT). Screening for these three founder mutations alone is now part of routine clinical practice for Ashkenazi Jewish individuals. These three mutations account for 98–99% of identified mutations and are carried by about 2.6% (1/40) of the Ashkenazi Jewish population. With rare exceptions, few pew panels besides the Ashkenazi were found for epidemiological utility.

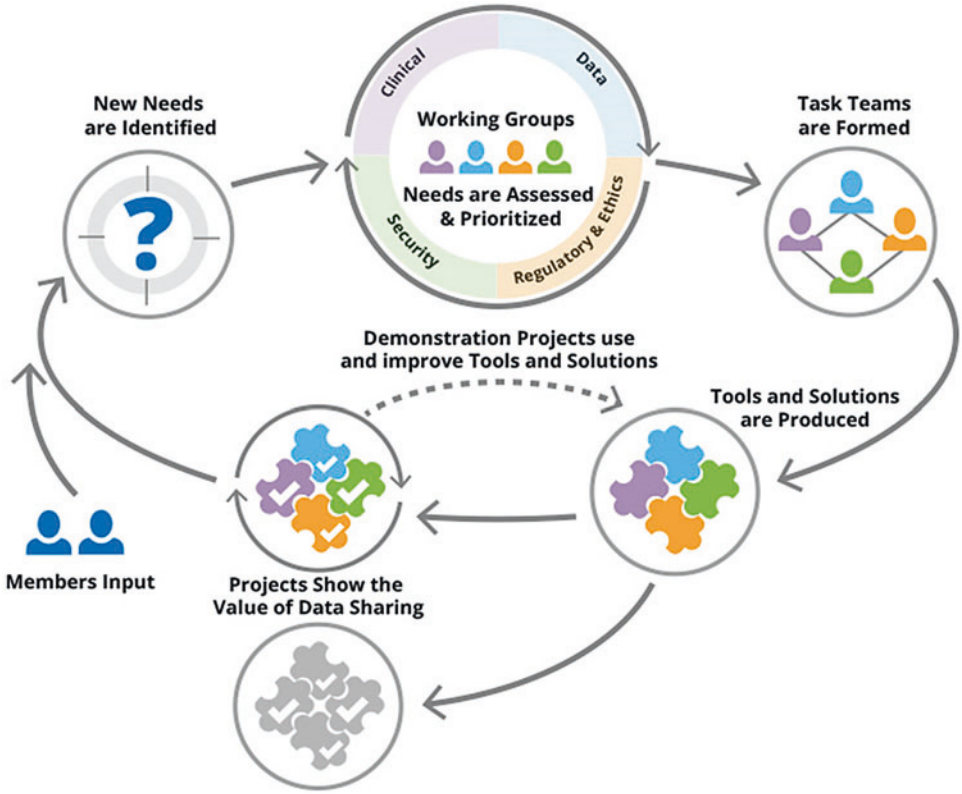
Large rearrangements are frequent in few ethnic groups (Sluiter and van Rensburg, 2010) and very infrequent in others (Solano et al. 2016). These striking differences draw attention to the import of panels from apparently similar populations. This is a key issue in many aspects: (a) clinicians and patients may be misinformed, even in cases with accomplished genetic counseling; (b) when a panel is the first analysis, in a health system, insurance may reject further analyses in the same line, that is, twice the analysis "of the same genes," which might also be inaccurate, as a full sequencing test is required after a non-mutation has been detected in a panel; (c) if health insurance covered both analyses (the panel of mutations and the full sequencing), 97% of the patients analyzed for the recurrent mutations would need full sequencing of *BRCA1/2*, which is even economically nonconvenient; and (d) attention needs to be drawn to the correct interpretation of results, as "normal" is considered equivalent to "uncompleted analysis" at two levels: the restricted number of mutations analyzed and the limitations of the *BRCA1/2* analysis itself, a restraining concept for the initial analysis of a complex genetic study.

This is an important point to be considered with caution in order to provide the best health care possible, mostly in emerging countries where the supporting economy is frequently in crisis and low cost studies are attractive. There is a real need for the implementation of a highly supported medical care in an ethic and genetic basis for every study. This will render profits from funds invested in health, mostly in the prevention of high costs for cancer treatments and the analysis in hereditary cancer, to be used in prevention (first goal) and early detection.

The identification of *BRCA1* and *BRCA2* mutation carriers and individualized risk assessment is an important procedure growing in clinical importance, since management protocols for mutation carriers become well established (NCCN Guidelines for detection, prevention and risk reduction) and proven life-saving, risk-reducing preventive medical interventions exist. Once mutation is identified in a given family, a very informative predictive (or presymptomatic) genetic test can be offered virtually to all adult family members. Moreover, genetic testing is becoming the powerful therapeutically predictive tool, as new targeted therapeutic opportunities.

Future and Perspective

Since the cloning and characterization of *BRCA1* in the mid-1990s mutational screening of the breast cancer susceptibility genes *BRCA1/2* leads to the identification of numerous pathogenic variants such as frameshift and nonsense variants, as well as large genomic rearrangements. The screening moreover identifies a large number of variants, for example, missense, silent, and intron variants, which are classified as variants of unknown clinical significance owing to the lack of causal evidence. Variants of unknown clinical



BRCA1 and BRCA2, Fig. 1 How the Global Alliance for Genomics and Health (GA4GH) works (The graphic is reprint with permission from GA4GH)

significance can potentially have an impact on splicing, and therefore, functional examinations are warranted to classify whether these variants are pathogenic or benign. The identification of variants of unknown clinical significance makes genetic counseling of patients and their families complicated and generates a big challenge.

This challenge was taken by two very well-known organizations that open a great opportunity to advance in the study of the significance of a large number of mutation in the *BRCA1/2* genes and their implication in diagnosis and treatment of hereditary cancer. The organizations are the "**Global Alliance for Genomics and Health (GA4GH)"** and the "**Human Variome Project (HVP)**."

The Global Alliance was formed to help accelerate the potential of genomic medicine to advance human health. It brings together over 400 leading institutions working in healthcare, research, disease advocacy, life science, and information technology. The partners in the Global Alliance are working together to create a common framework of harmonized approaches to enable the responsible, voluntary, and secure sharing of genomic and clinical data.

The work of the Global Alliance is critical to realizing the potential of recent technological advances that make possible the large-scale collection of data on genome sequencing and clinical outcomes. To seize this extraordinary opportunity, it is often necessary to ask questions that span individual datasets. The Global Alliance is working to alter the current reality where data are kept and studied in silos, and tools and methods are non-standardized and incompatible. The BRCA Challenge of the Global Alliance for Genomics and Health aims to advance understanding of the

genetic basis of breast cancer and other cancers by pooling data on BRCA genetic variants from around the world, bringing together information on sequence variation, phenotype, and scientific evidence. Improved understanding of genetic variation in these genes has the potential to improve patient diagnoses and prevention of disease (Fig. 1).

The Human Variome Project (HVP) is an international attempt to catalogue all human genetic variation relevant to a wide range of genetic disorders and drug responses. The goal of the Human Variome Project is to be an all-inclusive global collaboration to collect genetic variation and its corresponding phenotype for ultimate annotation onto the human genome. The project will also create a resource that can become a repository of all information on genetic influence on disease.

In the last years, there have been created formal groups of investigators pursuing concentration of the efforts in elucidate topics regarding the correlation between the genetic findings and clinical application with deep bioinformatic and statistical analysis. The two most related to BRCA1/2, as described in their web pages, are:

(a) The Consortium of Investigators of Modifiers of BRCA1/2, CIMBA, is a collaborative group of researchers working on genetic modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers. The aim of CIMBA is to provide sufficient sample sizes to allow large scale studies in order to evaluate reliably the effects of genetic modifiers.
(b) Evidence-based Network for the Interpretation of Germline Mutant Alleles, ENIGMA. ENIGMA is an international consortium of investigators focused on determining the clinical significance of sequence variants in *BRCA1*, *BRCA2*, and other known or suspected breast cancer genes, to provide this expert opinion to global database and classification initiatives, and to explore optimal avenues of communication of such information at the provider and patient level.

In the area of genomics, the high interaction of the different disciplines, including bioinformatics, made in the few last years an immense development of knowledge and open an enormous perspective for a faster improvement impacting in the human health.

B

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BRCA1/BRCA2-Containing Complex, Subunit 1

▶ BRCA1 and BRCA2

BRCA1/BRCA2-Containing Complex, Subunit 2

▶ BRCA1 and BRCA2

Brca2

▶ BRCA1 and BRCA2

BRCAI

▶ BRCA1 and BRCA2

BRCC1

▶ BRCA1 and BRCA2

BRCC2

▶ BRCA1 and BRCA2

Breast and Ovarian Cancer Susceptibility Gene, Early Onset

▶ BRCA1 and BRCA2

Breast and Ovarian Cancer Susceptibility Protein 1

▶ BRCA1 and BRCA2

Breast Cancer 1

▶ BRCA1 and BRCA2

Breast Cancer 1, Early Onset

▶ BRCA1 and BRCA2

Breast Cancer 2

▶ BRCA1 and BRCA2

Breast Cancer 2 Tumor Suppressor

▶ BRCA1 and BRCA2

Breast Cancer 2, Early Onset

▶ BRCA1 and BRCA2

Breast Cancer Antiestrogen Resistance Protein 1 (BCAR1)

▶ p130Cas

Breast Cancer Susceptibility Protein BRCA2

▶ BRCA1 and BRCA2

Breast Cancer Type 1 Susceptibility Protein

▶ BRCA1 and BRCA2

Breast Cancer Type 1 Susceptibility Protein Homolog

▶ BRCA1 and BRCA2

Breast Cancer Type 2 Susceptibility Protein Homolog

▶ BRCA1 and BRCA2

Breast Tumor Kinase (Brk)

▶ Protein Tyrosine Kinase-6 (PTK6)

BRF1

▶ Tristetraprolin (ZFP36) and TIS11B (ZFP36-L1)

BRG1

▶ SWI/SNF Chromatin Remodeling Complex

BRL-Ras

▶ Rab7a in Endocytosis and Signaling

BRM

▶ SWI/SNF Chromatin Remodeling Complex

BRPK

▶ PTEN-Induced Kinase 1 (PINK1)

Bruton Agammaglobulinemia Tyrosine Kinase

▶ BTK

Bruton's Tyrosine Kinase

▶ BTK

Brx1

▶ PITX2 (Pituitary Homeobox Gene 2)

BSCL3

▶ Caveolin-1

BSPI

► Osteopontin (*Spp1*)

BTG/TOB

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Synonyms

BTG1; BTG2 (TIS21, PC3); BTG3 (ANA, TOB5); BTG4 (PC3B); TOB1 (TOB, Transducer of ERBB2); TOB2 (Transducer of ERBB2 2)

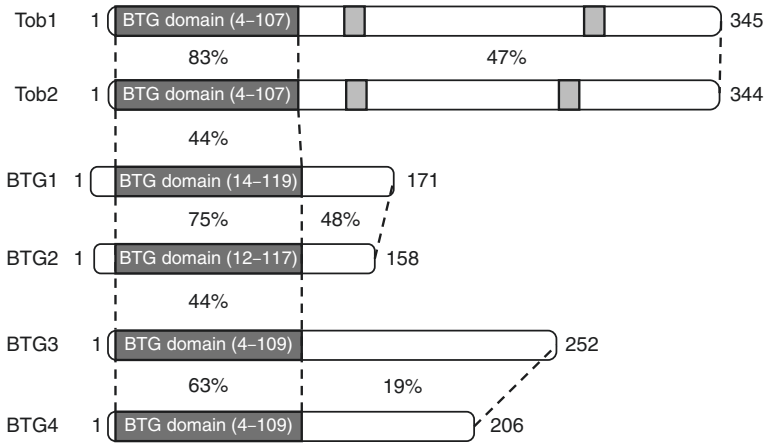
Historical Background

The human BTG/Tob proteins form a small family of six proteins, which share a conserved N-terminal domain and antiproliferative activity (Matsuda et al. 2001; Tirone 2001; Winkler 2010). BTG2 was discovered first by two laboratories: as the immediate/early response gene PC3 in rat PC12 cells stimulated with nerve growth factor (NGF) and as TIS21 in mouse 3T3 fibroblasts in response to treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA). The discovery of BTG1 (B-cell translocation gene 1) as a gene involved in a chromosomal translocation associated with chronic lymphocytic leukemia suggested the presence of a new family of antiproliferative genes. These findings were extended by the discovery of TOB1, which was found as an interacting protein of the ErbB2 tyrosine-kinase receptor (HER2). The remaining three members BTG3 (ANA), BTG4 (PC3B), and TOB2 were identified based on sequence homology of the conserved N-terminal domain. The preferred gene names by the Human Genome Nomenclature Committee are BTG1, BTG2, BTG3, BTG4, TOB1, and TOB2.

Regulation of Gene Expression: mRNA Deadenylation

The conserved N-terminus is known as the BTG domain (Pfam number PF07742; also known as APRO domain) and comprises 104–106 amino acids. The C-terminal regions are less conserved and confer additional functions to the family members. Sequence analysis of both the BTG domain and the C-terminal regions suggests that Tob1 and Tob2 as well as BTG1 and BTG2 are highly similar, whereas BTG3 and BTG4 are more distantly related (Fig. 1). The BTG/Tob proteins are implicated in the regulation of gene expression by at least two distinct mechanisms.

The best characterized role of the BTG/Tob proteins in gene expression is mediated via the BTG homology domain, which interacts with the Caf1 subunit of the Ccr4-Not complex, which is encoded by *CNOT7* or *CNOT8*. The highly similar CNOT7 and CNOT8 proteins are deadenylase enzymes, which shorten and remove the poly (A) tails of cytoplasmic mRNA resulting in translational repression and mRNA degradation (Mauxion et al. 2009; Winkler 2010). The interaction of all BTG/Tob proteins with either CNOT7 and/or CNOT8 is experimentally confirmed, and a specific role in the regulation of deadenylation and mRNA degradation was demonstrated for all proteins except BTG4. BTG1, BTG2, TOB1, and TOB2 can interact with the poly(A)-binding protein PABPC1. In case of BTG1 and BTG2, this interaction is mediated by the BTG domain (Stupfler et al. 2016). By contrast, Tob1 and Tob2 contain PAM2 motifs in their C-terminal regions, which allow them to interact with poly(A) binding protein 1 (PABPC1) (Ezzeddine et al. 2007; Funakoshi et al. 2007). During termination of translation, several proteins containing a PAM2 motif are consecutively recruited to the mRNA by PABPC1: following binding of the translation termination complex eRF1-eRF3 and the PAN2-PAN3 deadenylase, Tob1 recruits the Ccr4-Not deadenylase via



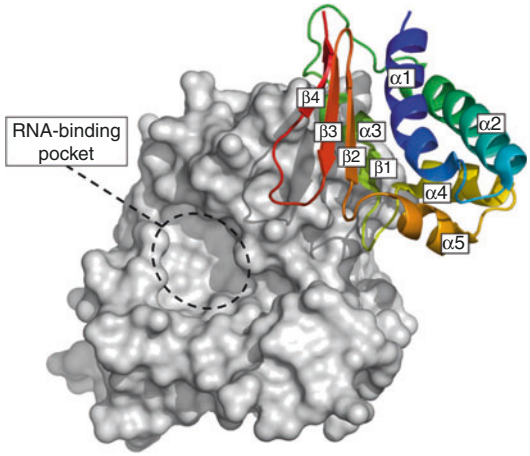
BTG/TOB, Fig. 1 Schematic overview of the human BTG/Tob protein family. The approved gene names used by the human genome nomenclature committee are used. Indicated are the N-terminal BTG/Tob domains (*dark gray*) and PAM2 motifs (*gray*). The pair-wise percentage identities were determined using the Clustalw2 multiple sequence alignment program. The length of the proteins (amino acids) and the position of the BTG domain are also indicated

interactions between the conserved BTG domain and the CNOT7 and CNOT8 deadenylase subunits. This sequence of events implicates Tob1 – as well as the related Tob2 protein – in mRNA deadenylation coupled to termination of translation. Alternatively, Tob1 can be recruited to specific mRNAs by sequence specific RNA-binding proteins. For example, cytoplasmic polyadenylation element-binding protein 3 (CPEB3) binds Tob1 resulting in mRNA destabilization (Hosoda et al. 2011).

Several protein structures illuminate the molecular details of the interaction between BTG/Tob proteins and the CNOT7/CNOT8 deadenylase enzymes. The BTG domain is characterized by two long antiparallel α-helices in the N-terminus of the domain that are part of a four-helix bundle and three β-sheets at the C-terminus of the domain (Fig. 2). Comparison of the structure of the free BTG domain of Tob1 with the domain in complex with the CNOT7 deadenylase indicates that the BTG domain does not undergo significant rearrangements upon binding. The RNA-binding, catalytic site of the CNOT7 deadenylase appears to be separated from the residues important for binding to the BTG domain. In agreement with this, binding of the BTG domain of Tob1 does not influence the catalytic activity of the CNOT7 deadenylase (Horiuchi et al. 2009).

Regulation of Gene Expression: Transcription

In addition to their role in mRNA deadenylation, BTG/Tob proteins can regulate gene expression at the level of transcription (Matsuda et al. 2001; Tirone 2001; Winkler 2010). Several reports point to the ability of the BTG/Tob proteins to interact with DNA-binding transcription factors and modulate their ability to bind their cognate DNA sequence elements. Both BTG1 and BTG2 can interact with Hoxb9, a homeobox DNA-binding transcription factor, through their extreme N-terminus (residues 1–14). This interaction enhances the ability of Hoxb9 to bind to its consensus DNA sequence. Thus, this may increase the transcription rates of Hoxb9 target genes, which may contribute to the antiproliferative function of BTG1 and BTG2. Tob1 and Tob2 have the most extensive C-terminal regions within the protein family. This region of Tob1 mediates interactions with a number of Smad transcription



BTG/TOB, Fig. 2 Structure of the BTG domain of Tob1 in complex with the Caf1/CNOT7 deadenylase enzyme. The representation was generated using structure 2d5r deposited in the Protein Data Bank (PDB) using Pymol (www.pymol.org). The BTG domain of Tob1 is represented by a multicolored cartoon. Indicated are the five α-helices and four β-sheets. The surface of the Caf1/CNOT7 deadenylase enzyme is represented in *gray*. *Circled* is a deep pocket that binds poly(A) RNA and corresponds to the catalytic center

factors, altering their ability to bind to DNA. As a consequence, Tob1 regulates the expression of Smad target genes, such as the cytokine IL-2 promoter in quiescent T-cells. BTG3 presents a third example of this mode of action: BTG3 can interact with E2F1, a transcription factor important for S-phase entry and cell cycle progression. BTG3 binds E2F1 through its N-terminal region, which, in this case, inhibits DNA binding of the E2F1, thereby reducing the overall transcription rate of E2F1-responsive promoters and cell proliferation.

Finally, BTG1 and BTG2 can interact with protein arginine methyl-transferase 1 (PRMT1) through a short β-sheet region (also known as Box C) just outside the BTG domain, which is not conserved in other BTG/Tob proteins. PRMT1 specifically methylates the arginine 3 residue of histone H4 in vitro and in vivo, which facilitates subsequent acetylation of histone H4 tails by p300 and gene activation. Thus, this raises the possibility that BTG1 and BTG2 could be involved in the regulation of chromatin modifications.

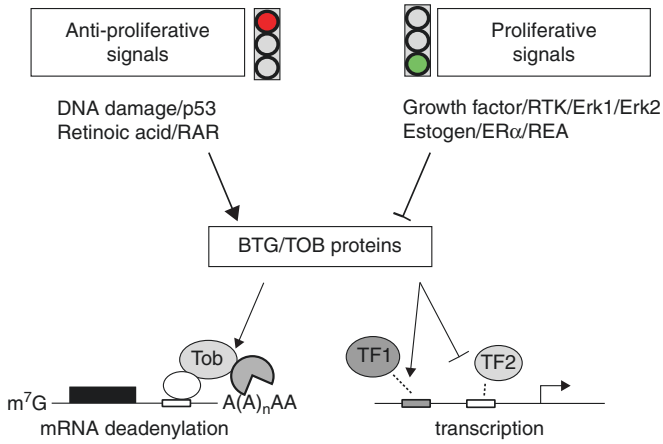
Effectors of Signaling Pathways

There are a variety of different signaling pathways that exploit the antiproliferative properties of BTG/Tob proteins either positively or negatively by regulating the cellular levels of these proteins by transcriptional and post-translational mechanisms. Both Tob1 and BTG2 are phosphorylated upon stimulation with growth factors by the Erk1/Erk2 kinases at serine residues in the C-terminus. This results in subsequent deactivation, which – in the case of Tob1 – leads to increased cyclin D1 expression and enhanced activation of CDK4, driving cell cycle progression and cell proliferation.

In MCF7 cells (an estrogen receptor-expressing breast cancer cell line), BTG2 mRNA can be regulated both positively and negatively by signaling through nuclear receptor transcription factors. BTG2 expression is activated when MCF7 cells are treated with retinoic acid through direct binding of the retinoic acid receptor (RAR)/RXR heterodimers to three retinoic acid response elements (RARE) in the BTG2 promoter region. Conversely, BTG2 expression is reduced when MCF7 cells are treated with estrogen through estrogen receptor ERα and its corepressor REA.

BTG2 and BTG3 are both downstream targets of the p53 signaling pathway. Both proteins are direct transcriptional targets for p53 and play a role in the p53-mediated response to DNA damage (Rouault et al. 1996; Ou et al. 2007). In embryonic mouse fibroblasts, BTG2 plays critical role in suppressing transformation through oncogenic Ras by acting as a downstream effector of p53 (Boiko et al. 2006). BTG2 expression down-regulates cyclin D1, cyclin E1, and the phosphorylation of retinoblastoma (Rb) slowing cell cycle progression and preventing cellular transformation.

Finally, Tob1 and BTG2 are implicated in signaling of TGF-family members through Smad transcription factors. This was demonstrated in both quiescent T-cells activated by CD28, which impinges on TGF-β signaling, and in bone-forming osteoblast cells upon stimulation by bone morphogenic protein (BMP) 2, a TGF-family member (Fig. 3).



BTG/TOB, Fig. 3 Signaling pathways impinge on BTG/Tob proteins. Both antiproliferative and proliferative signals impinge on BTG/Tob family members by upregulation/activation or inhibition, respectively. In turn, BTG/Tob proteins can participate in the regulation of gene expression by deadenylation (*left*) or transcriptional mechanisms (*right*). See text for further details

Bone Formation: Tob1 and Tob2

The generation of mice containing null alleles of Tob1, Tob2, Btg2, and Btg3 uncovered a role for these proteins in bone formation and resorption (Yoshida et al. 2003; Park et al. 2004; Ajima et al. 2008; Miyai et al. 2009). The contrasting phenotypes observed in Tob1 and Tob2 knockout mice are of particular interest. Mice lacking Tob1 are apparently normal but display increased bone volume and bone density. Interestingly, in a mouse model for estrogen deficiency–induced osteoporosis, the increased bone mineralization in Tob1 null mice can compensate for bone loss associated with induced osteoporosis since ovariectomized Tob1 knockout mice have a bone mineral density and volume comparable to (sham operated) control mice (Usui et al. 2004). The increased bone density in Tob1$^{-/-}$ mice is due to enhanced bone formation, and osteoclast parameters are unchanged as compared to control mice. A similar increase in bone density is observed in mice lacking the Cnot7 deadenylase (Washio-Oikawa et al. 2007). As observed in Tob1 null mice, Cnot7 knockout mice do not display altered osteoclast parameters suggesting that the role of Tob1 in bone formation is mediated via its interactions with the CNOT7 deadenylase subunits of the Ccr4-Not complex.

By contrast, mice lacking Tob2 display decreased bone mass due to an increased number of differentiated osteoclast cells. Tob2 interacts with the vitamin D receptor and reduces expression of RANKL, a vitamin D-induced gene. In agreement with this notion and the observation that osteoclast parameters are unaltered in CNOT7 knockout mice, Tob2 is a repressor of vitamin D-induced osteoclast formation (Ajima et al. 2008).

Cancer and Tumorigenesis

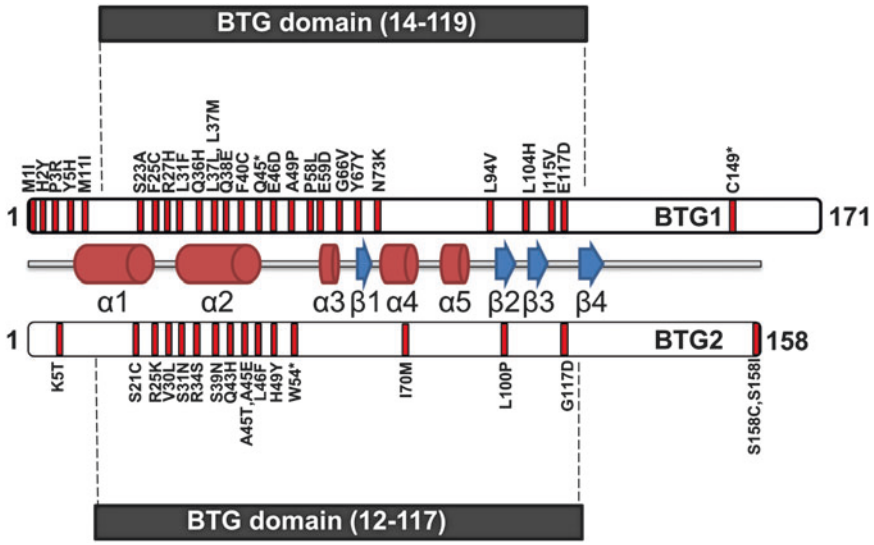
The discovery of BTG2 as an effector of the tumor suppressor function of p53, as well as the critical role of Tob1 in Ras-mediated transformation, strongly implicates these BTG/Tob proteins as important cellular components that contribute to the prevention of tumorigenesis (Rouault et al. 1996; Suzuki et al. 2002; Boiko et al. 2006). In agreement with this notion, expression of BTG/Tob genes is reduced or undetectable in a variety of clinical cancer samples (Table 1). In particular, the presence of increased levels of phosphorylated, inactive Tob1 and the absence of Tob1 protein levels correlate with tumor grade in a panel of lung cancer samples. Similarly, expression of BTG3 is reduced in the majority of

BTG/TOB, Table 1 Relationship of BTG/Tob expression and cancer

Gene	Cancer		References
TOB1	Lung	Decreased expression/increased phosphorylation[b]	Iwanaga et al. 2003. Cancer Lett 202:71–79
	Lung	Spontaneous tumor formation[a]	Yoshida et al. 2003. Genes Dev 17:1201–1206
	Lymph node	Spontaneous tumor formation[a]	*Ibid.*
	Liver	Spontaneous tumor formation[a]	*Ibid.*
	Breast	Increased expression associated with poor prognosis	Helms et al. 2009. Cancer Res 69:5049–5056
	Thyroid	Decreased mRNA expression[b]	Ito et al. 2005. Cancer Lett 220:237–242
	Pancreatic	Induced expression inhibits tumorigenesis in nude mice	Yanagie et al. 2009. Biomed Pharmacother 3:275–286
BTG1	Leukemia	Reduced/undetectable expression[b]	Cho et al. 2004. Proteomics 4:3456–3463 Waanders et al. 2012. PLoS Genet 8:e1002533 Xie et al. 2014. Cancer Genetics 207:226–230
	Lymphoma	Somatic mutations[b]	Morin et al. 2011. Nature 476:298–303 Lohr et al. 2012. PNAS 109:3879–3884 Zhang et al. 2013. PNAS 110:1398–1403
	Waldenström macroglobulinemia	Somatic mutations and deletion[b]	Hunter et al. 2014. Blood 123:1637–1646
	Gastric	Low expression associate with poor prognosis[b]	Kanda et al. 2014. Dig Dis Sci 60:1256–1264
BTG2	Breast	Reduced expression and relocalization (nuclear to cytoplasm) [b]	Kawakubo et al. 2006. Cancer Res 66:7075–7082
	Renal	Reduced mRNA levels[b]	Struckmann et al. 2004. Cancer Res 64:1632–1638
	Prostate	Low/undetectable mRNA levels[b]	Ficazzola et al. 2001. Carcinogenesis 22:1271–1279
	Brain	Induced expression inhibits medulloblastomas (transgenic mice)	Farioli-Vecchioli et al. 2007. FASEB J 21:2215–2225
	Lymphoma	Somatic mutations[b]	Morin et al. 2011. Nature 476:298–303 Lohr et al. 2012. PNAS 109:3879–3884 Love et al. 2012. Nat Genet. 44:1321–1325 Zhang et al. 2013. PNAS 110:1398–1403 Fukumura et al. 2016 Acta Neuropathol 131:865–875
BTG3	Lung	Increased lung tumor formation[a]	Yoneda et al. 2009. Cancer Sci 100:225–232
	Lung	Reduced expression in adenocarcinoma samples[b]	*Id.*
	Renal	Reduced mRNA expression[b]	Majid et al. 2009. Carcinogenesis 30:662–670
BTG4	Colon	Reduced mRNA expression[b]	Toyota et al. 2008. Cancer Res 68:4123–4132
	Leukemia	Methylation associated with good prognosis[b]	Irving et al. 2011. Epigenetics 6:300–306

[a]Observations made using mouse knock-out models
[b]Observation made using human clinical cancer samples and biopsies

lung cancer cell lines and clinical samples derived from lung cancer patients. Furthermore, BTG1 and BTG2 are frequently found to be mutated in leukemia and non-Hodgkin lymphomas (Fig. 4; Table 1). Such mutations are seemingly present in a mutually exclusive manner as compared to p53 mutations, suggesting a causative role as a component of the p53 pathway in this type of cancer.



BTG/TOB, Fig. 4 Mutations identified in BTG1 and BTG2 in non-Hodgkin lymphoma. Mutations in BTG1 and BTG2 are identified by whole-exome and RNA sequencing data from over 100 non-Hodgkin lymphomas. In some cases, both alleles contained mutations. Indicated are schematic representations of BTG1 and BTG2, the location of the BTG domain, and the presence of secondary structure elements based on the crystal structure of BTG2 (PDB structures 3dju, 3djn and 3e9v)

It is yet unknown how the identified mutations in BTG1 and BTG2 interfere with the function of the encoded gene products.

An important role for BTG/Tob proteins in the suppression of tumorigenesis is further evident from mouse knockout models. Disruption of Tob1 in mice results in susceptibility to a variety of cancers, including lung tumors, which is also observed in mice lacking BTG3 (Table 1). Thus, a direct role of several BTG/Tob proteins in the suppression of tumorigenesis and cancer development has been demonstrated in a number of cases. However, there are a few notable exceptions. For example, TOB1 expression is increased in EGF- and HER2-positive breast cancer (Table 1). In this case, TOB1 was highly phosphorylated, which may counteract the antiproliferative function of the unphosphorylated protein.

Summary

The understanding of the function and mechanisms through which the BTG/Tob proteins act has rapidly advanced in the past few years. The best characterized role of the BTG/Tob proteins is mediated by the interaction of the BTG homology domain with the CNOT7 and CNOT8 deadenylase subunits of the Ccr4-Not complex, which impacts on mRNA deadenylation. In addition, BTG/Tob proteins are also involved in the regulation of transcription and, possibly, the establishment of histone H4 modifications through the interactions of BTG1 and BTG2 with the PRMT1 methyltransferase. Mouse models have uncovered the importance of these proteins in the biology of bone and cancer. Reduced expression of BTG/Tob proteins is observed in a variety of clinical samples, and mutations in BTG1 and BTG2 are found in non-Hodgkin lymphoma. It remains to be determined whether BTG/Tob proteins regulate cell proliferation through mRNA degradation or transcriptional mechanisms or both. Furthermore, there are still many questions with respect to unique and/or redundant roles of the individual BTG/Tob proteins.

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BTG1

▶ BTG/TOB

BTG2 (TIS21, PC3)

▶ BTG/TOB

BTG3 (ANA, TOB5)

▶ BTG/TOB

BTG4 (PC3B)

▶ BTG/TOB

BTK

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Synonyms

Atk; ATK/PKB; Bpk; Bruton agammaglobulinemia tyrosine kinase; Bruton's tyrosine kinase; Xid

Historical Background

Bruton's tyrosine kinase (BTK), a member of the Tec family of nonreceptor kinases, is expressed in all hematopoietic cells except T and NK cells and functions in many different signaling pathways (Table 1). It functions as a crucial signaling molecule downstream of many receptors, including the B cell receptor (BCR) on B lymphocytes. Loss-of-function mutations in the *Btk* gene were shown to drive X-linked agammaglobulinemia (XLA), an inherited immunodeficiency disease marked by near absence of peripheral B cells and circulating immunoglobulins (Ig), first described by Dr. O.C. Bruton in 1952. Since this discovery, many striking findings have contributed to our understanding of the role of Btk in B cell development and function (Fig. 1).

Similar to humans, mutations in the *Btk* gene also underlies the milder X-linked immunodeficiency (XID) phenotype in the CBA/N mouse strain. The effects of these mutations are largely limited to the B cell lineage, stressing the importance of Btk in B cell biology. Besides XLA and XID, a role for BTK has also been described in the context of oncogenic signaling and more recently in autoimmune disease. Several inhibitors of BTK have shown great efficacy in treatment of patients with various B cell malignancies, such as chronic lymphocytic leukemia (CLL) and mantle cell leukemia (MCL). In addition, mouse models have shown that a B cell–intrinsic dysregulation of signaling can induce systemic autoimmune disease. These studies indicate that BTK expression levels and activity may be very relevant in B cell malignancies and systemic autoimmune disease.

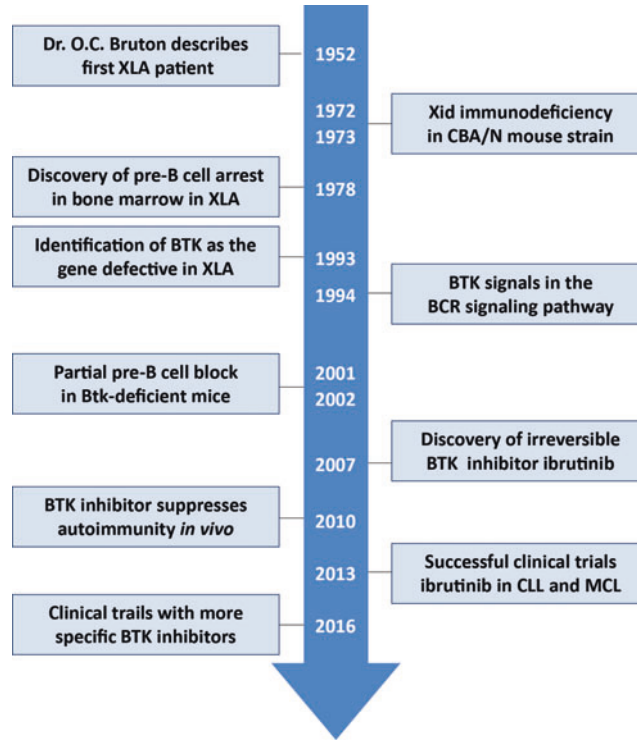
BTK, Table 1 Btk is involved in signaling pathways downstream of various receptors on different immune cell types

Receptor pathway	Cell type(s)
Pre-BCR	Pre-B cells
BCR	B cells
CXCR4	Pre-B, B cells
CD38	Activated B cells
Epo-R	Erythrocytes
TRAIL-R1	Erythrocytes
FcεR	Mast cells, basophils
FCγR	Myeloid cells
GPVI	Platelets
IL-5R	B cells, eosinophils, basophils
IL-6R	Activated B cells, plasma cells
TLR	Myeloid cells, B cells
M-CSFR	Macrophages
CD303 (BDCA-2)	Plasmacytoid dendritic cells
HGF/c-MET	Dendritic cells
fMLFR	Neutrophils

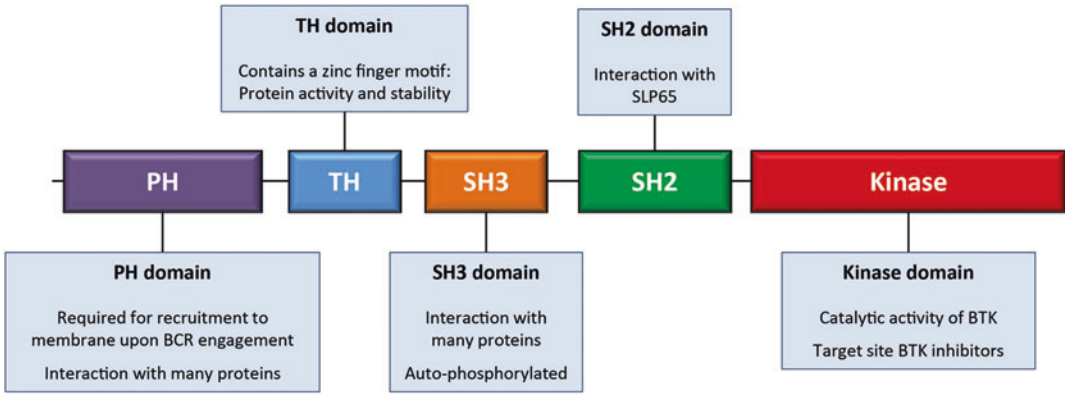
R receptor, *Epo* erythropoietin, *GPVI* collagen receptor glycoprotein VI, *IL* interleukin, *TLR* toll-like receptor, *TRAIL* tumor necrosis factor (TNF)-related apoptosis-inducing ligand, *HGF* hepatocyte growth factor, *fMLFR* formyl-methionyl-leucyl-phenylalanine receptor

BTK in B Cell Receptor Signaling

BTK is a cytoplasmic signaling molecule that is evolutionarily highly conserved and has a structure similar to SRC family kinases. The BTK protein consists of five domains (Fig. 2) (Rawlings and Witte 1995). The pleckstrin homology (PH) domain is involved in the recruitment of cytoplasmic BTK to the cell membrane upon receptor activation, and the Tec homology (TH) domain contains a zinc finger motif important for the stability of the protein. The Src homology (SH) 2 and 3 domains are involved in binding of BTK to many other proteins, including the linker molecule SLP65. In addition, the SH3 domain contains the autophosphorylation site



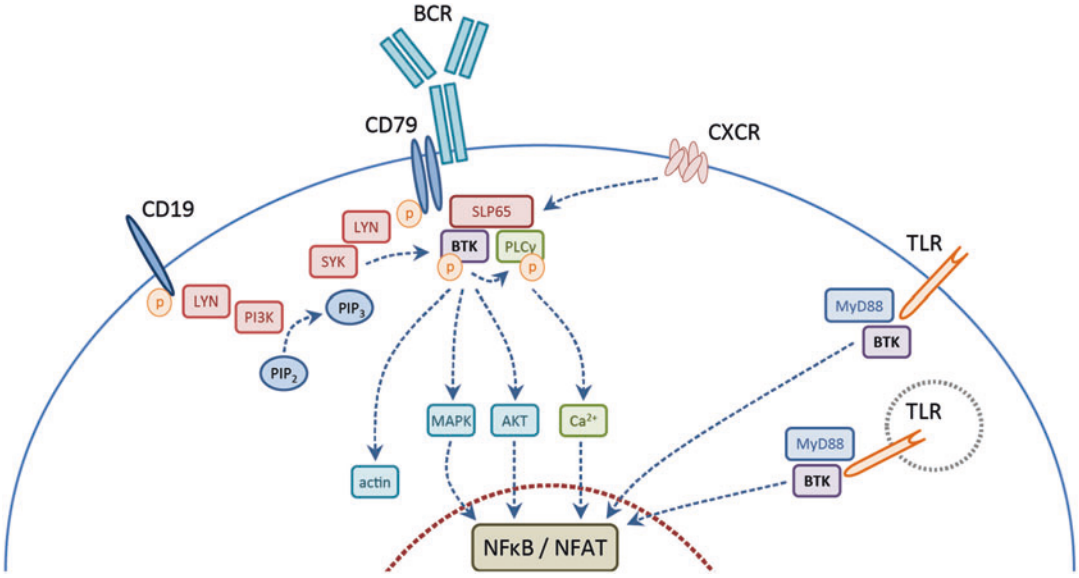
BTK, Fig. 1 Key discoveries in XLA, XID, and BTK research



BTK, Fig. 2 BTK protein structure

Y223. Finally, BTK has a kinase domain that harbors the catalytic capacity of BTK, containing the phosphorylation site Y551 that activates the protein. The kinase domain is also the target site of BTK inhibitors.

BTK plays a key role in BCR signaling (Fig. 3) (Aoki et al. 1994; de Weers et al. 1994), which provides crucial survival signals in circulating mature B cells and – upon antigen recognition – induces proliferation and terminal differentiation of B cells (Corneth et al. 2016). Moreover, BTK signals downstream of the pre-BCR, which is an immature form of the BCR that acts as a checkpoint during B cell development in the bone (Corneth et al. 2016). Upon triggering of the BCR, the Src family tyrosine kinase Lyn will phosphorylate the immunoreceptor tyrosine-based activation motifs



BTK, Fig. 3 BTK in BCR signaling

(ITAMs) of the BCR complex components CD79a/b, resulting in the recruitment of another tyrosine kinase called Syk. Lyn also phosphorylates the cytoplasmic tail of CD19, which is a coreceptor of the BCR. This will lead to the recruitment and activation of phosphoinositide 3-kinase (PI3K). Activated PI3K generates PIP_3, which can recruit BTK to the cell membrane by interacting with the PH domain. Subsequently, Lyn and activated Syk together can fully activate BTK by phosphorylation at Y551. In addition, activated Syk will phosphorylate the linker SLP65, which is crucial for the formation of a signaling complex. BTK and its downstream target phospholipase Cγ2 (PLCγ2) will then be able to bind phosphorylated SLP65 with their SH2 domains, and BTK can phosphorylate PLCγ2. This multiprotein complex is involved in the activation of many pathways, such as calcium mobilization, mitogen-activated protein kinases (MAPK) signaling, NF-κB translocation, and actin remodeling. Furthermore, it has been shown that BTK can interact with other signaling molecules such as Akt, which can also be initiated by PI3K-mediated activation upon CD19 stimulation. Akt signaling induces survival and proliferation of B cells (Corneth et al. 2016).

Upon BCR signaling, BTK protein levels in B cells are increased. It is not fully understood how BTK levels are regulated, but it is clear that BTK can induce its own transcription in an NFκB dependent way and that microRNA-185 is involved in posttranslational regulation (Corneth et al. 2016). Regulation of BTK levels is vital for normal B cell function. Subphysiological expression levels of BTK cannot restore the BTK-deficient phenotype in mice whereas physiological levels can. Furthermore, enhanced expression of BTK leads to enhanced activation of B cells and the development of autoimmunity in mice.

BTK in Other Signaling Pathways

In addition to BCR signaling, BTK plays a major role in many other signaling pathways (Table 1). Toll-like receptor (TLR) function has been described to depend on the expression of BTK (Rawlings et al. 2012). For example, Btk-deficient B cells show decreased activation following TLR4 stimulation with LPS compared to normal controls. Upon triggering, TLRs recruit adaptor molecules such as myeloid differentiation primary response gene 88 (MyD88) or TIR domain-containing adaptor protein inducing

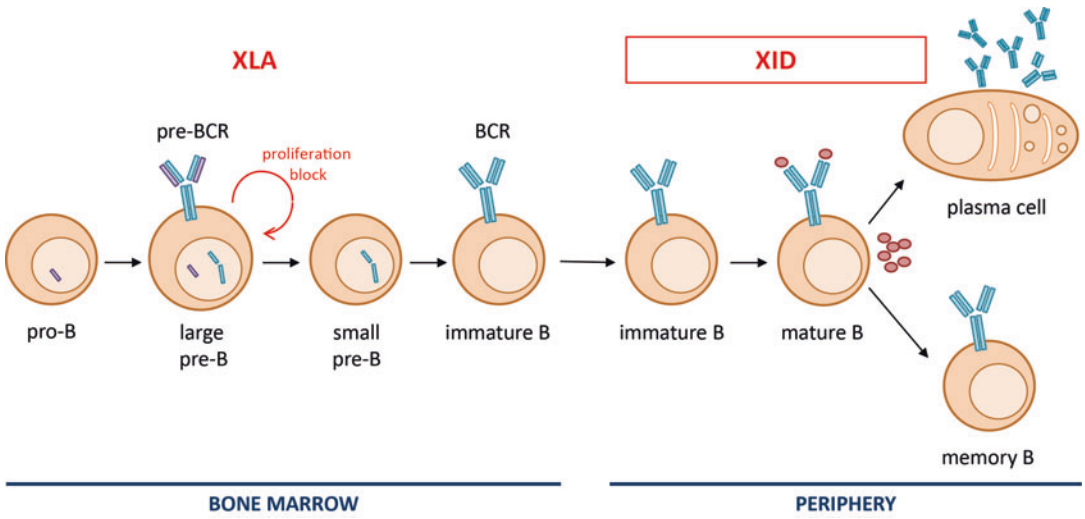
interferon-β (TRIF). Signaling via these adaptor proteins leads to the activation of interferon regulatory factor 3 (IRF3) and translocation of NF-κB, providing proliferation and survival signals. BTK is able to interact directly with TIR domains of the TLRs, but also with adaptor molecules MyD88 and TRIF and other downstream signaling molecules, although the domain of BTK that interacts with these molecules is still unknown. Furthermore, BTK has been shown to mediate synergistic signaling between the BCR and TLR9, which is an endosomal TLR that recognizes nuclear material. Synergistic signaling of the BCR with TLRs provides a strong survival signal for B cells and has been linked to development of autoimmune diseases (Rawlings et al. 2012).

BTK is also involved in chemokine-receptor signaling (de Gorter et al. 2007), in particular downstream of CXCR4 and CXCR5. Chemokine receptors belong to the family of G protein-coupled receptors and signal via G protein subunits, which can be bound by the PH and TH domain of BTK (Tsukada et al. 1994). Homing of B cells to lymph nodes was hampered in Btk-deficient mice, a process in which chemokine receptors play a key role. Furthermore, BTK inhibitors induce lymphocytosis in CLL patients by drawing malignant cells out of their (survival) niche in the lymph nodes and into the circulation (Hendriks et al. 2014).

BTK-mediated signaling has been described in Fc receptor signaling, which is not limited to B cells, but also affects monocytes and macrophages. Depending on the nature of the Fc receptor, signaling through BTK may activate a B cell or induce its apoptosis. In addition, BTK may also play a role in CD38 and CD40 signaling; however, the exact role of BTK in these pathways is less well defined (Corneth et al. 2016).

BTK in Immunodeficiency

Mutations in the *BTK* gene in humans are the underlying cause of the severe primary immunodeficiency disease, X-linked agamaglobulinemia (XLA) (Tsukada et al. 1993; Vetrie et al. 1993). XLA, which was first described in 1952 by Dr. O. C. Bruton (1952), is the most common primary immunodeficiency with a reported incidence of 1/380,000 live births in the USA. BTK has a crucial function in pre-BCR signaling and therefore in the associated checkpoint during B cell development (Corneth et al. 2016). Early in B cell development (Fig. 4), functional gene recombination events within the Ig heavy chain gene locus result in surface expression of the heavy chain, which marks a crucial checkpoint.



BTK, Fig. 4 B cell development. Defects in XLA and XID are indicated

In the pre-BCR complex, the Ig heavy chain protein is associated with the invariant surrogate light chain proteins that have homology to Ig light chains. Expression of the pre-BCR induces clonal proliferation of large pre-B cells and subsequently their developmental progression to the stage of resting, small pre-B cells. In human pre-B cells, the pre-BCR-mediated expansion and differentiation is crucially dependent on BTK (Corneth et al. 2016). In the bone marrow of boys with XLA, the number of pre-B cells expressing intracellular Ig heavy chain is rather variable but generally reduced. These pre-B cells are significantly smaller in XLA patients than in healthy controls, which is in agreement with a crucial function of BTK in the induction of proliferative expansion of pre-B cells that Ig heavy chain in their cytoplasm. But even those pre-B cells present in XLA patients appear to have a developmental block, since very few pre-B cells undergo Ig light chain recombination. In healthy individuals, the transition from large cycling to small resting pre-B cells is marked by the initiation of Ig κ and λ light chain rearrangement. Following successful Ig light chain rearrangement, the pre-B cells progress to the immature B cell compartment, in which the BCR is checked for autoreactivity. If these immature B cells do not recognize antigen, they leave the bone marrow (Fig. 4). Taken together, BTK deficiency leads to a severe block in early B cell development in the bone marrow at the pro- to pre-B cell stage, resulting in an almost complete absence (<1%) of mature B cells in the circulation (Pearl et al. 1978) (Fig. 4). As a consequence, there are no plasma cells and very low levels of immunoglobulins in the periphery. Those few B cells that do remain have an immature IgM^{hi} phenotype and harbor BCRs that are auto- or poly-reactive; however, autoimmune diseases in these patients are relatively rare.

The gene encoding BTK is located on the X-chromosome. Therefore, heterozygous female carriers of a BTK mutation are healthy, whereas affected males present with recurrent infections of the airways, the gastrointestinal tract, and the skin caused by parasites and encapsulated bacteria. B cells of female carriers all express the unaffected X-chromosome and have inactivated the affected chromosome. This is explained by the phenomenon of random X-chromosome inactivation that takes place in every female somatic cell early in embryogenesis, whereby in female XLA carriers developing B cells that harbor the defective *BTK* gene on their active X chromosome have a selective disadvantage. This is not the case for other cell types that express BTK, indicating that the defect in XLA is B cell-intrinsic. Furthermore, because T cells and NK cells do not express BTK and are therefore unaffected, viral infections do not cause severe problems in patients (Corneth et al. 2016).

XLA is a very heterogeneous disease. Many mutations that cause loss of function of BTK have been described in all domains, except the SH3 domain containing the autophosphorylation Y223 tyrosine residue. In addition, no correlations have so far been made between specific mutations and clinical or immunological symptoms. XLA patients require life-long treatment with intravenous Ig and antibiotics, but when on sufficient treatment they are relatively healthy, indicating that the effects of loss of BTK are mostly restricted to humoral immunity (Corneth et al. 2016). It has been proposed that – based on promising findings in animal models – XLA forms a good candidate for gene therapy replacing current noncurative treatment.

In contrast to human XLA patients, *xid* CBA/N mice, which harbor a mutation in the Btk gene, present with the milder X-linked immunodeficiency (XID) phenotype (Amsbaugh et al. 1972). Homozygous Btk-deficient mice show normal B cell development in the bone marrow. However, heterozygous females show loss of Btk-deficient B cells beyond the pre-B cell stage, indicating a selective advantage of Btk-sufficient B cells similar to human B cells (Hendriks et al. 1996). Transition through the pre-B cell stage is delayed in Btk-deficient compared to wild-type B cells, consistent with the role for Btk at the pre-BCR checkpoint. Furthermore, Btk is actively involved in light chain rearrangement and λ immunoglobulin light chain usage is reduced in Btk-deficient B cells (Corneth et al. 2016).

B cells are present in Btk-deficient mice in the circulation, although they are ~50% reduced in

number compared to normal control mice (Fig. 4). They retain an immature $IgM^{hi}IgD^{lo}$ phenotype and show impaired activation and differentiation in vitro. They fail to proliferate upon IgM or IgD stimulation and cannot obtain an activated phenotype upon IgM stimulation. BCR-mediated survival signals are decreased in Btk-deficient B cells, and they are more sensitive to apoptosis due to lower expression of the survival proteins BCL2 and BCL-XL. However, they do respond normally to Phorbol myristate acetate/ionomycin stimulation, which bypasses the BCR (Corneth et al. 2016).

Follicular and marginal zone B cell numbers in the spleen are reduced in Btk-deficient mice, although the proportions of these populations are relatively normal. In contrast, B1 B cells, which are a specific subset of self-renewing B cells of mainly fetal origin with a specific BCR repertoire, are completely absent in the spleen and peritoneal and pleural cavities. As a consequence, IgM and IgG3 isotype antibody levels are decreased in Btk-deficient mice, which can be explained by the lack of natural antibodies produced by B1 cells, but other isotypes are normally present. Btk-deficient mice fail to show a B cell response to thymus independent TI antigens, which is thought to be dependent on B1 cells. In addition, Btk-deficient mice have reduced antigen-specific antibody levels upon primary immunization with thymus dependent TII antigens. However, secondary immunization mounts a normal memory response, suggesting that Btk is not crucial for germinal center, memory B cells, or plasma cell formation. In contrast with the observed normal T cell-dependent responses to model antigens in adjuvants, Btk-deficient mice have reduced numbers of GC B cells in their draining lymph nodes following pulmonary infection with influenza virus (Corneth et al. 2016).

As in humans, the defect in Btk-deficient mice is restricted to humoral immunity. Infections with pathogens which require the presence of natural antibodies will lead to more severe disease. However, Btk-deficient mice will develop less severe disease upon infections with pathogens that induce the production of harmful antibodies or primarily infect B1 cells (Corneth et al. 2016).

BTK in Cancer

Btk has been implicated in both murine and human leukemia and lymphoma (Hendriks et al. 2014). Murine Btk-deficient pre-B cells show increased proliferation in vitro. Although Btk deficiency alone does not lead to tumor formation in vivo, combined deficiency with SLP65 enhances pre-B cell leukemia in mice compared to SLP65 single mutants, showing tumor suppressive capacity of Btk in pre-B cells which was independent of its kinase function. Mutations in BTK have been found in human childhood pre-B cell acute lymphoblastic leukemia (pre-B ALL), but these were all mutations affecting kinase functions of BTK; a single XLA patient with pre-B ALL has been described. On the other hand, overexpression of Btk in murine B cells leads to decreased susceptibility to apoptosis. Again overexpression of Btk alone did not lead to tumor development but did increase the incidence and mortality rate of mice in a chronic lymphatic leukemia (CLL) mouse model. Interestingly, in this model, deficiency of Btk prevented tumor development, clearly illustrating the differential roles for Btk in pre-B cells and mature B cells. Although it is still unclear whether mutations in BTK can cause B cell tumors in humans, these data show the importance of correct regulation of expression levels of BTK.

BTK protein expression is enhanced in several B cell malignancies, including CLL and mantle cell lymphoma (MCL), and in some patients, phosphorylated BTK is also highly expressed (Hendriks et al. 2014). Because BTK is crucial for B cell survival and proliferation, great effort has been undertaken to develop specific inhibitors targeting BTK. Several of these inhibitors have already shown impressive efficacy in human B cell malignancies in vitro and in vivo. Treatment with ibrutinib, the first FDA approved small molecule inhibitor of BTK approved in the clinic, significantly reduced survival and proliferation of primary tumor cells and tumor cell lines in vitro. Phosphorylation of PLCγ2, Akt, and ERK, important downstream targets of BTK, was reduced in these cultures. Importantly, not only viability of the cells was affected but also adhesion and migration, through inhibition of BTK dependent

chemokine receptor signaling. In CLL and MCL, this is considered the main mode of action of BTK inhibition (Byrd et al. 2013; Wang et al. 2013). Upon BTK treatment, patients exhibit lymphocytosis, caused by an egress of malignant cells from the lymph nodes. Upon leaving the lymph nodes, tumor cells lose important survival signals provided by stromal cells, rendering them susceptible to apoptosis. In addition, tumor cells lose the cell intrinsic proliferation signals mediated through BCR signaling induced phosphorylation of PLCγ2 and Akt, which may also contribute to the successful elimination of cancer cells (Hendriks et al. 2014).

BTK inhibition may also affect TLR signaling or the interaction between BCR and TLR signaling in tumors (Hendriks et al. 2014). In Waldenström's macroglobulinemia patients, who frequently harbor an activating mutation in MyD88, BTK is often constitutively active. BTK inhibition was shown to limit interaction of BTK and MyD88 in these patients and to induce apoptosis in vitro. However, in vivo, it is unclear whether BTK inhibition works primarily through inhibition of the TLR signaling pathway or whether inhibition of the BCR and chemokine receptors is more important.

Apart from affecting tumor cells, BTK inhibition also limits tumor development by targeting the tumor cell survival niche (Hendriks et al. 2014). In multiple myeloma (MM), a plasma cell-derived tumor, BTK inhibition crucially affects osteoclasts in the bone marrow that provide essential survival signals to MM cells, including CCL3, an important marker for disease progression. Ectopic expression of BTK was found in nonhematological tumors, including prostate cancer and breast cancer cell lines. Inhibition of BTK in these tumors shows promising results, suggesting that the role of BTK in aberrant cell proliferation is not limited to the hematopoietic lineage.

Although BTK inhibition has shown impressive efficacy in lymphoma patients, not all patients respond well to this therapy. In some tumors, mutations in BTK or other genes were shown to promote resistance to BTK inhibitors (Chiron et al. 2014). These mutations may be present before onset of treatment, but mutations have been shown to arise during treatment, although it is unclear whether treatment itself may promote these mutations. To overcome this therapy resistance, new treatment strategies are being developed, including novel more selective inhibitors, including Acalabrutinib (Byrd et al. 2016) for treatment of CLL, that are specifically designed to improve on the safety and efficacy of BTK inhibition. Moreover, inhibitors of multiple pathways are combined and now being tested in the clinic. Indeed, combinations of BTK inhibitors with PI3K inhibitors or inhibitors of the Akt pathway have shown better results than monotreatment (Woyach et al. 2014).

BTK in Autoimmunity

B cells are involved in many autoimmune diseases, and B cell intrinsic defects have been shown to be sufficient to induce autoimmunity in mice (Corneth et al. 2016). The discovery that BTK plays a crucial role in the selection of pre-B cells during development and in activation of mature B cells in the periphery prompted studies into the role of BTK in autoimmune diseases. Early studies in mice showed an important role for Btk in the formation of autoreactive antibodies. When the XID mutation was crossed into the lupus-prone NZWxNZB or MRL.lpr/lpr background, spontaneous autoantibody formation and kidney damage were dramatically reduced. Interestingly, stimulation of B cells from these mice with TLR ligands did induce the production of nonautoreactive antibodies. Similarly, Btk deficiency in the NOD mouse model of diabetes prevented the development of autoantibodies without affecting total antibody levels in serum of mice (Corneth et al. 2016).

Studies with NOD mice harboring an insulin-reactive BCR transgene showed that BTK deficiency affects only mature cells in the periphery as insulin specific pre-B cells in the bone marrow were unaffected. Similarly, expression of low levels of the constitutively active E41K-BTK mutant, which allows for B cells survival past the pre-B cell stage, enhances B cells survival and activation, leading to a rapid enhanced formation of IgM plasma cells producing autoreactive

antibodies. These data indicate that BTK expression and activation levels affect mature B cells and may be involved in peripheral B cell selection (Corneth et al. 2016).

Overexpression of human BTK specifically in B cells in mice leads to a spontaneous autoimmune phenotype resembling systemic lupus erythematosus (SLE) and Sjögren's syndrome. Mice first develop spontaneous germinal centers and plasma cells in the spleen, followed by an increase in memory B cells and plasma cells in the bone marrow. Plasma cells produce autoreactive antibodies leading to antibody deposition in the kidneys and immune infiltrates of the kidneys, salivary glands, and lungs. This phenotype depends strongly on T cells, as crosses with CD40 ligand-deficient mice, inhibiting B-T cell interaction, abrogated the disease. However, these mice did still develop IgM-autoreactive antibodies, suggesting that BTK may be involved in a two-step induction of autoreactivity, by enhancing survival of autoreactive B cells and subsequent induction of the germinal center response. Importantly, the phenotype depended on BTK kinase activity, as a kinase inactive BTK mutant did not develop autoimmunity, and inhibition of BTK kinase activity by ibrutinib prevented the formation of spontaneous germinal centers (Corneth et al. 2016).

Because these studies show the involvement of Btk in B cell mediated autoimmunity, Btk inhibition has been studied extensively in mouse autoimmune models. In collagen-induced arthritis, a mouse model for rheumatoid arthritis (RA), Btk inhibition before onset of disease completely prevented arthritis development, and treatment after onset greatly decreased disease severity. Similar to Btk-deficient mice in autoimmune models, inhibition of Btk affected the formation of autoantibodies, but nonautoimmune antibodies in serum remained present. Of note, the efficacy of Btk inhibition in this model may be partly due to the important role for Fc-mediated signaling in monocytes and macrophages, which is also dependent on Btk. In addition, in several models of murine lupus, Btk inhibition limits the formations of autoantibodies and prevents or decreases levels of kidney damage, significantly improving survival of mice (Honigberg et al. 2010; Corneth et al. 2016).

BTK expression in human autoimmune patients has not yet been extensively studied, although some studies indicate a pathogenic role for BTK. In RA patients, phosphorylated BTK levels correlate with rheumatoid factor (RF) titers and are increased in anti-citrullinated-protein-antibody (ACPA) positive patients, indicating a link between activation of BTK and autoantibody production. In addition, BTK signaling was required for IL-21 expression by B cells, which is important for the maintenance of tertiary lymphoid follicles involved in autoantibody production. Furthermore, upstream signaling molecule SYK was more highly expressed in blood of RA patients. In SLE patients, expression of downstream target ARID3A was shown to be correlated with disease severity. The promising results of BTK inhibition in mouse autoimmunity studies and the finding that BTK inhibitors are very well tolerated by leukemia patients with limited side effects have prompted several clinical trials of BTK inhibitors in human autoimmune diseases that are currently underway (www.clinicaltrials.gov).

Summary

BTK is a signaling molecule expressed in many hematopoietic cells but most crucially involved in B cell development in the bone marrow and activation and terminal differentiation of peripheral B cells. As it is involved in many signaling pathways, deregulated BTK expression can lead to a number of clinical diseases. BTK deficiency in humans leads to XLA, a severe X-linked immunodeficiency affecting humoral immunity. Male with this defect suffer from severe recurrent infection due to loss of peripheral B cells. In mice, Btk deficiency leads to a milder phenotype with decreased numbers of B cells and impaired humoral immunity. BTK signaling is crucially involved in the proliferation, migration, and adhesion of leukemic cells in several B cell-derived

malignancies. Inhibition of BTK in these patients leads to expulsion of cells from their survival niche and discontinuation of intrinsic survival signals and is now a very successful new therapeutic approach in the clinic. In addition, increased expression of Btk in mice can induce an autoimmune phenotype, and BTK has been implicated in human autoimmune diseases. Ongoing clinical trials will reveal the potential of BTK inhibitors in autoimmune patients.

See Also

- ▶ AKT
- ▶ NF-κB Family
- ▶ PI3K

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BUB1

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Synonyms

BUB1A; BUB1L

Related Molecules in the Encyclopedia

► Monopolar Spindle 1 (Mps1); ► AURORA Kinases; ► BUBR1

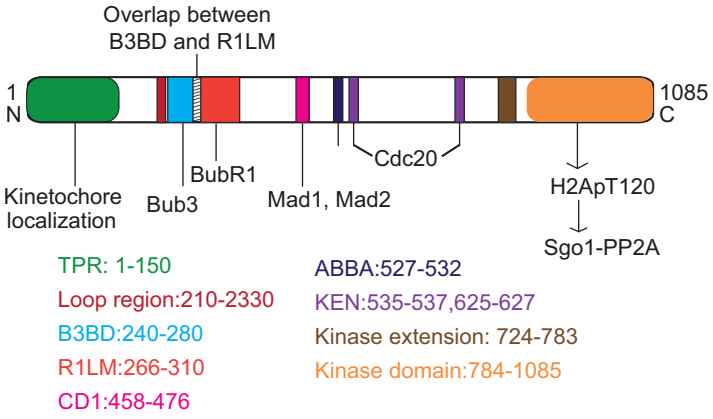
Historical Background

Bub1 was originally discovered as a gene required for cell cycle arrest during mitosis in response to the microtubule depolymerizing drug benzimidazole in the model organism *Saccharomyces cerevisiae* (Hoyt et al. 1991). Mutant yeast were unable to arrest and inhibit the budding process at the end of mitosis, a marker for cell cycle progression, hence the name *Budding Uninhibited* by *Benzimidazole* 1 (BUB1). Through its capacity to contribute to mitotic arrest, BUB1 functions as an integral component of the spindle assembly checkpoint (SAC), a surveillance mechanism that delays mitotic progression until all kinetochores are properly attached to microtubules, and aligned at the spindle equator in metaphase. Since its original discovery in budding yeast, a SAC function for BUB1 has been verified in all model organisms studied to date (reviewed in (Elowe 2011). Soon after its discovery, hBUB1 was characterized as a Serine/Threonine kinase able to autophosphorylate and constitutively associate with another of the original BUB proteins, BUB3 (*Budding Uninhibited* by *Benzimidazoles 3*). It was recognized early on that BUB3 plays an important role in BUB1 recruitment to kinetochores (Roberts et al. 1994; Taylor et al. 1998) which are macromolecular structures that assemble onto centromeres at each mitosis and form both the signaling platform for the SAC as well as the major microtubule binding interface on dividing chromosomes (reviewed (Cheeseman 2014)). The human *Bub1* gene maps to chromosome 2 at 2q14, includes 25 exons (NCBI gene ID 699) (Cahill et al. 1999), and was identified by virtue of its homology to budding yeast BUB1 (Cahill et al. 1998). Indeed mutants of this gene were identified in panel of chromosomally instable colorectal cancers. This led to an enormous body of work that aimed to explore mutations in SAC genes in various cancers. While it is now recognized that complete inactivation of the SAC is incompatible with cell survival, this early work was nevertheless influential in that it revived interest in the role of aneuploidy in the development of cancer, and set the pace for much of the more recent work exploring the relationship between mitotic deregulation, chromosome segregation defects, aneuploidy, and cancer (reviewed in (Gordon et al. 2012).

Structural Aspects of BUB1

The BUB1 N-Terminus: A Kinetochore Localization Module

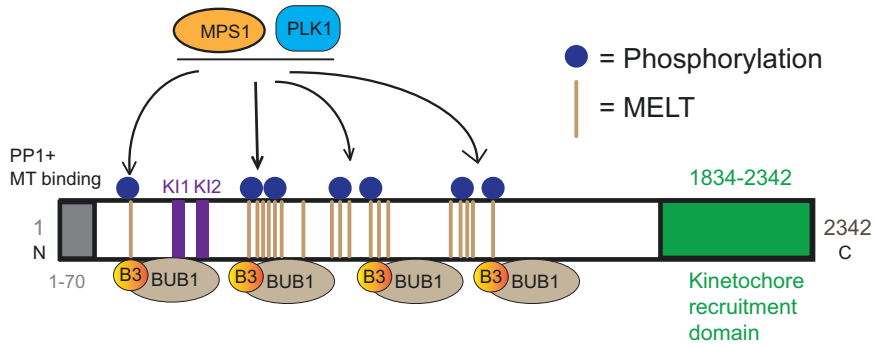
The BUB1 protein has several well-characterized and highly conserved structural motifs (Fig. 1). At the very N-terminus of BUB1 orthologs is the TPR (tetratricopeptide repeat) domain – which consists of a triple tandem repeats of 34 amino acids – that bears strong structural similarity to the TPR domains of the paralog protein BUBR1 (*Budding Uninhibited by Benzimidazole Related 1*) and MPS1 (*MonoPolar Spindle 1*), suggesting a common evolutionary ancestor for this domain (Bolanos-Garcia and Blundell 2011; Lee et al. 2012). A short region termed as "loop region" follows the TPR domain and is implicated in BUB3 binding to KNL1 (*Kinetochore Null 1*, also known as Blinkin or AF15q14 in humans),



BUB1, Fig. 1 Bub1 structural domains in humans: Bub1 structural domains shown with respective functions and recruitment targets. At N-terminus of Bub1 is a tetratricopeptide repeat (TPR) that interacts with Knl1. The BUB3-binding domain (B3BD)-mediated direct interaction with BUB3. R1LM (BubR1 localization motifs) is needed for direct binding of BubR1 to Bub1 and its kinetochore recruitment. The middle region contains conserved motif1 that is required for SAC and Mad1, Mad2 recruitment, while KEN boxes and ABBA motifs are need for Cdc20 binding and kinetochore recruitment. C-terminal region comprises kinase extension or N-terminal extension domain required for Bub1 activation and a serine/threonine kinase domain whose activity is required for chromosome congression and Sgo1 recruitment in humans. The numbers represent amino acids for each region. "N" and "C" are amino-terminus and carboxy-terminus, respectively

a kinetochore scaffold protein and a major receptor for SAC signaling molecules (Kiyomitsu et al. 2007). Interestingly, the equivalent loop from BUBR1 cannot substitute for the BUB1 loop in this context (Primorac et al. 2013). The difference between BUB1 and BUBR1 is likely due to their divergent loop sequences which is of functional interest; while the BUB1 loop promotes its recruitment to kinetochores, in BUBR1, this loop may instead be required for interaction with the APC/C (*Anaphase Promoting Complex/Cyclosome*), a large E3 ubiquitin ligase that is the target of the SAC and the MCC (*Mitotic Checkpoint Complex*), the principal inhibitory complex of the APC/C (Overlack et al. 2015).

The TPR domain of BUB1 is followed by the BUB3-binding domain (B3BD, also known as the GLEBS (Gle2- Binding Sequence)), a short conserved stretch of 40 amino acids between residues 240 and 280 (hBUB1 numbering) that forms extensive interactions with the β-propeller structures of BUB3 (reviewed in (Bolanos-Garcia and Blundell 2011)). Both the TPR and B3BD are implicated in the kinetochore docking of BUB1, which occurs through direct interaction between BUB1, BUB3, and KNL1 (Taylor et al. 1998; Kiyomitsu et al. 2007). Since the identification of KNL1 (Kiyomitsu et al. 2007), the mechanism of BUB1 binding to kinetochores has been a matter of intense research. KNL1 orthologs contain multiple and often degenerate copies of a short motif known as MELT (for the consensus sequences in hKNL1, Met-Glu-Leu-Thr) (Fig. 2). These motifs are phosphorylated at the Threonine residue by the MPS1 kinase in most species and become direct recognition motifs for BUB3 in complex with BUB1 kinase (London et al. 2012; Shepperd et al. 2012; Primorac et al. 2013). Thus, BUB1 is recruited to kinetochores by upstream phosphorylation of KNL1 by MPS1. Some nematode lineages such as the model organism *C. elegans* however do not have an MPS1 homologue. Here, the mitotic kinase PLK1 (Polo-Like Kinase 1) is the major kinase of MELT motifs (Espeut et al. 2015). Recent work also suggests that in human cells, PLK1 may cooperate with MPS1 in MELT phosphorylation and BUB1 recruitment (von Schubert et al. 2015). BUB1 binding to kinetochores is further enhanced by interaction with KI1 (*Lys-IIe 1*) motif of KNL1 (Krenn et al. 2014). Several TΩ (T= Threonine, Ω = Tyrosine/Phenylalanine) motifs, similar to



BUB1, Fig. 2 BUB1 kinetochore recruitment: KNL1 acts as an anchor for BUB1 at kinetochores. At N-terminus, KNL1 binds microtubules (MT), while C-terminus residues 1834–2342 are required for recruitment to kinetochores. KNL1 contains consensus motifs known as MELT motifs. At least 19 MELT motifs have been identified in humans. MPS1 and PLK1 share phosphorylation of these motifs which are read by BUB3 in complex with BUB1. BUB1 also interacts with KI1 of Knl1 which enhances BUB1 binding to kinetochores. Once at kinetochores, BUB1 recruits its downstream targets to kinetochore

KI1 motif, have been identified that are present in close proximity to MELTs and contribute to hBUB1 kinetochore recruitment (Vleugel et al. 2013). KI motifs are however poorly conserved and may have evolved in higher eukaryotes to allow for more fine-tuned regulation of the BUB1-BUB3 interaction with KNL1. BUB1 recruitment to kinetochores is critical for its function in SAC signaling as it serves to scaffold the kinetochore loading of other checkpoint signaling molecules (Fig. 2), as discussed below.

The BUB1 Middle Region Contains the SAC Signaling Motifs

BUB1 contains two KEN (Lys-Glu-Asn) boxes (residues 535–537 and 625–627) required for the interaction with and phosphorylation of CDC20 (*Cell Division Cycle 20*), an activating and specificity-determining co-factor of APC/C (Kang et al. 2008; Jia et al. 2016). Earlier reports identified BUB1 KEN boxes as degradation motifs required for BUB1 destruction by the APC/C (Qi and Yu 2007). However, a later investigation reported a requirement for KEN boxes in hCDC20 binding and phosphorylation in synergy with PLK1, which was proposed to be essential for SAC activation (Jia et al. 2016). Recently, a motif termed as ABBA (*Cyclin* A*, BUBR1, BUB1, and Acm1*, also known as the Phe-box, A-box; BUB1 residues 527–532) was shown to contribute to the BUB1-CDC20 interaction (Di Fiore et al. 2015). Deletion of BUB1 residues encompassing this region caused a reduction in CDC20 kinetochore localization (Di Fiore et al. 2015; Vleugel et al. 2015), although a similar role has been proposed for the ABBA motif of the BUB1 paralog BUBR1 (Lischetti et al. 2014). Another important segment in the middle region is the conserved motif1(CD1) (hBUB1 residues 458–476) and is required for SAC function likely through mediating recruitment of MAD (*Mitotic Arrest Deficient*) 1 and 2 (Klebig et al. 2009).

The BUB1 C-Terminus Includes a Highly Conserved Serine/Threonine Kinase Domain

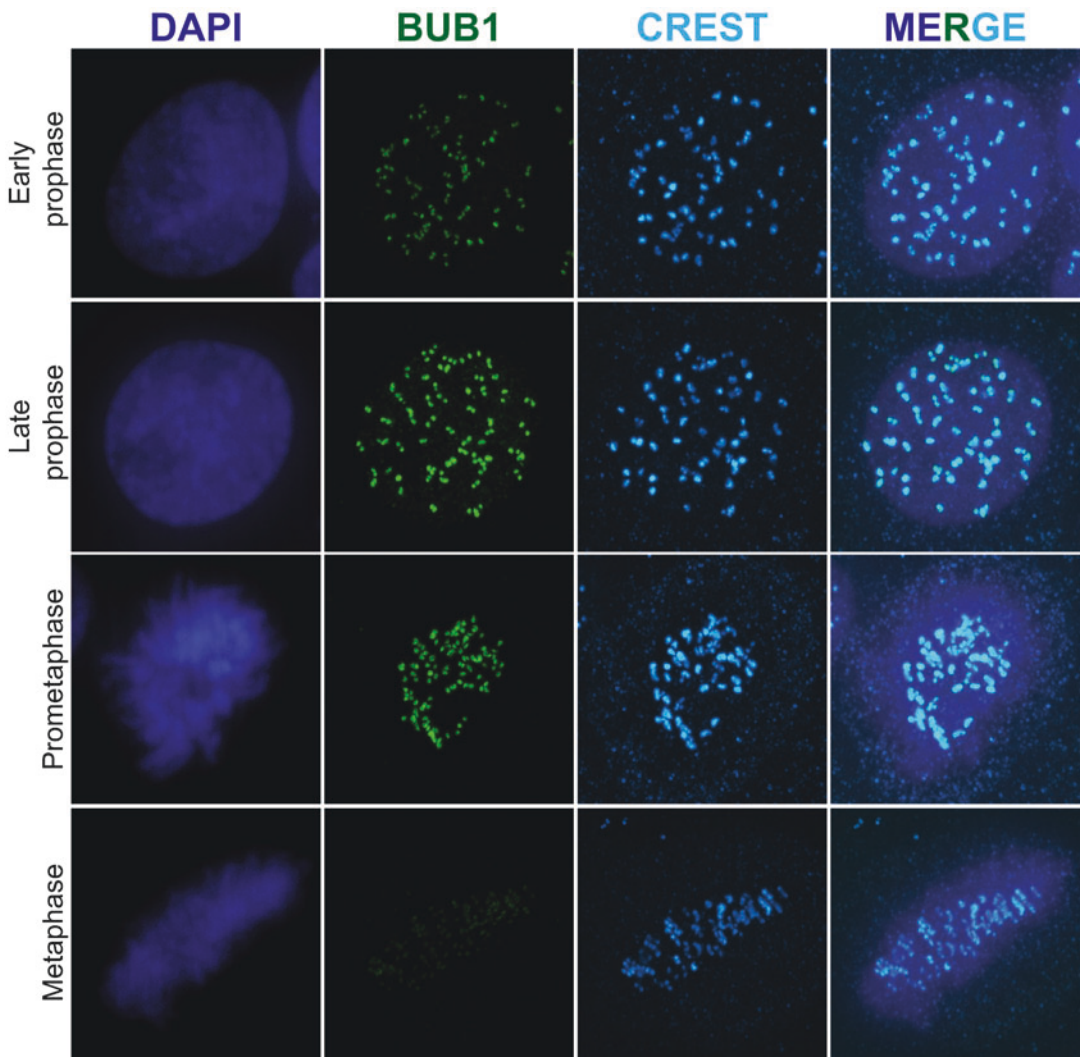
At the C-terminus, BUB1 has a kinase extension region (amino acids 724–783) required for the activation of BUB1 followed by the kinase domain (amino acids 784–1085), which contributes to chromosome congression and alignment (Kang et al. 2008; Klebig et al. 2009), and potentially the SAC (Tang et al. 2004; Ricke et al. 2011; Ricke et al. 2012; Jia et al. 2016). Two phosphosubstrates of BUB1 have been identified so far in addition to its autophosphorylation (See below). BUB1 phosphorylates Histone H2A at S121 which then allows SGO protein binding and recruitment to kinetochores in fission yeast (Kawashima et al. 2010). Similarly mouse and human H2A phosphorylation by BUB1at the

equivalent residue has also been reported (Ricke et al. 2012; Liu et al. 2013). A single mutation in Sgo1 (K492A) abolishes the interaction between SGO1 and H2ApT120 (Liu et al. 2013), suggesting that it is the direct site of interaction between these two proteins. On the other hand, CDK1 (cyclin dependent kinase 1) phosphorylates SGO1 at T346, which is required for SGO1 interaction with cohesin, a protein complex needed for sister chromatid cohesion (Liu et al. 2013). The mutation of both sites (K492A and T346A) abolishes SGO1 localization at chromosomes. Hence, both H2AT120 phosphorylation by BUB1 and cohesin binding promote SGO1 recruitment to inner centromeres (Liu et al. 2013). SGO proteins form a complex with PP2A (protein phosphatase 2A) that removes phosphorylation of cohesin subunits to prevent premature sister chromatid separation; thus, BUB1 kinase activity is essential for cohesion protection at centromeres through recruitment of SGO-PP2A complex (Kitajima et al. 2006; Tang et al. 2006). BUB1 phosphorylation of H2AT120 has also been suggested to contribute to SAC functioning through proper localization and activation of the AURORA B kinase at centromeres (Ricke et al. 2012). The second direct substrate of BUB1 kinase activity is CDC20 which contains, at least six sites in its N-terminus potentially phosphorylated by BUB1. Mutation of these sites to alanine causes inefficient APC/C inhibition in vitro and SAC defects in vivo, as measured by early mitotic exit compared control cells (Tang et al. 2004). Recent work suggest that PLK1 may cooperate with BUB1 to phosphorylate these sites, demonstrating yet another potential redundancy in SAC kinase signaling (Jia et al. 2016).

Regulation of BUB1 Kinetochore in Early and Late Mitosis

BUB1 is a stable kinetochore protein in fission yeast and mammalian cells as demonstrated by its relatively slow turnover and exchange at unattached kinetochores compared to other SAC proteins like MAD2, BUBR1, and MPS1 (Howell et al. 2004; Shah et al. 2004; Rischitor et al. 2007). Autophosphorylation has been implicated in restricting hBUB1 turnover at kinetochores (Asghar et al. 2015). Mutation of a single autophosphorylation site to alanine (T589A) increases hBUB1 kinetochore turnover, resulting in an increase in cytoplasmic BUB1 levels and ectopic phosphorylation of its substrate H2AT120, at chromosome arms, and consequently ectopic recruitment of the H2ApT120 binding partner SGO1, resulting in aberrant chromosome congression and sister chromatic cohesion. Artificially stabilizing this BUB1 mutant at kinetochores refocuses H2ApT120 and SGO1 levels back to centromeres (Asghar et al. 2015).

BUB1 begins to accumulate at kinetochores at the start of prophase (Fig. 3), with its levels peaking at prometaphase and gradually diminishing during metaphase when correct attachments between kinetochores and microtubules are established and after which the silencing of the SAC signal occurs. Final loss of BUB1 from the kinetochores occurs during early anaphase (Sharp-Baker and Chen 2001). How BUB1 is removed from kinetochores after chromosome segregation is not well understood, and this area of inquiry has been recently explored. Evidence suggests that BUB1 could be removed from kinetochores by the Dynein motor protein in human cells (Silva et al. 2014). ATP depletion in cells affects Dynein cargo release without affecting Dynein activity; indeed, BUB1 accumulated at spindle poles in ATP depleted human cells (Silva et al. 2014). This was further confirmed when either Dynein or spindly, a recruiter of Dynein at kinetochores, was depleted from cells, which resulted in loss of BUB1 localization to spindle poles, thus confirming that BUB1 is a Dynein cargo (Silva et al. 2014). Another mechanism through which BUB1 may be removed from kinetochores was initially described in budding yeast and implicates PP1 (*protein phosphatase 1*), a known player in SAC silencing (Vanoosthuyse and Hardwick 2009; Rosenberg et al. 2011). In this organism, BUB1 was stripped from kinetochores in a PP1-dependent manner, through complex formation with the PP1 adaptor subunit Fin1 (Bokros et al. 2016). Cells in which Fin1 protein expression was abrogated displayed



BUB1, Fig. 3 BUB1 kinetochore localization: Immunofluorescence images are shown in which expression of BUB1 in HeLa is monitored during mitotic progression. Anti-BUB1 and anti-CREST (a centromere marker) antibodies were used to stain mitotic cells. The BUB1 signal can be detected as early as prophase during which BUB1 has a clear punctate signal on kinetochores. As cells traverse mitosis, the signal strength increases and is the strongest during prometaphase. The BUB1 signal begins to diminish in metaphase and is lost completely during anaphase (not shown). DAPI is used to stain the chromosomes and to mark the mitotic stage

abnormal BUB1 signal after anaphase entry, demonstrating the important role of Fin1 and PP1 in BUB1 protein removal from kinetochores and SAC silencing in budding yeast (Bokros et al. 2016). More recently a prominent function during mitotic progression has been demonstrated for the protein phosphatase 2A (PP2A), and in both budding yeast and human cells, it has been suggested that complex interplay between PP1 and PP2A promotes SAC silencing by removing SAC protein including BUB1 from kinetochores (Espert et al. 2014; Nijenhuis et al. 2014).

Activation of BUB1 Kinase

The crystal structure of the active form of BUB1 kinase domain (Kang et al. 2008) and, more

recently, the structure of the active autophosphorylated (pS969) kinase have been reported (Lin et al. 2014). The BUB1 kinase domain deviates from canonical kinase domain found in other kinases such as PKA (protein kinase A) in certain aspects (Lin et al. 2014). For example, the canonical motifs at the catalytic and activation segments are slightly different. The canonical HRD is modified into HGD, the DFG into DLG, and the APE into CVE. Moreover, BUB1, as discussed above, has an extended kinase activation domain also known as an N-terminal extension domain which forms extensive interactions with N- and C-lobe of kinase domain to stabilize it. The mode of activation of BUB1 kinase domains by kinase extension domain is much like cyclins in activating CDKs, and mutations in this kinase extension domain severely attenuate kinase activity of BUB1(Kang et al. 2008). The structural comparison of unphosphorylated and phosphorylated BUB1 (BUB1pS969) showed that there are no major differences between the two structures except in P+1 loop of activation segment. Structural rearrangements in this region after autophosphorylation at S969 act as a molecular switch required for activation of BUB1 kinase (Lin et al. 2014). Autophosphorylation of S969 is needed for kinase activity towards H2A yet it is dispensable for CDC20 phosphorylation which could be due to differences in binding affinity of CDC20 and H2A phosphoresidues with the activation segment of BUB1 (Lin et al. 2014). In addition to localization, the TPR domain of BUB1 was proposed to induce long range activation of C-terminal kinase domain as mutagenesis of this region produced less effective BUB1 kinase activity (Krenn et al. 2012; Ricke et al. 2012). However, later studies using similar methods did not support this mode of activation (Lin et al. 2014; Asghar et al. 2015).

Regulation of SAC by BUB1

BUB1 is a genuine component of the SAC, and its role in SAC has been confirmed and studied in several model organisms including fission yeast, budding yeast, frog, worm, fruit fly, mouse, and humans (Roberts et al. 1994; Taylor and McKeon 1997; Basu et al. 1998; Bernard et al. 1998; Sharp-Baker and Chen 2001; Tang et al. 2004; Encalada et al. 2005). In these studies, depletion or structural mutations in BUB1 cause precocious exit from mitosis. For example, in humans, mutations in TPR domain and BUB3 binding domain caused SAC defects (Klebig et al. 2009). The role of BUB1 kinase activity in SAC function remains controversial. As mentioned above, one target of BUB1 kinase activity for SAC activation is CDC20 (Tang et al. 2004). CDC20 binding to KEN boxes allows for its phosphorylation by hBUB1 and hPLK1 for SAC activation (Jia et al. 2016). Thus, a nonkinase region of hBUB1 may be necessary for kinase activity during SAC activation. BUB1 kinase activity may also promote SAC activity through H2A-T120 phosphorylation and timely AURORA B localization and activation (Ricke et al. 2012). Nevertheless, others have found that kinase-inactivating mutations in the BUB1 catalytic domain do not affect the strength of the SAC (Klebig et al. 2009; Perera and Taylor 2010; Vleugel et al. 2015).

The major role of BUB1 kinase in SAC function may lie in its ability to function as a kinetochore scaffold for downstream proteins (Johnson et al. 2004; Rischitor et al. 2007; Klebig et al. 2009), including BUBR1, MAD1, MAD2, BUB3, SGO, CENP (centromere protein)-E and -F, CDC20 and RZZ (Rod–Zwilich–Zw10) complex (Johnson et al. 2004; Kang et al. 2008; Klebig et al. 2009; Kawashima et al. 2010; Zhang et al. 2015). Distinct structural regions on BUB1 have been implicated in its scaffolding functions. The role of BUB1 in kinetochore recruitment of BUBR1 has been reported in humans (Johnson et al. 2004; Klebig et al. 2009). A central region of BUB1 protein following the TPR domain (residues 266–311), termed as the R1LM (BUBR1 localization motif), is involved in direct pseudo-symmetrical BUBR1 binding and kinetochore recruitment (Overlack et al. 2015; Zhang et al. 2015). In addition to BUBR1 recruitment, a region containing amino acids 430–530 binds and recruits components of RZZ (Rod–Zwilich–ZW10), a

complex required for MAD1 and MAD2 protein recruitment and SAC (Zhang et al. 2015). Furthermore, depletion of BUB1 severely reduces ZW10 and Zwilch recruitment to kinetochores, which suggests that BUB1 is required to recruit the entire RZZ complex (Zhang et al. 2015). BUB1 is also required for kinetochore recruitment of MAD1 and MAD2, likely through the CD1 region as mutation of CD1 causes reduction in MAD1 and MAD2 kinetochore localization (Klebig et al. 2009). Furthermore, a conserved RLK (Arg-Leu-Lys) motif of MAD1 is implicated in its interaction with BUB1 and kinetochore recruitment in humans (Kim et al. 2012).

BUB1 and Chromosome Congression

Chromosome congression is the process of chromosome alignment at the spindle equator during a symmetric mitosis. BUB1 is required for this as depletion of BUB1 or structural mutations that reduce BUB1 kinetochore localization cause defects in chromosome alignment (Johnson et al. 2004; Fernius and Hardwick 2007; Logarinho et al. 2008). However, the requirement of kinase activity for chromosome congression is controversial. Expression of BUB1 mutants devoid of kinase activity did not rescue chromosome congression defects caused by BUB1 depletion, demonstrating the importance of BUB1 kinase activity for chromosome congression (Vanoosthuyse et al. 2004; Klebig et al. 2009). However, this remains controversial and other studies in mice and humans did not support the above findings (Perera and Taylor 2010; Baron et al. 2016).

BUB1 and Cancer

Most solid tumors exhibit aneuploidy, a state defined by a number of chromosomes that deviates from the norm for a given species (Weaver and Cleveland 2006). Although aneuploidy may arise due to several contributing factors, in the context of cell division, chromosome cohesion, SAC, and microtubule attachment defects are often observed in aneuploid cells (Gordon et al. 2012). However, the SAC, a signaling cascade particularly essential for cell survival, is rarely fully defective in human tumors, and it has been suggested that an imbalance in SAC signaling in aneuploid tumors contributes to chromosomal instability (*CIN*), which reflects a higher rate of chromosome gain or loss (Schvartzman et al. 2010). In agreement with this, complete abrogation of SAC causes early development arrest in mouse models and lethality in several tumors; thus, a weakened SAC is detected in many tumors (Weaver and Cleveland 2006; Schvartzman et al. 2010). However, SAC overactivation manifested by abnormal delay in APC/C inhibition can also contribute to CIN due to accumulation of lagging chromosomes and merotelic attachments (Schvartzman et al. 2010). Indeed, BUB1 MAD2 overexpression has been reported in breast cancer patients (Wang et al. 2015), and this overexpression is associated with poor survival and tumor aggressiveness. Reduction of BUB1 and MAD2 expression was sufficient to reduce invasive nature of some tumor cells (Wang et al. 2015). *hBub1* is also overexpressed in several human lymphomas, and *Bub1* overexpression in mice causes increased chromosome segregation defects due to AURORA B kinase hyperactivation (Ricke et al. 2011).

Although mutations in the SAC genes are not very common (Gordon et al. 2012), one mutation identified in *Bub1* results in an amino acid substitution (A130S, in the Bub1 kinetochore localization module) and leads to defects in SAC, chromosome congression and SGO1, BUBR1 and CENP-F recruitment (Klebig et al. 2009). Thus, both structural mutations and abnormal expression of BUB1 might contribute to cancer.

Recent evidence suggests that BUB1 kinase activity plays a role in TGF-β (transforming growth factor-β) signaling in lung and breast cancer cells (Nyati et al. 2015). TGF-β is ubiquitously expressed and involved in many cellular processes related to growth, cell proliferation, and differentiation and its deregulation is associated with cancer (Weiss and Attisano 2013). BUB1 binds to TGFBRs (transforming growth factor

beta receptor) at cell membranes and mediates TGF-β signaling through its kinase activity (Nyati et al. 2015). These results show a novel pathway that requires BUB1 kinase activity, which might contribute to cell migration and invasion of tumor cells. In this context, inhibition of BUB1 activity could provide a therapeutic strategy against tumor metastasis. Efforts to date have yielded 2 classes of BUB1 kinase inhibitors: an adenine analog 2OH-BNPP1 and the benzylpyrazole compounds, BAY-320, and BAY-524 (Kang et al. 2008; Baron et al. 2016). Interestingly, 2OH-BNPP1-mediated BUB1 inhibition attenuated TGFβ signaling, suggesting that this may be a viable therapeutic avenue in cancers with hyperactive TGFβ signaling (Nyati et al. 2015). BAY-320 and BAY-524 treatment presented antiproliferative effects in combination with the microtubule-stabilizing and chemotherapeutic drug Paclitaxel (Baron et al. 2016). These studies support further examining the potential use of BUB1 kinase inhibitors for cancer treatment.

Summary

The BUB1 kinase was initially discovered in yeast for its role in mitotic progression and the SAC. Later, it was also identified in other model organisms including fruit fly, frogs, worms, mice, and humans. BUB1 coordinates its activity with other SAC components to delay mitotic progression until correct kinetochore-microtubule attachments are established. Although most studies agree that BUB1 kinase activity is dispensable for its role in the SAC, this remains controversial and the role of the kinase domain may well be context dependent. BUB1's scaffolding function however is clearly required for the SAC. BUB1 is one of the first SAC proteins to dock at kinetochores to recruit a number of other SAC proteins and mitotic regulators including (but not limited to) BUB3, BUBR1, MAD1, MAD2, SGO, and PP2A. BUB3, BUBR1, MAD, and RZZ are recruited as a result of direct interactions with BUB1(Elowe 2011), while SGO and PP2A are recruited indirectly via BUB1 phosphorylation of H2AT120 or through secondary interactions (e.g., a PP2A pool is recruited through BUBR1 (Kawashima et al. 2010; Suijkerbuijk et al. 2012)). BUB1 phosphorylation of H2AT120 is also required for proper chromosome congression likely, through promoting proper recruitment of AUROR B and SGO proteins. Finally, BUB1 expression is deregulated in several tumors and has a role in tumor progression and is being actively explored as a potential target for therapeutic intervention.

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BUB1A

▶ BUB1
▶ BUBR1

BUB1Beta

▶ BUBR1

BUB1L

▶ BUB1

BUBR1

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Synonyms

Bub1A; BUB1beta; hBUBR1; MAD3L; MVA1; SSK1

Related Molecules

▶ APC; ▶ BUB1; ▶ CDC25; ▶ PP2A.

Historical Background

The *BUB1B* gene encodes the protein budding uninhibited by benzimidazole-related 1 (BUBR1), a vital mitotic pseudokinase of the Spindle Assembly Checkpoint (SAC). This signaling pathway is responsible for delaying anaphase onset until all chromosome are properly attached to microtubules originating from opposing poles of the mitotic spindle, and prevents errors in chromosome segregation, which can lead to aneuploidy and chromosome instability, a pathogenic state with the potential to drive oncogenesis (Holland and Cleveland 2009; Kops et al. 2005).

Human BUBR1 was discovered through sequence-database searches in a study that aimed to identify the relationship between chromosomal instability, the SAC, and neoplasia (Cahill et al. 1998). BUBR1 is considered to be the human homolog of yeast Mitotic Arrest Deficient (MAD) 3 protein, although it was first thought to be the second human homolog of a related kinase from budding yeast, budding uninhibited by benzimidazole 1 (BUB1), which resulted in some confusion in the nomenclature. Spindle checkpoint genes were indeed initially identified in the budding yeast *Saccharomyces cerevisiae*. MAD3 was discovered in a screen of essential genes necessary for SAC activity and proper cell cycle progression at the end of mitosis (Li and Murray 1991). Mutant yeasts with compromised SAC activity did not arrest in the presence of the microtubule poison benomyl, and died due to chromosomal instability followed by apoptosis, allowing the identification of three *mad* genes required for SAC activation, *mad1–3*. Other essential SAC genes are the *Bub* genes, which were discovered in a similar screen and around the same time as the *mad* genes. In this case, the microtubule disrupter benzimidazole was used in the screen to block the final stages of cell division in budding yeast; the mutants that continued to divide and form

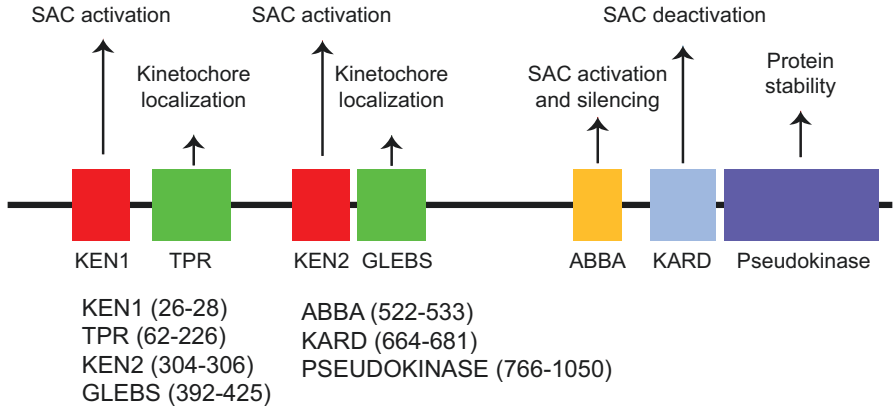
progeny buds were dubbed *budding uninhibited by benzimidazole* mutants (Hoyt et al. 1991). Subsequent to their identification in budding yeast, efforts were initiated to identify the human homologs of the SAC genes. Similarities at the amino acid level between hBUBR1 and budding yeast BUB1 led to its initial classification as a BUB1 homologue (Cahill et al. 1998). However, there are critical differences between the pseudokinase domain of hBUBR1 and the bona fide kinase domain of BUB1 from yeasts as well as higher eukaryotes, and these two domains have only 20% identity. Moreover, whereas the N-terminal region of hBUBR1 and *S. cerevisiae* BUB1 display 26% similarity, the N-terminal region of hBUBR1 is 35% similar to budding yeast MAD3 (Taylor et al. 1998). Consequently, it is now well established that BUBR1 is the human homologue of yeast Mad3.

In order to explain the similarity among hBUBR1 and budding yeast BUB1 and MAD3, it was suggested that human BUB1 and BUBR1 originated from a common gene – *MADBUB*, present in the last eukaryotic common ancestor (LECA). This protein presents two essential SAC domains with distinct functions required for the checkpoint arrest: a sequence containing a lysine(K)-glutamate(E)-asparagine(N) (KEN) box and a kinase domain. The *MADBUB* gene undertook different paths through evolution: it either suffered a gene duplication event on nine different occasions or remained relatively intact. It has been suggested that around 20–50 million years ago *MADBUB* suffered a duplication where its domains – and function – were passed on to two distinct genes (Murray 2012; Suijkerbuijk et al. 2012a; Vleugel et al. 2012). In an example of subfunctionalization, in both the fission yeast *Schizosaccharomyces pombe* and the very distantly related *S. cerevisiae*, the BUB1 protein retained the kinase domain, while MAD3 lost the kinase domain but preserved the KEN box domains.

On the other hand, in the majority of vertebrates, BUBR1 homologs retained both the KEN box domains and a degenerate kinase-like domain. Substitutions and posttranslational modifications of this domain gave rise to a lively debate in the literature as to whether BUBR1 in higher eukaryotes can function as a true kinase. Although it is now generally accepted that hBUBR1 is indeed a pseudokinase and cannot perform a phosphotransfer reaction, it is noteworthy that at least in one model organism, *Drosophila melanogaster*, BUBR1 has retained catalytic function, although no substrates have been identified to date (Buffin et al. 2007; Rahmani et al. 2009; Suijkerbuijk et al. 2012a). Despite the controversy surrounding the pseudokinase domain, in all organisms where it has been tested, BUBR1 is a crucial player in SAC signaling (Chan et al. 1999; Li and Murray 1991; Musacchio and Salmon 2007; Rahmani et al. 2009; Wong and Fang 2007). Both human and mouse BUBR1 have also been shown to play a role in chromosome congression to the metaphase plate and in the stabilization of kinetochore-microtubule attachments (Elowe et al. 2010; Lampson and Kapoor 2005; Park et al. 2013; Suijkerbuijk et al. 2012b; Touati et al. 2015; Wei et al. 2010; Xu et al. 2013).

Structural Diversity of MAD3/BUBR1 Orthologs

A MADBUB-like protein has been identified in a number of species, including *M. brevicollis*, *B. dendrobatidis*, *C. neoformans*, *N. crassa*, *D. discoideum*, and *P. infestans* (Vleugel et al. 2012). This protein presents five conserved domains: two KEN box domains; a tetratricopeptide repeat (TPR) domain; a BUB3-binding domain (B3BD, also known as the Gle2-binding-sequence (GLEBS) domain); and a kinase domain. After gene duplication, the BUBR1 homolog in budding and fission yeast, nematodes, *A. thaliana* and *N. gruberi* lost the C-terminal kinase domain, yielding MAD3-like proteins. Insects and vertebrates, however, maintained the kinase domain giving rise to BUBR1-like proteins, which as noted above degenerated into a catalytically inactive pseudokinase domain in most higher eukaryotes (Murray 2012; Suijkerbuijk et al. 2012a; Vleugel et al. 2012). Thus, the distinguishing feature of



BUBR1, Fig. 1 Domain architecture of BUBR1. BUBR1 is a modular protein with a number of distinct domains that regulate its functions. The KEN box associates directly with CDC20 and promotes MCC formation, APC/C inhibition, and thus plays a central role in SAC signaling. The TPR and B3BD are important for BUBR1 kinetochore localization as they promote its binding to KI2 and MELT motifs of KNL1, respectively. The ABBA motif is a secondary CDC20 recruitment motif found in both BUBR1 and its paralog BUB1. This motif in BUBR1 has been proposed to be important for both promoting and extinguishing SAC signaling. The phosphorylated KARD is responsible for BUBR1 association with a pool of PP2A-B56 which plays an important role in chromosome alignment and SAC silencing. At the very C-terminus of BUBR1 is the pseudokinase domain, mutations of which destabilize the protein

BUBR1 orthologs throughout evolution is the presence of the two N-terminal KEN boxes.

Human BUBR1 has a number of distinct protein interaction domains and motifs, in addition to the characteristic KEN boxes (Fig. 1). Its N-terminal region largely mimics the N-terminal structure of the ancestral protein: It starts with a helix-loop-helix motif (HLH), where one of the two KEN box motifs is localized; three TPR motifs; a destruction (D)-box; and the B3BD with a characteristic internal loop that is distinct from that of the BUB1 B3BD. The rest of hBUBR1 diverges from the MAD3 and has acquired additional functionality in line with the more complex mitosis of higher eukaryotes. The middle region of BUBR1 includes an internal CDC20-binding domain (IC20BD), also known as the ABBA-motif, or phenylalanine-containing motif (Phe-box). Finally, the C-terminus of hBUBR1 is characterized by the kinetochore alignment and regulatory domain (KARD) and the pseudokinase domain.

Functions of the Distinct Domains of hBUBR1

The first KEN box motif of BUBR1 and MAD3 proteins is crucial for the interaction with CDC20, which is the target of the SAC, and the formation of the Mitotic Checkpoint Complex (MCC), the effector of the SAC (Burton and Solomon 2007; King et al. 2007). The second KEN box motif is required for direct inhibition of APC/C, a large E3 ubiquitin ligase that when activated by CDC20 (APC/C^{CDC20}) is the major target of the SAC. This KEN box most likely functions by blocking the recruitment of substrates to the APC/C^{CDC20} (Lara-Gonzalez et al. 2011). Recently, it was demonstrated that the loop region of the BUBR1 BUB3-binding domain may also be required for optimal formation of the MCC and APC/C binding (Overlack et al. 2015).

The TPR and B3BD contribute to BUBR1 kinetochore localization, which is required for SAC activation. This recruitment is complex and appears to require multiple interaction interfaces on BUBR1, as well as multiple docking partners, including the obligate BUBR1 binding partner BUB3, and the BUB3-BUB1 dimer, which directly binds to and recruits BUB3-BUBR1. These collectively dock on KNL1 (Kinetochore null protein 1), which has recently emerged as the major SAC signaling platform at kinetochores (Kiyomitsu et al. 2007; Krenn et al. 2014;

Primorac et al. 2013; Vleugel et al. 2013). Binding of BUBR1 to kinetochores absolutely requires the direct interaction between BUBR1 and BUB1 mediated through residues 271–409 of hBUB1 and 362–571 of hBUBR1 (Primorac et al. 2013). Both of these regions lie downstream of the BUB3 binding domain in their respective proteins. This pseudosymmetric complex between BUB1 and BUBR1 is further stabilized by a number of additional interactions. The TPR domain of BUBR1 binds to the second lys-Ile (KI2) motif of KNL1 (Kiyomitsu et al. 2011; Krenn et al. 2014; Krenn et al. 2012), although in vivo, this interaction is not necessary for BUBR1 kinetochore docking (Krenn et al. 2012). On the other hand, the pool of BUB3 associated with BUBR1 is essential for BUBR1 kinetochore recruitment; and although its function is not entirely clear, it may play a role in direct recruitment to BUB1 (D'Arcy et al. 2010; Overlack et al. 2015), or may mediate further interaction with KNL1 phosphorylated Met-Glu-Leu-pThr (MELT) motifs (Primorac et al. 2013).

In the central region of BUBR1 is another broad motif that promotes BUBR1 interaction with CDC20, and that was independently identified by several groups, resulting in multiple names for it in the literature. These include the Phe-box (residues 528–531) (Diaz-Martinez et al. 2015); IC20BD (residues 490–560, which has a core from residues 530 to 535) (Lischetti et al. 2014); and the ABBA motif (residues 528–533), named after its presence in human A-type cyclins, BUBR1, BUB1, and in budding yeast Acm1 (Di Fiore et al. 2015). Although this region mediates CDC20 binding and APC/C inhibition, its role in the spindle checkpoint-mediated arrest remains unclear (Diaz-Martinez et al. 2015; Di Fiore et al. 2015; Lischetti et al. 2014). It has been implicated in recruiting CDC20 to kinetochores (Di Fiore et al. 2015; Han et al. 2014) and in SAC silencing, presumably by competing with the KEN-boxes for CDC20 binding (Lischetti et al. 2014). In addition, this region may compete with cyclin A for its binding to CDC20, thus explaining how this cyclin is promptly degraded at mitotic exit (Di Fiore et al. 2015).

In the C-terminal region of hBUBR1 lies the kinetochore Attachment Regulatory Domain (KARD) followed by the pseudokinase domain. At the core of the KARD is a Leu-Xaa-Xaa-Ile-Xaa-Glu motif, which binds to the concave side of the B56 pseudo-HEAT repeats of the phosphatase PP2A-B56 (Wang et al. 2016). The association between B56 and BUBR1 KARD has been suggested to recruit a subpopulation of the PP2A phosphatase to the outer kinetochore to counteract the activity of the AURORA B kinase (Nijenhuis et al. 2014). This interaction explains, at least in part, the role of BUBR1 in the regulation of many mitotic functions including chromosome congression, kinetochore-microtubule attachment stability, and the termination of SAC signaling (Espert et al. 2014; Kruse et al. 2013; Suijkerbuijk et al. 2012b; Xu et al. 2013).

The function of the BUBR1 kinase domain has garnered considerable controversy. The presence of a conventional catalytic triad led to its initial classification as an active kinase in humans, mice, and in *Xenopus laevis* (Cahill et al. 1998). Early studies suggested that BUBR1 kinase activity is required to fully activate the SAC (Kops et al. 2004; Malureanu et al. 2009; Mao et al. 2003), although others argued that catalytic activity of BUBR1 was dispensable for the SAC (Chen 2002; Elowe et al. 2007). Recent results however suggest that phenotypes observed in BUBR1 kinase inactivating mutants are probably due to destabilization of BUBR1, and not the mutation of the kinase domain per se, and catalytic activity previously attributed to BUBR1 has been suggested to be a result of a contaminating kinase (Suijkerbuijk et al. 2012a).

Recent work based on the structural homology with hBUB1 and other active kinases provides mechanistic insight into the inactivation of BUBR1 and demonstrates the presence of residues or potential posttranslational modifications that would render it inactive (Suijkerbuijk et al. 2012a). More specifically, BUBR1 presents two conserved deviations from conventional kinases, a substitution in the Gly-rich loop for large and negatively charged residues (Asp at the equivalent of Gly52 and Leu at the equivalent Gly55 of PKA), and two substitutions at the catalytic loop (Ser and Cys at the equivalent

of Lys168 and Asn171 of PKA, respectively). In addition, in two vertebrates, *A. carolinensis* and *D. rerio*, substitutions in the catalytic triad ($Asp882^{BUBR1}$) render BUBR1 in these species a conventional pseudokinase. In the fruit fly *Drosophila melanogaster*, BUBR1 resembles the fly BUB1 more closely and, consequently, is thought to present a catalytically active BUBR1 protein (Suijkerbuijk et al. 2012a). Despite the lack of catalytic activity, the BUBR1 kinase domain is important for protein stability (Suijkerbuijk et al. 2010). Remarkably, mutations and truncations associated with this domain have been found in patients suffering from Mosaic Variegated Aneuploidy (MVA) (Hanks et al. 2004; Matsuura et al. 2006). This disorder is characterized by high tumor formation and shorter life span due to low BUBR1 levels resulting from its instability (Suijkerbuijk et al. 2010). Furthermore, the substitution of residues at the catalytic triad, used to produce an inactive kinase, is known to destabilize the protein, as in the MVA syndrome (Suijkerbuijk et al. 2012a). Whether the BUBR1 pseudokinase domain has functions unrelated to the protein homeostasis remains to be seen.

Posttranslational Modifications

PLK1 Is a Major Regulatory Kinase of BUBR1

BUBR1 is phosphorylated at a number of sites that collectively play an important role in its function and regulation, with polo-like kinase 1 (PLK1) being arguably the most important BUBR1 kinase identified to date. Human BUBR1 is phosphorylated at Thr620 and Ser670 by Cdk1 (Elowe et al. 2007, 2010; Huang et al. 2008). Phosphorylation of Thr620 forms a polo-box domain docking site, which promotes PLK1 binding to BUBR1. Subsequently, PLK1 phosphorylates BUBR1 at S676 (Elowe et al. 2007), T680 (Suijkerbuijk et al. 2012b), and Thr792 and Thr1008 (Matsumura et al. 2007). Phosphorylation of BUBR1 at Ser670, Ser676, and Thr680 in the KARD promotes the binding of PP2A-B56 (Kruse et al. 2013; Suijkerbuijk et al. 2012b; Xu et al. 2013). Phosphorylation of Thr1008 and Thr792 by PLK1 was thought to activate BUBR1 kinase activity to promote proper chromosome alignment (Matsumura et al. 2007). However, in light of recent evidence that BUBR1 is a pseudokinase, a re-examination of the function of these phosphosites is required. In a similar fashion, the budding yeast AURORA B kinase IPL1 and the PLK1 ortholog CDC5 is believed to phosphorylate MAD3 on unattached kinetochores in this organism (Rancati et al. 2005). In *Xenopus*, BUBR1 phosphorylation by PLK1 homologue, PLX1, creates the 3F3/2 epitope (Wong and Fang 2007), long considered a marker for lack of kinetochore tension that establishes a link between the mechanical regulation of chromosome segregation and a biochemically controlled sensor such as the SAC (Ahonen et al. 2005; Nicklas et al. 1995). In human cells however, the 3F3/2 epitope appears to be distinct from BUBR1 (Elowe et al. 2007).

BUBR1 also interacts with the BRCA2 (*BReast CAncer susceptibility gene 2*) and PCAF (*P300/CBP-associated factor*) complex, an acetyltransferase responsible for acetylating BUBR1 at Lys250. As part of the MCC, BUBR1 is potentially prone to ubiquitination by the APC/C^{CDC20}. Acetylation of BUBR1 is believed to protect it from APC/C^{CDC20}-mediated ubiquitination and destruction by the proteasome (Choi et al. 2009, 2012; Yekezare and Pines 2009). Upon chromosome biorientation and SAC silencing, BUBR1 is deacetylated which allows for sumoylation of BUBR1 at the previously acetylated Lys250 residue. Sumoylated BUBR1 promotes its release from kinetochores and thus potentially mediates SAC silencing (Yang et al. 2012a, b).

Functions of BUBR1 during Mitosis

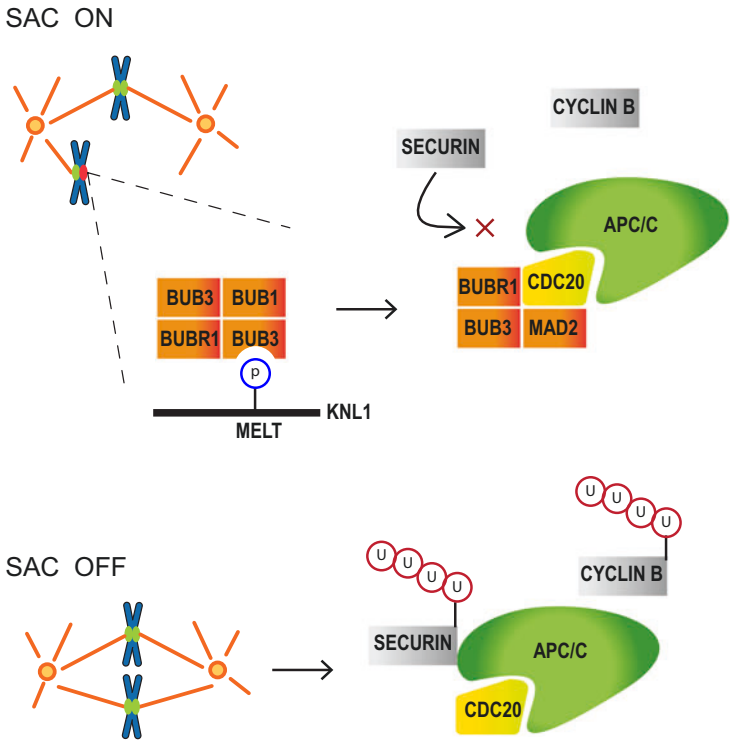
BUBR1 contributes to several functions during mitosis: mitotic checkpoint complex (MCC) formation and spindle checkpoint activation, chromosome alignment, formation of proper kinetochore-microtubule attachments, and most recently spindle assembly checkpoint termination.

Spindle Assembly Checkpoint Activation and MCC Formation

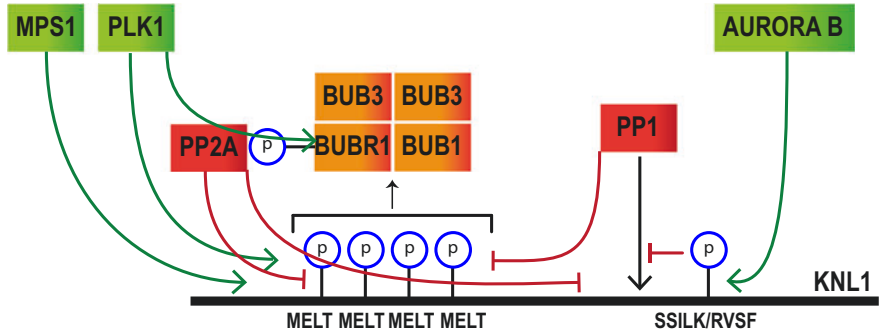
The highly conserved N-terminal region of BUBR1 is directly involved in SAC activation and delay of anaphase onset. In vitro and on its own, the N-terminal region of BUBR1 is sufficient to inhibit the ubiquitination activity of the APC/C^{CDC20}, and this inhibitory effect is further enhanced in the presence of MAD2 (Fang 2002; Tang et al. 2001). In vivo, BUBR1 and MAD2 likely function together as part of the MCC, together with BUB3 and CDC20, although the exact nature of the final APC/C^{CDC20} inhibitor remains disputed. The MCC functions through the sequestration of CDC20 molecules, or through direct competition with APC/C^{CDC20} substrates such as CYCLIN B and SECURIN, effectively delaying their ubiquitination and destruction (Fig. 2) (Foley and Kapoor 2013). Importantly, and as noted above, the KEN box motifs of BUBR1 are crucial for MCC activity (Burton and Solomon 2007; Lara-Gonzalez et al. 2011), and mutation of these in budding yeast, mouse fibroblasts, fruit flies, and human cancer cells abrogates SAC activity. The IC20BD has also been implicated in SAC function, perhaps through bulk recruitment of CDC20 to kinetochores (Lischetti et al. 2014), although it has been shown that this may also be mediated by a similar domain in BUB1 in certain contexts (Jia et al. 2016).

Regulation of Kinetochore-Microtubule Attachments and SAC Silencing

The congression of chromosomes to the cell equator relies on the dynamics of kinetochore-microtubule attachments, a dynamic process in which BUBR1 plays an important regulatory role (Kops et al. 2010; Lampson and Kapoor 2005). As noted above, BUBR1 at kinetochores is phosphorylated in the KARD which promotes recruitment of the PP2A protein phosphatase through its B56 specificity subunit (Kruse et al. 2013; Suijkerbuijk et al. 2012b; Xu et al. 2013). This phosphatase, together with the PP1 phosphatase (which is recruited to Arg-Val-Ser-Phe (RVSF) and [Ser-Gly]Ile-L.-Leu-Lys([SG] ILK) motifs of KNL1 (Liu et al. 2010)), is



BUBR1, Fig. 2 The function of BUBR1 in the SAC. At nonbioriented chromosomes, BUBR1 localizes to the phosphorylated MELT motif at kinetochore protein KNL1, where it promotes the activation of the SAC. The ultimate step of SAC signaling is the formation of the SAC effector, the MCC, composed of BUBR1, BUB3, MAD2, and CDC20. This complex is responsible for directly inhibiting the APC/C^{CDC20}, resulting in the delay of sister-chromatid segregation and mitotic exit until all chromosomes are properly attached to microtubules originating from opposing poles of the mitotic spindle in a bioriented manner



BUBR1, Fig. 3 BUBR1-mediated priming of SAC silencing and stabilization of kinetochore-microtubule attachments. BUBR1 is phosphorylated by PLK1, which promotes the recruitment of PP2B56. Subsequently, this phosphatase dephosphorylates KNL1 and, consequently, promotes PP2A and PP1 kinetochore tethering and counteracts MPS1, PLK1, and AURORA B activity. This mechanism is responsible for promoting kinetochore-microtubule attachments and SAC silencing

responsible for opposing SAC kinases at kinetochores, and in particular the kinases AURORA B and Monopolar Spindle 1 (MPS1). AURORA B promotes microtubule attachments turnover by phosphorylating key factors that control microtubule dynamics and attachment at kinetochores (Cheeseman et al. 2006; Iimori et al. 2016; Lan et al. 2004; Welburn et al. 2010). As a consequence, the balance between AURORA B activity and BUBR1-recruited PP2A-B56 is important for ensuring proper microtubule attachment dynamics at kinetochores and hence proper chromosome congression and alignment. In terms of SAC silencing, the current model suggests that a PP2A-B56 and PP1 relay results in dephosphorylation of KNL1 at key motifs that ultimately result in the removal of SAC components from kinetochores and termination of SAC signaling. The major motifs of KNL1 that are targeted are pMELT motifs whose phosphorylation by MPS1 is necessary for recruitment of the BUB3-BUB1-BUB3-BUBR1 complex, and the RVSF and [SG]ILK motifs which are PP1 binding sites, and whose phosphorylation by AURORA B inhibits PP1 docking. In this context, BUBR1 recruitment to kinetochores and phosphorylation by PLK1 essentially primes the recruitment of the PP2A-B56, which ultimately creates a negative feedback loop causing the delocalization of SAC proteins, and SAC silencing (Fig. 3) (Espert et al. 2014; Nijenhuis et al. 2014).

Mitotic Timer

The minimum time from nuclear envelope breakdown until anaphase onset is also regulated by BUBR1 and MAD2 (Meraldi et al. 2004). The MCC is also found in interphase, and this interphase MCC is thought to be responsible for the basal inhibition of the APC/C^{CDC20} in early mitosis before kinetochores fully mature (Sudakin et al. 2001). This function of BUBR1 appears to be dependent on the first KEN box, as flies expressing a mutant KEN box and lacking MAD2 exhibit an accelerated undisturbed mitosis (Rahmani et al. 2009).

Nonmitotic Functions of BUBR1

BUBR1 also plays an important function in meiosis; in meiosis I, BUBR1 functions much like it does in mitosis (Homer et al. 2009); it promotes kinetochore-microtubule attachments and SAC activation (Wei et al. 2010). Additionally, *Drosophila* BUBR1 is responsible for the integrity of the synaptonemal complex (SC) (Malmanche et al. 2007), which is important for maintaining homologous chromosomes attached. Interestingly, older oocytes present decreased levels of BUBR1 protein compared to younger ones. Consequently, BUBR1 is thought to be an important factor explaining why older women present increased rate of anomalous chromosome

segregation, which is associated with miscarriages and a higher probability of whole chromosome aneuploidy (Touati et al. 2015).

Summary

BUBR1 is an essential protein for the Spindle Assembly Checkpoint signaling, where it acts as an effector in the Mitotic Checkpoint Complex to inhibit APC/C^{CDC20} and prevent mitotic exit. It has also an important role in the congression of chromosomes to the metaphase plate and in stabilizing microtubule attachments. Recent studies have also highlighted a potential role for BUBR1 in SAC silencing. An important novel study indicates that BUBR1 in higher eukaryotes possesses no catalytic activity but has nevertheless retained a pseudokinase domain that is apparently required for maintaining the stability of the protein. In the future, it will be interesting to determine whether this domain plays any additional roles during mitosis.

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B

Butyrate Response Factor 1

► Tristetraprolin (ZFP36) and TIS11B (ZFP36-L1)

BY55

► CD160

Bypass Sod1p Defects (Bsd2p) (*Saccharomyces cerevisiae*)

► NDFIP1 and NDFIP2

BZP

► ZEB1 (Zinc Finger E-Box Binding Homeobox 1)