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Regulation of transcription factor function by phosphorylation

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Abstract. Changes in protein phosphorylation represent a mechanism that is frequently employed by cells to regulate transcription factor activity. In response to alterations in the extracellular environment, signal transduction pathways target transcription factors, transcriptional coregulators and chromatin-modifying factors, leading to their phosphorylation by protein kinases or dephosphorylation by protein phosphatases. These modifications either positively or negatively regulate transcription factor activity to facilitate a program of gene expression that results in appropriate changes in cell behavior. Protein phosphorylation and dephosphorylation can directly regulate distinct aspects of transcription factor function, including cellular localization, protein stability, protein-protein interactions and DNA binding. The phosphorylation-dependent modulation of the activities of transcriptional coregulators and chromatin-modifying factors can also control transcription factor activity. Here we review recent studies that have led to a better understanding of the mechanisms by which protein phosphorylation and dephosphorylation governs transcription factor function.

Key words. Transcription factor; protein kinase; protein phosphatase; phosphorylation; signal transduction.

Introduction

Exposure of cells to extracellular signaling molecules or to changes in the extracellular environment causes changes in gene expression that lead to appropriate physiological responses. This occurs by integrating multiple intracellular signaling pathways to control the activity of transcription factors. The most common regulatory mechanism, particularly for rapid alterations in transcription factor activity, involves protein phosphorylation and dephosphorylation [1, 2]. The signal transduction systems that are responsible for regulating the phosphorylation state of transcription factors and transcriptional coregulators are an area of intense interest, as they represent potential targets for therapeutic intervention in a number of diseases. The elucidation of the different molecular mechanisms by which these signal transduction pathways control transcription factor function is therefore an important goal.

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Protein phosphorylation and dephosphorylation can regulate transcription factor function by at least five different mechanisms: (i) by controlling the length of time transcription factors (or protein kinases and protein phosphatases that regulate their activity) spend in the nucleus; (ii) by targeting transcription factors or their coregulators for proteolytic degradation; (iii) by modulating protein-protein interactions between transcription factors, coregulators and the basal transcription complex; (iv) by regulating transcription factor DNA binding; and (v) by modifying chromatin structure (fig. 1). In this review we will discuss recent progress towards understanding the role of protein phosphorylation in regulating these different aspects of transcription factor function.

Regulation of cellular location

In order to interact with their target DNA sequences, transcription factors need to be located in the cell nucleus. Many transcription factors are constitutively

nuclear and are phosphorylated and dephosphorylated by protein kinases and protein phosphatases within the nucleus. However, a number of transcription factors shuttle between the cytoplasm and the nucleus, and in many cases this process is regulated by protein phosphorylation/dephosphorylation. The nucleocytoplasmic shuttling of transcription factors is an active process that relies on the recognition of nuclear localization signals (NLSs) and nuclear export signals (NESs) within the transcription factor primary sequence by proteins of the nuclear import and export machinery [3, 4]. Protein phosphorylation and dephosphorylation can regulate the accessibility of the NLSs and NESs to nuclear import and export proteins in at least two ways: (i) by masking or unmasking the transcription factor signal sequences directly, or (ii) by modulating the binding of the transcription factor to other regulatory proteins which mask or unmask the signal sequences.

The phosphorylation-dependent nucleocytoplasmic shuttling of the nuclear factor of activated T-cells (NF-AT) family of transcription factors provides an excellent example of a transcription factor integrating both positive and negative regulatory signals from multiple signaling pathways to control its cellular location [5, 6]. Nuclear import of NF-AT is induced by dephosphorylation mediated by the calcium-dependent protein phosphatase calcineurin (also called protein phosphatase 2B) [5] (fig. 2A). In response to a rise in intracellular calcium levels, calcineurin is activated and dephosphorylates a group of serine residues within the NH_2-terminal domain of NF-AT. Dephosphorylation exposes one or more NLSs on NF-AT, resulting in its nuclear import [7] (fig. 2A). The action of calcineurin on NF-AT is opposed by protein kinases which phosphorylate the same group of serine residues [6] (fig. 2A). The protein kinases responsible may differ depending on either the signaling pathways that are activated, or the particular NF-AT isoform. Glycogen synthase kinase-3 (GSK-3) has been identified as important for phosphorylation of the isoform NF-ATc following its initial phosphorylation by an unknown kinase(s) [8], whereas the NF-AT4 isoform is reported to be phosphorylated by two ki-

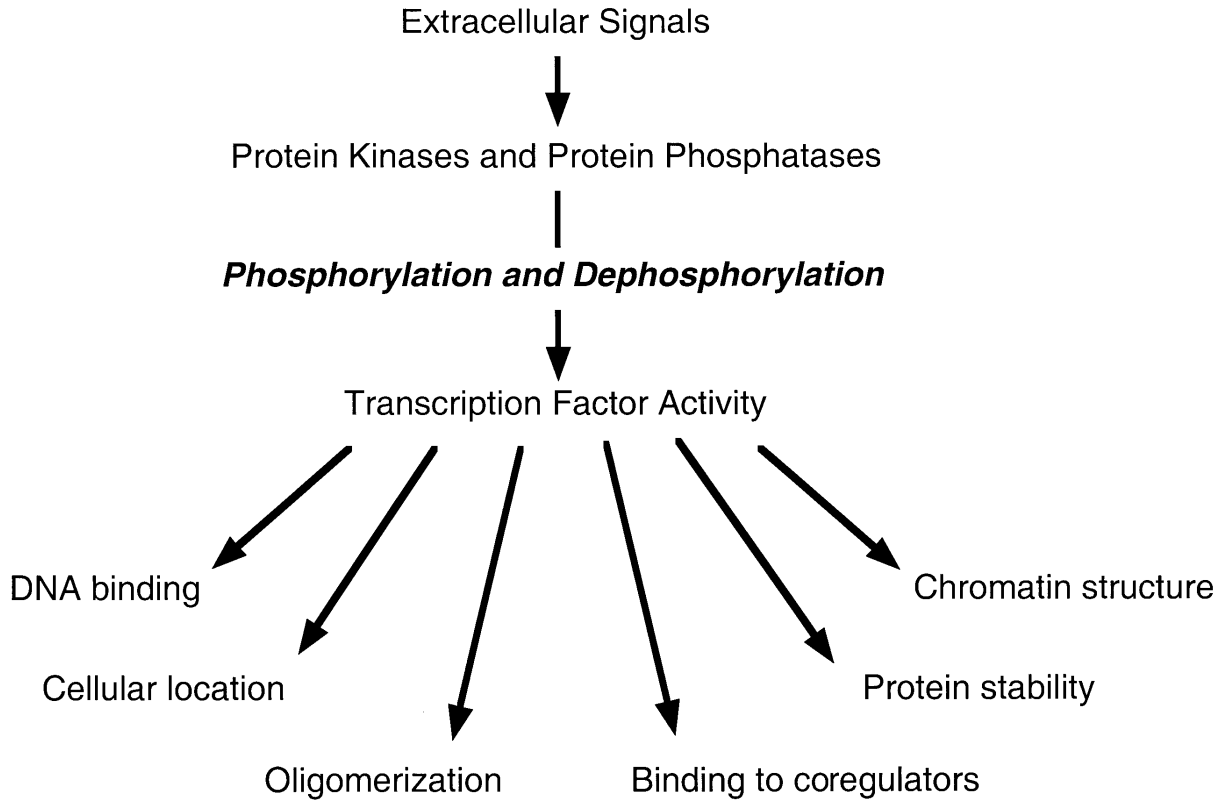

Figure 1. Protein phosphorylation and protein dephosphorylation regulate the activities of transcription factors through multiple mechanisms. In response to extracellular signals, the phosphorylation state of transcription factors and coregulators is modulated by the activities of protein kinases and protein phosphatases. These modifications either positively or negatively affect transcription factor activity by regulating distinct aspects of transcription factor function.

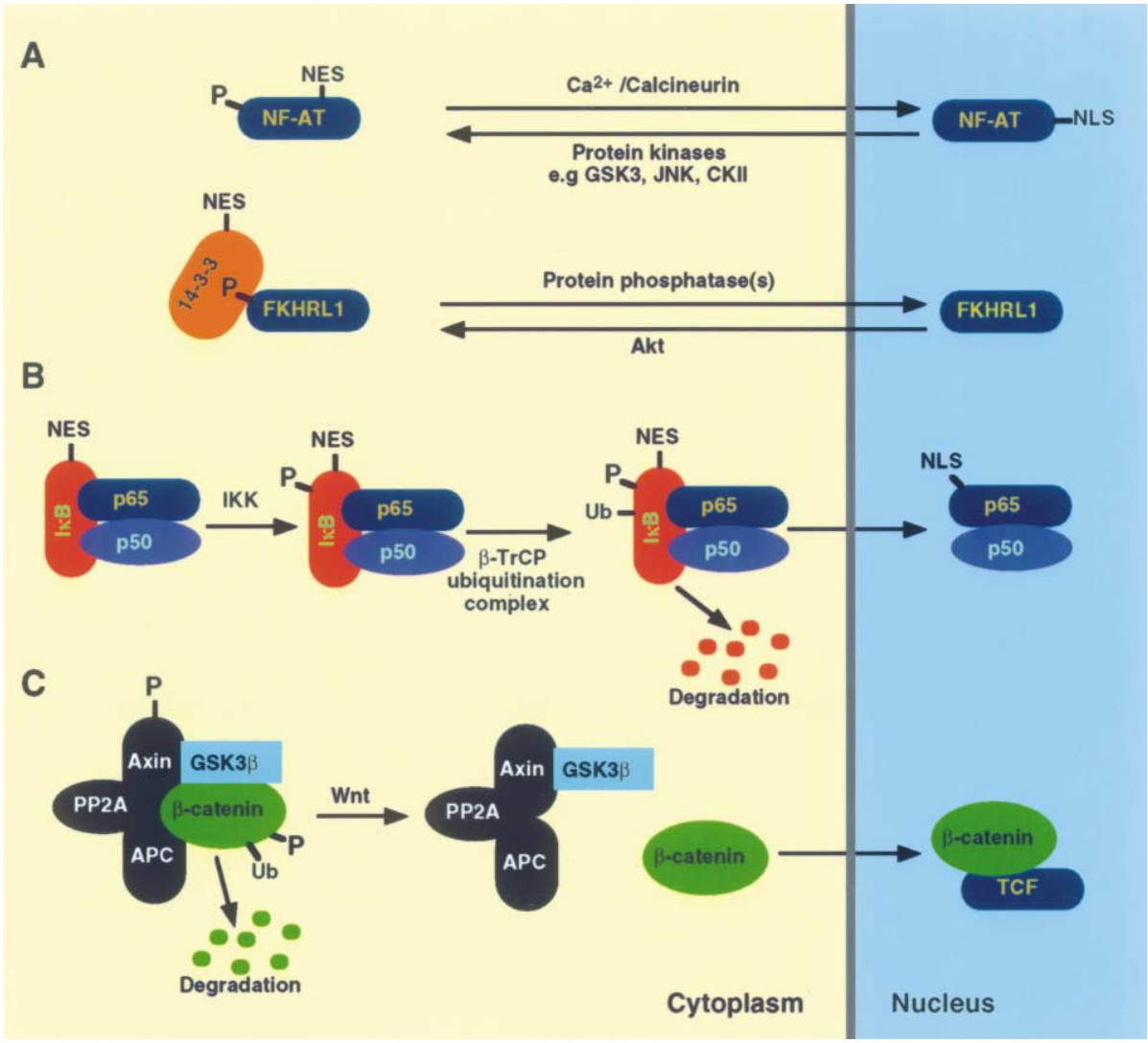

Figure 2. Phosphorylation-dependent regulation of the nucleo-cytoplasmic transport and protein degradation of transcription factors. (*A*) The dephosphorylation of the NH_2-terminal region of NF-AT by calcineurin exposes one or more NLSs on NF-AT and leads to its nuclear import [6, 7]. The phosphorylation of the same group of sites unmasks an NES on NF-AT, resulting in its nuclear export [7–10]. The Forkhead family transcription factor FKHRL1 forms a complex with 14-3-3 proteins following its phosphorylation by Akt protein kinase [11]. The complex is retained in the cytoplasm by 14-3-3, which has an exposed NES. The dephosphorylation of FKHRL1 disrupts the complex and allows nuclear import of FKHRL1. (*B*) The heterodimeric transcription factor NFκB (p65 and p50) is localized to the cytoplasm by the anchoring protein IκB [15]. In response to signals produced during infection, IκB is phosphorylated by the IKK complex [16] and is degraded by ubiquitin-mediated proteolysis [36–40]. This unmasks an NLS in the p65 subunit of NFκB, resulting in its nuclear import [15]. (*C*) The coactivator β-catenin is bound in a complex with APC, Axin, PP2A and GSK3β [44]. The phosphorylation of β-catenin by GSK3β leads to its ubiquitin-mediated degradation [41, 42]. In response to Wnt signals, GSK3β activity is downregulated, and both axin and β-catenin are dephosphorylated, leading to the disruption of the complex and the translocation of β-catenin into the nucleus, where it binds to the transcription factor TCF [44–47].

nases, the mitogen-activated protein (MAP) kinase JNK [9] and casein kinase-1α [7]. It is proposed that phosphorylation of NF-AT leads to the exposure of an NES resulting in nuclear export mediated by the exportin protein Crm1 [7, 10]. It is likely that additional protein kinases and phosphatases contribute to regulating the localization of NF-AT proteins.

Protein phosphorylation also mediates the nuclear export of members of the Forkhead family of transcription factors [11, 12]. The serine/threonine kinase Akt

(also called protein kinase B) phosphorylates FKHRL1 and FKHR1, resulting in their nuclear export [11, 12] and association with 14-3-3 proteins in the cytoplasm [11], thereby negatively regulating their transcriptional activity (fig. 2A). The protein phosphatase(s) responsible for the dephosphorylation of FKHRL1 and FKHR1 in the cytoplasm to allow their re-entry into the nucleus is not known. 14-3-3 proteins may play a widespread role in regulating the cellular location of proteins. They contain an NES that is recognized by the exportin protein Crm1 [13] and is responsible for mediating the cytoplasmic retention of phosphorylated binding partners such as FKHRL1. However, the binding site for Crm1 overlaps with the phosphoserine recognition site in 14-3-3 [14], suggesting that the role of 14-3-3 in nucleocytoplasmic trafficking is more complex. It has been proposed that the binding of phosphorylated proteins, such as FKHRL1, to dimeric 14-3-3 could mask the NES within one of the bound 14-3-3 monomers, but would not affect the NES of the second 14-3-3 monomer [14]. Such a model would account for the cytoplasmic localization of the 14-3-3/FKHRL1 complex and could also explain how 14-3-3 could target proteins to the nucleus [14]. If both NES within the 14-3-3 dimer were masked by binding to proteins, then the localization of the complex could be controlled by an NLS within the binding partners. The complex would remain nuclear until dephosphorylation released one of the 14-3-3 proteins, thereby exposing its NES [14].

The function of regulatory proteins that control transcription factor localization can also be regulated by phosphorylation. For example, the heterodimeric transcription factor nuclear factor-κB (NF-κB) is regulated by binding to the cytoplasmic anchoring protein inhibitor of NF-κB (IκB) [15] (fig. 2B). The binding of IκB to NF-κB masks the NLS located on the p65 subunit of the NF-κB heterodimer, thereby preventing translocation of NF-κB to the nucleus [15]. In response to signals produced during infection, IκB is phosphorylated on two serine residues within its NH_2-terminal region (Ser-32 and Ser-36 in isoform IκBα) by the IκB-kinase (IKK) complex [16]. The phosphorylation of these serine residues targets IκB for ubiquitination and its rapid degradation by the 26S proteosome [15] (fig. 2B). The NLS on the p65 subunit of NF-κB is therefore exposed, allowing transport of the NF-κB heterodimer to the nucleus [15] (fig. 2B). Interestingly, some components of NF-κB heterodimers can mimic the actions of IκB. p50 and p52 are derived from large precursor proteins of 105 and 100 kDa, respectively [15]. These precursor proteins can bind to p65 and mask its NLS via their extended COOH-terminal domains, resulting in the cytoplasmic retention of NF-κB. The cleavage and degradation of the COOH-terminal domains to produce the smaller p50 and p52 proteins can be regulated by phosphorylation. Both IKKs and the TPL-2 protein kinase have been reported to mediate the phosphorylation-directed proteolysis of the COOH-terminal domain of p105 [17, 18].

In addition to regulating the cellular localization of transcriptional activators, protein phosphorylation also regulates the nuclear import and export of transcriptional repressors. Genetic studies in *Drosophila melanogaster* have demonstrated that the development of the R7 photoreceptor neurons in the eye is controlled by the Sevenless receptor-tyrosine kinase, which signals through the RAS/MAP kinase pathway [19]. The non-phosphorylated form of the ETS-domain repressor Yan (also called Aop) binds to composite ETS-AP1 sites in the promoters of genes required for differentiation of the precursor cells into R7 photoreceptor neurons [20]. Upon activation of the Sevenless signaling pathway, Yan is phosphorylated by MAP kinase, thereby relieving its inhibitory effect and resulting in its exclusion from the nucleus. Simultaneously, MAP kinase phosphorylates the positive regulator Pointed-P2, which binds to the ETS-AP1 site, leading to increased transcription [21, 22]. Yan also participates in regulating *decapentaplegic* (Dpp) expression during dorsal closure in response to a different signaling pathway [23]. *Drosophila* JNK (DJNK) phosphorylates and inactivates Yan, and also phosphorylates the positive regulator DJun [23]. In these two examples, distinct phosphorylation events mediated by the same signaling pathway are responsible for both relieving inhibition and activating transcription from the same promoter site. Similar mechanisms are likely to exist in mammalian cells where the RAS/ERK MAP kinase pathway targets the ETS-domain repressor ERF, leading to its exclusion from the nucleus [24], and also positively regulates a number of other ETS-domain transcription factors [25].

The examples we have described thus far feature transcription factors whose location can be regulated by intracellular signaling pathways initiated at the cell membrane. The STAT (signal transducers and activators of transcription) and SMAD (*sma-mothers against dpp*) families of proteins behave both as intracellular signaling molecules and transcription factors. They are activated within receptor complexes at the cell membrane and translocate to the nucleus where they bind to their target sites in gene promoters. STAT proteins are activated by many cytokines and growth factors [26, 27]. The ligand-mediated dimerization of cytokine and growth factor receptors results in tyrosine phosphorylation of the receptor by its intrinsic kinase activity, or by associated tyrosine kinases such as the Janus kinases (JAKs) [26, 27]. STAT proteins are then recruited to the receptors via binding of their SH2 domain to the phos-

phorylated tyrosine residues, and are themselves phosphorylated on a single tyrosine residue by JAKs or other nonreceptor tyrosine kinases. The tyrosine-phosphorylated STAT proteins form homo- or heterodimers and are translocated to the nucleus by an unknown mechanism, where they bind DNA and activate transcription [26, 27]. The importance of regulating STAT dimerization is underlined by a recent report that demonstrates that constitutive STAT3 dimers mediate cellular transformation [28]. SMAD proteins are activated by members of the transforming growth factor-β (TGF-β) family of cytokines [29]. Certain SMAD family proteins directly associate with, and are substrates of, the TGF-β family receptor kinases. The resulting serine phosphorylation of the receptor-bound SMADs allows their association with the common SMAD binding partner SMAD4 in oligomeric complexes which translocate to the nucleus [29]. The nuclear import of SMAD complexes can be opposed by phosphorylation at different serine residues mediated by the RAS/ERK MAP kinase pathway in response to growth factor stimulation of cells [30].

The signaling molecules that target transcription factors can also be regulated at the level of nuclear import and export in response to extracellular signals. For example, the MAP kinase ERK2 is predominantly localized to the cytoplasm in resting cells (possibly retained there by binding to its activator MEK1 [31]). Following mitogenic stimulation of cells, activated ERK dimerizes [32] and translocates to the nucleus, where it phosphorylates its transcription factor substrates [33]. The protein phosphatase Cdc25, an inducer of mitosis that regulates cyclin B/Cdc2 activity, is retained in the cytoplasm by binding to 14-3-3 proteins in response to signals that induce growth arrest [13, 34, 35]. The activity of transcription factors can therefore be modulated by both controlling their localization and that of the protein kinases and phosphatases that target them.

Regulation of proteolysis

Signal-dependent degradation of transcription factors or their coregulators represents an important mechanism of transcriptional regulation [36]. Ubiquitin is covalently attached to the target protein by a group of enzymes (E1, E2 and E3) that serve to activate ubiquitin and ligate it to specific lysine residues [37, 38]. The ubiquitinated proteins are then degraded by the 26S proteosome [37].

A well-studied example of a transcription factor that is regulated by phosphorylation-dependent proteolysis is the previously discussed IκB-NF-κB complex. IκB phosphorylated by the IKK complex is recognized by a receptor component of the E3 ubiquitin ligase, β-TrCP (also termed FWD1) [39, 40], which acts to couple IκB with the ubiquitination enzymes (fig. 2B). β-TrCP has also been implicated in targeting the ubiquitination complex to the coactivator β-catenin [41, 42], which is a component of the Wnt signal transduction pathway [43, 44]. In the absence of Wnt signals, β-catenin can be found in a complex with adenomatous polyposis coli (APC), axin, protein kinase GSK3β and protein phosphatase 2A (PP2A) [44] (fig. 2C). It is proposed that GSK3β phosphorylates axin and promotes its binding to β-catenin [45, 46]. The axin-bound β-catenin is then phosphorylated by GSK3β at multiple sites within its NH_2-terminal region to form a recognition site for β-TrCP and the ubiquitination complex [41, 42] (fig. 2C). Activation of the Wnt signaling pathway results in the downregulation of GSK3β activity and dephosphorylation of axin and β-catenin (possibly mediated by PP2A), leading to the release of β-catenin from the complex and its increased stability [44, 46]. The stabilized β-catenin translocates by an unknown mechanism, to the nucleus where it binds to members of the lymphoid enhancer factor-1/T-cell factor (LEF1/TCF) family of transcription factors and regulates their transcriptional activity [47] (fig. 2C). The importance of phosphorylation-dependent regulation of β-catenin stability is underlined by the presence of mutations in the critical phosphoacceptor sites of β-catenin in several types of human cancer [48, 49].

The p53 tumor suppressor is an example of a transcription factor that is targeted for degradation depending on its phosphorylation state. This can occur by at least two independent mechanisms. The Mdm2 oncoprotein binds to the NH_2-terminal transactivation domain of p53 and inhibits its transcriptional activity [50]. In addition, Mdm2 can function as an E3 ubiquitin ligase [51, 52] and mediate ubiquitin-dependent proteolytic degradation of p53 [50] (fig. 3). The phosphorylation of serine residues within the p53 transactivation domain (in particular Ser-15 by ATM protein kinase [53, 54]) upon treatment with DNA damage-inducing agents can reduce the affinity of p53 for Mdm2, resulting in the increased stability and transcriptional activity of p53 [50, 55]. The second mechanism appears to be Mdm2-independent. It has been proposed that the binding of the nonactivated form of the MAP kinase JNK to the NH_2-terminal region of p53 targets it for ubiquitin-mediated proteolytic degradation [56] (fig. 3). Activated JNK can phosphorylate the p53 NH_2-terminal region leading to the stabilization of p53 and, in vitro at least, can block Mdm2 binding to p53 [56, 57]. JNK MAP kinase has been proposed to regulate a number of its transcription factor substrates similarly to p53. Nonactivated JNK binds to c-Jun, JunB and ATF2 and targets these factors for ubiquitin-mediated proteolytic degradation [58, 59]. The phosphorylation of these fac-

tors by activated JNK protects them against degradation [58, 59]. Phosphorylation of some transcription factors, rather than protecting against degradation, may be required to facilitate degradation. The basic helix-loop-helix transcription factor MyoD is phosphorylated at consensus cyclin-dependent kinase (CDK) sites which target it for ubiquitin-mediated degradation, implicating this as a mechanism for coordinating MyoD activity with changes in the cell cycle [60].

Regulation of transactivation and interactions with coregulators

Transcription factors interact with many different proteins including other transcription factors, coregulators, and components of the basal transcription complex. These interactions can potentially be regulated by protein phosphorylation. Many transcription factors contain phosphorylation-dependent transcriptional activation domains and, in a few cases, transcriptional repression domains. The precise mechanisms by which phosphorylation of these domains potentiates or represses transcriptional activity are still poorly understood, but it is likely that they modulate the affinity of the transcription factor for coactivators, corepressors or the basal transcription complex. This could occur either: (i) by direct blocking of an interaction by a phosphorylated residue, (ii) by a phosphorylation-induced conformational change that unmasks or masks a binding surface or (iii) by phosphorylation causing the dissociation of an inhibitor molecule to unmask a binding surface.

Protein phosphorylation can regulate the interaction of transcription factors with coactivators and corepressors as well as the activity of the coregulators themselves. The transcription factor cAMP-response element binding protein (CREB) is phosphorylated on Ser-133 by

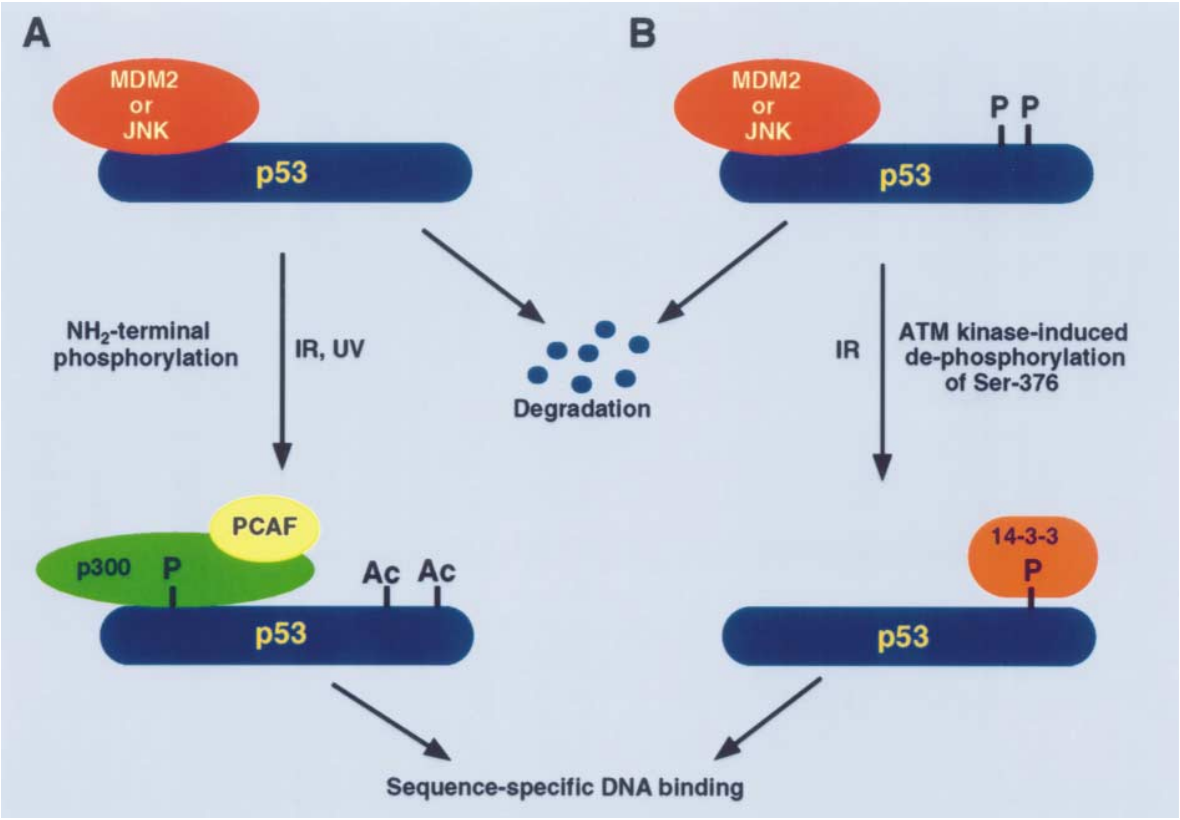

Figure 3. Regulation of p53 sequence-specific DNA binding by phosphorylation. Two proposed mechanisms are illustrated. (*A*) In nonirradiated cells p53 is bound by Mdm2 or JNK MAP kinase, which facilitate its ubiquitin-mediated degradation [50–52, 56]. Upon irradiation of cells, the NH_2-terminal domain of p53 is phosphorylated, leading to enhanced binding of the HATs p300 and PCAF [81]. These enzymes acetylate the COOH-terminal domain of p53, resulting in increased sequence-specific DNA binding [81]. (*B*) In nonirradiated cells, p53 is phosphorylated at Ser-376 and Ser-378 within its COOH-terminal domain. In irradiated cells Ser-376 is dephosphorylated by a mechanism dependent on the ATM protein kinase [80]. p53 phosphorylated on just Ser-378 is recognised by 14-3-3 proteins, which associate with p53 and enhance its binding to DNA [80].

many protein kinases [including protein kinase A (PKA), calcium/calmodulin-dependent kinase-II (CaMKII), CaMKIV and components of the MAP kinase signaling pathways] in response to diverse stimuli [61]. Phosphorylation of Ser-133 allows the recruitment of the coactivator CREB-binding protein (CBP), which is proposed to link CREB with components of the basal transcription complex, leading to increased transactivation [61, 62]. However, the phosphorylation of Ser-133 and the recruitment of CBP appear insufficient to mediate robust CREB-activated transcription. A recent study proposes that the phosphorylation of CBP itself is also required. A transactivation domain was identified in CBP that is phosphorylated by CaMKIV in response to an increase in nuclear calcium concentration, leading to potentiated CREB-induced transcriptional activity [63]. The phosphorylation of CBP by other protein kinases may also regulate its activity. Both PKA and ERK MAP kinase phosphorylate CBP in vitro and enhance its transactivation potential [64, 65], although the in vivo role of this phosphorylation remains to be clarified.

Interestingly, CaMKII phosphorylation of CREB does not result in CBP recruitment as it phosphorylates an additional site on CREB (Ser-142) [66], which destabilizes the CREB-CBP interaction [67]. This provides an example of protein phosphorylation negatively regulating the interaction of a transcription factor with a coactivator. Because both CaMKIV and CaMKII are activated by an increase in intracellular calcium, the same signal can both positively and negatively regulate CREB activity. Exactly how the actions of these two kinases are integrated to modulate CREB activity is not clear. It is also possible that CaMKII phosphorylation of CREB could be switched from a negative to a positive regulatory effect by the action of a protein phosphatase that specifically targets Ser-142 [66, 67].

Since its initial characterization as a CREB coactivator, CBP and the highly related p300 protein have been demonstrated to bind and enhance the activity of many transcription factors [62, 64]. Distinct regions of CBP/p300 mediate binding to different transcription factors, suggesting the possibility that CBP/p300 could simultaneously bind more than one transcription factor and serve to integrate signals from different signaling pathways. Such a model has recently been proposed for integrating the STAT and SMAD signal transduction pathways [68]. The cytokines leukemia inhibitory factor (LIF) and bone morphogenic protein-2 (BMP2) signal through the STAT and SMAD pathways, respectively, and act synergistically on primary fetal neural progenitor cells to induce astrocyte formation. p300 binds to both STAT3 and SMAD1 and coordinates the synergistic response to LIF and BMP2 [68].

While CBP/p300 mainly function as phosphorylation-dependent transcriptional coactivators, the retinoblastoma (RB) protein is an example of a corepressor that is regulated by phosphorylation [69]. RB is a tumor suppressor that arrests cells at the G1/S cell cycle boundary by repressing the transcription of genes required for transition into S phase [70]. RB associates with a number of transcription factors, the best characterized of which are members of the E2F group [71]. RB binds to the transactivation domain of E2F and represses its activity [70, 71]. Prior to the G1 to S phase transition, RB is phosphorylated on multiple sites by a combination of CDKs. This disrupts the RB-E2F complex and releases the repression [69]. RB dephosphorylation, possibly mediated by the PP1 family of protein phosphatases, occurs during late M phase, resulting in its re-recruitment to E2F-bound promoter complexes and the repression of E2F-mediated transcription [69].

Regulation of the basal transcription complex

The basal transcription complex consists of RNA polymerase II and the general transcription factors (GEFs) [72]. Three of these factors (TFIID, TFIIF and TFIIH) have been demonstrated to possess associated protein kinase activities. TFIID is composed of the TATA-binding protein (TBP) and many TBP-associated factors (TAFs) [72]. The largest of these, $TAF_{II}250$, contains two independent serine/threonine protein kinase domains and has been reported to phosphorylate the RAP74 subunit of TFIIF [73, 74]. TFIIF itself has recently been shown to possess an associated serine/threonine kinase activity which may modulate its activity [75]. The cyclin-dependent kinase CDK7 can be a component of TFIIH [76]. It phosphorylates the COOH-terminal domain of RNA polymerase II and regulates transcriptional elongation [76]. In addition, TFIIH has been shown to phosphorylate the RAP74 subunit of TFIIF [76] and the transcription factor E2F-1, leading to its degradation [77]. Whereas the function of these protein kinase activities and the role of protein phosphorylation in regulating the basal transcription complex are poorly understood at present, it is highly probable that GEFs represent direct targets of signaling pathways which can modulate their activities.

Regulation of DNA binding

The binding of transcription factors to promoter sites can be regulated directly or indirectly by protein phosphorylation. Many DNA binding domains are basic in character, so phosphorylation within or nearby these domains introduces a negative charge which may be incompatible for efficient DNA binding. For example,

the DNA binding activity of the zinc-finger-containing Wilms' tumor gene product WT1, a transcriptional repressor, is inhibited by PKA phosphorylation of two serine residues within the zinc-finger region [78]. While in most cases the phosphorylation of DNA binding domains inhibits DNA binding, the phosphorylation of the POU-domain transcription factor T-cell factor β1 (TCFβ1) by JNK MAP kinase at residues within the DNA binding domain appears to enhance its ability to bind to its promoter sites [79].

DNA binding activity of transcription factors may also be regulated indirectly by phosphorylation at residues remote from the DNA binding domain. The phosphorylation-dependent oligomerization of the STAT or SMAD families, which facilitates their nuclear import, is also essential for DNA binding [26, 27, 29]. Multiple mechanisms are proposed to regulate DNA binding of p53 in response to genotoxic stress [50]. In nonirradiated cells p53 is phosphorylated at two serine residues within its COOH-terminal region (Ser-376 and Ser-378). Upon irradiation Ser-376 is dephosphorylated by a mechanism dependent upon the ataxia telangectasia-mutated (ATM) protein kinase, creating a consensus binding site for 14-3-3 proteins [80] (fig. 3B). The binding of 14-3-3 to p53 is proposed to cause a conformational change in p53 that enhances its sequence-specific DNA binding [80]. A second potential mechanism involves the phosphorylation of NH_2-terminal serine residues on p53, which increases the affinity of p53 for a complex containing the p300 coactivator and the histone acetyltransferase (HAT) PCAF [81] (fig. 3A). p300 (which itself possesses HAT activity) and PCAF acetylate specific lysine residues within the COOH-terminal region of p53, which promotes sequence-specific DNA binding [81] (fig. 3A). In the latter example, protein phosphorylation facilitates the recruitment of other protein-modifying enzymes to regulate transcription factor function.

The DNA binding activity of the bZIP transcription factor c-Jun is proposed to be coordinately regulated by both direct and indirect phosphorylation events. The phosphorylation of c-Jun on three COOH-terminal residues located close to the DNA binding domain is reported to inhibit DNA binding [2]. The phosphorylation of the NH_2-terminal transactivation domain by JNK MAP kinase [82] causes a conformational change in c-Jun that facilitates the dephosphorylation of the COOH-terminal residues, and results in increased DNA binding [83].

Regulation of chromatin structure

Changes in the structure of chromatin can dramatically affect gene transcription by regulating the accessibility of promoter binding sites to transcription factors [84]. These changes are mediated by multisubunit ATP-dependent remodeling complexes or by covalent modification of the nucleosomes [84]. Recent studies indicate that remodeling complexes and chromatin modifying enzymes are under signal-dependent regulation. During mitosis the SWI/SNF remodeling complex is inactivated by phosphorylation, resulting in a repressive chromatin structure. As cells leave mitosis, SWI/SNF is activated by dephosphorylation and alters the chromatin structure to facilitate transcription [85]. Core histones within the nucleosome are acetylated by HATs resulting in a relaxation of chromatin structure and increased transcription [86, 87]. The action of HATs is opposed by the action of histone deacetylases (HDACs), which remove the acetyl groups, allowing the formation of a repressive chromatin structure [86, 87]. The balance between the activities of HATs and HDACs appears to be controlled by signal transduction pathways. Global histone H4 hyperacetylation can be induced by a number of extracellular stimuli, probably mediated by MAP kinase signaling pathways [88]. It has also been demonstrated that the activity of specific HATs can be increased or decreased by phosphorylation. The HAT activity of the coactivator CBP is increased following phosphorylation by an unknown protein kinase (possibly cyclin E-Cdk2) in a cell-cycle dependent manner [89], whereas phosphorylation of the Gcn5 coactivator by DNA-protein kinase decreases its HAT activity [90]. Transcriptional repressors can participate in regulating chromatin structure through HDACs. RB can recruit an HDAC to the RB-E2F complex to repress transcription [91, 92]. The phosphorylation of RB would dissociate the RB-E2F-HDAC complex, leaving E2F free to recruit HATs, such as CBP, and activate transcription.

Histones are also direct targets of protein kinases and are phosphorylated during mitosis and in response to growth factors [93–95]. Genetic studies in the protozoan *Tetrahymena thermophila* have linked phosphorylation of histone H3 at Ser-10 to chromosome condensation and segregation during mitosis and meiosis. [96]. However, the precise function of these phosphorylation events in regulating gene expression has yet to be defined.

Targeting of protein kinases and protein phosphatases to transcription factors

How substrate specificity is achieved by signal transduction pathways is an important question. The phosphorylation of a protein by a protein kinase is determined by a consensus sequence surrounding the

phosphoacceptor site. However, often these consensus sequences appear to be highly degenerate or limited to one or two amino acids. Therefore additional specificity determinants must exist. One of these determinants appears to be complex formation between the protein kinase and the substrate. Indeed, it has been demonstrated that many protein kinases associate with the transcription factors they phosphorylate. JNK MAP kinase binds to its substrates c-Jun, JunB, ATF2 and Elk-1 through a specific docking domain termed the δ or D domain, and this binding is required for efficient substrate phosphorylation [82, 97–100]. The transcription factor JunD, which contains suitable phosphoacceptor sites, is poorly phosphorylated by JNK as it does not possess a JNK docking domain [98, 99]. However, upon heterodimerization with c-Jun, Jun D can be trans-phosphorylated by JNK bound to c-Jun [99]. This suggests a novel regulatory mechanism whereby transcription factors can recruit protein kinases to transphosphorylate other targets. δ/D domains, as well as additional types of docking domains, mediate the binding of ERK and p38 MAP kinase family members to transcription factors [100–103], and docking domains for other protein kinase families have been reported [104]. Protein phosphatases may also be targeted to their transcription factor substrates by direct association. Calcineurin binding to NF-AT has been demonstrated to be required for efficient dephosphorylation of NF-AT [105]. The binding of protein kinases and protein phosphatases to transcription factors is therefore a critical determinant of substrate specificity.

The binding of signaling molecules to transcription factors may play additional phosphorylation-independent roles in regulating transcription. As mentioned previously nonactivated JNK targets transcription factors for proteolytic degradation [56, 58]. It has also been proposed that the protein phosphatase calcineurin may compete for binding to NF-AT4 with the nuclear export protein Crm1 and block nuclear export of NF-AT4 [10]. Nonactivated protein kinases bound to transcription factors can also act as transcriptional repressors. This has been demonstrated genetically in the yeast *Saccharomyces cerevisiae* where nonactivated Kss1p MAP kinase binds in a complex with the transcription factor Ste12p at filamentation response elements and causes repression of Ste12p-mediated transcription [106–108]. Upon stimulation of the filamentation signaling pathway, Kss1p is activated, leading to the disruption of the complex and Kss1p phosphorylation of Ste12p. This results in increased Ste12p-mediated transcription. Therefore, depending on its own activation state, a protein kinase may act as a repressor or an activator of a transcription factor.

Concluding remarks

In this review, we have discussed how protein phosphorylation and dephosphorylation control various aspects of transcription factor function. Many transcription factors are phosphorylated on a number of residues which may act either cooperatively or antagonistically to regulate a single or multiple functions. Transcription factors therefore integrate the different signaling pathways that regulate these functions to determine their cellular activities. Coactivators and corepressors also integrate signals from multiple signaling pathways, and in addition they may possess or recruit chromatin-modifying activities (such as HAT or HDAC activity), thereby linking multiple aspects of gene regulation.

Over the past few years our understanding of the mechanisms by which signaling pathways regulate gene expression has been underpinned by studies using genetically tractable model organisms, including yeast, fruitflies and worms. In the future, our understanding will be greatly aided by new and expanding technologies. The development of mammalian cell lines lacking components of signal transduction pathways, combined with the analysis of global gene expression, will allow the signal transduction networks that are essential for the expression of particular genes to be mapped. In addition, the deletion of mouse genes encoding protein kinases and protein phosphatases that target particular transcription factors, as well as targeted mutations of the codons encoding the phosphoacceptor sites on transcription factors, will provide genetic evidence for the importance of these signaling molecules in regulating particular functions of a transcription factor.

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