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Regulation of transcription factor activity by interconnected, post-translational modifications

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Highlights

- Transcription factors integrate extracellular signals
- Transcription factor PTMs underlie signaling mechanisms to nucleus
- PTMs control every aspect of transcription factor function
- Multiple signaling pathways converge at level of transcription factors
- Sequential transcription factor PTMs allow for coincidence detection

Transcription factors comprise just over 7% of the human proteome and serve as the gatekeepers of cellular function, integrating external signal information into gene expression programs that reconfigure cellular physiology at the most basic levels. Surface-initiated, cell signaling pathways converge on transcription factors, decorating these proteins with an array of post-translational modifications (PTMs) that are often interdependent, being linked in time, space, and combinatorial function. These PTMs orchestrate every activity of a transcription factor over its entire lifespan—from subcellular localization to protein–protein interactions, sequence-specific DNA binding, transcriptional regulatory activity, and protein stability—and play key roles in the epigenetic regulation of gene expression. The multitude of PTMs of transcription factors also offers numerous potential points of intervention for development of therapeutic agents to treat a wide spectrum of diseases. We review PTMs most commonly targeting transcription factors, focusing on recent reports of sequential and linked PTMs of individual factors.

General overview of PTMs of transcription factors

Post-translational modifications regulate every aspect of transcription factor function and coordinate access of RNA polymerases to promoter templates. Site-specific, DNA-binding transcription factors (SSTFs) serve to nucleate repressor, activator, enhancer, or silencer complexes and associated enzymatic activities. To coordinate these activities, often with great spatial, temporal, and tissue-specific precision required of developmental and cell-cycle programs, the full range of cellular post-translational modifications (PTMs) of SSTFs may occur. In many cases, these PTMs occur as individual, isolated events and these modifications dictate some aspect of transcription factor function. In other cases, individual PTMs on proteins are sequentially linked—that is, one PTM may promote (or inhibit) the establishment of a second-site PTM within the same protein. These two PTMs are "linked" or "interconnected," and as we describe below, this interconnectedness can be exploited therapeutically in the treatment of disease.

Among the more prominently studied PTMs of transcription factors are phosphorylation, sumoylation, ubiquitination, acetylation, glycosylation, and methylation. The analysis presented below (Figure 1) suggests that most of these PTMs occur on transcription factors at about the same rate as seen with other proteins, with the notable exceptions of ubiquitination, glycosylation, and sumoylation, which are found on transcription factors with moderately decreased, moderately increased and greatly increased frequencies, respectively (Figure 1). There is no obvious logic why ubiquitination would be somewhat lower or glycosylation somewhat higher among transcription factors. The many-fold increased incidence of sumoylation among transcription factors may reflect

a real biological phenomenon. Alternatively, it is possible that this modification, which has historically been difficult to detect in native proteins, may be over-represented in transcription factor data sets due to a relative lack of information concerning this modification among non-nuclear proteins.

PTMs may alter SSTF subcellular localization (transport into or out of the nucleus), stability, secondary structure and DNA binding affinity, or tertiary structure and association with co-regulatory factors. PTMs of SSTFs are of particular interest as a means of altering transcriptional regulatory activity of these proteins. Many excellent reviews have focused on the varied effects of transcription factor phosphorylation [2,3], sumoylation [4], ubiquitination [5], acetylation [6], and glycosylation [1,7]. In this review, we provide a few examples of small-mass modifications, including phosphorylation, acetylation, methylation, and glycosylation, and then focus on the larger modifications of sumoylation and ubiquitination, highlighting some examples of interconnected or sequentially-dependent modifications. Specific information about known PTMs of all proteins can be found at http://www.phosphosite.org [1–3] and information about sequentially linked PTMs in proteins can be accessed at the PTMcode website (http://ptmcode.embl.de) [4,8]. Both websites are actively curated and exceptionally informative.

Phosphorylation

Phosphorylation is a gateway PTM; easily detected, phosphorylation is often the first PTM to be studied when looking at regulation of protein activity. Rapidly reversible phosphorylation, a ubiquitously utilized mechanism to transduce extracellular signals to the nucleus, may affect transcription factor stability, location, structure and/or protein–

interaction network (Figure 2), all of which may impact target gene expression. Phosphorylation may also regulate the status of other PTMs in a time-dependent sequence that, for some transcriptional regulatory proteins may culminate in degradation. Straightforward examples of transcription factor regulation by single- or double-site phosphorylation leading to well-characterized, binary effects are described elsewhere [2,3,5].

Transcription factors may harbor multiple phosphorylation sites, serving as points of convergence of signaling pathways initiating at the plasma membrane. As such, transcription factors may function as coincidence detectors in which two or more pathways must be activated before gene transcription is altered. For example, TORC2 is normally a cytosolic protein in insulinoma cells that is activated to translocate to the nucleus by dephosphorylation, after which it becomes associated with the CREB transcriptional complex. TORC2 translocation requires both high glucose and incretin receptor activation to increase intracellular calcium and cAMP levels, respectively. Both calcium-induced activation of calcineurin and inhibition of the SIK2 serine/threonine kinase by cAMP are required to mediate TORC2 dephosphorylation at distinct sites to allow for translocation [2,3,6,9].

Multiple phosphorylation sites may also serve as tunable signal regulators with incremental phosphorylation leading to changes in amplitude of gene expression. The MSN2 transcription factor in yeast processes different stress responses by “tunable” accumulation in the nucleus. Phosphorylation of eight serines clustered within at least two regulatory domains of MSN2 leads to complex translocation kinetics and differentially tuned responses to stressors. Osmotic stress produces a single pulse of nuclear localization,

glucose limitation induces sporadic pulses of nuclear localization, and oxidative stress produces sustained nuclear localization [4,10]. These differential responses are produced by dual regulation of both nuclear import and nuclear export rates by different phosphorylation states of MSN2.

A cooperative signaling response is another potential function of clusters of multiple phosphorylation sites in an SSTF. Although not a new story, the response of NFAT to dephosphorylation in T cells is the best-detailed example of multi-site phosphorylation/ dephosphorylation providing a steep response curve for conformational change in response to signaling (reviewed by [5,11]). Dephosphorylation of NFAT by the calcium-sensitive phosphatase calcineurin allows for nuclear accumulation and transcriptional activity. Nuclear accumulation results from an alteration in the balance between nuclear import and export. Progressive dephosphorylation of 13 phosphorylation sites alters the likelihood that the NFAT nuclear localization signal subdomain is exposed and that the nuclear export signal is hidden due to a phosphorylation-dependent change in the folding energy of the protein [6,12,13]. This mechanism appears to provide a tightly defined, calcium concentration threshold for activation.

Although the above examples are all of multisite phosphorylation affecting nuclear localization in various ways, multisite phosphorylation of SSTFs may have other effects. A multisite phosphorylation gradient progressively impacts the stability and ubiquitination of ATF4 in cell cycle control, allowing for ATF4 to exert dose-dependent regulation of target genes in neurogenesis [7,14]. Dephosphorylation of RUNX1 by the tyrosine phosphatase SHP2 alters RUNX1 protein–protein interactions within the megakaryocyte nucleus, increasing association with the SWI/SNF chromatin remodeling complex, reducing

association with other factors such as GATA1, and promoting development of megakaryocytes [2,3,15].

O-GlcNAcylation

Many transcription factors, in particular, but also other nuclear proteins, as well as cytosolic proteins are extensively modified by addition of β-D-*N*-acetylglucosamine (GlcNAc; Figure 2). The modification of serine and threonine by this monomeric GlcNAc moiety is distinguished from other forms of glycosylation by its relatively small size, subcellular location, and dynamic nature. GlcNAc addition and removal occurs in both the nucleus and cytoplasm, targeting the same motifs as phosphorylation, with which it competes. This reciprocal regulation is seen both on the nuclear factor substrates and on the enzymes affecting this regulation. Kinases are overrepresented among *O*-GlcNAcylation substrates [16] and the enzyme responsible for adding GlcNAc is itself phosphoactivated by a kinase that is regulated by *O*-GlcNAcylation. *O*-GlcNAcylation of CaMKIV on Ser189 limits phosphoactivation on a nearby Thr200 [7]. Similarly, *O*-GlcNAcylation of CK2 on Ser347 antagonizes phosphorylation at Thr344 and alters both stability and substrate specificity of the enzyme [17].

Much of the transcriptional proteome is modified by *O*-GlcNAcylation and the effects of this PTM on transcription factors and transcriptional regulatory proteins, including RNA polymerase II itself, may be either positive (via protein stabilization) or negative (via inhibition of transcriptional activation) [7,18,19].

O-GlcNAcylation occurs as a consequence of the activities of the single biosynthetic (*O*-GlcNAc transferase) and catabolic (*O*-GlcNAcase) enzymes in this pathway, and the availability of the high-energy donor substrate, UDP-GlcNAc [7]. Flux through the

biosynthetic side of the *O*-GlcNAcylation pathway is dictated by nutrients (glucose), insulin, and cellular stress, and it has been proposed that alterations in the levels *O*-GlcNAcylation of transcription factors may underlie certain pathological aspects of metabolic diseases, such as diabetes, as well as neurodegenerative diseases and cancer [7].

Acetylation

Lysine residues are positively charged at physiological pH and acetylation of these residues, which generates an uncharged amide, may logically be expected to reduce the affinity of the SSTF for DNA. Accordingly, acetylation inhibits interaction of FOXO1 with the glucose-6-phosphatase promoter [20]. However, the data show an evolving picture that is substantially more complex as deacetylation of FOXO1 inhibits its interaction with the *BIM* promoter in transfected cells [21]. Acetylation of FOXO1 increases its nuclear localization in skeletal muscle, and this results in enhanced regulation of FOXO1 target genes [22]. These findings indicate the consequences of alterations in the acetylation status of FOXO1 are both promoter-specific and influenced by cell type, highlighting the complexity of this relatively simple modification.

Methylation

Arginine methylation may alter the transcriptional regulatory activity of SSTFs by altering the protein–interaction network of these factors. For example, methylation of RUNX1 by the arginine methyltransferase PRMT1 inhibits binding of this SSTF to the co-repressor SIN3A and promotes de-repression of RUNX1 target genes [23]. Similarly, methylation of C/EBPβ regulates its interaction with Mediator and SWI/SNF co-activator complexes, and alters C/EBPβ-mediated activation of myeloid and adipogenic target genes

[24]. Reversible lysine modification of SSTFs, including NF-κB, STAT3, p53, and pRb, appears to be catalyzed on promoter templates by the same enzymes that place this modification on and remove it from the core histones [25] The effect of lysine methylation on the transcriptional regulatory activity of these proteins varies with the protein and the context within the protein [25].

Sumoylation

Reversible modification by small ubiquitin-like modifier (SUMO) proteins, SUMO1–4, can profoundly affect the activities, nuclear or sub-nuclear localization, and/or the protein–protein interaction network of SSTFs [4,26]. Protein sumoylation couples a glycine residue in the carboxyl terminus of the activated SUMO protein to the ε-amino group of an acceptor lysine in the target protein, resulting in a covalent, but highly labile, isopeptide bond. The majority of known SUMO-acceptor sites in target proteins conform to the sequence ψKx(D/E), where ψ corresponds to an aliphatic, hydrophobic amino acid and "x" can be any amino acid [4,27].

Importantly, the 10 kDa SUMO moiety is far larger than other common PTMs such as phosphorylation, acetylation, GlcNAc, and methylation, and larger also than ubiquitin. For example, mono-sumoylation of a 50 kDa transcription factor increases the size, and presumably the surface area, of that factor by 20%. The presence of multiple sites of mono-sumoylation and/or formation of SUMO chains at one or more these sites can easily double the mass and surface area of this transcription factor and alter the ability of the factor to participate in protein-protein interactions, which play a deterministic role in the activities of SSTFs.

Sumoylation of transcription factors is most often associated with enhanced transcriptional repressive activity [26], and several underlying mechanisms have been described or proposed. First, the presence of a large SUMO moiety may serve as a platform for interaction with proteins containing SUMO interaction motifs (SIMs; reviewed in [28]). Recruitment of the LSD1/CoREST1/HDAC complex to chromatin requires functional interaction of the CoREST1 SIM with SUMO2/3 [29], suggesting that sumoylation of a template-associated factor(s) nucleates assembly of a transcriptionally repressive complex. Second, sumoylation plays a key role in assembly and function of Polycomb group bodies [30], which serve as localized hubs of transcriptional repression [26]. Finally, sumoylation may directly enhance the enzymatic activity of DNA methylating enzymes, such as DNMT1 [31], which promote transcriptional repression.

Sumoylation stimulates the transcriptional activity of—or at least dampens transcriptional repression mediated by—a handful of transcription factors whose dysregulation has been linked to developmental defects and cancer, but the underlying mechanisms remain unknown. Sumoylation appears to stimulate transcriptional activation mediated by Ikaros [32], p53 [33], PAX6 [34], and BCL11B [35]. In the latter case, sumoylation of BCL11B results in recruitment of p300 to the BCL11B-NuRD complex and subsequent transcriptional activation of a BCL11B target gene [35]. Thus, sumoylation of BCL11B serves as a switch that converts BCL11B from a repressor to an activator of transcription, and this has relevance in the T-cell developmental program [35]. Finally, sumoylation of MBD1 inhibits its interaction with the histone-lysine methyltransferase SETDB, impairing repression mediated by the MBD complex [36].

Mono-sumoylated transcription factors may be further sumoylated to a state of poly-sumoylation, which may promote subsequent ubiquitination by E3 ligases harboring SUMO-Targeted Ubiquitin Ligase (STUbL) activity [37,38]. STUbL proteins, such as RNF4, harbor multiple SIMs that presumably dock with poly-sumoylated proteins via a multimerized SUMO–SIM interface [39,40]. STUbL proteins link sumoylation and ubiquitination pathways by catalyzing simultaneous hydrolysis of SUMO adducts and poly-ubiquitination of the target protein, preceding proteosomal degradation (see below).

Ubiquitination

There are many parallels between protein ubiquitination and sumoylation. Both modifications involve multiple processing steps that produce an active adduct, which is competent for transfer to target proteins, a reaction carried out by the cognate ligase in each pathway. Although consensus sequences surrounding the point of attachment diverge, SUMO and ubiquitin moieties share a common linkage to the lysine side chain of substrates [41]. Both SUMO and ubiquitin modify target proteins with a single copy at a single (mono-sumoylation or –ubiquitination) or multiple (multi-mono-sumoylation or –ubiquitination) lysine residues. SUMO and ubiquitin are both capable of chain extension at sites of modification, producing poly-sumoylated or –ubiquitinated target proteins [39,42,43]. Sumoylation and ubiquitination alter the functional properties of SSTFs in a myriad of overlapping ways [42].

Although the role of ubiquitination in protein degradation is well known, ubiquitination contributes to transcriptional processes via both proteolytic and non-proteolytic mechanisms. In general, mono-ubiquitination alters signaling mechanisms and/or activities of SSTFs [42,43]. For example, ubiquitination of FOXO4 drives nuclear

translocation and stimulates transcriptional activation mediated by this factor [44]. In this case, ubiquitination may alter the interaction network of the target SSTFs, either by inhibiting basal protein-protein interactions or facilitating those involving ubiquitinated factors with proteins harboring ubiquitin binding domains [43]. In this way, ubiquitination likely facilitates nucleation of large protein complexes that are competent for transcriptional activation. Termination of the activity of ubiquitinated transcription factors may proceed by at least two pathways. First, the mono-ubiquitinated protein may continue to become more extensively ubiquitinated, producing a poly-ubiquitinated species, which is then subjected to proteosomal degradation. The kinetics of poly-ubiquitination would presumably dictate the active lifetime of the ubiquitinated transcription factor complex. Second, the ubiquitinated transcription factor may serve as a substrate for de-ubiquitinating enzymes, known as DUBs [45], the availability and catalytic activity of which determine the active lifetime of the ubiquitinated transcription factor complex.

The role of ubiquitination-induced proteolytic processing of transcription factors is equally complex. Poly-ubiquitination of SSTFs generally promotes degradation via the proteosomal system, and this dictates cellular levels and activities of SSTFs over time. However, the corollary is not always true: ubiquitination-stimulated proteolysis is necessary for transcriptional activation mediated by several types of SSTFs [46], and recruitment of the proteosomal machinery to the promoter template is integral to the transcriptional activation process [47]. This topic, "activation by destruction," was recently reviewed by Geng and colleagues [42], who suggested that the transcriptional machinery marks particular SSTFs by phosphorylation when these factors are "spent." They further suggested that phosphorylation of spent transcription factors both locks the proteins in an

inactive state and recruits the proteosomal machinery to degrade the ubiquitinated transcription factor *in situ*. This proteolytic action of the ubiquitin-proteosomal system would then facilitate recruitment of non-marked transcription factor to the template for additional rounds of transcription.

Finally, ubiquitination does not necessarily doom transcription factors to the proteosomal system and degradation. For example, ubiquitination and subsequent, limited proteolytic processing of NF-κB is required for maturation and activation of this transcription factor (reviewed in [42]).

Interconnected PTMs

PTMs are often progressive with examples of alterations in phosphorylation leading to increased (or decreased) sumoylation, sumoylation leading to ubiquitination through the action of STUbL proteins, phosphorylation affecting acetylation, *etc.,* in kinetically orchestrated sequences over the functional life of the protein. PTMs may also interact reciprocally as well as sequentially. Attempts have been made to quantitate the frequency of multiple, interconnected PTMs in the proteome using sequential isolation techniques and mass spectrometry [48,49]. In these studies, inhibiting the proteasome to reduce ubiquitination altered approximately 3% of all phosphorylations. Beltrao *et al*. used a computational evolutionary approach across the proteomes of 11 eukaryotic species to predict likely interconnected PTMs, and identified regulatory "hot spots" in protein sequences [50]. We provide a few examples of interconnected, transcriptionally relevant PTMs of SSTFs below.

Phosphorylation targets degradation

Absent in quiescent cells but essential for proliferation, c-MYC is regulated by a tight cycle of phosphorylation-driven control of activity and degradation. This cycle starts with ERK-mediated phosphorylation of Ser62, which is essential for transcriptional activity and required for subsequent GSK3-mediated phosphorylation of Thr58 (reviewed by [51]). These phosphorylations orchestrate, both individually and in combination, specific interactions that bring active c-MYC to target promoters while assuring rapid inactivation and degradation. Efficient association of PIN1, a peptidylprolyl isomerase that catalyzes production of *trans*-Pro63-MYC, requires Thr58 phosphorylation. This c-MYC conformation rapidly moves to target gene promoters [52] where it specifically recruits transcriptional coactivators and becomes multiply acetylated [53]. Phosphorylation on both sites is required for ubiquitination by AXIN2-scaffolded E3 ligase SCF^{Fbw7}, which targets c-MYC for proteosomal degradation. In addition to being more active, the *trans*-Pro63 conformation is also a substrate for *trans*-directed PP2A-mediated dephosphorylation of phospho-Ser62-MYC, resulting in inactivation of the protein. This normal cycle is disrupted in phosphonegative Thr58 mutants that are frequently observed in tumors and result in active phospho-Ser62-MYC accumulation [51].

Deacetylation promotes sumoylation

Competing for the same substrate target lysines, acetylation and sumoylation are competitively antagonist modifications. Regulation of protein sumoylation can occur through the action of deacetylases, which render the ε-amino groups accessible at sumoylation consensus sites. The tumor suppressor HIC1 is a SSTF required for mammalian development and silenced in many tumors. Sumoylation of HIC1 Lys314 is required for transcriptional repressor activity without affecting nuclear or subnuclear

localization. Lys314 is also a substrate for acetylation by p300/CBP. Deacetylation of Lys314 by SIRT1 or HDAC4 increases sumoylation and thus the repressor activity of the protein [54].

Phosphorylation promotes sumoylation

Phosphorylation regulates the sumoylation and acetylation of some MEF2 proteins. The MEF2 transcription factors are required for myogenesis [55], and neuronal morphogenesis [56]. For MEF2A, MEF2C, and MEF2D, phosphorylation, acetylation and sumoylation are associated with regulation of transcriptional activity in several different tissues [57–59]. For each MEF2 factor, a phosphorylation-dependent sumoylation switch is present with the consensus motif of ψKxExxS/T. This motif is present in other transcription factors [60], including PPARγ2, HSF, and STAT1. Postsynaptic morphogenesis in cerebellar granule neurons is promoted by sumoylation and inhibited by acetylation of Lys403 of MEF2A. The phosphorylation status of Ser408 plays a key role in the phospho-SUMO switch, and ultimately the transcriptional regulatory activity of MEF2A. Ser408 of MEF2A is phosphorylated under basal or non-stimulated conditions and this promotes sumoylation at Lys403, which extinguishes the transcriptional activation activity of this factor. Dephosphorylation of phospho-Ser408, which appears to be catalyzed by the neuronal activity- and calcium-dependent phosphatase calcineurin, promotes a sumoylation to acetylation switch at Lys403, restoring the ability of MEF2A to activate expression of target genes [59].

Similarly to MEF2A, MEF2D sumoylation is dependent upon CDK5-mediated phosphorylation of Ser444 and sumoylation is opposed by calcineurin. Sumoylation of

Lys439 of MEF2D inhibits its transcriptional regulatory activity and the ability to potentiate myogenesis. [61].

Phosphorylation-linked desumoylation

The transcriptional regulatory protein BCL11B provides a fascinating example of sequential, linked, and reversible PTMs with a clear transcriptional outcome. BCL11B exists as an ensemble of phosphorylated, sumoylated, and unmodified protein species under basal conditions in primary mouse thymocytes (Figure 3). The protein becomes rapidly phosphorylated by at least two MAP kinases, ERK1/2 and p38 [35], immediately following initiation of phorbol ester treatment. Within five minutes all three states of the BCL11B protein collapse into a species that is multiply phosphorylated co-incident with extensive desumoylation of the protein. The latter is due to the presence of a "phospho–deSUMO" switch within the BCL11B protein, the mechanistic basis of which is the phospho-BCL11B-dependent recruitment of SENP1 to the BCL11B complex and subsequent hydrolysis of SUMO-BCL11B [35]. Hydrolysis of SUMO-BCL11B is followed by a cycle of dephosphorylation and re-sumoylation, the latter of which is required for recruitment of the transcriptional co-activator p300 to SUMO-BCL11B complex and subsequent induction of expression of *Id2*, a gene that is repressed by BCL11B under basal conditions [35,62]. Finally, prolonged stimulation results in extensive poly-ubiquitination, perhaps via the action of an unidentified STUbL protein, and proteasomal degradation [35]. This pathway of intricate PTMs appears to serve as a molecular switch that converts the transcriptional repressor BCL11B into an activator of target gene expression, whereas the terminal step of poly-ubiquitination and proteosomal degradation likely serves as a mechanism of signal termination.

Phosphorylation-linked acetylation and ubiquitination

As described above, FOXO1, a negative regulator of insulin sensitivity [63], is a target for acetylation affecting interaction with target genes. Acetylation also promotes phosphorylation of FOXO1 by the insulin-dependent protein kinase B (PKB)/AKT, rendering a previously acetylated FOXO1 more sensitive to insulin signaling [20]. Progressive phosphorylation of FOXO1, first by PKB/AKT and then by CK1, reduces nuclear localization and DNA binding by FOXO1 [64–68], further reducing transcriptional regulatory activity [69]. Phosphorylation of FOXO1 and retention in the cytosol in insulin-sensitive, hepatic cells ultimately leads to its ubiquitination and proteasomal degradation [70].

Phosphorylation alters methylation

Prior to activated by extracellular signals C/EBPβ is maintained in a transcriptionally inactive state that is promoted by CARM1-mediated dimethylation of Arg3, which prevents interaction with SWI/SNF and Mediator complexes. MAP kinase-mediated phosphorylation of Thr235 induces a conformational changes that destabilizes CARM1–C/EBPβ interaction. Upon demethylation, C/EBPβ interacts productively with both SWI/SNF and Mediator complexes to induce transcription of target genes [24].

Interconnectedness of PTMs and possible clinical interventions

PTMs can be biomarkers of disease states and their utility in assessing and monitoring diseases of misregulation—cancer—is an emerging clinical priority. Our arsenal of clinically useful, PTM-directed drugs are few in general, and those that affect the PTM status of SSTFs are even more rare, likely because most compounds that affect PTMs

lack specificity. However, the linkages between PTMs for any given disease state, once discerned, have the potential to identity novel drug targets. Several real and hypothetical drugs that impact transcription factor PTMs have been described and representative examples of these are summarized in Table 1. Of these, As_2O_3 is the one compound that most obviously targets linked PTMs, and does so on the oncogenic fusion protein PML-RARα [71]. The presence of PML-RARα is causative for acute promyelocytic leukemia, a subtype of acute myeloid leukemia, and the goal of chemotherapy is to eliminate this fusion protein from promyelocytes. As_2O_3 binds to the PML portion of the fusion protein and promotes oligomerization with subsequent sumoylation of the fusion protein. In this case, sumoylation of PML-RARα is linked to poly-ubiquitination via the action of the STUbL protein RNF4 [37,38]. Poly-ubiquitinated PML-RARα is then degraded via the proteasome, clearing the cell of this oncogenic protein and restoring the differentiative capacity of the affected promyelocytes [40].

Concluding remarks

Although the evidence is just beginning to accumulate on the frequency of multiply-modified proteins, it seems likely that evolving techniques, such as highly sensitive and quantitative mass spectrometry, will undercover many more examples. Multiple PTMs expand the possibilities for scalable transcriptional regulatory activity, and sequential, interdependent modifications allow for time-dependent control of gene expression in response to an initial stimulus with downstream effects that may play out over an extended time frame. Owing to the great importance of post-translational modification in dictating every aspect of transcription factor function and the key role that these proteins play in

disease, it is perhaps not surprising that PTMs of transcription factors have become an attractive target for the development of therapeutic agents to treat a wide variety of diseases. Table 1 provides representative examples of current and future drug classes that target various transcription factor PTMs.

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Table 1. Transcription factor PTMs as therapeutic targets

PTM affected	Target Protein	Potential Use	Comments	Refs
Phosphorylation	Tyrosine kinases	Treatment of cancer, rheumatoid arthritis,	Inhibition of tyrosine kinases by drugs, such as imatinib, ruxolitinib, and tofactinib, directly inhibits phosphorylation of STAT proteins, and indirectly inhibits phosphorylation of other transcription factors, including c-JUN, Rb1, Tp73, YAP1, and β-catenin.	[72]
	Cyclin-dependent kinase 5 (CDK5)	Type 2 diabetes mellitus	Inhibition of CDK5-mediated phosphorylation of peroxisome proliferator-activated receptor γ (PPARγ) may be useful for T2DM.	[73]
	Peptidyl-prolyl *cis–trans* isomerase NIMA-interacting 1 (PIN1)	Treatment of cancer, including cancer of the breast	PIN1 isomerizes the pSer118–Pro119 bond of estrogen receptor α (ERα), increasing ligand-independent transcriptional activation mediated by ERα and inhibiting proteosomal-dependent degradation of the phospho-activated receptor. PIN1 expression is also elevated in some breast cancers that exhibit poor outcomes and numerous PIN1 inhibitors are in development.	[52,74–76]

	Calcineurin (protein phosphatase 3)	Immunosuppressant	Calcineurin activity is required to dephosphorylate and promote translocation of the transcription factor NFAT from the cytosol to the nucleus. Inhibition of calcineurin by FK506 (tacrolimus) prevents nuclear translocation of NFAT in T lymphocytes, leading to reduced expression of IL-2 and suppression of adaptive immunity.	[11]
	SMAD phosphatases	Spinal cord injury	Phosphorylated members of the SMAD family of transcription factors promote motor neuron axonal outgrowth. Inhibition of SMAD phosphatases may prolong the active lifetime of phospho-SMAD proteins.	[77,78]
Sumoylation	PML-RARα	Acute promyelocytic leukemia	Arsenic Trioxide (As_2O_3) promotes oligomerization and sumoylation of the PML portion of the PML-RARα fusion protein, with subsequent ubiquitination of the protein via SUMO-targeted ubiquitin ligases (STUbL proteins) and proteosomal degradation.	[71]

	SENP proteins	Treatment of cancer	Inhibition of SENP proteins by betulinic acid and related compounds may prolong the lifetime of sumoylated transcription factors, such as SP1.	[79,80]
Ubiquitination	p53	Treatment of cancer	Compounds such as Nutlin-3 bind to the MDM2 binding pocket of p53, inhibiting p53-MDM2 interaction and MDM2-mediated ubiquitination of p53, and prolonging the lifetime of activated p53.	[81]
Methylation	Co-activator-associated arginine methyltransferase 1 (CARM1)	Treatment of cancer, potentially other disorders	Indole and/or pyazole inhibitors of the catalytic function of CARM1 may interrupt signaling by estrogen receptors in breast cancer, or other transcription factors in other hormone-dependent tumors.	[82,83]
O-GlcNAcylation	*O*-GlcNAc transferase (OGT)	Treatment of cancer; particularly of the breast	Inhibition of OGT may sensitize breast cancer cells to tamoxifen therapy.	[84]
	O-GlcNAc hydrolase (*O*-GlcNAcase)	As yet unknown	Inhibition of *O*-GlcNAcase by compounds, such as Thiamet-G, may be a means of altering the *O*-GlcNAc/phosphorylation reciprocal balance toward *O*-GlcNAc. This may serve to turn off transcription	[85,86]

			factors that require phosphorylation for activity. A similar observation has been made in the case of Tau kinases, which become hyperphosphorylated in Alzheimer's disease and drive tau-mediated neurodegeneration.	
Acetylation	Histone acetyltransferases (HATs)	Treatment of cancer, HIV	Garcinol, a natural compound, inhibits HAT activity of p300/CBP and may be useful to reprogram histone and transcription factor modifications that are characteristic of disease processes, such as cancer and HIV.	[87,88]

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Figure Legends

Figure 1. Relative enrichment of PTMs in transcription factors. Values are the relative ratio of PTMs identified in human transcription factors compared to those identified in non-transcription factor human proteins and are plotted to show the divergence from equality for the indicated PTM. Blue bars indicate the degree of over-enrichment in transcription factors and the red bar indicates the degree of under-ubiquitination. PTM data was drawn from the PhosphoSitePlus database [1]. Abbreviations: Ub, ubiquitination; Ac, acetylation; Me, methylation; PO_4, phosphorylation; OG, *O*-GlcNAcylation, and SU, sumoylation.

Figure 2. Mechanisms by which PTMs may alter transcription factor activity. To alter gene expression, phosphorylation may affect the secondary structure of a transcription factor (Δ Conformation) to reveal binding sites or alter affinity, increase or decrease protein degradation (Δ Stability), increase or decrease the nuclear occupancy and thus access to DNA (Localization), alter affinity for DNA regulatory regions (DNA Binding), or alter the modification of the factor by other PTMs including acetylation, sumoylation, methylation, *O*-GlcNAcylation, or ubiquitination.

Figure 3. Sequential regulation of BCL11B by phosphorylation and sumoylation. BCL11B, constitutively in the context of the NuRD repressor complex (NuRD), is subject to modification by kinases, phosphatases (PPTase), sumo-ligating enzymes (UBC9), sumo proteases (SENPx), and sumo-dependent ubiquitin-targeted ligases (StUBL) that alter its

activity at the *Id2* oncogene promoter over a 60 min time frame following mouse thymocyte stimulation. Termination of the stimulated signal involves ubiquitin-targeted degradation by the proteasome complex.

Glossary
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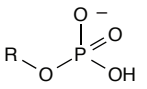
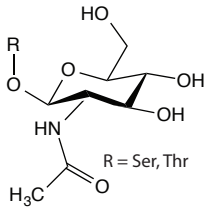
O-linked Modifications

Phosphorylation: Addition of a phosphate group through an ester bond to polar amino acids results in increased negative charge in the vicinity of the modification.
Glycosylation: Addition of an *O*-linked N-acetylglucosamine monosaccharide to polar amino acids through a β-linkage. Competitive with phosphorylation, glycosylation does not alter the charge of the protein.



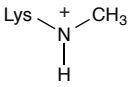
R = Ser, Thr, Tyr



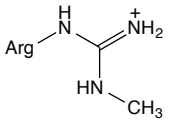
R = Ser, Thr

N-linked Modifications

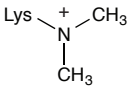
Methylation: Addition of CH_3 to basic amino acids results in increased hydrophobicity. Unlike acetylation, mono-, di, and tri-methylation of lysine is not charge neutralizing but increases the effective radius of the positive charge by replacing hydrogens with bulky methyl groups.



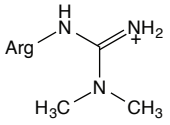
Mono-methyl lysine



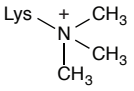
Mono-methyl arginine



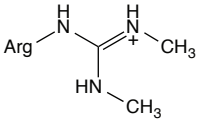
Di-methyl lysine



Asymmetric di-methyl arginine

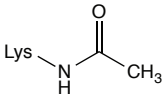


Tri-methyl lysine

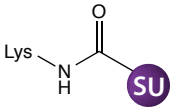


Symmetric di-methyl arginine

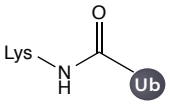
Acetylation: Acetylation of lysine groups is charge neutralizing and competitive with ubiquitination and sumoylation.

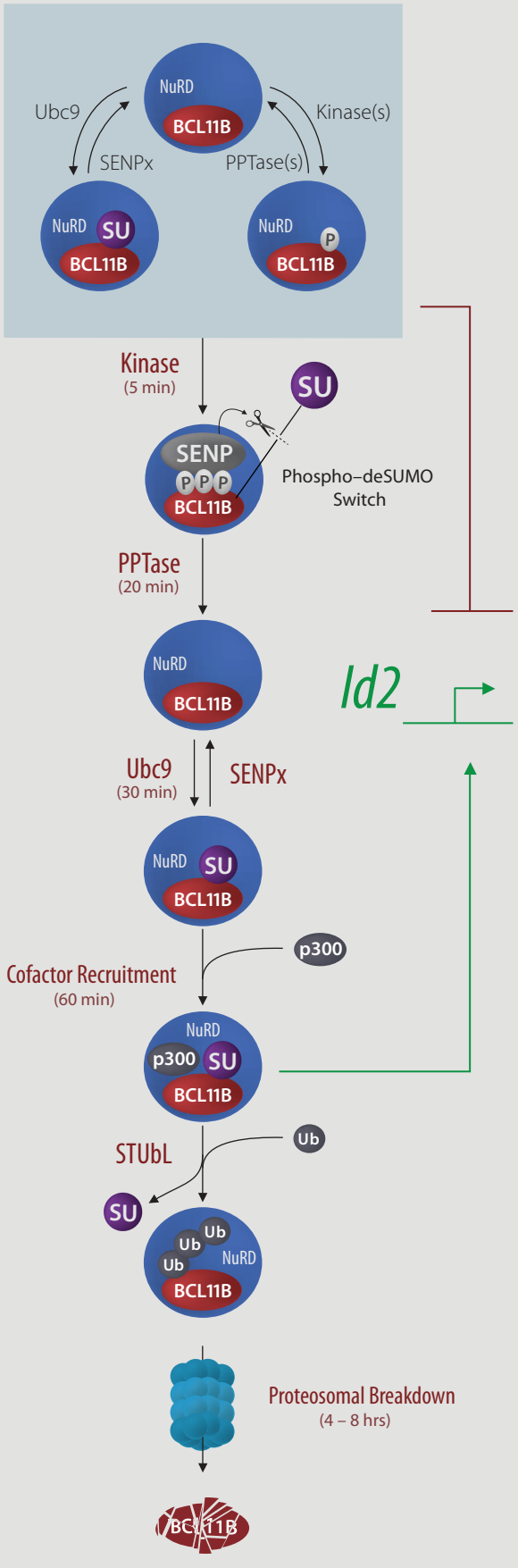


Sumoylation: Addition of one or many ~100 amino acid peptides through a highly labile ε-amino isopeptide bond to lysine results in greatly increased protein bulk. Poly-sumoylation can occur via lysines within the SUMO moiety.



Ubiquitination: Addition of one or many ~76 amino acid peptides through an ε-amino isopeptide bond to lysine or through a peptide bond to the amino terminus. Poly-ubiquitination can occur through different lysines in the ubiquitin peptide to form a variety of chains.




NuRD
BCL11B
Ubc9
SENPx
Kinase(s)
PPTase(s)
NuRD
SU
BCL11B
NuRD
P
BCL11B
Kinase
(5 min)
SU
SENP
P P P
BCL11B
Phospho–deSUMO
Switch
PPTase
(20 min)
NuRD
BCL11B
Id2
Ubc9
(30 min)
SENPx
NuRD
SU
BCL11B
p300
Cofactor Recruitment
(60 min)
NuRD
p300
SU
BCL11B
STUbL
Ub
SU
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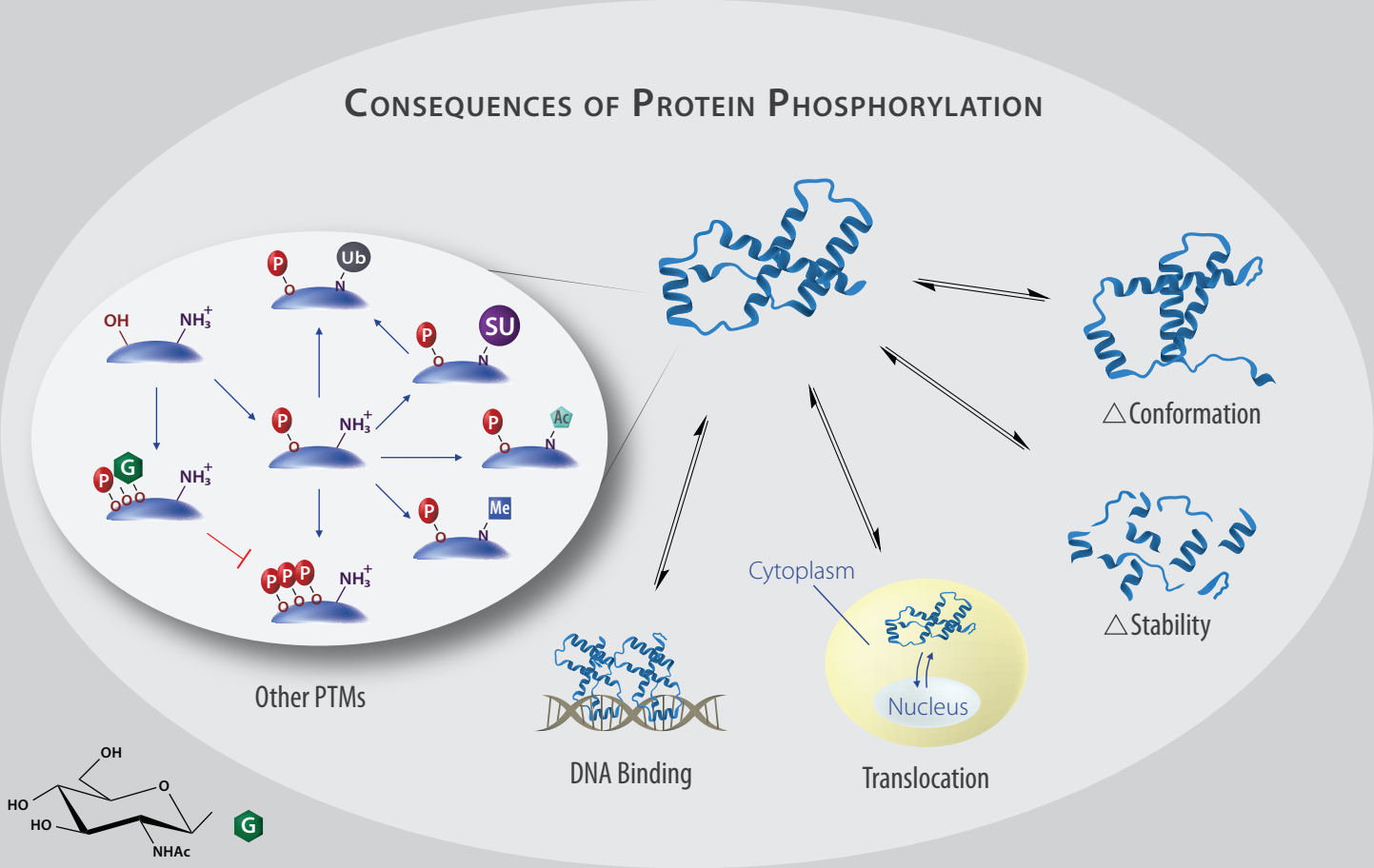
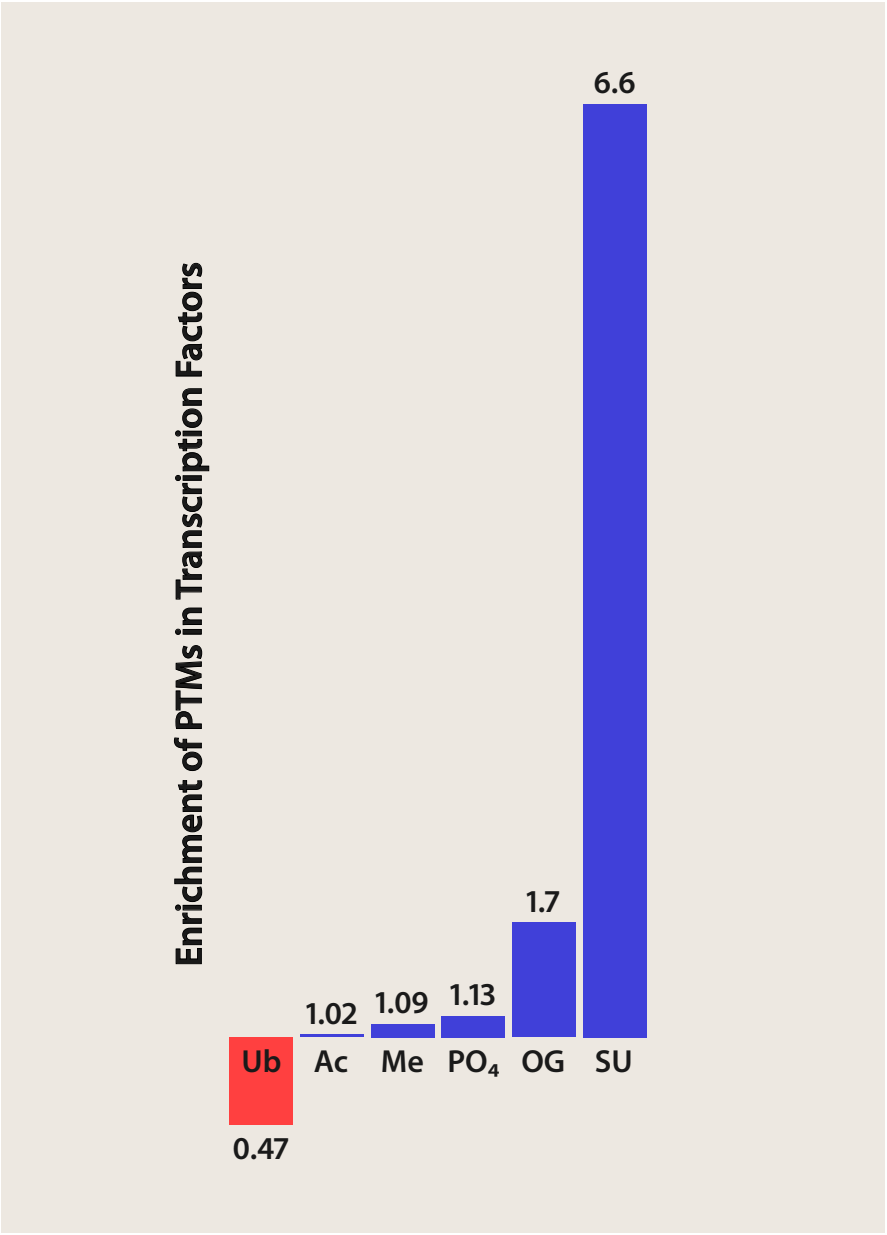
NuRD
BCL11B
Proteosomal Breakdown
(4 – 8 hrs)
BCL11B


Consequences of Protein Phosphorylation
Ub
SU
Ac
Me
G
OH
NH3+
Other PTMs
△Conformation
△Stability
Cytoplasm
Nucleus
DNA Binding
Translocation
HO
HO
NHAc


Enrichment of PTMs in Transcription Factors
6.6
1.7
1.02
1.09
1.13
Ub
Ac
Me
PO4
OG
SU
0.47