The Ins and Outs of MYC Regulation by Posttranslational Mechanisms

The proteins of the MYC family are key regulators of cell behavior. MYC, originally identified as an oncogene, affects growth, proliferation, differentiation, and apoptosis of cells through its ability to regulate a significant number of genes. In addition, MYC governs events associated with tumor progression, including genetic stability, migration, and angiogenesis. The pleiotropic activities attributed to MYC and their balanced control require that the expression and function of MYC is tightly controlled. Indeed, many different pathways and factors have been identified that impinge on MYC. The protein is subject to different posttranslational modifications, including phosphorylation, ubiquitinylation, and acetylation. Here we discuss the latest developments regarding these modifications that control various aspects of MYC function, including its stability, the interaction with partner proteins, and the transcriptional potential.

Few genes and their products have stimulated the extent of interest and research activities comparable to what MYC genes and proteins have done and still do. A strong driving force in sustaining the long lasting efforts to understand the function and regulation of MYC is its potent role as an oncprotein in human tumors. The first myc gene emerged almost 30 years ago when it was realized that several highly transforming chicken retroviruses had captured a cellular sequence that transformed myeloid cells among others, hence the name myc for myelomonocytic leukemia (1, 2). In mammals several MYC genes, including MYC (formerly c-MYC), gene and protein symbols as proposed by the Human Genome Organisation Gene Nomenclature Committee (www.gene.ucl.ac.uk/nomenclature/index.html), MYCN, and MYCL, have been identified. All three have been implicated in the genesis of human malignancies and suffer in many instances from different genetic alterations, including translocations and amplifications (for a more detailed overview see supplemental Fig. S1) (3, 4). In addition the MYC promoter is targeted by multiple signal transduction cascades, including the WNT, RAS/RAF/MAPK, JAK/STAT, transforming growth factor β, and NF-κB pathways, that are deregulated in cancer cells and contribute to enhanced MYC expression (5, 6). These tumor-associated alterations in general deregulate and enhance MYC expression. Because MYC proteins function primarily as transcription factors, the consequences of these alterations result in the deregulation of MYC target genes and subsequent effects on cell behavior and in the inability to down-regulate MYC expression to levels sufficiently low for a cell to exit the cell cycle and enter a quiescent state or to differentiate in response to appropriate signals.

MYC is a member of the MYC/MAX/MAD network of the basic region/helix-loop-helix/leucine zipper (bHLHZ) domain transcriptional regulators (for a summary of the domain structure see Fig. 1). MYC proteins form obligatory heterodimers with MAX, and these complexes bind to specific E-box DNA sequences with the consensus 5′-CACGTG (7). The transactivation domain (TAD) is localized at the N terminus, containing two highly conserved elements, MYC box (MB) I and II, that are particularly relevant for MYC regulation and cofactor recruitment, respectively (Fig. 1). Within the MYC/MAX/MAD network, MYC proteins are antagonized by several bHLHZ proteins collectively referred to as MAD proteins (for an overview of the network see Fig. S2A) (8). As an activator MYC recruits a number of different cofactors that possess the capability to control chromatin structure, acetylate core histones as well as transcriptional regulators, and regulate polymerase complexes (summarized in Fig. S2B) (9). Recent studies have demonstrated that MYC activates all three RNA polymerases (10). In addition to its role as an activator, MYC also has the ability to repress genes (summarized in Fig. S2C) (11). In contrast to MYC, the members of the MAD family function as transcriptional repressors at least in part by recruiting mSin3-histone deacetylase complexes (summarized in Fig. S2D) (8).

Various studies, including several microarray expression analyses, have demonstrated that MYC proteins control the expression of many target genes (11–14). The resulting consequences on many different aspects of cell behavior and cell fate demand that this protein is precisely controlled. Too little or too much MYC protein and/or activity may severely affect proper functioning of cells and as a consequence affect their proliferation, differentiation, and apoptosis, which may result in disease. As pointed out above this is most evident in cells that produce too much MYC, which is associated with tumorigenesis. Thus it is not surprising that the expression and function of this protein is regulated at multiple levels. The expression of the MYC gene is regulated by a wide variety of signals that control promoter activity, RNA polymerase elongation, and mRNA processing and collectively determine where, when, and how much MYC is synthesized (6).

Here we will focus on mechanisms further downstream that impinge on MYC. The protein is subject to interdependent
posttranslational modifications, including phosphorylation, acetylation, and ubiquitinylation, suggesting that MYC proteins directly integrate the information of different signal transduction pathways.

**MYC Is Phosphorylated at Multiple Sites**

MYC proteins are phosphorylated at multiple sites distributed over the entire protein (Fig. 1). The first and major sites were identified almost 20 years ago within two areas, the acidic region and near the basic region, and are substrates of protein kinase CK2, an enzyme that has broad biological activities including a role in tumorigenesis (15, 16). Nevertheless the functional relevance of these CK2 phosphorylation sites on MYC has remained elusive. However, in a recent study a correlation between CK2 activity and MYC protein levels was demonstrated, suggesting that CK2 stabilizes MYC (17). Although it is not clear whether this is a direct effect, the CK2 sites within the acidic region might be relevant. This region lies within a PEST consensus sequence that has been shown to be involved in neuronal differentiation (15, 20) and the contribution of RAS signaling to MYC expression levels at the G0 to G1 transition will need further studies. It should also be noted that the role of MAPK in phosphorylation of Ser-62 has been disputed (29).

Once Ser-62 is phosphorylated, Thr-58 of MYC becomes a substrate for GSK3 (24). Most substrates of this enzyme must first be phosphorylated by another kinase at a Ser or Thr amino acid four residues C-terminal of the GSK3 site (35). This "priming phosphate" binds specifically into a pocket of the substrate recognition domain of GSK3 and explains why MYC and other substrates require this phosphorylation for the precise positioning of the phosphorylation site. Thus phosphorylation of Thr-58 appears to be strictly dependent on prior phosphorylation of Ser-62. Similar findings were made for MYCN (36). Phosphorylation at Ser-54 by CDK1 leads to Thr-50 phosphorylation, the two sites correspond to Ser-62 and Thr-58 in MYC, respectively, which enhances MYCN turnover during mitosis in neuronal cells. This is suggested to be relevant for neuronal differentiation.

**FIGURE 1. Structure-function analysis of MYC.** TAD, aa 1–150; / and I refer to two conserved MYC boxes (aa 45–63 and 129–141, respectively); A, acidic region (aa 242–261); NLS, aa 320–328; bHLHZ, aa 355–435, domain that mediates binding to MAX and sequence-specific DNA binding; P, main phosphorylation sites in MYC with relevant kinases (the red arrow indicates that Thr-58 phosphorylation by GSK3 requires prior phosphorylation at Ser-62); Ac, acetylation sites with relevant HAT enzymes (the following lysines are acetylated: 143, 148, 157, 275, 317, 323, 371, and 417). Additional phosphorylation sites are mentioned in the text. The regions important for recruiting proteins or protein complexes discussed in the text are indicated. The functional relevance of these MYC-interacting proteins is summarized by the color code.
Finally it should be noted that phosphopeptide mapping has revealed additional phosphorylation sites in MYC (37). These include Ser-71, Ser-82, Ser-162 or -164, Ser-293, and possibly Ser-343/344. With the exception of Ser-162 and Ser-343, all other sites contain a Pro at the +1 position and are thus potential substrates for Pro-directed kinases. At present kinases possibly involved in modifying these phosphorylation sites and their functional relevance have not been defined.

**Phosphorylation of N-terminal Sites Regulates MYC-dependent Gene Transcription**

Because of the location of the phosphorylation sites at Thr-58 and Ser-62 within the TAD, it was speculated early on that these sites are involved in the regulation of gene transcription. However, these findings have been controversial (2). Although the differences between the published studies could not be clarified, the recent findings connecting these phosphorylation sites to protein turnover indicate that distinct effects might have been mingled, potentially explaining the inconclusive results. More recently this discussion has been revived. It has been suggested that Ser-62 phosphorylation modulates gene expression (26). In response to oxidative stress Ser-62 becomes phosphorylated, which correlates with increased recruitment of MYC to specific promoters but not with stabilization. It remains to be determined what the role of Ser-62 phosphorylation is in promoter selection or whether the phosphorylation is the consequence of targeting MYC to specific DNA sequences.

Furthermore MYC mutated at Thr-58 fails to activate the expression of BIM, a proapoptotic BH3-only BCL2 family member, resulting in reduced apoptosis and enhanced tumorigenesis (38). Although MYC stimulates apoptosis by regulating many different genes (39, 40), MYC-stimulated expression of BIM is particularly relevant for repression of tumor development (41). BIM functions as a tumor suppressor, at least in MYC-driven B cell leukemia. This is independent of the status of the p53 tumor suppressor pathway that is viewed as an important mediator of MYC-induced apoptosis (39, 40). It remains to be determined how MYC controls the expression of the BIM gene and whether it is a direct MYC target. Nevertheless the data suggest that the frequent mutations of the MYC coding sequence in Burkitt lymphoma uncouple proliferative and apoptotic effects at least in part by differential regulation of BIM. Together these findings suggest that phosphorylation at Thr-58 and/or Ser-62 affects promoter selection of MYC. It will be interesting to unravel the underlying mechanism.

**Phosphorylation Regulates Ubiquitinylation of MYC**

A significant body of evidence points to the importance of the phosphorylation sites at Thr-58 and Ser-62 within MBI in controlling MYC protein stability. As indicated above, phosphorylation of Ser-62 stabilizes MYC, whereas upon phosphorylation of Thr-58 a series of events are induced that lead to MYC degradation (Fig. 2A). Once both sites are phosphorylated, the PIN1 prolyl isomerase promotes access of protein phosphatase 2A to MYC and dephosphorylates Ser-62 (42, 43). The Thr-58 only phosphorylated form of MYC is recognized by the F-box protein FBW7. This protein is a subunit of one SKP1-CUL1-F-box protein (SCF) ubiquitin–protein isopeptide ligase complex that stimulates polyubiquitinylation and subsequent proteasomal degradation of MYC (44–48). The relevance of the two phosphorylation sites in controlling MYC stability is supported by the altered stability of tumor-derived MYC mutant proteins and is in agreement with the notion that FBW7 possesses tumor suppressor activity (24, 49).

From these findings a model has been developed that sug-

![FIGURE 2. Regulation of MYC by posttranslational mechanisms. A, phosphorylation at Ser-62 by Pro-directed kinases (P-K) is a prerequisite for GSK3- (P-K)-dependent dephosphorylation of MYC at Thr-58. This triggers binding of PIN1, subsequently protein phosphatase 2A (PP2A)-dependent dephosphorylation of Ser-62, and then recruitment of SCF-FBW7 to the Thr-58-phosphorylated MYC. SCF-FBW7 polyubiquitinylates MYC (branching through Lys-48), leading to its proteasomal degradation. B, the SCF-SKP2 complex interacts with MYC at promoters. This results in the activation of transcription, i.e. SKP2 is a MYC cofactor, and subsequently in polyubiquitinylination and proteasomal degradation of MYC. Thus the activation and subsequent inactivation/degradation of MYC are tightly coupled. C, the recruitment of CBP/p300 HAT cofactors to promoters by MYC stimulates transcription. Modification of MYC by HectH9 results in polyubiquitinylination with Lys-63 branching. This does not lead to degradation but rather enhances the interaction with CBP/p300. D, MYC is acetylated by at least three different HAT enzymes. Although the functional consequences of individual acetylations are not known in detail, this modification can interfere with ubiquitinylination because specific lysine residues can be potentially both ubiquitinylated and acetylated. Thus MYC acetylation may interfere with the activities summarized in A–C. In addition HectH9-dependent ubiquitinylation may stimulate MYC acetylation by enhancing CBP/p300 recruitment.](image-url)
gests a dual role for RAS signaling (24): because RAS stimulates phosphorylation of Ser-62 through MAPK, the subsequent phosphorylation of Thr-58 has to be prevented to avoid subsequent rapid degradation of MYC by the mechanism summarized above. Therefore it was suggested that RAS inhibits the activity of GSK3 through the phosphatidylinositol 3-kinase-AKT pathway, resulting in MYC proteins phosphorylated only at Ser-62. This model is consistent with the finding that mutations of Ser-62 stabilize MYC, possibly due to the lack of Thr-58 phosphorylation by GSK3 (the substrate lacks the priming phosphate). Nevertheless it is worth considering a role of Ser-62 phosphorylation in stabilizing MYC independent of Thr-58. Because most likely several kinases can phosphorylate Ser-62, it seems counterintuitive that all the Ser-62 targeting signals would enhance MYC turnover. Also the need to dephosphorylate Ser-62 suggests that this site might possess additional functions. If Ser-62 by itself is indeed a stabilizing signal, it will be challenging to establish the underlying molecular mechanism.

Ubiquitinylation of MYC Has Multiple Functions

In addition to the phosphorylation-induced SCF-FBW7-dependent degradation, MYC is also turned over in response to polyubiquitinylation by the SCF-SKP2 ubiquitin-ligase complex (Fig. 2B) (50, 51). Presently it is not known whether the interaction of SKP2 with MYC requires any signal. Importantly, however, this interaction serves at least one additional function. SKP2 as well as other subunits of the SCF-SKP2 complex are recruited to MYC-regulated promoters and seem to be relevant for MYC-dependent gene transcription. The emerging model suggests that SCF-SKP2, after interacting with MYC, results in ubiquitinylation that first activates the transcriptional potential of MYC and subsequently triggers its proteasomal degradation, thereby limiting the transcriptional potential of this proto-oncoprotein to a potentially narrow time window (Fig. 2B). This model is in line with the observation that the stability of transcriptional activators is inversely proportional to the power of their TAD, a correlation that is dependent on ubiquitinylation (52).

It is not entirely clear how the SCF-SKP2 complex stimulates MYC function. One possibility is that components of the SCF-SKP2 complex or of the proteasome function as coactivators. In support of this, recent findings demonstrate that the 19 S lid of the proteasome can recruit SAGA, a chromatin remodeling complex (53). Another possibility is that ubiquitinylation of MYC modulates cofactor recruitment, one candidate being the positive transcription elongation factor P-TEFb (54). In support of such a model, ubiquitinylation of LexA-VP16 stimulates the interaction with P-TEFb and activates transcription (55). P-TEFb can also be recruited to promoters through its binding partner Brd4. This bromodomain protein binds to acetylated histones (56, 57) and thus represents an additional possibility of how MYC, through its ability to mediate core histone acetylation (11), may recruit P-TEFb to promoters. It is conceivable that the two suggested mechanisms synergize.

Recently a third ubiquitin-ligase, HECTH9, was identified as a modulator of MYC (58). This enzyme polyubiquitinylates MYC by catalyzing the branching through Lys-63 rather than Lys-48 (Fig. 2C). This Lys-63-dependent polymerization does not signal degradation but rather appears to function by enhancing CBP/p300 recruitment. Although these cofactors can interact with bacterial MYC in vitro (59), in cells HECTH9-dependent ubiquitinylation enhances binding, recruitment to promoters, and affects the gene expression pattern.

Acetylation Interferes with Ubiquitinylation of MYC

Several of the cofactors or cofactor complexes that interact with MYC possess histone acetyltransferase (HAT) activity (Fig. S2B). These HAT enzymes, including CBP/p300, Tip60, and mammalian mGCN5, have been shown to regulate histone acetylation upon recruitment by MYC to specific promoters (11). However, MYC does not simply serve as a platform for these cofactors but is itself a substrate of these HATs (Fig. 2D) (59, 60). These HATs modify different lysines, some of which have been identified either in response to co-expressed mGCN5 or p300 or by in vitro acetylation with p300 (Fig. 1) (60–62). At least one of the lysines, Lys-323, located within the NLS, is modified by both p300 and mGCN5. Acetylated lysines can serve as docking sites for proteins and can be recognized by bromodomains (63). At present it is unclear whether any of the acetylated lys residues in MYC are binding sites for specific interaction partners. It was conceivable that acetylation of Lys-323 might affect NLS function. However co-expression of mGCN5 did not alter the subcellular localization of MYC. Furthermore binding of MYC to MAX was tested because Lys-417, acetylated by mGCN5, is located within the leucine zipper. Again no alterations could be measured (60).

Because lysines can be modified by both ubiquitinylation and acetylation, these two modifications can potentially interfere with each other (Fig. 2D). Indeed stimulation of acetylation decreases ubiquitinylation of MYC and enhances its stability (59–61). In addition some of the identified acetylation sites overlap with the proposed ubiquitinylation sites of HECTH9 (58), suggesting that acetylation may also affect the recruitment of CBP/p300, the binding of which is enhanced upon Lys-63-linked polyubiquitinylation (Fig. 2D). Thus these studies suggest that ubiquitinylation and acetylation are tightly connected, not only in regulating MYC protein stability but potentially also in controlling the binding of interacting proteins.

Conclusions

The studies on MYC function and regulation have provided a wealth of information that indicates multiple signaling pathways converging on MYC. Many of these pathways control directly the function of MYC by posttranslational means, involving phosphorylation, acetylation, and ubiquitinylation. These modifications in turn define interactions with other proteins that regulate the stability of MYC and modulate its molecular functions as a transcriptional regulator. Some of the modifications are interconnected, resulting in a cross-talk of phosphorylation and ubiquitinylation and of acetylation and ubiquitinylation. It will be a challenge for the future to catalogue all the different posttranslational modifications, to understand their functional consequences, and to define their cross-talk. Because MYC is deregulated in the majority of human tumors, the MYC/MAX complex, but also the signaling
pathways and the enzymes that modify and control MYC, should be considered as potential therapeutic targets.

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