nature REVIEWS
MOLECULAR CELL BIOLOGY

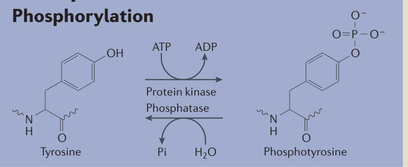
Reading protein and phospholipid modifications with interaction domains

Bruce T. Seet, Caesar Lim and Tony Pawson

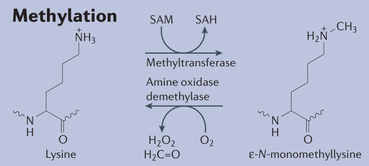
Cells continuously receive a wide range of external and internal signals, and the cellular responses to changing conditions are commonly mediated by the reversible covalent modification of existing molecules such as proteins and phospholipids. Proteins can be altered by a diverse set of post-translational modifications that include phosphorylation, methylation, acetylation, ubiquitylation and hydroxylation. These modifications often function in combination to control the activities of individual proteins and multiprotein signalling pathways. Each of these modifications can be recognized by specific protein-interaction domains, which therefore read out the dynamic state of the proteome and transmit this information to the molecular machines that organize cellular behaviour. Such modification-dependent interactions can be configured to produce complex forms of regulation that involve, for example, autoregulation, cooperative interactions, sequential interactions, inhibition and mutually exclusive interactions.

Example modification reactions

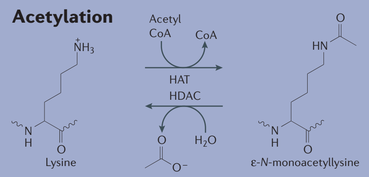
Phosphorylation



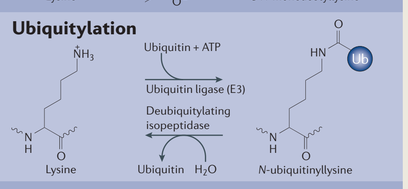
Methylation



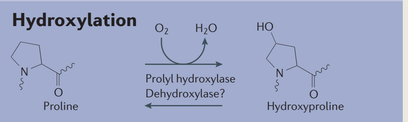
Acetylation



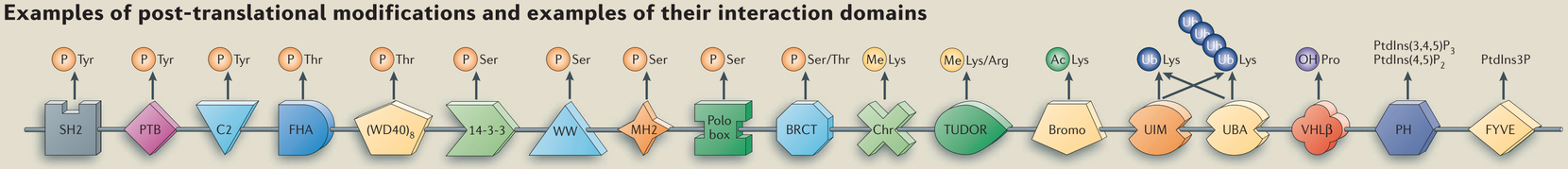
Ubiquitylation



Hydroxylation

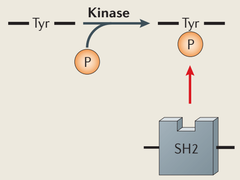


Examples of post-translational modifications and examples of their interaction domains

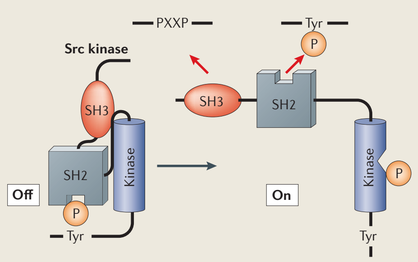


Regulatory properties of post-translational modifications and their interaction domains

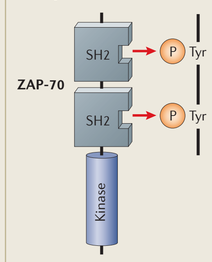
Inducible interaction



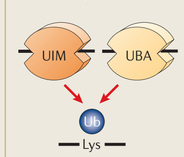
Intramolecular regulation



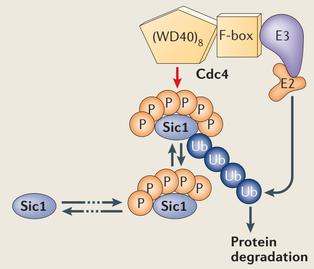
Cooperative



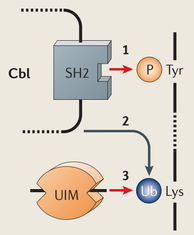
Convergent



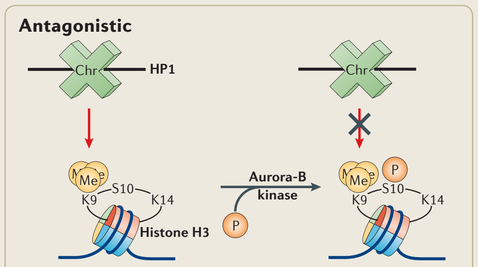
Multi-site switch



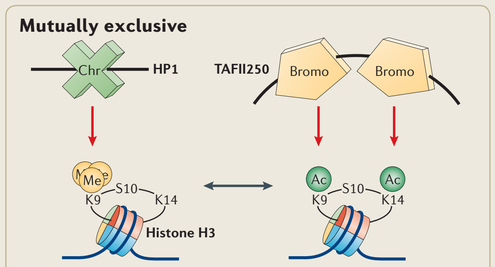
Sequential



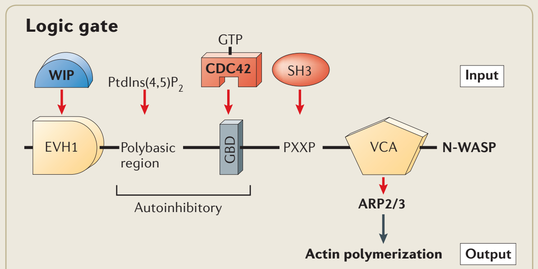
Antagonistic



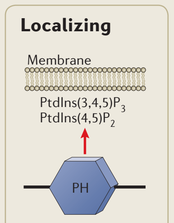
Mutually exclusive



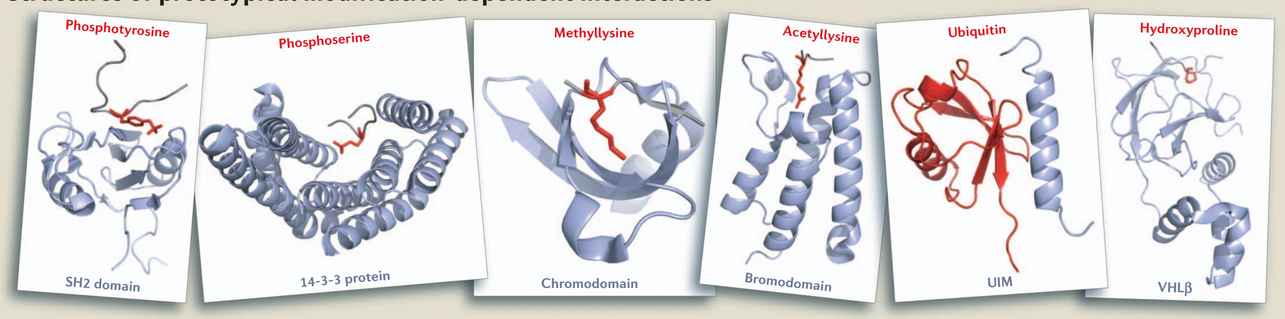
Logic gate



Localizing



Structures of prototypical modification-dependent interactions



Ac–Lys, acetylated lysine; ARP2/3, actin-related protein-2/3 complex; BRCT, breast-cancer-susceptibility protein-1 C-terminal; Bromo, bromodomain; C2, conserved region-2 of protein kinase C; Cbl, Casitas B-lineage lymphoma proto-oncogene (an E3 ubiquitin ligase); Cdc4, cell-division cycle-4; CDC42, cell-division cycle-42; Chr, chromodomain; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; EVH1, Enabled/VASP homology-1; FHA, forkhead-associated; FYVE, 'Fab1, YOTB, Vac1, EEA1'; GBD, GTPase-binding domain; GCN5, general control of amino-acid-synthesis protein-5; GRB2, growth-factor-receptor-bound protein-2; HAT, histone acetyltransferase; HDAC, histone deacetylase; HP1, heterochromatin protein-1; Me–Arg, methylated arginine; Me–Lys, methylated lysine; MH2, MAD-homology-2; N-WASP, neuronal Wiskott–Aldrich syndrome protein; OH–Pro, hydroxylated proline; PH, pleckstrin-homology; Pi, inorganic phosphate; P–Ser, phosphoserine; PTB, phosphotyrosine-binding; PtdIns3P, phosphatidylinositol-3-phosphate; PtdIns(4,5)P_2, phosphatidylinositol-4,5-bisphosphate; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate; P–Thr, phosphothreonine; P–Tyr, phosphotyrosine; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SH2, Src-homology-2; SH3, Src-homology-3; Sic1, substrate inhibitor of cyclin-dependent protein kinase-1; TAFII250, TATA-binding protein-associated factor-II250; UBA, ubiquitin-associated; Ub–Lys, ubiquitylated lysine; UIM, ubiquitin-interacting motif; VCA, verprolin-homology, cofilin-homology and acidic; VHLβ, von Hippel–Lindau protein-β; Vps27, vacuolar protein sorting protein-27; WIP, WASP-interacting protein; ZAP-70, ζ-chain (T-cell receptor)-associated protein kinase 70 kDa.

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Post-translational modifications

Dynamic modifications of proteins and phospholipids create binding sites for interaction domains, which are frequently components of multidomain regulatory proteins. A number of post-translational modifications (PTMs) are shown — phosphotyrosine (P–Tyr), phosphothreonine (P–Thr), phosphoserine (P–Ser), methylated lysine (Me–Lys), methylated arginine (Me–Arg), acetylated lysine (Ac–Lys), ubiquitylated lysine (Ub–Lys), polyubiquitylated lysine and hydroxylated proline (OH–Pro). A number of interaction domains that selectively recognize these modifications are depicted as icons (top centre panel). There are usually several different classes of domain that can bind the same PTM. Typically, each interaction domain has a binding pocket for the modified residue and also interacts with flanking amino acids. The structures of selected domains that are bound to P–Tyr, P–Ser, Me–Lys, Ac–Lys, ubiquitin and OH–Pro are depicted (bottom centre panel; Protein Data Bank accession codes: 1JYR for the interaction between the SH2 domain of GRB2 and P–Tyr; 1QJA for the interaction between a 14-3-3 protein and P–Ser; 1Q3L for the interaction between the chromodomain of HP1 and Me–Lys; 1E6I for the interaction between the bromodomain of GCN5 and Ac–Lys; 1Q0W for the interaction between the UIM of Vps27 and ubiquitin; and 1LM8 for the interaction of VHLβ with OH–Pro).

By contrast to the simpler modifications, ubiquitin is a folded protein domain that is most often attached through its C-terminal glycine to a lysine side chain in a host protein. A single lysine can be modified by a single ubiquitin or by several linked ubiquitins, and the ubiquitin modification can be recognized by the ubiquitin-binding domains of other proteins (for example, UIMs and UBA domains).

Similar to proteins, phospholipids can be dynamically modified by phosphorylation. For example, the inositol headgroup of phosphoinositides can be phosphorylated at several sites, and this creates binding sites at membranes for interaction domains such as PH and FYVE domains.

The same protein can potentially be modified at several sites by different types of PTM, which leads to complex forms of regulation (middle centre panel):

- ***Inducible interaction*:** In the simplest case, a PTM induces the binding of an interaction domain.
- ***Intramolecular regulation*:** A PTM can also create an intramolecular interaction that regulates the activity of a protein, as for the Src tyrosine kinase.
- ***Cooperative*:** PTM-dependent interactions can be cooperative, as shown for a protein that has tandem SH2 domains that bind selectively to a doubly phosphorylated site.
- ***Convergent*:** Several different classes of domain can converge on the same PTM.
- ***Multi-site switch*:** A threshold number of modifications (for example, six phosphorylation sites on Sic1) are required to induce a stable association (in this case, with Cdc4, which leads to Sic1 ubiquitylation).
- ***Sequential*:** PTMs and their resulting interactions can be interdependent and occur sequentially. For example, protein phosphorylation can recruit an E3 ubiquitin ligase, which can then create a ubiquitin-dependent interaction.
- ***Antagonistic*:** Two adjacent PTMs can be antagonistic, with one blocking the capacity of its neighbour to bind to an interaction domain.
- ***Mutually exclusive*:** Two different PTMs and their corresponding interactions can be mutually exclusive, for example, the methylation or acetylation of the same lysine.
- ***Logic gate*:** A protein that is regulated by several protein–protein and protein–phospholipid interactions can function as a logic gate, such that it is only activated in the presence of several upstream signals.
- ***Localizing*:** Phospholipids can induce the membrane recruitment of proteins that contain the appropriate interaction domain.

Together, these protein and phospholipid modifications and their recognition by interaction domains work in concert to control many dynamic aspects of cellular function, including signal transduction, cytoskeletal architecture, cell-cycle checkpoints, DNA-damage responses, chromatin organization and gene expression.

LINKED REVIEW ARTICLE

Seet, B. T., Dikic, I., Zhou, M.-M. & Pawson, T. Reading protein modifications with interaction domains. *Nature Rev. Mol. Cell Biol.* 23 June 2006 (doi:10.1038/nrm1960).

FURTHER READING

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