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Review

Biogenesis of cytosolic and nuclear iron–sulfur proteins and their role in genome stability ☆

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ABSTRACT

Iron–sulfur (Fe–S) clusters are versatile protein cofactors that require numerous components for their synthesis and insertion into apoproteins. In eukaryotes, maturation of cytosolic and nuclear Fe–S proteins is accomplished by cooperation of the mitochondrial iron–sulfur cluster (ISC) assembly and export machineries, and the cytosolic iron–sulfur protein assembly (CIA) system. Currently, nine CIA proteins are known to specifically assist the two major steps of the biogenesis reaction. They are essential for cell viability and conserved from yeast to man. The essential character of this biosynthetic process is explained by the involvement of Fe–S proteins in central processes of life, e.g., protein translation and numerous steps of nuclear DNA metabolism such as DNA replication and repair. Malfunctioning of these latter Fe–S enzymes leads to genome instability, a hallmark of cancer. This review is focused on the maturation and biological function of cytosolic and nuclear Fe–S proteins, a topic of central interest for both basic and medical research. This article is part of a Special Issue entitled: Fe/S proteins: Analysis, structure, function, biogenesis and diseases. Guest Editors: Roland Lill, Joan Broderick, and Dennis Dean.

1. Introduction

Iron–sulfur (Fe–S) clusters are small inorganic protein cofactors that function as electron carriers in redox reactions, catalysts in chemical reactions, regulatory sensors of environmental conditions, sulfur donor for other cofactors, and devices for stabilization of protein domains [1,2]. In eukaryotic cells, Fe–S proteins can be found in the mitochondria, cytosol and nucleus where they perform many functions that are essential for life. The importance of Fe–S proteins is explained by their participation in numerous reactions including mitochondrial energy production, amino acid biosynthesis, cofactor biosynthesis, tRNA modification, and various aspects of protein translation [3–5]. Moreover, Fe–S cluster biogenesis is directly linked to nuclear genome stability. In 2009, Gottschling and colleagues demonstrated that genomic instability arises from mitochondrial dysfunction because of defects in Fe–S cluster biogenesis during a 'crisis' elicited during the loss of mitochondrial DNA [6]. Consistent with that finding, different nuclear DNA metabolism enzymes, such as DNA primase and DNA polymerases [7,8], ATP-dependent DNA helicases [9–11] and DNA glycosylases [12] have been shown to coordinate a functionally crucial Fe–S cofactor. A selective list of DNA metabolism enzymes that depend on a Fe–S cluster for function is provided in Table 1. Several diseases such as xeroderma pigmentosum [13], Fanconi anemia [14–16] and the Warsaw breakage syndrome [17] have been linked to mutations in these DNA metabolism Fe–S enzymes, making them an important target for cancer research.

Maturation of all cellular Fe–S proteins strictly depends on mitochondria which harbor the conserved iron–sulfur cluster (ISC) assembly machinery that was inherited from the bacterial ancestor of the organelles [18,19]. The mitochondrial biogenesis pathway has been reviewed recently in depth and therefore will be addressed only briefly here [20–23]. Biogenesis of cytosolic and nuclear Fe–S proteins additionally requires the mitochondrial export system with the ABC transporter ABCB7 as its central component and the cytosolic iron–sulfur protein assembly (CIA) machinery. Both systems are conserved in virtually all eukaryotes and are usually essential for cell viability. Research during recent years has provided us with an insight into the molecular inventory involved in cytosolic–nuclear Fe–S protein biogenesis. Here, we will summarize the components and molecular mechanisms that achieve Fe–S protein assembly in the eukaryotic cytosol and nucleus. Even though the majority of the CIA components has first been identified and characterized in yeast, a mechanistically similar system operates in human cells. In addition to an overview of the general process, we therefore will highlight the peculiarities of the CIA maturation process in human cells. In the second part of the review, we will provide a

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Table 1
Human DNA metabolism enzymes with Fe–S cofactors, their yeast counterparts and connected diseases.
Cellular functions of the individual DNA metabolism enzymes are described in detail in the text. Note that in this review mainly human nomenclature is used.

Human	Yeast	Function	Connected disease
CHLR1	Chl1	Helicase, sister chromatid cohesion, heterochromatin organization	Warsaw breakage syndrome
DNA2	Dna2	Helicase/nuclease, DNA repair, Okazaki fragment maturation, telomere maintenance	
FANCJ	Absent	Helicase, repair of DNA interstrand crosslinks	Fanconi anemia
MUTYH	Absent	DNA glycosylase, base excision repair	
NTHL1	Ntg2	DNA glycosylase, base excision repair	
POLA	Pol1	Catalytic subunit of polymerase α, DNA replication	
POLD1	Pol3	Catalytic subunit of polymerase δ, DNA replication	
POLE1	Pol2	Catalytic subunit of polymerase ε, DNA replication	
PRIM2	Pri2	Subunit of DNA Primase, DNA synthesis and double-strand break repair	
RTEL1	Absent	Helicase, regulation of telomere length, anti-recombinase	Hoyeraal-Hreidarsson syndrome
XPD	Rad3	Helicase, nucleotide excision repair	Xeroderma pigmentosum, Cockayne syndrome, Trichothiodystrophy

brief synopsis of the intimate link between the Fe–S protein maturation process and the maintenance of genome stability which is of central interest for basic science and has important medical implications. This topic includes a description of the function of several important Fe–S proteins participating in various aspects of DNA metabolism, such as DNA replication and repair.

2. A brief general overview on eukaryotic Fe–S protein biogenesis

Fe–S clusters can be assembled on purified proteins in vitro from ferrous iron and sulfide under anaerobic conditions [24]. However, since the late 1990s it is well established that biogenesis of these cofactors within a eukaryotic cell is catalyzed by different proteinaceous machineries (for recent reviews see [18,25–28]). The synthesis of Fe–S clusters and their subsequent insertion into apoproteins is an essential process involving some 30 known proteins. Fe–S protein biogenesis is initiated by the mitochondrial ISC assembly machinery which is involved in the biogenesis of virtually all cellular Fe–S proteins including those located in the cytosol and nucleus [29,30]. The 17 known ISC components are conserved from yeast to man, and several mitochondrial diseases have been linked to the process (reviewed by [20–22,27]). Maturation of cytosolic–nuclear Fe–S proteins additionally depends on a yet unknown, sulfur-containing compound (termed X-S) that is provided by the mitochondrial ISC assembly system and delivered by the ISC export machinery to the cytosol where the CIA machinery completes biogenesis [31–33]. Functional insights into the molecular mechanism of (non-plant) Fe–S protein biogenesis have mainly been gained from yeast, but the process occurs along a similar pathway in virtually all eukaryotes including human cells. In this review, we will present a combined view on the components and mechanisms assisting the Fe–S protein biogenesis in yeast and human cells. Due to extensive studies on human DNA metabolic enzymes with a bound Fe–S cluster we will use the human nomenclature for the CIA maturation factors and for the Fe–S target proteins.

The ISC and CIA components are not structurally related, yet they assist the biogenesis processes in mitochondria and the cytosol along similar biosynthetic principles [18]. The overall Fe–S protein biogenesis processes can be dissected into two main steps. First, a Fe–S cluster is assembled de novo on a scaffold protein where it is bound in a transient fashion. The synthesis reaction requires a sulfur donor, iron, and the supply of electrons. Second, the loosely bound Fe–S cluster is released from the scaffold protein and may be transiently bound by dedicated targeting factors that finally facilitate the specific insertion into the polypeptide chains of target apoproteins. Each of these steps is accomplished by dedicated ISC or CIA components, some of which depend on low molecular mass cofactors. In a nutshell, mitochondrial Fe–S protein biogenesis starts with the synthesis of a [2Fe–2S] cluster on the scaffold protein ISCU. This reaction requires the cysteine desulfurase complex NFS1-ISD11 as a sulfur donor, frataxin for stimulation of sulfur transfer and possibly iron acquisition, and the redox chain NADPH-ferredoxin reductase–ferredoxin for sulfur reduction to sulfide [34–37]. The cluster is then released from ISCU by a dedicated ATP-dependent Hsp70–Hsp40 chaperone system to become transiently associated with the glutaredoxin GLRX5 which holds the cluster in a glutathione (GSH)-dependent fashion [28,38]. These components are termed the core ISC system because they are also essential for extra-mitochondrial Fe–S protein biogenesis. The final maturation steps in mitochondria involve the synthesis of a [4Fe–4S] cluster by virtue of the ISCA and IBA57 proteins, and the dedicated cluster insertion into the diverse polypeptide chains of target Fe–S proteins by the cluster transfer proteins NFU1 and IND1. In the next part of this review we will focus on the maturation of cytosolic and nuclear Fe–S proteins and the role of the CIA machinery.

3. Biogenesis of cytosolic and nuclear Fe–S proteins is a two-step process depending on mitochondrial function, the CIA machinery and a glutaredoxin

Biogenesis of cytosolic and nuclear Fe–S proteins strictly depends on the core ISC assembly machinery (Fig. 1) [29,30,33,39,40]. The participation of mitochondria in that process (Table 2) explains the indispensable character of these organelles for the viability of eukaryotes [18,26,41]. Mitochondria generate a sulfur-containing compound (X-S in Fig. 1) and deliver it to the cytosol to allow formation of extra-mitochondrial Fe–S cofactors. The ABC transporter ABCB7 mediates the export reaction of the X-S molecule, a process which is dependent on glutathione (GSH) and the intermembrane space protein ALR [30,42–44]. GSH may be part of the exported molecule, because recently solved crystal structures of yeast Atm1 and its bacterial homolog both contain bound GSH in a positively charged binding pocket [45,46]. The role of the export machinery including recent studies on the potential Atm1 substrate have been addressed in depth elsewhere ([33,47,48]). Another partner in the export function may be the FAD-dependent sulfhydryl oxidase ALR. Its well-established function is in the introduction of disulfide bridges into mitochondrial preproteins during their MIA40-dependent import into the intermembrane space [49].

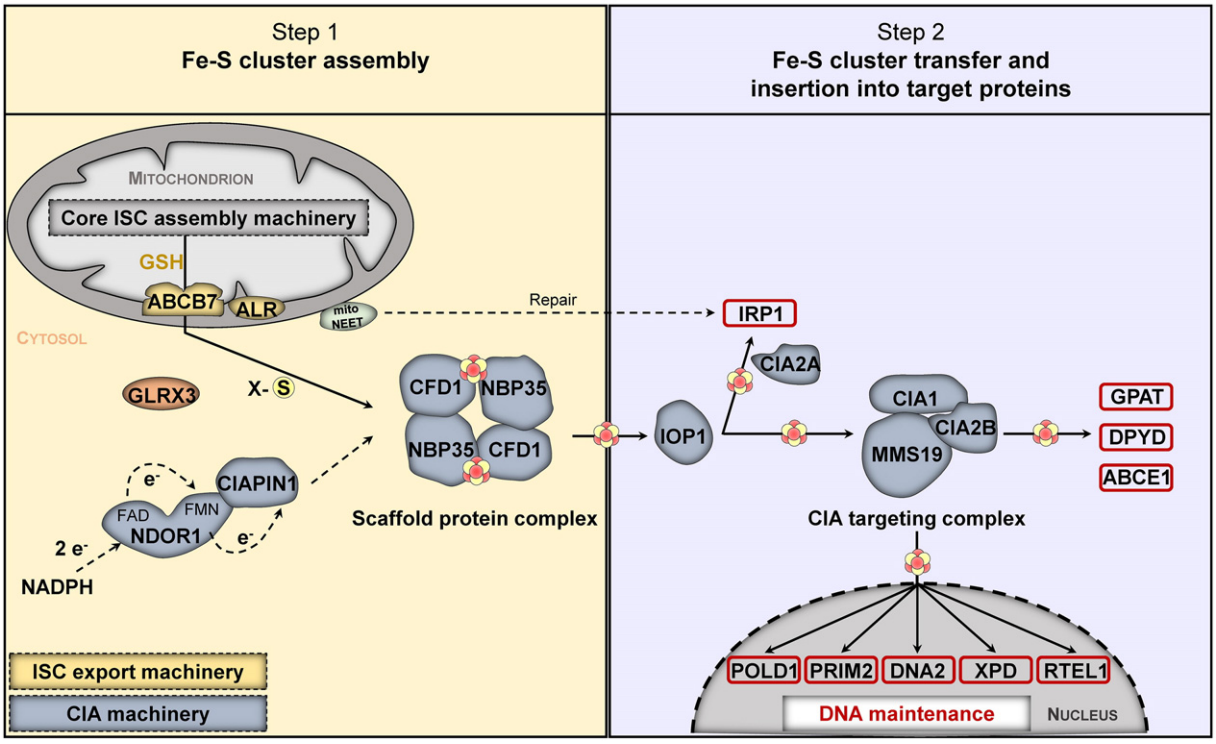

Fig. 1. A working model for the maturation of cytosolic and nuclear Fe–S proteins. The process depends on the interplay between the mitochondrial core ISC (iron–sulfur cluster) assembly machinery, the ISC export machinery and the CIA (cytosolic iron–sulfur protein assembly) machinery and can be dissected into two major steps. In the first step, a bridging [4Fe–4S] cluster is assembled and loosely bound to the scaffold complex consisting of CFD1 and the Fe–S protein NBP35. This [4Fe–4S] cluster assembly depends on a sulfur-containing compound (X-S) that is generated by the core ISC machinery and exported to the cytosol via the ABC transporter ABCB7. The export reaction further requires the function of the intermembrane space sulfhydryl oxidase ALR and glutathione (GSH). Electrons are required for the assembly reaction. They are provided by NADPH, the diflavin protein NDOR1 and the Fe–S protein CIAPIN1. Additionally, the biogenesis of the Fe–S clusters depends on the multidomain glutaredoxin GLRX3, yet its exact function and site of action is unknown to date. In the second step, the newly assembled Fe–S cluster is transferred from the scaffold protein complex onto apoproteins. This transfer is performed by the CIA targeting complex consisting of CIA1, CIA2B and MMS19, which mediate direct interactions with dedicated apoproteins. The CIA factor IOP1, itself an Fe–S protein, fulfills an intermediary function within the transfer step and interacts with both the early and late parts of the CIA machinery. The CIA2B homolog CIA2A is specifically required for maturation of iron regulatory protein 1 (IRP1). Its oxidatively labile [4Fe–4S] cluster may be repaired with the help of the [2Fe–2S] protein mitoNEET of the mitochondrial outer membrane. Cytosolic target Fe–S proteins matured by these machineries execute diverse cellular functions, e.g., in nucleotide metabolism (GPAT, DPYD) or translation initiation and termination (ABCE1). Nuclear Fe–S proteins conduct functions in DNA replication (POLD1, PRIM2, DNA2) as well as in DNA repair processes (XPD) or the regulation of telomere length (RTEL1) thus contributing to the maintenance of genome integrity.

After export of the molecule X-S to the cytosol, the nine known components of the CIA machinery conduct the assembly of the Fe–S cluster and its insertion into extra-mitochondrial target proteins (Table 2) [31,32,50]. In the following, we will discuss the two main steps of cytosolic–nuclear Fe–S protein assembly in more detail.

3.1. Requirements for [4Fe–4S] cluster assembly on the CFD1–NBP35 scaffold protein complex

An initial step of cytosolic Fe–S protein biogenesis is the assembly and transient binding of a [4Fe–4S] cluster on the cytosolic scaffold

Table 2
Protein components required for the maturation of cytosolic and nuclear Fe–S proteins. Human and yeast proteins are listed. Note that maturation of cytosolic and nuclear Fe–S proteins also depends on components of the mitochondria-located ISC assembly machinery. The ISC components are not depicted here but briefly described in the text. Fe–S cluster-containing biogenesis proteins are indicated.

Human	Yeast	Fe–S cluster types	Function
ISC export component			
ABCB7	Atm1		Export of X-S from mitochondria
ALR	Erv1		Unknown function in Fe–S cluster biogenesis
CIA component			
CFD1	Cfd1	Bridging [4Fe–4S] with Nbp35	Scaffold protein for formation of a [4Fe–4S] cluster
NBP35	Nbp35	Bridging [4Fe–4S] with Cfd1; N-terminal [4Fe–4S]	Scaffold protein for formation of a [4Fe–4S] cluster
CIAPIN1	Dre2	[2Fe–2S]; [4Fe–4S]	Electron transfer
NDOR1	Tah18		Electron transfer
IOP1	Nar1	2 [4Fe–4S]	Adaptor protein to connect early- and late-acting CIA components
CIA1	Cia1		Transfer and insertion of Fe–S clusters into target proteins (CIA targeting complex)
CIA2B	Cia2		Transfer and insertion of Fe–S clusters into target proteins (CIA targeting complex)
MMS19	Met18		Transfer and insertion of Fe–S clusters into target proteins (CIA targeting complex)
CIA2A	Absent		Specific maturation factor of IRP1
Iron trafficking protein			
GLRX3	Grx3/4	Bridging [2Fe–2S], GSH-coordinated	Iron trafficking?

protein complex comprised of the two P-loop NTPases CFD1 and NBP35 [51–54] (Fig. 1). The two proteins are related in sequence and form a heterotetrameric complex that coordinates two different kinds of [4Fe–4S] clusters [55]. A loosely bound [4Fe–4S] cluster is coordinated by a conserved CX_2C motif found at the C-terminus of both proteins. The [4Fe–4S] cluster can bridge the two complex subunits. The second [4Fe–4S] cluster binds at the N-terminus of NBP35 where it is tightly associated to a ferredoxin-like $CX_{13}CX_2CX_5C$ motif and essential for NBP35 function. Several in vitro and in vivo experiments confirmed the scaffold function of the CFD1–NBP35 complex by providing evidence for both the de novo assembly and transient binding of the bridging [4Fe–4S] cluster [52,55,56]. The differential lability of the two Fe–S clusters associated with the CFD1–NBP35 complex was underlined by a pulse-chase experiment with ^{55}Fe labeled yeast cells [56]. These data showed that only the N-terminal cluster is stably associated with NBP35. The C-terminal [4Fe–4S] cluster was readily lost over time. The loose binding of this latter cluster may be an important determinant for its transfer to and incorporation into dedicated target proteins in the second major step of biogenesis (see below).

Cytosolic Fe–S protein biogenesis depends on the supply of electrons, similar to the role of ferredoxin in the mitochondrial process [37]. The electron transfer chain of the CIA system is composed of NADPH, the diflavin protein NDOR1, and the Fe–S protein CIAPIN1 [57,58] (Fig. 1). The yeast homolog of CIAPIN1 termed Dre2 (Table 2) was found to be synthetically lethal with the deletion of the two mitochondrial iron importers Mrs3–Mrs4 and was biochemically characterized as a component of the CIA machinery [59]. The domain structure of yeast Dre2 encompasses a N-terminal S-adenosylmethionine (SAM) methyltransferase-like domain which is not known to bind SAM. The domain is connected by a flexible linker to a C-terminal Fe–S domain [60,61]. This latter domain harbors two pairs of four conserved cysteine residues which were suggested to coordinate one [2Fe–2S] and one [4Fe–4S] cluster [31,58,59]. As shown by co-immunoprecipitation and several high-throughput studies CIAPIN1 physically interacts with NDOR1, an essential protein containing NADPH-, FAD- and FMN-binding domains [62]. NDOR1 was characterized as CIA factor and shown to be part of the electron transfer chain [58]. EPR studies using the yeast counterparts unraveled that electrons are transferred from NADPH via the FAD and FMN centers of Tah18 to the [2Fe–2S] cluster of Dre2 (Table 2). The precise destination of the electrons in the CIA pathway is still unclear. However, it has been observed that in the absence of yeast Tah18 and Dre2 the maturation of the N-terminal [4Fe–4S] cluster of Nbp35 is impaired [55].

Cytosolic–nuclear Fe–S protein biogenesis additionally requires the cytosolic multidomain glutaredoxin GLRX3 (Fig. 1), yet its precise function and site of action in the pathway remains to be determined [63,64]. Yeast Grx3 was shown to be involved in cellular iron regulation, because in the presence of a bound [2Fe–2S] cluster it attenuates the transcription factors Aft1–Aft2 that regulate about 40 genes of the so-called iron regulon [65]. Further, yeast Grx3 and Grx4 are involved in the maturation of di-iron proteins such as ribonucleotide reductase [64,66]. Hence, it was speculated that these cytosolic monothiol glutaredoxins play a general role in iron trafficking. Because of its broad role in various aspects of cellular iron metabolism, we do not consider GLRX3 as a dedicated CIA protein. It is possible, but hitherto not experimentally addressed, that GLRX3 may transfer its [2Fe–2S] cluster to the CFD1–NBP35 complex that then may convert it to a [4Fe–4S] cluster.

3.2. The role of the CIA targeting complex in the transfer and insertion of newly assembled Fe–S clusters into dedicated apoproteins

The second major step of cytosolic Fe–S protein biogenesis involves the release of the newly assembled and transiently bound [4Fe–4S] cluster from the CFD1–NBP35 scaffold complex followed by its transfer and subsequent insertion into dedicated apoproteins [67–70] (Fig. 1). This reaction requires the coordinated function of IOP1 (iron-only hydrogenase-like protein) and the CIA targeting complex composed of CIA1, CIA2B (one of the two human homologs of yeast Cia2), and MMS19. The latter components undergo direct interactions with numerous cytosolic and nuclear Fe–S target proteins [10,11,71–75]. CIA1, CIA2B and MMS19 form binary and ternary complexes in yeast and mammalian cells underscoring the conserved function of this late-acting CIA unit. The CIA protein IOP1 exhibits sequence homology to bacterial [FeFe] hydrogenases, yet the active center of this enzyme is missing in IOP1 [76,77]. Nevertheless, IOP1 coordinates two [4Fe–4S] cofactors that are similar to those in hydrogenases [68,78]. Studies on the yeast homolog Nar1 showed that these clusters are bound to N- and C-terminal motifs with four conserved cysteine residues each. Both motifs are crucial for the function of this CIA component.

The presence of Fe–S clusters on some of the CIA proteins allowed a staging of the cytosolic Fe–S protein biogenesis pathway. Depletion of the four early-acting CIA factors in yeast resulted in a maturation defect of the Fe–S protein Nar1 (human IOP1; Table 2), whereas depletion of the three CIA targeting complex components had no effect on Fe–S cluster insertion [52,56,58,68]. This finding combined with functional data and co-immunoprecipitation studies led to the assumption that IOP1-Nar1 connects early- and late-acting components of the CIA machinery by a yet unknown mode of action [10,11,51,67,68,71,73]. The WD40-repeat protein CIA1 represents the putative docking site of the CIA targeting complex. Structural analysis of the *Saccharomyces cerevisiae* CIA1 homolog presented seven blades pseudo-symmetrically arranged around a central axis [72]. Site-directed mutagenesis studies identified the conserved, surface-exposed residue R127 as a potential docking site for other components of cytosolic Fe–S protein assembly. However, so far it is not fully understood how CIA1 recognizes its complex partners and thus mediates the formation of the CIA targeting complex by interacting with CIA2B and MMS19.

The function of CIA2 is conserved in eukaryotes [73,75,79]. Depletion of the human CIA2B or of yeast Cia2 has a strong effect on the maturation of cytosolic and nuclear Fe–S proteins. Critical for its function is a reactive Cys residue, but whether the residue is modified during the CIA2 reaction cycle is unknown. Structural information on members of the CIA2 protein family is available but does not allow predictions on the protein's molecular function [80]. The HEAT repeat protein MMS19 (also called Met18 in yeast) is the largest CIA protein and its deletion in yeast and mammalian cells is associated with a multitude of phenotypes, e.g., methionine auxotrophy, impaired chromosome segregation and increased sensitivity to DNA damage [81–84]. These pleiotropic phenotypes of MMS19-deficient cells were difficult to associate with one molecular function for a long time (see below). With the identification of MMS19 as component of the CIA machinery a longstanding mystery was resolved [10,11]. MMS19 as part of the CIA targeting complex mediates the interactions with and maturation of many target Fe–S proteins that in turn are participating in a multitude of cellular processes, e.g., methionine biosynthesis in yeast, DNA synthesis (POLD1, PRIM2), DNA repair (XPD, DNA2) and regulation of telomere length (RTEL1). The defect of these latter enzymes to a large extent explains the different phenotypes observed upon MMS19 depletion (see below).

4. Peculiarities of the human CIA targeting complex components

Despite the high conservation of the CIA components and the similar mechanisms of cytosolic Fe–S protein assembly in eukaryotes, two peculiarities for the late-acting human CIA proteins were identified. First, the different human CIA targeting complex components exhibit a high specificity for the maturation of dedicated Fe–S proteins [10,11,73]. Second, human cells, in contrast to yeast, express two Cia2-like proteins termed CIA2A and CIA2B (Table 2) [73]. While CIA2B serves as a general Fe–S protein maturation factor and thus acts as the yeast Cia2 ortholog, CIA2A was shown to strongly influence the cellular iron status of human cells. These two aspects are discussed in the following.

4.1. Differential requirement of CIA targeting complex components for the maturation of selected target proteins

A differential and highly specialized requirement of the three CIA targeting complex components for the maturation of the individual target Fe–S apoproteins in human cells was reported [10,11,73]. CIA1, CIA2B, and MMS19 form various binary and ternary sub-complexes, and thus the three components seem to dynamically interact (Fig. 2). The various sub-complexes may receive the [4Fe–4S] clusters assembled on CFD1–NBP35 and deliver them in a target-specific fashion to dedicated cytosolic Fe–S proteins such as glutamine phosphoribosylpyrophosphate amidotransferase (GPAT), dihydropyrimidine dehydrogenase (DPYD) and the ATP-binding cassette protein ABCE1 or nuclear Fe–S proteins such as XPD and POLD1. The ternary CIA targeting complex appears to have the broadest substrate spectrum mediating maturation of numerous Fe–S proteins such as DPYD, ABCE1 and XPD. MMS19 plays only a minor role in the maturation of GPAT, while CIA2B is not crucially required for DNA polymerase δ (POLD1) assembly [73]. This functional data was nicely corroborated by proteomic studies unraveling the selective binding of one, two or three components of the CIA targeting complex to different target Fe–S proteins [10,11,71,73,74]. The proteomic list of CIA1, CIA2B, and MMS19 interaction partners may potentially contain additional cytosolic or nuclear Fe–S proteins and new CIA factors. The high targeting specificity of the different late-acting, human CIA constituents is additionally underscored by the specific requirement of CIA2A for the dedicated maturation of iron regulatory protein 1 (IRP1) [73]. In contrast to CIA2B, its homolog CIA2A binds to only few partners including IOP1 of the CIA machinery and the DNA polymerase subunit POLE1. Surprisingly, no direct interaction of CIA2A was reported for IRP1, despite the functional involvement of CIA2A in IRP1 maturation.

4.2. Involvement of the human CIA machinery in cellular iron homeostasis

The impact of the human CIA system on cellular iron homeostasis is twofold and mainly connected to CIA2A (Fig. 3). First, as already mentioned CIA2A is specifically required for the maturation of cytosolic aconitase, the holoform of IRP1 which, together with IRP2, is a key regulator of mammalian iron homeostasis [73]. Both IRP1 and IRP2 conduct their regulatory functions via complex post-transcriptional mechanisms (for exhaustive reviews see Ref. [85,86]). Upon iron starvation IRP1 loses its [4Fe–4S] cluster enabling the apoform to bind to messenger RNA stem-loop structures called iron-responsive elements (IREs). IRP1 binding differentially regulates the efficiency of translation or the stability of mRNAs. This in turn modulates the expression of proteins involved in iron trafficking, storage and utilization. Under iron-replete conditions the equilibrium between apo- and holo-IRP1 is shifted towards the Fe–S cluster form, whose assembly strictly depends on the early-acting CIA components and CIA2A, but not on CIA2B or MMS19 [73]. Hence, intracellular iron homeostasis is regulated by the efficiency to assemble cytosolic Fe–S clusters. A recent study suggests that an oxidatively damaged [4Fe–4S] cluster may be repaired with the help of the [2Fe–2S] protein mitoNEET located at the mitochondrial outer membrane possibly adding another layer of regulation (Fig. 1) [87].

Second, CIA2A binds to and stabilizes IRP2, the other important regulatory factor for intracellular iron supply and distribution (Fig. 3) [27,73,85,86]. IRP2 does not contain a Fe–S cluster but its protein level is regulated in an iron- and oxygen-dependent fashion. Under iron-replete conditions IRP2 is polyubiquitinated by the E3 ubiquitin ligase FBXL5 which responds to iron and oxygen levels via its hemerythrin domain thus resulting in proteasomal degradation of IRP2 [88,89]. Under low iron or oxygen conditions FBXL5 is destabilized and degraded leading to increased levels of IRP2. CIA2A can tightly bind to and stabilize

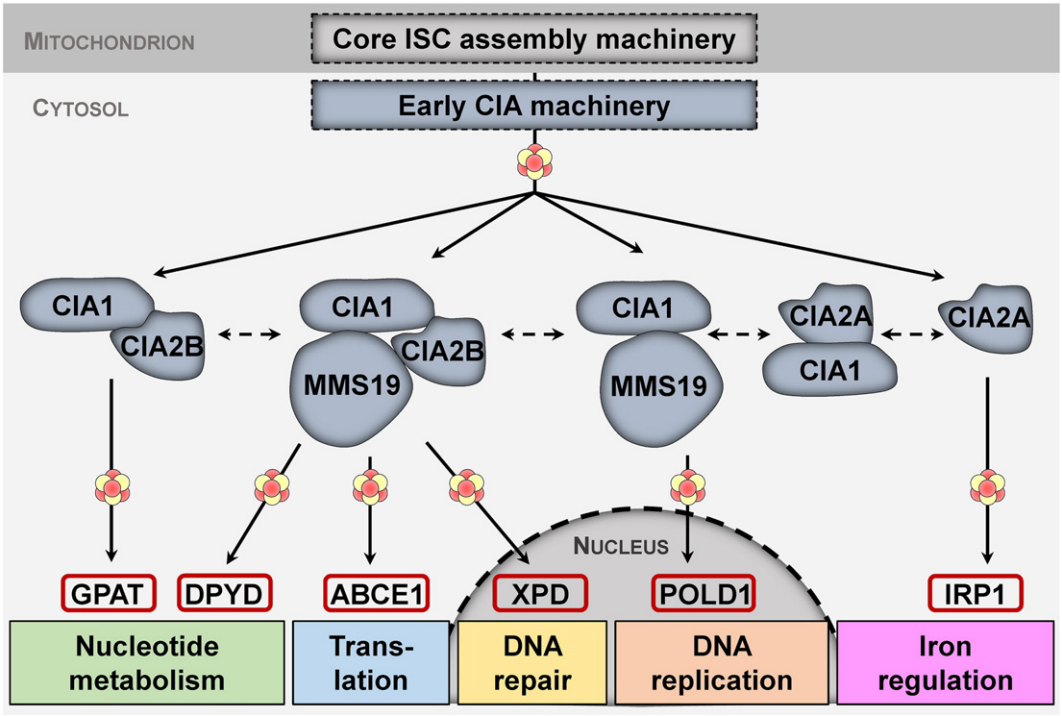

Fig. 2. Different late-acting human CIA proteins mediate the target-specific maturation of Fe–S apoproteins. The figure presents the current view of the target specificity of the late-acting CIA components. The [4Fe–4S] clusters are delivered from the early step of cytosolic Fe–S cluster assembly (cf. Fig. 1) to dedicated cytosolic–nuclear target Fe–S proteins with the help of the late-acting CIA components CIA1, CIA2B, and MMS19. These CIA proteins form several sub-complexes. The majority of the Fe–S proteins (e.g., DPYD, ABCE1, XPD) are matured with the help of the trimeric targeting complex consisting of CIA1, CIA2B (functional orthologue of yeast Cia2) and MMS19. In contrast, maturation of GPAT and POLD1 was hardly affected by the absence of MMS19 or CIA2B, respectively. The human CIA system possesses a second Cia2 isoform termed CIA2A that is not found in yeast. This CIA component is specifically required for the maturation of IRP1, thus contributing to the regulation of iron homeostasis in human cells (see also Fig. 3). No dedicated function has been assigned to a CIA2A–CIA1 subcomplex. The respective cellular functions of the individual Fe–S target proteins are depicted in the bottom to indicate the importance of Fe–S protein biogenesis for many aspects of cell homeostasis and viability.

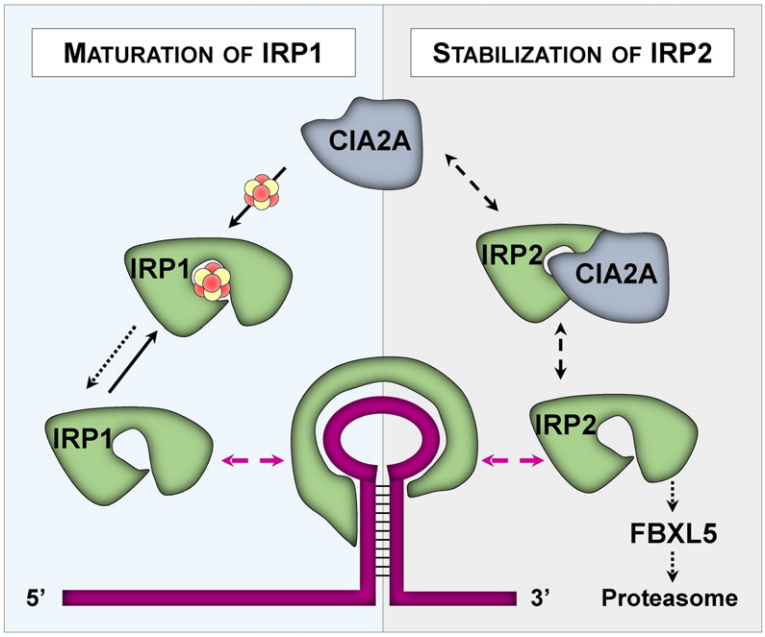

Fig. 3. CIA2A performs a dual role in human iron homeostasis. In mammalian cells the iron regulatory proteins IRP1 and IRP2 are key components of regulating the intracellular iron supply and distribution. IRP1 is a cytosolic aconitase whose activity depends on the coordination of a [4Fe–4S] cluster. Maturation of this Fe–S protein depends on the mitochondrial ISC assembly and export systems, and further involves the early-acting CIA components and the late-acting CIA factor CIA2A, while the CIA targeting complex is dispensable. Upon iron starvation or during oxidative stress IRP1 loses its cluster. The resulting apoform, after undergoing a major conformational change, can bind to iron-responsive elements (IREs; magenta line) of mRNAs which encode proteins involved in iron trafficking, storage and utilization. IRP–IRE binding can alter the translation efficiency or stability of the mRNA. IRP2 does not contain a Fe–S cluster but is regulated by iron- and oxygen-dependent proteasomal degradation mediated by the E3 ubiquitin ligase FBXL5. Under iron-replete conditions IRP2 is poly-ubiquitinated and degraded, whereas it is stable under low iron conditions. IRP2 can additionally be stabilized by binding to CIA2A demonstrating the dual role of CIA2A in iron homeostasis.

IRP2 to prevent it from FBXL5-mediated proteasomal degradation. Moreover CIA1 interacts with the CIA2A–IRP2 complex, yet depletion of CIA1 had no major effect on the cellular iron metabolism [73]. The role of the CIA1–CIA2A complex thus remains to be elucidated. In summary, the CIA protein CIA2A performs a dual function as a dedicated Fe–S cluster maturation factor for IRP1 and as a stabilizer for IRP2, and hence appears to integrate the functions of the two IRPs in intracellular iron homeostasis of human cells.

5. Intimate connection between cellular Fe–S protein assembly and genome integrity

A multitude of Fe–S proteins participate in essential processes such as DNA replication, DNA repair, transcription and chromosome segregation (Table 1). The Fe–S cofactors are predicted to be crucial for the proteins' functions, but so far the precise molecular role of the Fe–S cofactors in most of these proteins has not been resolved. Maturation of these DNA metabolic enzymes strictly depends on the mitochondrial core ISC machinery, the export machinery and the CIA system (Figs. 1 and 2). Both genetic and biochemical studies have shown that all stages of cellular Fe–S protein biogenesis, are intimately linked to the maintenance of genome integrity including sister chromatid cohesion [6,10,11,73,90]. This is most evident for the CIA factor MMS19. Before this protein has been characterized as part of the CIA targeting complex, it has been functionally linked to rather diverse aspects of DNA metabolism as described in detail below.

MMS19 was initially detected in two independent genetic screens in budding yeast as a gene required for methionine biosynthesis, nucleotide excision repair (NER) and RNA polymerase II transcription [84,91]. Whereas its role in methionine biosynthesis remained largely unexplored, as it is not a biosynthetic enzyme for methionine production, studies concerning its role in transcription and DNA repair were carried on. Intensive efforts in yeast showed that MMS19 is required for the transcription factor IIH (TFIIH) complex, which is needed for transcription and NER [83]. MMS19 itself is not part of the TFIIH complex. However, it was shown to be required to maintain normal cellular levels of the TFIIH component Rad3 (human XPD), which, according to a more recent investigation, is a Fe–S cluster-containing protein [82]. Consistent with these data, studies of the human MMS19 homolog also suggested a regulatory role in NER that is mediated by regulation of TFIIH function [92,93]. Additionally, MMS19 was found to form a TFIIH-independent complex with CIA1 and CIA2B that is involved in chromosome segregation and furthermore has a function in the regulation of telomere length [81,94].

The functional identification of MMS19 as a component of the CIA targeting complex allowed the explanation of virtually all these diverse phenotypes observed for MMS19-deficient cells [10,11]. The direct impact of MMS19 on DNA metabolism pathways was demonstrated in multiple ways. On the one hand, MMS19 and also other CIA targeting complex components were shown to physically interact with diverse enzymes required for DNA metabolism, like DNA polymerase subunits (POLD1, POLA1, and POLE1), DNA helicases (XPD, FANCJ, RTEL1), the DNA glycosylase NTHL1, the nuclease DNA2 and the DNA primase (PRI2). All these proteins contain a Fe–S cluster that is matured with the help of MMS19. On the other hand, functional studies unraveled that depletion of MMS19 leads to an inhibition of XPD incorporation into TFIIH which in turn affects DNA metabolism. Further, depletion of yeast CIA targeting complex components, including Met18/Mms19, increased Rad3 phosphorylation and caused a subsequent induction of Rad3-dependent gene expression [10]. Likewise, in human cells CIA protein depletion caused increased sensitivity of cell growth to DNA damage-causing agents such as UV irradiation or chemical mutagens. Hence, MMS19 can be described as a CIA maturation factor for multiple Fe–S proteins including some with an important function in various DNA metabolic pathways, In that sense, MMS19 participates in an indirect way in the maintenance of genome integrity. The fact that all these MMS19-linked phenotypical effects can likewise be elicited by depletion of both the mitochondrial

ISC and the other cytosolic CIA components clearly indicates the crucial functional role of the Fe–S cofactors coordinated by the mentioned DNA metabolism proteins.

Recently, another link between DNA metabolism and a CIA component was observed in a plant genetic study [95]. Functional inactivation of the *Arabidopsis* CIAPIN1 homolog termed AtDRE2 revealed an epigenetic role in maternal activation of DNA demethylation in the endosperm lineage. Likely, this phenotype is indirect as well, and is mediated by a Fe–S cluster-dependent DNA glycosylase. The function of AtDRE2 in Fe–S protein biogenesis has not yet been verified in this study.

6. An overview on cytosolic and nuclear Fe–S proteins with a role in various aspects of DNA metabolism

In the following we will describe several examples of DNA metabolic enzymes that coordinate a functionally important Fe–S cluster, and we will highlight the importance of Fe–S protein biogenesis for the maintenance of their Fe–S cluster.

6.1. Fe–S proteins involved in DNA replication

DNA replication is a fundamental process for maintaining genome integrity and depends on the coordinated activities of a large number of enzymes [96]. Replicative DNA polymerases cannot start DNA replication de novo but require a DNA template to build a complementary strand [97]. Essentially, the two parental DNA strands have to be separated by helicase activity. Moreover, initiation of DNA synthesis depends on the function of DNA primases that assemble short RNA primers which can be extended by the replicative DNA polymerases. The helicase–nuclease DNA2 mediates a role in Okazaki fragment processing which is essential for lagging strand replication [98]. Interestingly, all three types of these replication factors have been shown to depend on the coordination of Fe–S clusters to fulfill their particular function in this complex biosynthetic process.

Eukaryotic primase contains two subunits (PRIM1 and PRIM2) that are tightly associated with DNA polymerase α [99,100]. Although catalytic activity resides within the PRIM1 subunit, PRIM2 is equally essential for primase function [101]. Spectroscopic analysis of the large primase subunit (PRIM2) from human cells, yeast and Archaea demonstrated the binding of a [4Fe–4S] cluster [7,102]. Loss of this cofactor resulted in a reduced enzymatic activity underscoring the importance of the Fe–S domain for the initiation of primer synthesis. Mutation of the conserved lysine at position 314 was shown to interfere with primer synthesis and DNA binding [101,103]. This residue is part of a conserved, basic region that is buttressed by the [4Fe–4S] cluster as gathered from the high-resolution structure of the C-terminal domain of human PRIM2 [103]. Based on this finding it was suggested that the Fe–S cluster might play a role in organizing the protein surface to facilitate DNA binding [103].

The DNA polymerase complexes Pol α, Pol δ and Pol ε are essential for replication of double-stranded nuclear DNA in eukaryotes [104]. They belong to the class B family of DNA polymerases [105] and are composed of catalytic, regulatory and accessory subunits. Pol α in complex with DNA primase initiates the synthesis of short RNA primers that are extended by Pol δ and Pol ε for processive synthesis of the lagging and leading strands, respectively [106,107]. The catalytic subunits of Pol α, Pol δ and Pol ε (i.e. POLA, POLD1 and POLE1, respectively) are evolutionarily conserved and contain two C-terminal cysteine-rich motifs (CysA and CysB) which were predicted Zn^{2+}-binding sites [108,109]. A single point mutation within the CysB motif of yeast Pol3 was shown to be synthetically lethal with mutations in the essential CIA genes *NBP35*, *DRE2* and *TAH18* [110]. Although it was widely believed that both cysteine-rich motifs of DNA polymerases coordinate Zn^{2+} ions, this genetic finding pointed to the probable presence of a Fe–S cluster in Pol3. A combination of in vivo and in vitro approaches demonstrated the binding of a functionally essential [4Fe–4S] cluster to the CysB motifs of all three catalytic subunits of DNA polymerases [8]. In vivo ^{55}Fe radiolabeling assays as well as UV–Vis and EPR spectroscopic studies with purified proteins demonstrated the coordination of a Fe–S cluster rather than Zn^{2+} to the CysB motifs of all B-family DNA polymerases. Assembly and insertion of these essential Fe–S clusters are strictly dependent on the ISC and CIA machineries, explaining the synthetic lethality of the *pol3-13* allele and Fe–S biosynthetic genes [8,110]. Cysteine ligand mutagenesis in Pol3 leads to the loss of the cofactor and interactions with accessory subunits (Pol31–Pol32) of the Pol δ complex are abrogated. This demonstrates the important physiological role of the Fe–S cluster in polymerase complex formation [8]. It is clear from these studies that the Fe–S cluster plays an important structural role for folding the C-terminal domain in such a way that it can bind the accessory subunits. However, an additional mechanistic function of the Fe–S cluster for polymerase activity cannot be excluded.

DNA2 (DNA replication helicase/nuclease 2) is a multitasking enzyme involved in Okazaki fragment processing during DNA replication, double-strand DNA break repair and telomere maintenance [98,111,112]. The super-family 1 helicase DNA2 belongs to a class of enzymes that possess nuclease and helicase domains fused within the same polypeptide [113]. A sequence alignment with the bacterial AddAB helicase–nuclease complex which carries a [4Fe–4S] cluster, revealed a putative metal-binding motif in eukaryotic DNA2. Interestingly, the four cluster-coordinating cysteine residues in AddAB are distributed in the nuclease domain and not clustered together. This leads to the assumption that the Fe–S cluster might be involved in the overall conformational stabilization of the nuclease domain [113]. Comprehensive genetic and biochemical studies in yeast confirmed the Fe–S cluster binding by a conserved cysteine motif in DNA2 [114]. Mutation of any of the Fe–S cluster-coordinating cysteine residues not only diminished nuclease activity but also the ATPase function of DNA2, while DNA binding was not affected. A proline at position 504, which resides adjacent to the Fe–S domain, was shown to be important for Fe–S cluster stabilization. This clearly shows that the Fe–S cluster is mandatory for the physiological function of DNA2 in DNA replication and repair, most likely by mediating conformational changes that are required for coupling the nuclease and helicase activities.

6.2. Fe–S proteins involved in DNA repair

Base excision repair (BER) corrects DNA damage that arises from oxidation, deamination and alkylation [115]. DNA glycosylases are the key components of this repair mechanism and many of them were shown to coordinate a Fe–S cofactor [12,116–119]. During the repair process DNA glycosylases search for the presence of DNA lesions in a vast excess of normal bases leaving an abasic site that is further processed by downstream enzymes to restore the regular DNA structure [120]. The mechanism of BER is highly conserved between *Escherichia coli* and mammals. The *E. coli* endonuclease III (Endo III) which possesses both glycosylase and lyase activities has been the first reported example for being a DNA repair enzyme with a bound Fe–S cluster [116]. The coordination of the [4Fe–4S] cluster appears to be important for the interaction with the DNA phosphate backbone by positioning conserved basic residues [121]. MutY, structurally similar to Endo III, is another BER glycosylase that coordinates a [4Fe–4S] cluster [117]. *E. coli* MutY is an adenine DNA glycosylase and it was demonstrated that its Fe–S cluster is essential for the recognition of substrate DNA and the overall stability of the enzyme [122,123]. By the use of electrochemical methods it was demonstrated that DNA binding of Endo III and MutY shifts the redox potentials of the [4Fe–4S] clusters so that they can be oxidized more easily [124]. Based on these findings, a model was proposed in which DNA repair glycosylases harboring the redox-active [4Fe–4S] cluster may use the electron transport through the wire-like DNA double helix for sensing lesions within the DNA (see below) [125,126].

Until now the human homologs of this repair enzyme family have not been studied extensively. The mammalian MUTYH has been

shown to be involved in the repair of DNA damage upon oxidation which is consistent with the function of its bacterial homolog MutY [127]. Even though the presence of the Fe–S cluster has been recently documented, its exact function has not been addressed experimentally [128].

6.3. The XPD DNA-helicase family is characterized by a conserved Fe–S domain and linked to various diseases

Helicases are ATP-dependent molecular motors that unwind structured nucleic acids thereby influencing many different aspects of DNA metabolism [129]. A variety of processes depends on strand separation, e.g., DNA replication, DNA repair, transcription and the regulation of telomere length, thus explaining the general role of helicases in maintaining genome integrity [130–134]. Depending on the primary amino acid sequences, helicases are classified into different super-families [135].

XPD (xeroderma pigmentosum complementation group D; yeast Rad3) is the founding member of the XPD helicase family comprising several related super-family 2 DNA helicases such as Fanconi anemia complementation group J (FANCJ), DEAD/DEAH box helicase 11 (DDX11/ChlR1), and Regulator of Telomere Elongation 1 (RTEL1). All these proteins are linked to human diseases [136,137] (Table 1). The key characteristics of all family members is the conservation of four cysteine residues required for the coordination of a Fe–S cluster suggesting that binding of this cofactor is a conserved feature of these 5′-3′-DNA helicases.

XPD is part of the transcription initiation factor TFIIH that plays a fundamental role in nucleotide excision repair (NER) and basal transcription [138]. TFIIH is a multi-protein complex with ten subunits that consist of two functional subcomplexes, the core complex (XPB, p62, p52, p44, p34 and p8) and the CAK (CDK-activating kinase) complex (CDK7, cyclin H and MAT1). The helicase XPD mediates the bridging of these two subcomplexes underscoring the essential character of this protein.

Three different genetic disorders are connected to mutations within the human *XPD* gene, namely xeroderma pigmentosum (XP), Cockayne syndrome (CS) and Trichothiodystrophy (TTD). All three diseases are characterized by a skin hypersensitivity to sun exposure which is explained by a defective NER pathway that normally serves to repair bulky DNA adducts [13]. Initial biochemical and spectroscopic analyses of an archaeal XPD relative identified a [4Fe–4S] cofactor that is required for helicase activity. This breakthrough observation greatly helped to clarify the molecular differences underlying the different diseases caused by mutations in a single gene [9]. Structural insights confirmed the presence of the [4Fe–4S] domain that, together with an arch domain, forms a channel that can accommodate ssDNA [139–141]. Based on these findings the Fe–S domain might act as a molecular “ploughshare” analogous to the β-hairpin domain in the bacterial NER helicase UvrB that is physically responsible for separation of complementary strands [142,143]. Mutational analysis of the conserved Fe–S cluster-coordinating cysteine residues in archaeal XPD demonstrated the significance of the Fe–S domain integrity for helicase activity and/or stabilization of the protein structure [9,139,144]. Moreover, it was shown that binding of ssDNA and ATP hydrolysis is uncoupled from strand displacement in Fe–S domain mutants.

XP-causing mutations primarily inactivate the helicase without disrupting the protein structure and all residues whose exchange is reported to result in XP are conserved in archaeal XPD [9,139,140]. In contrast to the situation observed for XP mutations, only two out of twelve residues mutated in TTD are conserved in the archaeal protein [140]. The most common mutation in TTD patients is the R112H exchange resulting in a complete inhibition of XPD helicase activity and a concomitant defect in NER [145]. In the archaeal XPD sequences this residue is conserved as arginine or lysine, and positioned close to the first conserved Fe–S cluster-coordinating cysteine residue [9]. Functional data confirmed that this amino acid residue is an important structural component of the Fe–S domain, suggesting that the mutation might destabilize XPD to some extent. This finding underscores the structural role of the Fe–S cluster for helicase activity and shows the intimate link of Fe–S cluster biogenesis and the maintenance of genome stability. Other mutations were shown to influence TFIIH function either by weakening the interactions of XPD with subunits of the TFIIH complex or a direct destabilization of the helicase structure [140,145].

FANCJ was originally identified as a protein that binds to the breast cancer C-terminal (BRCT) repeats of BRCA1 and thus was named BACH1 (BRCA1-associated C-terminal helicase 1) [146]. Now, BACH1 is widely referred to as FANCJ because it was identified as the gene mutated in the J complementation group of Fanconi anemia, a genome instability disorder with elevated risk of developing cancer [14–16]. In humans, mutations in the *FANCJ* gene are additionally associated with early-onset breast cancer [146]. FANCJ is suggested to be a tumor suppressor and genome caretaker with functions in DNA double strand break repair and interstrand crosslink repair [147]. One *FANCJ* mutation related to Fanconi anemia leads to the amino acid substitution A349P [15,148]. This residue is not conserved within the XPD helicase family but resides adjacent to the fourth highly conserved Fe–S cluster-coordinating cysteine residue. Biochemical and functional analysis of the recombinant FANCJ-A349P protein demonstrated a diminished iron content and a defect in unwinding DNA substrates or in displacing DNA-bound proteins [148]. This suggests that integrity of the Fe–S domain might be essential for the catalytic activities of FANCJ.

DDX11/CHLR is the third member of the FANCJ/XPD family of helicases. Mutations in the human *CHLR1* gene are associated with the unique genetic disorder Warsaw breakage syndrome (WASB) [17]. Despite extensive genetic studies on the yeast and human *CHLR1* (chromosome loss mutation) genes the structural or functional significance of the highly conserved Fe–S domain within the respective proteins has not been addressed yet. Initial studies demonstrated that a mutation in *CHL1* in *S. cerevisiae* is linked to unusual mating phenotypes due to chromosome loss [149,150]. This finding was corroborated by the role of yeast Chl1 and its human homolog CHLR1 in sister chromatid cohesion [151–153]. The DNA-dependent ATPase activity and 5′-3′ helicase directionality of the human CHLR1 were confirmed biochemically and interactions with DNA replication factors, like CTF18–RFC (replication factor C) complex, PCNA (human proliferating cell nuclear antigen) and the flap endonuclease FEN1 were reported suggesting that the involvement of CHLR1 in lagging strand processing during DNA replication may be important for sister chromatid cohesion [154]. The patient-derived mutation abolishes helicase activity by perturbing its DNA binding and DNA-dependent ATPase activity [155].

RTEL1 (regulator of telomere length 1) is the fourth paralog of XPD sharing the Fe–S cluster-coordinating motif with highly conserved cysteine residues [9]. The *RTEL1* gene was originally detected in a genome-wide screen using two inter-fertile species of mice which differ in telomere length [156]. Inactivation of *RTEL1* gene expression in mice demonstrated the role of this gene product in telomere homeostasis [157]. While the *RTEL1* gene knockout was embryonically lethal, embryonic stem cells derived from these mice displayed telomere loss and other chromosomal abnormalities upon differentiation in vitro. Subsequent studies suggested a function of the essential helicase RTEL1 in unwinding a variety of DNA secondary structures, a finding which is also consistent with the close relation of RTEL1 to *Caenorhabditis elegans* DOG-1 (deletion of G-tracts) [158–161]. Mutations in *RTEL1* have recently been linked to the Hoyeraal-Hreidarsson syndrome (Table 1), but the impact of disease-associated mutations on the function or regulation of RTEL1 have not been addressed experimentally yet [162–164]. This issue and the biochemical analysis of RTEL1 with respect to the function of the Fe–S domain have to be addressed in future studies.

7. Putative molecular roles of Fe–S cofactors in DNA metabolic enzymes

The number of Fe–S cluster-containing enzymes involved in the maintenance of genome integrity is steadily increasing. Hence, resolving questions centered around the molecular function of their metal cofactors has become a major focus of research. For the majority of those DNA maintenance proteins their Fe–S cluster has been proposed to play a non-catalytic role in stabilizing the structure of the protein [165]. However, additional functions are not excluded. An attractive model was proposed by the Barton group suggesting that the redox-active Fe–S cofactors of DNA glycosylases are used to scan the genome for DNA damages [125,126,166]. This model is based on the fact that electron transport can occur over long molecular distances within the DNA duplex due to π-stacking of its aromatic base pairs [167,168]. The presence of mismatched or damaged DNA bases substantially disrupts the charge transfer within the π-stack [169]. Thus, DNA-mediated charge transfer could be a suitable model to explain how DNA repair enzymes use their Fe–S cofactors to scan the genome integrity and distinguish between intact and damaged bases [124–126]. A problem linked to the DNA binding of redox-active enzymes may be the possible Fe–S cluster oxidation and resulting release of free iron ions that potentially can generate reactive oxygen species. It therefore may be argued that it is dangerous to locate such redox-active cofactors in the proximity of DNA and put its integrity at risk. Stably bound Fe–S clusters would of course avoid such a problem.

Experiments performed with DNA glycosylases such as Endo III and MutY suggest that those enzymes may use the redox properties of their Fe–S clusters to cooperatively search for DNA lesions that disturb the DNA electron transport [170,171]. While a reduced Fe–S enzyme is weakly bound to DNA as it scans the genome, it can be tightly attached to DNA by oxidation as a result of oxidative stress or other DNA damaging agents. The oxidized enzyme can now receive an electron from a second loosely-bound reduced enzyme. In case of an error-free DNA, the electron transfer to the nearby oxidized glycosylase through the DNA helix is successful and leads to reduction of its $[4Fe–4S]^{3+}$ cluster. This event signals integrity of the intervening DNA sequence and facilitates the dissociation of the reduced glycosylase from the DNA. In contrast, in case of a DNA lesion the electron transport between the two flanking enzymes is impaired, and no glycosylase dissociation occurs due to a failure of reduction. The maintenance of the enzyme near the site of a DNA lesion may then initiate the repair process.

This model raises the question of how the oxidation of the glycosylase Fe–S cluster may initially occur. Guanine radicals are generated early during oxidative stress and were shown to directly oxidize the Fe–S clusters in bacterial MutY [171,172]. This suggests a redox activation of DNA repair proteins through DNA charge transport, with guanine radicals as oxidants of the DNA-bound repair proteins [171]. Additional evidence for such a coordinated DNA charge transport between different repair proteins in their search for DNA damages was provided by studies on the 5′-3′ helicase XPD [170]. Like DNA glycosylases this enzyme exhibits a DNA-bound redox potential that is physiologically relevant. XPD was shown to redistribute onto DNA strands containing a single base mismatch, which like a lesion inhibits charge transport. Further studies demonstrating the DNA-mediated signaling between XPD and Endo III and their coordinated relocalization onto mismatched strands support the idea that the function of repair proteins is generally based on carrying out DNA charge transport. Despite strong in vitro evidence corroborating the DNA charge transport model for a function of the DNA repair enzymes in the communication and scanning of the DNA strands for lesions, future studies will have to address the in vivo significance of this proposal.

8. Conclusions and perspectives

Cytosolic and nuclear Fe–S proteins participate in many essential processes of life, such as biosynthetic reactions, tRNA modifications, protein translation as well as the synthesis and maintenance of DNA. Recent research has greatly advanced our understanding of the assembly and insertion of Fe–S clusters into cytosolic and nuclear target proteins. Numerous proteins of the CIA machinery have been identified and characterized, and the role of the mitochondrial ISC machinery in this process has been established. However, the molecular mechanisms of the individual steps catalyzed by the CIA proteins are biochemically still ill-defined. Their elucidation will require a combination of structural, biochemical and cell biological approaches. Another important goal of future research will be the deciphering of the exact function of these inorganic cofactors in DNA metabolic enzymes. It is obvious that Fe–S clusters are required to stabilize the protein structure, but other, more intricate biochemical functions are suspected. In case of DNA repair enzymes strong in vitro evidence suggests that the redox sensitivity of these cofactors is exploited to allow these Fe–S proteins to communicate and cooperatively search for damaged DNA bases within the genome. It will be exciting to test the in vivo relevance of this hypothesis and to discover other possible functions of Fe–S clusters in DNA metabolic enzymes.

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