



Gene Transcription

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Transcription by RNA polymerases (pols) I and III is often abnormal in tumours. These pils make rRNA and tRNA, which provide about 95% of a cell's RNA content. The rates of transcription by pils I and III are major determinants of the capacity for cellular growth and so deregulation can have serious consequences. The goal of our work is to understand how and why deregulation occurs.

Transcription by pils I and III is stimulated by oncogene products

The growth potential of cells is influenced strongly by their content of ribosomes, the sites of protein synthesis. This reflects the fact that protein constitutes 80 to 90% of a cell's dry mass. The first step in ribosome production is the synthesis of rRNA by pils I and III. This is stimulated directly by c-Myc, a proto-oncogene product that is overexpressed in many types of tumour, including breast and colon cancers. Recent evidence indicates that the ability of c-Myc to stimulate pil III transcription can make a substantial contribution to its transforming activity in cell culture and mouse models. Several oncogenic kinases also stimulate tRNA and rRNA production by phosphorylating transcription factors that are required by pils I and III. An example is the mitogen-activated protein kinase Erk, which is abnormally active in about 30% of human cancers.

The consequences of stimulating pil III transcription

TFIIIB is a transcription factor that recruits pil III to its

genetic templates, including genes encoding tRNA and 5S rRNA that are essential for cell growth. TFIIIB is expressed at elevated levels in some cases of cervical cancer. We tested the impact on cells of raising TFIIIB expression and found that it can be unexpectedly profound. Changes provoked by elevating TFIIIB levels can include accelerated cell growth and proliferation, as well as oncogenic transformation. To our surprise, these same effects can be achieved by overexpressing an individual pil III product, the tRNA_i^{Met} that is required for initiation of polypeptide synthesis. Raising expression of tRNA_i^{Met} stimulated proliferation of immortalised mouse embryonic fibroblasts and transformed them to a state in which they formed foci in culture and tumours in mice (Fig. 1). These responses were not seen with a different tRNA. Levels of tRNA_i^{Met} that are sufficient to promote proliferation and transformation do not exceed

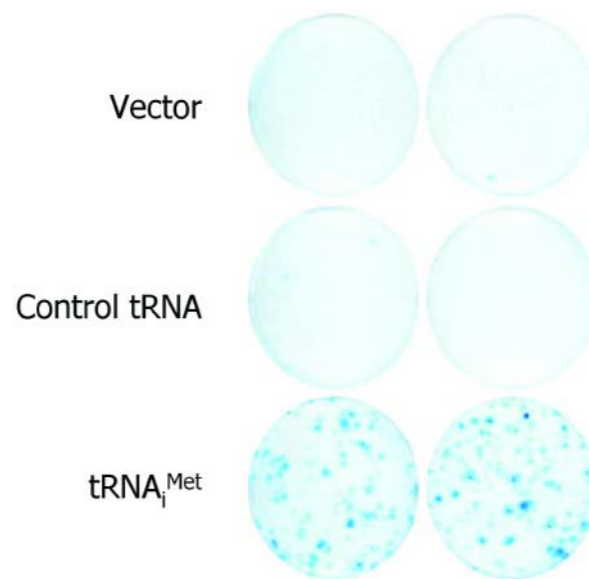


Fig. 1: Raising tRNA_i^{Met} expression can transform cells.

what is encountered *in vivo* in cancer cells. These observations convince us that the production of tRNA by pil III should be regarded as relevant to the biology of tumours.

We are keen to discover how tRNA_i^{Met} can transform cells. Its overexpression was found to increase polypeptide synthesis substantially, which suggests that it is rate-limiting for translation under the conditions of our assays. A significant increase in protein synthesis is expected to promote cell growth. As well as raising the global rate of translation, elevated tRNA_i^{Met} levels also cause selective changes in the expression of individual polypeptides. For example, it triggers the translational induction of c-Myc. This offers the possibility of a positive feedback loop, as c-Myc binds to TFIIIB and stimulates pil III transcription of tRNA genes. As c-Myc is oncogenic, its induction may be responsible for the transforming effect of tRNA_i^{Met}. We are currently testing this and other possibilities.

Tumour suppressors inhibit transcription by pils I and III

In healthy cells, transcription by pils I and III is inhibited by several tumour suppressors, including p53, Arf, RB and PTEN. This can provide a potent mechanism for growth restraint. Most, if not all cancers are expected to lose the function of one or more of these tumour suppressors, allowing the output of pils I and III to rise. This release from repression may be just as important as the activating effects of oncogene products in contributing to the elevated expression of pil I and pil III products in tumours. RB and p53 both bind directly to TFIIIB and prevent it functioning (Fig. 2). The fact that TFIIIB is targeted directly by these cardinal tumour suppressors provides a clear indication of the importance of restraining pil III output.

The observations that RB represses tRNA gene transcription and that translation can be limited by tRNA_i^{Met} availability lead to the prediction that RB should be able to inhibit translation. We confirmed this, in collaboration with Robb MacLellan's group at UCLA, by measuring the effect of RB on polypeptide synthesis in primary cardiomyocytes. Using this post-mitotic cell type avoided the complication of cell cycle changes. RB was found to block the protein synthesis that is required for cardiomyocytes to increase in size in response to growth stimuli. Using a combination of knockout and transgenic mice, we also showed that cyclin D2 controls the ability of RB to bind TFIIIB and repress tRNA expression when cardiomyocytes are stimulated to grow in the heart.

A potent pil III repressor

Maf1 is a repressor of pil III transcription that was discovered in yeast, where it inhibits pil III output in response to a broad range of stresses, including DNA damage and nutrient starvation. Mammals contain an

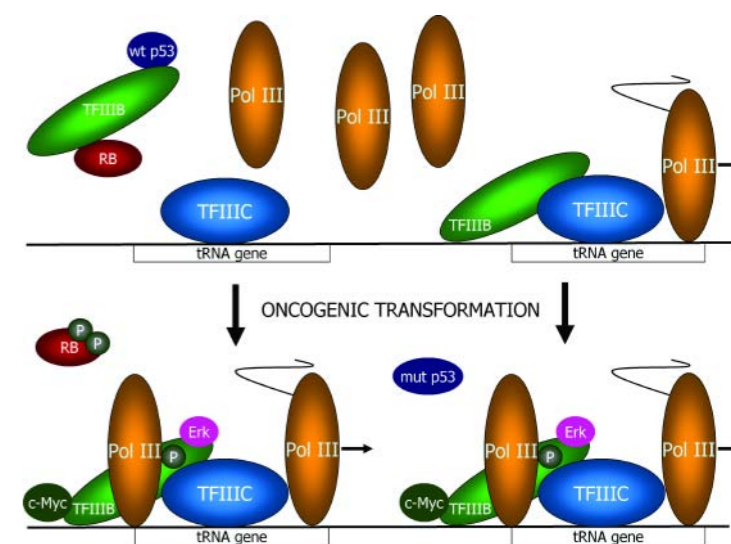


Fig. 2: Molecular changes that can increase pil III transcription in transformed cells.

orthologous factor that displays only limited blocks of sequence conservation to yeast Maf1, but may function in a similar manner. It has been shown to influence cell morphology and the anchorage-dependence of tumour cells. We have found that mammalian Maf1 binds tRNA and 5S rRNA genes and suppresses their expression. This reflects direct interactions of Maf1 with pil III and TFIIIB. We want to know how these interactions inhibit transcription and when they become important; is Maf1 activated by DNA damage or other stresses in human cells? We have evidence that it is required for pil III responsiveness to mTOR.

The mTOR pathway controls production of rRNA and tRNA

The mTOR signal transduction pathway regulates cell growth and proliferation. It functions primarily through changes to the protein synthesis machinery, stimulating translation initiation and ribosome accumulation. Production of rRNA and tRNA by pils I and III is responsive to the mTOR pathway. When cells are deprived of nutrients or growth factors, mTOR signalling is blocked. Many types of cancer have lost control of the mTOR pathway, which remains active regardless of conditions that would normally switch it off. Accordingly, mTOR inhibitors are in clinical trials to test for anti-cancer efficacy. We are studying how mTOR activates pil III transcription. It seems that this involves direct regulation of pil III transcription factors by mTOR in the nucleus. In contrast, synthesis of rRNA by pil I responds indirectly to the same signals, via kinases that lie downstream of mTOR.

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