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How the *c-myc* Promoter Works and Why It Sometimes Does Not

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Abstract

The *c-myc* promoter is regulated by scores of signals, transcription factors, and chromatin components. The logic integrating these multiple signals remains unexplored. Recent evidence suggests that activated MYC expression is regulated in several phases: 1) conventional transcription factors trigger transcription by the RNA polymerase II (pol II) paused within the proximal promoter region. Concurrently (and probably consequently), newly arrived chromatin-remodeling complexes mobilize a nucleo-some masking the far upstream element (FUSE), 1.7-kb upstream of the P2 start site; 2) binding by FUSE-binding proteins (first FBP3, then FBP); and 3) FBP-interacting repressor (FIR) binds FUSE and returns transcription to basal or steady-state levels. The recruitment and release of the FBPs and FIR is governed by FUSE-DNA conformation, itself controlled by dynamic supercoils propagated behind pol II. The organization and operation of the *c-myc* promoter make it difficult to inactivate, but sensitive to disturbances (translocations, viral insertions, amplification, and mutation) that disrupt the fine-tuning seen at its normal chromosomal context.

c-myc is critical to proliferation, growth, and apoptosis (1–5). Shut off of *c-myc* is required for differentiation and senescence. MYC interacts with the general transcription machinery, a variety of transcription factors, and with chromatin-modifying and -remodeling complexes (1). As many as 10% of genes are MYC targets (6–9). Although usually an activator, some genes are also repressed by MYC. MYC is rarely a dominating force; MYC adjusts the expression of most targets by small or even incremental amounts. *c-myc* expression is deregulated in most cancers (1,4,5,10) by chromosomal translocation, viral insertions, amplification, deletions, insertions, and/or mutation of *cis*-elements. MYC-coding mutations occur but are not obligatory for carcinogenesis; inappropriate expression of the wild-type protein suffices.

The best characterized chromosomal pathology deregulating *c-myc* occurs in Burkitt lymphoma (1). Burkitt translocations inevitably approximate *c-myc* on chromosome 8 with immunoglobulin (Ig) sequences from the κ, λ, or heavy-chain genes on chromosomes 2, 22, or 14, respectively. Some translocations occur within the body of the gene, but others occur hundreds of thousands of bases upstream or downstream of the major transcription start sites. Despite the juxtaposition with Ig regulatory sequences, overall *c-myc* expression within many tumors remains within the normal range (11,12). The derangement of *c-myc* in these cases may disturb the timing of expression or may lead to heterogeneous levels where elevated MYC in some cells is offset by reduced expression in others.

A series of mice with declining MYC expressed from its natural locus become progressively smaller (13). Cells making more MYC induce apoptosis in their neighbors even when the expression differential is slight (14,15). Such observations reveal the importance of uniform

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MYC levels throughout a developmental field. Whereas normal diploid human fibroblasts are immortalized by telomerase, cells haploinsufficient for *c-myc* are resistant to telomerase overexpression (16). So expressing MYC at the proper levels, times, and locations is very important.

c-myc mRNA and protein are short lived (approximately 20–30 minutes, each) and rare (1–5). Usually, *c-myc* is expressed at low levels, as low as one transcript per cell. Resting fibroblasts contain just 500 molecules of MYC protein, rising to 5000 upon activation (17). In embryonic tissues, *myc* mRNA levels have been estimated to be five per cell (18). Tumor cells express a much broader range of *c-myc* RNA and protein than normal cells. The instability of MYC protein and RNA together with small numbers of mRNA would seem to compel stochastic variation between cells (19). If such fluctuations are deleterious to normal physiology and homeostasis, mechanisms must exist to precisely control and safeguard *c-myc* expression. Though MYC levels are also controlled posttranscriptionally, our focus is on how the *c-myc* promoter works.

c-myc is transcribed from promoters, P1 and P2, separated by 150 bp. Most transcripts initiate at P2. When translocations displace P1 and P2 in Burkitt lymphomas, transcription starts at cryptic sites within intron I (1–5). *c-myc* and *Drosophila hsp70* were the first genes shown to harbor promoter-paused RNA polymerase II (pol II) (20,21). In vitro, pol II, either alone or in complex with general transcription factors, enters elongation upon the addition of the ninth nucleotide (22). In vivo, pausing at *c-myc* and *hsp70* promoters continues beyond +9, so either the reaction pathway is different (less probable) or some superimposed mechanism delays promoter escape (more probable). A variety of multi-subunit complexes remain associated with pol II from preinitiation complex (PIC) formation to initiation to promoter escape to elongation (23,24). Because its promoter is almost always loaded, the crucial step in *c-myc* activation is mobilization of the paused polymerase, not the PIC assembly.

Recent work in my own laboratory and that of other investigators has focused on certain DNA elements in the *c-myc* promoter (eg, CCCTCCCCA tandem repeats [CT elements] and far upstream element [FUSE]) and transcription factors binding to the promoter (eg, transcription factor IIH [TFIIH], FBP-interacting receptor [FIR]). The insights gleaned from these studies (selected findings are summarized in Box 1; references were omitted because of space constraints) have greatly enhanced our understanding of 1) the assembly of the transcription complex at the *c-myc* promoter, transcription initiation, and promoter escape; 2) the mechanism that limits the noise at low-level *c-myc* expression: prepromoter escape transitions that involve TFIIH, activators of TFIIH (such as FUSE-binding proteins [FBPs]), and repressors of TFIIH (such as FIR); 3) the underlying reason why the *c-myc* promoter executes a stereotypical pulse of expression peaking 2–3 hours poststimulation followed by either shutoff and return to G0 or decline to an intermediate steady-state level of gene expression if the cell reenters the active cell cycle; 4) the role of single-stranded DNA, mechanical (torsional) stress, and supercoils at the *c-myc* promoter; and 5) the utilization of conventional transcription factors for an “ignition system” of the *c-myc* promoter that subsequently turns control over to a “servomechanism” of *c-myc* expression. The reader is referred to the primary literature for details.

No reporter gene, transfection system (transient, stable, or episomal), or transgenic mouse has yet been devised that faithfully recapitulates the physiological responses of the *c-myc* promoter to its many activating signals (1–3). Either important *cis*-elements are missing or there are context-dependent requirements for *c-myc* transcription that can only be maintained at the normal locus. Nonetheless, the principles extracted from experimental model systems of *c-myc* expression have been helpful to understand the transcriptional regulation of the endogenous, normal *c-myc* gene, which, in turn, is a precondition for appreciating the mechanism that deregulates *c-myc* in the context of a chromosomal translocation, in which the

panel of *cis*-elements that recruit factors to the *c-myc* promoter would be profoundly altered and promoter output would be reprogrammed. There has been much progress, but we have a long way to go before the regulation of normal and rearranged *c-myc* can be fully understood.

Key transcription factors and DNA elements regulating c-myc expression

General TFIIH

- Contains 10 subunits in two subcomplexes. Includes the cyclin-activating kinase and the core subcomplex, which includes two helicases (XPB and XPD gene products).
- Serves a central role during the early phases of transcription, between transcription initiation and promoter escape. Before promoter escape, TFIIH receives input from activators (eg, FBP) and repressors (eg, FIR).
- Is mutated in XPB disease, leading to elevated levels of cell-to-cell variability of MYC.

CT-element

- Located in the *c-myc* proximal promoter, 100–150 bp 5′ of P1. Structurally responsive to mechanical stress. Can form single-stranded, slipped mismatched, H-triplex, G-quadruplex, and I-DNA structures in vitro.
- Binds an array of factors specific for different DNA conformations, including Sp1, MAZ1, hnRNPK, CNBP, nm23/puf60, and hnRNPA1. Sp1 is rapidly recruited following activation of serum-starved fibroblasts.
- Is nucleosome-free throughout the *c-myc* activation cycle, which may help to insure accessibility.

FUSE

- Located 1.7-kb upstream of the P2 promoter. Melts in cells expressing *c-myc*, thus exposing functional groups for recognition by FBP and FIR.
- When *c-myc* is off, a FUSE-masking nucleosome prevents melting. To melt FUSE, this nucleosome must be removed, repositioned, or remodeled. This likely involves Swi/Snf.
- Following melting, FBP3 is the first FBP family member recruited to FUSE. Approaching peak output, FBP3 is exchanged for FBP, which recruits FIR, which also binds single-stranded FUSE. FIR then decreases transcription, FUSE renatures, FBP is discharged, and FIR persists until a new *c-myc* cycle begins.

FBP = FUSE-binding protein; FIR = FBP-interacting repressor; FUSE = far upstream element; TFIIH = transwcription factor IIH.

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