

c-Myc and eIF4F Constitute a Feedforward Loop That Regulates Cell Growth: Implications for Anticancer Therapy

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Abstract

The Myc/Max/Mad family of transcription factors and the eukaryotic initiation factor 4F (4F) complex play fundamental roles in regulating cell growth, proliferation, differentiation, and oncogenic transformation. Recent findings indicate that the role of Myc during cell growth and proliferation is linked to an increase in eIF4F activity in a feedforward relationship, providing a possible molecular mechanism of cell transformation by Myc. Developing therapeutics to inhibit eIF4F and/or Myc could be a potential treatment for a wide range of human cancers. [Cancer Res 2009;69(19):7491–4]

Background

The *c-myc* oncogene is the prototypical member of the Myc/Mad/Max transcription factor network, which regulates divergent cellular functions such as proliferation, differentiation, and apoptosis. All members of this group share a common N-terminal transcriptional interacting domain and a C-terminal basic helix-loop-helix leucine zipper (bHLHzip) DNA binding motif. Although *c-Myc* (hereafter referred to as Myc) is not found to homodimerize *in vivo*, it readily heterodimerizes with Max, which is both necessary and sufficient for Myc transcriptional activity (1). Max has alternative dimerization partners, in particular Mad and Mnt, which act to antagonize Myc *trans*-activation. The Myc-Max complex binds to the promoters of their target genes in a sequence-specific manner, mostly at canonical (5' CACGTG³) E-box sites, but sometimes at other noncanonical related E-box-like sequences (2, 3).

Like many classical oncogenes, *c-myc* was first identified as *v-myc*, an avian retroviral oncogene, whose expression induces myelocytomas and carcinomas in chickens (4). Its human homolog was subsequently cloned and found to be overexpressed in wide range of human tumors including colon carcinomas, small lung carcinomas, breast carcinomas, and prostate cancers, as well as being the dominant oncogene found in chromosomal translocations of non-Hodgkin's Burkitt's lymphomas (5). Indeed, Myc today remains one of the most widely studied oncogenes in all of cancer biology. In mammalian cells *in vitro*, expression of Myc is rapidly induced following mitogenic or cytokine stimulation and down-regulated during cellular differentiation. One of its more obvious biological effects is to drive cellular proliferation, which is sufficient to allow quiescent cells to reenter the cell cycle (6). However, in the absence of growth factors, not only does Myc promote cell division, but it also induces apoptosis, mainly through

the ARF/Mdm2/p53 tumor suppression pathway (7). This has led to the notion that one of the key events to allow for the full oncogenic potential of Myc is a secondary genetic lesion needed to suppress its pro-apoptotic activity.

Recently, in an effort to further dissect the molecular pathways regulated by Myc, several genome-wide screens have been used to identify transcriptional targets of Myc. cDNA microarrays combined with chromatin immunoprecipitation have revealed that expression of up to 10 to 15% of all genes may be affected by Myc (8, 9). Although the obvious caveats apply when analyzing array data sets, one of the more consistent categories of genes up-regulated by Myc is cell growth and protein synthesis. Myc regulates all aspects of protein synthesis, increasing components of ribosome biogenesis and tRNA levels and key factors involved in translation initiation and elongation. The fact that regulation of protein synthesis is controlled by Myc was suggested by the findings that loss of *myc* severely impairs cell growth in both *Drosophila* and mammalian cells (10), whereas its overexpression clearly increases overall protein production, most notably in resting B lymphocytes (11).

mRNA translation can be separated into three distinct stages: initiation, elongation, and termination. The translation initiation phase begins with recruitment of a 43S ribosomal complex to the 5'-methylguanosine cap of mRNAs, proceeds with its scanning along the mRNA until it encounters the message's first start codon, and ends with joining of 60S large ribosomal subunit. It is the cap-dependent ribosomal binding step that, under normal circumstances, is thought to be rate-limiting. This step is stimulated by eukaryotic initiation factor (eIF) 4F, a complex consisting of three subunits: eIF4E, the least abundant of all initiation factors (12) that binds directly to the mRNA cap structure; eIF4A, an RNA helicase that prepares the mRNA template for ribosome loading; and eIF4G, a large molecular scaffold that bridges the 43S ribosome preinitiation complex to the mRNA. The aforementioned gene array screens have implicated all three members of eIF4F as well as other initiation factors, including eIF2 and eIF3 subunits, to be under Myc regulation. Although eIF4E has previously been shown to be regulated by Myc (13), it is unknown whether the other members are true targets or simply nonfunctional binding artifacts. We have shown recently that eIF4AI and eIF4GI are *bona fide* Myc targets, suggesting that the way Myc stimulates translation may be through its ability to up-regulate the rate-limiting step of translation initiation (14).

Myc Stimulates Protein Synthesis by Up-regulating eIF4F Expression

We used an inducible form of Myc, in which native Myc is fused to a mutated estrogen receptor (MycER; ref. 15). This chimeric protein is nonfunctional, but becomes fully active by addition of 4-hydroxytamoxifen (4-OHT), eliciting a transcriptional response that closely resembles endogenous Myc. In our study, we exploited

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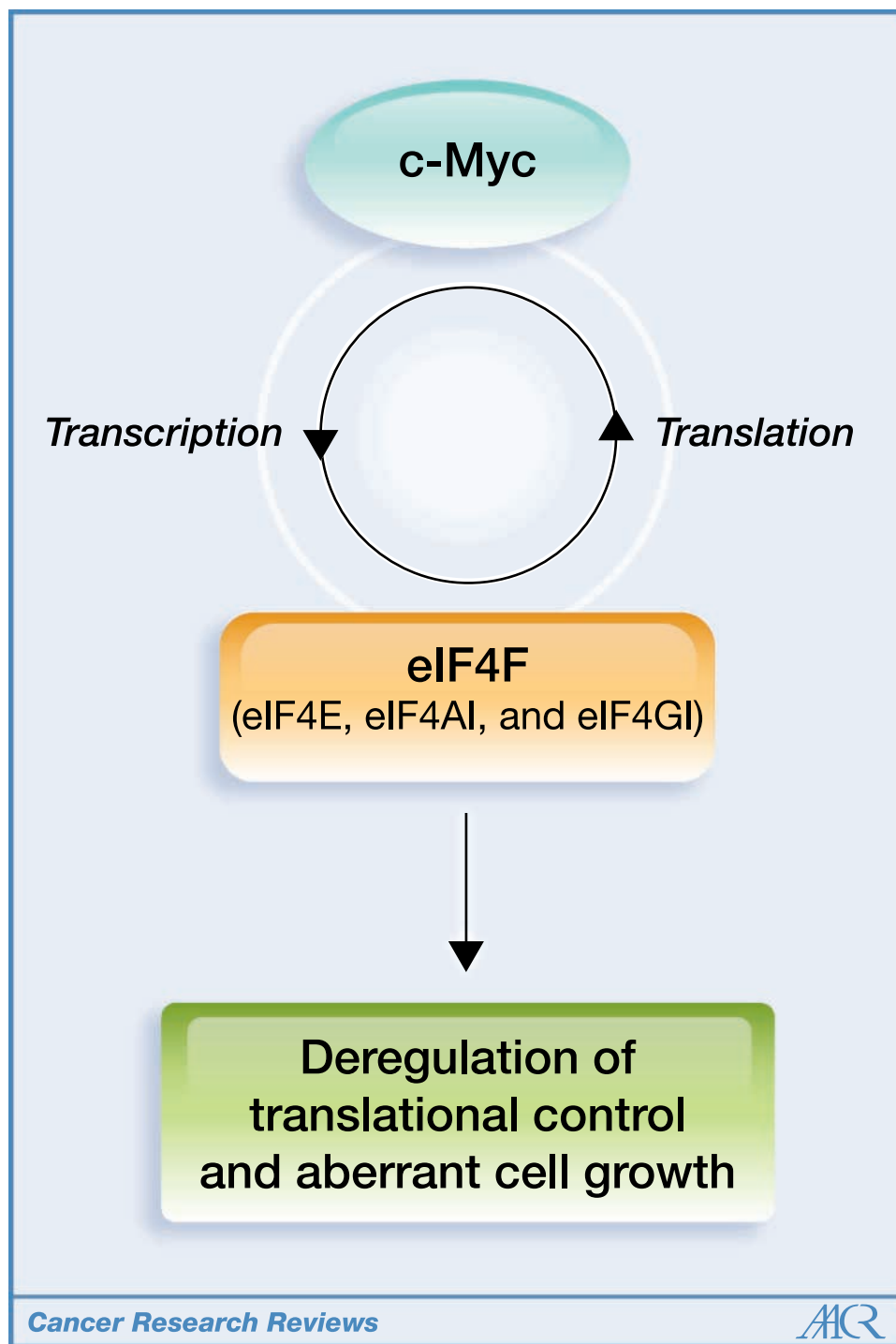


Figure 1. Model outlining a positive feedforward loop between c-Myc and eIF4F. Myc-induced activation of translation via eIF4F provides for increased synthesis and function of Myc. Deregulation of translational control could be one of the molecular mechanisms by which Myc causes tumorigenesis.

this system to assess the expression kinetics of verified (eIF4E and ornithine decarboxylase) and predicted (eIF4A1, eIF4G1) targets of *myc* upon stimulation by 4-OHT in the MycER NIH3T3 cell line. Using a variety of different transcriptional assays (time course of Northern blots, nuclear run-off, chromatin immunoprecipitation), we found that eIF4A1 and eIF4G1 are indeed directly up-regulated by MycER *in vivo* with a corresponding increase in total functional eIF4F complex formation. Conversely, Mad overexpression down-regulated all eIF4F components, both at the RNA and protein levels and in heterologous promoter binding assays. Stimulation by

4-OHT also resulted in an obvious increase in total protein synthesis, as measured by ^{35}S methionine incorporation and total polysome content. These results suggest that an increase in Myc levels affects mRNA translation at least partially through increasing eIF4F levels. This was further corroborated upon examination of tumors derived from the E μ -Myc transgenic mouse model, a mouse model of Burkitt's lymphoma. The expression of eIF4F was also elevated, leading to the idea that increased translation initiation via eIF4F could be one of the molecular mechanisms by which Myc causes tumorigenesis.

The Expression of Myc Is Regulated by eIF4F

One of the consequences of elevated eIF4F levels is not necessarily an increase in bulk protein synthetic output, but rather a more selective increase in the translation of poorly translated mRNAs. These mRNAs are characterized by lengthy, G-C rich, highly structured 5-UTRs and often encode proteins whose levels are to be tightly maintained: anti-apoptotic proteins such as survivin or Mcl-1, or angiogenesis molecules such as VEGF or HIF1- α or growth factors such as cyclin D1, ornithine decarboxylase, and Myc (16). And this is precisely what was seen upon MycER activation: endogenous Myc levels increased as eIF4E levels increased and decreased when eIF4E levels were stably knocked down. This effect was translationally controlled as measured by a shift in the distribution of endogenous *myc* mRNA across polysomal fractions. Not surprisingly, knockdown of eIF4E did not affect global protein synthesis, which is consistent with the notion that eIF4E exerts gene-specific effects. These results imply that Myc-induced activation of translation via eIF4F provides for increased synthesis and function of Myc, establishing a positive feedforward (feedback) loop (more on this below; Fig. 1).

The Myc/eIF4F Feedforward Loop Has Implications in Tumorigenesis

So why turn to the regulation of translation initiation when Myc already has such extensive transcriptional control? For one, deregulation of translation initiation has been widely found in the pathogenesis of cancer. eIF4E was the first translation initiation factor to be documented as an oncogene. eIF4E overexpression has been reported in a wide variety of tumor types, leukemias, lymphomas, and cancers of breast, colon, bladder, lung, prostate, and head and neck (17). Recent work has shown cooperation between eIF4E and Myc in an $E\mu$ -Myc transgenic model, in which overexpression of eIF4E not only accelerates lymphomagenesis, but aggravates the neoplastic phenotype and leads to resistance to chemotherapy (18). Other members of eIF4F have also been implicated in cancer. eIF4AI is overexpressed in melanoma and hepatocellular carcinoma, whereas overexpression of eIF4GI in NIH3T3 fibroblasts allows for anchorage-independent growth *in vitro* and makes them able to form tumors *in vivo* (17). Moreover, it was reported that high levels of eIF4F complex were essential to maintain the malignant phenotype in human mammary epithelial cells (19).

In our study, we observed a coordinated increase in all three subunits of eIF4F when Myc activity is stimulated. This may be required for the cell to gain full functional eIF4F activity. Formation of eIF4F is governed by mTOR signaling. During periods of low growth stimulation or metabolic stress both eIF4E and eIF4A are bound in inactive complexes: eIF4E by eIF4E binding proteins (4E-BPs) and eIF4A by a tumor suppressor gene product Pdc4, both of which compete with eIF4G for binding. This limits eIF4F complex formation and attenuates translation initiation. However, in response to mitogens or nutrient availability, both 4E-BP and Pdc4 become hyperphosphorylated; the former directly by mTOR, causing added negative charges which liberate eIF4E for eIF4G binding (20), and the latter by S6K1, a downstream target of mTOR, targeting

Pdc4 for degradation by the proteasome (21). This suggests the cell might be exploiting an already available signaling network that is sensitive to internal metabolic changes in order to fine-tune Myc's proliferative regulation. By coupling Myc in a feedforward loop to eIF4E is to inextricably link it to mTOR regulation, a way to quickly adapt to changes in cellular environment such that regulation begins with a rapid, initial eIF4E translational response and is followed by a slower Myc transcriptional response. It also means that mutations that permanently activate mTOR signaling will aggravate a Myc-dominated setting, precisely those conditions found in tumor growth. But there is an upside: this tumor progression will be especially sensitive to inhibition of translation initiation. Myc can also regulate mTOR-dependent assembly of eIF4F as it has recently been found to inhibit transcription of tuberous sclerosis 2 (TSC2; ref. 22), a suppressor of mTOR activation. Hence, Myc regulates both production of eIF4F subunits and their assembly.

We have previously found that *Tsc2*^{+/-}*E μ -Myc* lymphomas arise much more rapidly as consequence of constitutively active mTOR activity and are sensitive to rapamycin treatment (23). In this study we observed a similar sensitivity, but noted a pronounced effect on Myc mRNA translation. Also, treatment of the *Tsc2*^{+/-}*E μ -Myc* lymphomas with hippuristanol, a small molecule inhibitor of eIF4A (24), also inhibited Myc expression. Inhibiting translation initiation is emerging as a viable chemotherapeutic approach to cancer and targeting this pathway using anti-sense oligonucleotides to eIF4E (25), or a small molecule inhibitor of eIF4F activity (26), has shown promise in mouse models of cancer.

Concluding Remarks

Myc promotes cell growth by regulating translation initiation, the rate-limiting step in protein synthesis. Activation of Myc is predicted to result in deregulated expression of the translation initiation factors eIF4E, eIF4AI, and eIF4GI, hence elevating eIF4F levels and influencing translation initiation rates. Regulators of transcription and translation that affect Myc function (e.g., Mad1 or antisense approaches) or eIF4F activity (e.g., mTOR) are expected to act as rheostats during normal growth and development to fine tune the outcomes of the Myc/eIF4F feedforward loop. Uncoupling of this loop by mutations or perturbations in the expression of regulatory factors would circumvent these checkpoints and could represent a mechanism to fuel neoplastic growth. Hence, components of the translational machinery, such as eIF4F and signal transduction pathways involved in regulating translation initiation, such as mTOR, represent promising targets for cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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