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# A tRNA with Oncogenic Capacity

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**Overexpression of Brf1, a transcription factor of the RNA polymerase III apparatus, can transform cells in vitro and cause tumor formation in vivo. Marshall et al. (2008) now show that one of the transcriptional products of RNA polymerase III, the initiator tRNA$^{Met}$, mediates this effect, revealing an unexpected role for this tRNA in tumorigenesis.**

RNA polymerase III (pol III) is the largest RNA polymerase with 17 subunits. The products of pol III-driven transcription include the untranslated transcripts tRNA and 5S rRNA that are essential for translation. It is well established that pol III transcription factors are frequently overexpressed in a variety of cancers (White, 2004), but it has been unclear whether pol III is a causative factor in cancer because recurrent mutations in its subunits or associated transcription factors have not been found in tumors.

Reporting in this issue, Marshall et al. (2008) now reveal that overexpression of the pol III-specific transcription factor Brf1 in a variety of cultured cell lines results in their transformation as shown by formation of foci, growth in soft agar, and formation of tumors when injected into mice. To confirm that Brf1 acts through pol III transcription, the authors performed an epistasis experiment in which they reduced the level of another component of the pol III apparatus, RPC39, that interacts with Brf1 to recruit the polymerase to its genetic templates. They partially depleted RPC39 (which is normally present in excess) using small-interfering RNA and saw no effect on tRNA levels or on proliferation of cells with normal levels of Brf1, but they did see abrogation of proliferation induced by overexpression of Brf1. Furthermore, partial depletion of Brf1 in transformed HCT116 colon carcinoma cells lacking the tumor suppressor p53 inhibited foci formation and impaired cell proliferation. Marshall and colleagues then asked whether a component of the translation initiation complex and one of the transcriptional products of pol III, the initiator tRNA$^{Met}$ (tRNA$_i^{Met}$), could cause transformation when overexpressed. They show convincingly that transfection of cells with a plasmid encoding tRNA$_i^{Met}$ caused transformation, whereas overexpression of tRNA$^{Met}$ encoding internal methionines failed to do so. Therefore, increased levels of the translation initiator tRNA$_i^{Met}$ can tip the balance leading to transformation.

Translational control of protein synthesis has recently been recognized as an important route to oncogenic transformation. This emanates primarily from the unraveling of signaling pathways converging on translational control (Bilanges and Stokoe, 2007) and from the discovery that microRNAs, implicated in tumorigenesis, serve as key modulators of mRNA stability and translation (Zhang and Coukos, 2006). Evidently, translational control of mRNAs plays a more prominent role in regulating which proteins are to be synthesized than previously assumed. From a conceptual viewpoint, regulation of protein synthesis at the level of translation makes sense as it permits the cell to respond swiftly to quickly changing conditions. An elaborate control system has been laid down during evolution to achieve this. During embryogenesis, translational control of protein synthesis enables the production of the vast amounts of proteins needed for accelerated growth. This is accompanied by

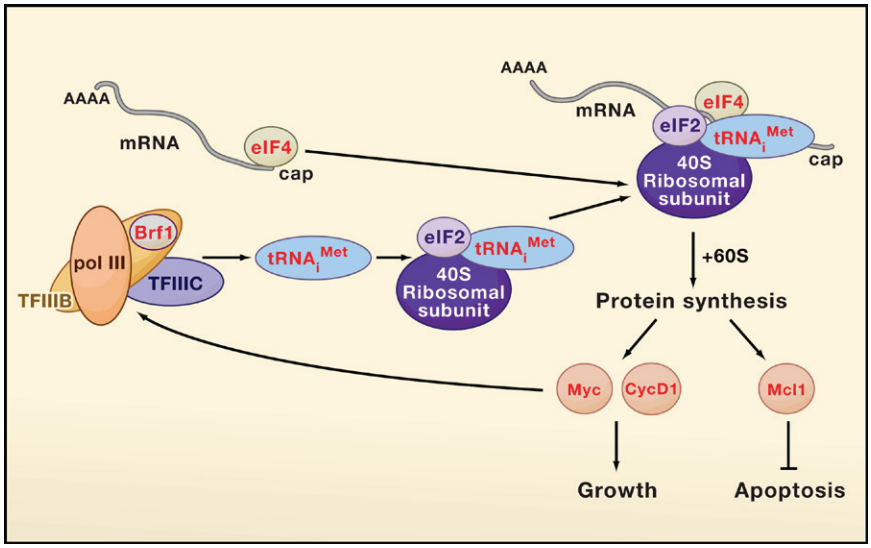


**Figure 1. Translation Initiation as a Control Node in Tumorigenesis**
Translation initiation factors such as eIF2 and eIF4, the structure of mRNAs, and the translation initiator tRNA$_i^{Met}$ are important determinants of the translational control of protein synthesis. Alterations in their expression or activation state can promote tumorigenesis by the selective translation of mRNAs that confer on tumor cells the ability to become transformed and to proliferate. Components labeled in red are known to have oncogenic potential; the pol III-specific transcription factor Brf1 and the pol III transcriptional product tRNA$_i^{Met}$ now can be added to this list (Marshall et al., 2008).

energy metabolism based on aerobic glycolysis rather than oxidative phosphorylation, which allows utilization of a larger fraction of the energy carriers as building blocks for macromolecules. Most tumor cells also exploit this strategy to promote their own growth (Christofk et al., 2008).

The phosphoinositide-3 kinase (PI3K) signaling pathway is best known for its capacity to regulate cell growth and proliferation in response to growth factors and changes in nutrient availability (Manning and Cantley, 2007), thereby playing a pivotal role in the regulation of protein synthesis. Components of the pathway are very frequently mutated in cancer. A critical arm of this pathway regulates cap-dependent translation through release of the initiation factor eIF4E from 4E-BP, which sequesters eIF4E. This permits eIF4E to bind together with other eIF4 subunits to the methyl 7-guanosine triphosphate ($m^7$GTP) cap at the 5′ end of the mRNA (Richter and Sonenberg, 2005). This complex has increased affinity for the preinitiation complex formed by $tRNA_i^{Met}$ and eIF2, other initiation factors, and the 40S ribosomal subunit (Figure 1). Both the binding of eIF4E to the cap and the formation of a complex between $tRNA_i^{Met}$, eIF2, and GTP are critical for the initiation of translation and are subject to extensive control. The initiation factor eIF4E can act as an oncogene when overexpressed or phosphorylated on serine 209 (Wendel et al., 2007). Also, the overexpression of the translation elongation factor eEF1A, which is involved in the recruitment of amino-acylated tRNAs to the ribosome, can cause transformation (Tomlinson et al., 2005), indicating that both translation initiation and elongation are important regulatory nodes with multiple inputs that can alter the efficiency of mRNA translation. The effects are both quantitative and qualitative: overall protein synthesis may be augmented, and the regulatory circuit may permit a shift toward the preferential assembly and translation of mRNA subsets with particular structural features. As a result mRNAs encoding cell-cycle proteins and antiapoptotic proteins (such as Myc, cyclin D1, and Mcl1) can be preferentially translated, providing an explanation for why overexpression or phosphorylation of distinct initiation factors or overexpression of $tRNA_i^{Met}$ can promote transformation (Figure 1).

A tumor cell can escape from growth control through a range of genetic and epigenetic alterations. Almost invariably, mutations are found in the pathways that respond to or influence nutrient supply thereby providing energy as well as the building blocks needed for protein, lipid, and nucleic acid synthesis. But this is also the Achilles heel of tumors and is exploited clinically through targeting of the tumor's vasculature (Ferrara and Kerbel, 2005). Recently, new strategies have been proposed to specifically attack the energy metabolism of tumor cells by inhibiting the M2 variant of pyruvate kinase. This variant is selectively expressed by many tumor cells and blocking it forces tumors to shift from aerobic glycolysis to oxidative phosphorylation, thereby limiting tumor growth (Christofk et al., 2008).

As Marshall et al. (2008) elegantly show in their new study, impairing cap-dependent translation initiation might be another approach for killing tumors by abrogating synthesis of proteins that are critical for their growth and proliferation. In this context, partial inhibition of pol III-dependent transcription is of particular interest. Inhibiting an oncogene by targeting different pathways that converge on its expression or activity has the attraction that the toxic effects are limited while an effective reduction in oncogene function can still be achieved. Inhibition of the PI3K signaling pathway is one way to impair the synthesis of specific subsets of proteins. So far, inhibition of this pathway has focused on blocking the activity of several key components such as PI3K, Akt, and the mTOR signaling complex. Inhibition of these and other components in the pathway can be relatively toxic or turn out to be ineffective as complex feedback loops can lead to compensatory activation of other components. Inhibiting a critical arm of the PI3K signaling pathway far downstream is therefore very attractive, especially if this can be achieved through several independent routes. The Marshall et al. study presents a new option for such an approach by limiting formation of the translation preinitiation complex by blocking synthesis of $tRNA_i^{Met}$. It will be particularly intriguing to explore combinations of inhibitors that target $tRNA_i^{Met}$ by impairing pol III transcription, eIF4E-dependent translation initiation, and pyruvate M2 kinase. Using combinations of inhibitors targeting different processes and pathways may provide a synergy that will boost the therapeutic index of the drug combination. Time will tell whether this optimism is justified.

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