Flipping the Myc switch

When certain cells differentiate, Myc in Myc–Max heterodimers is replaced by Mad or Mxi, generating heterodimers that suppress transcription by interacting with the repressor Sin3.

Members of the *myc* family of proto-oncogenes are deregulated in a variety of human malignancies. Amplification of N-*myc*, L-*myc* and c-*myc* genes is found in several types of human cancer. Furthermore, chromosomal translocations involving c-*myc* and point mutations in the c-*myc* coding region frequently occur in Burkitt's lymphoma. Some insight into the function of Myc proteins has come from the identification of two motifs in their carboxyl termini that are also present in a number of transcription factors: the basic-helix–loop–helix (bHLH) and leucine zipper (Zip) motifs (Fig. 1). These motifs are required for sequence-specific DNA binding and protein dimerization. In addition, the amino terminus of Myc proteins includes a strong and highly conserved transactivation domain (Myc box 1), and a second conserved motif (Myc box 2) that appears to be involved in transcriptional repression [1]. Importantly, both Myc box 1 and Myc box 2 are required for cell transformation. These observations indicate that Myc proteins are transcription factors that exert their effect on cellular physiology by activation of some genes and suppression of others.

The activity of c-*myc* is regulated at several levels. First, when quiescent cells are stimulated by growth factors, c-*myc* transcription is rapidly and transiently induced. In exponentially growing cells, c-*myc* mRNA and protein levels are invariant. However, they decline following the induction of differentiation. In addition to regulation at the level of transcription, both transactivation and DNA binding by c-Myc are highly regulated. The c-Myc transactivation domain is modulated in two different ways. Phosphorylation of residues Thr58 and Ser62 within the Myc transactivation domain (Fig. 1) occurs in a highly cell-cycle-regulated fashion [2]. In Burkitt's lymphoma, residue Thr58 is often mutated, resulting in enhanced c-Myc transforming activity. This suggests that phosphorylation at Thr58 negatively regulates c-Myc. In contrast, mutation of Ser62 severely reduces the transforming activity of c-Myc, suggesting that phosphorylation of this residue positively regulates c-Myc [3]. Myc is also regulated by the retinoblastoma-related protein p107, which binds to the transactivation domain of c-Myc and greatly inhibits its ability to activate transcription [4,5].

A third level of Myc regulation comes from its ability to form heterodimers with other DNA-binding proteins. Sequence-specific DNA binding by Myc proteins requires dimerization to a second bHLH–Zip protein, known as Max [6] (Fig. 1). Myc–Max heterodimers can activate the expression of reporter genes that carry a CACGTG consensus Myc–Max DNA-binding site [7]. Max can also form homodimers that bind the same DNA site. As Max lacks a transactivation domain, Max homodimers do not activate transcription, but rather repress CACGTG-containing promoters by competition with Myc–Max heterodimers and other transcription factors (such as USF and TFE3) for binding to the same site. High levels of Max expression, generating excess Max–Max homodimers, can thus suppress c-Myc transactivation. Under physiological conditions, Myc–Max heterodimers are favored over Max–Max homodimers. Induction of c-Myc synthesis is therefore thought to result in a shift from Max–Max homodimers to Myc–Max heterodimers.

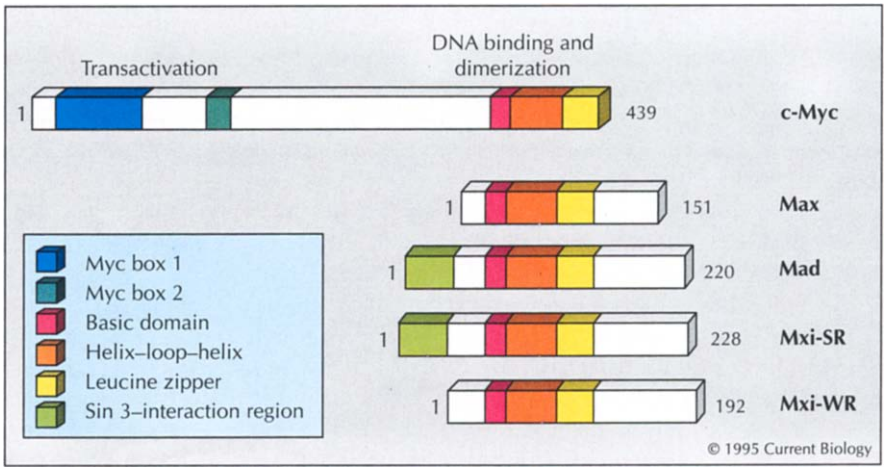

Fig. 1. Schematic representation of the factors that can bind the CACGTG Myc DNA-binding site. See text for details.

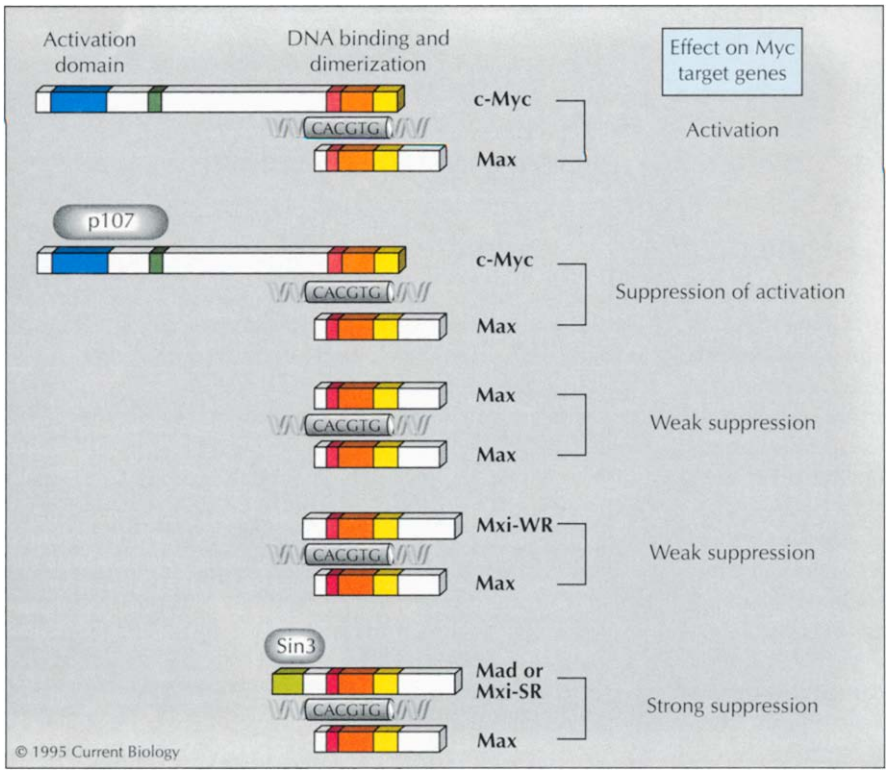

Fig. 2. Possible complexes and activities of complexes on the CACGTG Myc-binding site. Activation of Myc target genes occurs when a Myc–Max heterodimer binds to the CACGTG site in a Myc-responsive promoter. c-Myc transactivation can be modulated by amino-terminal phosphorylation or by binding of the retinoblastoma-related protein p107 to the c-Myc transactivation domain. Max–Max and Mxi-WR–Max dimers compete with Myc–Max heterodimers for binding to the same site, leading to weak suppression of the CACGTG element. Strong suppression on this element is seen when the Sin3 transcriptional repressor is recruited to the site by ternary complex formation with either Mxi-SR or Mad proteins.

Two new players in this game of musical chairs around the CACGTG motif have recently been identified [8,9]. Mad and Mxi are related bHLH-Zip proteins that both form heterodimers with Max, but not with other bHLH-Zip proteins. Both Mad–Max and Mxi–Max heterodimers bind the Myc–Max consensus site [8,9]. Like Myc-Max heterodimers, Mad–Max heterodimers are favored over Max–Max homodimers [9]. Because Mad lacks a transactivation domain, it cooperates with Max to repress transcription of a Myc-site-containing reporter plasmid [9]. Consistent with this antagonistic effect of Mxi and Mad on c-Myc, both Mxi and Mad have been found to suppress cotransformation by c-*myc* plus *ras* of rat primary embryo fibroblasts.

When myeloid cells are induced to differentiate, dramatic changes are observed in the protein complexes that bind to the c-Myc DNA-binding site. The mRNA and protein levels of the central character, Max, are invariant during cell division and differentiation [10]. In contrast, c-Myc levels decline following the induction of monocytic differentiation, whereas both Mxi and Mad levels rise sharply [8,10]. These changes in protein expression levels cause a reshuffle in the protein partners that bind to Max. In undifferentiated U937 myeloid leukemia cells, only Myc–Max heterodimers are found. Within 2 hours following induction of monocytic differentiation, Mad is first detected in complexes with Max, and, after 48 hours, only Mad–Max heterodimers remain in fully differentiated U937 cells [10]. These data indicate that dynamic changes occur on the Myc-recognition site during monocytic differentiation. Whether the change from Myc–Max to Mad–Max heterodimers is sufficient, alone, to induce differentiation remains to be investigated.

Two recent papers shed further light on the significance of this shift from Myc–Max to Mad–Max or Mxi–Max heterodimers [11,12]. Using yeast two-hybrid interaction screening — in which proteins that potentially interact are identified by their ability to bring DNA-binding and transcriptional activation domains together on the promoter of a reporter gene — the groups of Eisenman [11] and DePinho [12] isolated proteins that interact with Mad. Two of these are related mammalian homologs of the yeast transcriptional repressor Sin3, named mSin3A and mSin3B. A DNA-binding ternary complex of Mad, Max and Sin3 can be assembled *in vitro*. Both Sin3 homologs interact with an amino-terminal region of Mad thought likely to be α-helical in structure: disruption of this domain by insertion of two helix-breaking prolines leads to loss of mSin3 interaction. Significantly, the ability of Mad to suppress a promoter containing consensus Myc–Max binding sites correlates with its ability to bind mSin3 [11]. These data suggest that, during differentiation, the CACGTG motif switches from being a positive element to a negative element by recruitment of a transcriptional repressor to the Myc–Max binding site, thereby silencing Myc-responsive genes in differentiated cells (Fig. 2).

The motif that mediates binding of Mad to mSin3 is also present in the amino terminus of Mxi. Importantly, two Mxi proteins (Figs 1 and 2) can be generated from a single *mxi* gene by alternative mRNA splicing — one version, Mxi-SR, that contains the Sin3-interaction domain, and a shorter version of the protein, Mxi-WR, that lacks the Sin3-interaction surface [12]. When the proteins were compared for their ability to suppress c-*myc*-plus-*ras*-mediated transformation of rat embryo fibroblasts,

only Mxi-SR had strong transformation-repressing activity, whereas Mxi-WR only weakly interfered with c-*myc*-plus-*ras* transformation [12]. Thus, by differential splicing of Mxi pre-mRNA, cells can fine-tune the degree of Myc DNA binding-site suppression.

This dual regulation — activation and suppression mediated via the same site — is reminiscent of the regulation of E2F, another transcription factor with growth-promoting activity. Free E2F promotes S-phase entry by stimulating the expression of genes involved in cell-cycle progression. In contrast, a complex between E2F and the retinoblastoma protein acts as an active repressor on the same site [13]. This may indicate that non-proliferating cells need actively to suppress the expression of growth-stimulatory genes by recruitment of transcriptional repressors to their promoters. It will be of interest to see what the effect is of loss of mSin3 gene function on cell proliferation. It will probably not be too long before we find out.

References

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