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Transcription by RNA polymerases (pols) I and III is often abnormal in tumours. These pols make rRNA and tRNA, which provide approximately 95 percent of a cell's RNA content. The rates of transcription by pols 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.

Characterising the pol III transcriptome

In collaboration with Keji Zhao's lab at NIH and Kevin Struhl's lab at Harvard, we have determined which genes are bound by pol III and its associated transcription factors, TFIIB and TFIIC (Barski *et al.*, Nature Struct. Mol. Biol. 2010; 17: 629, Moqtaderi *et al.*, Nature Struct. Mol. Biol. 2010; 17: 635). This identified some novel targets. In addition, it revealed that many established pol III targets, such as tRNA genes, are transcribed in a cell type-dependent manner. This was unexpected, because tRNA genes were thought to be ubiquitously active. We found that the chromatin associated with active tRNA genes is modified differently from the chromatin at inactive genes. Extensive lists of chromatin acetylation and methylation events were investigated for their correlation with gene activity. In most cases, chromatin modifications that correlate with pol III transcriptional activity are similar to those found at genes transcribed by pol II, although there are some exceptions. Our data suggest that pol III transcription may be regulated by many of the established chromatin acetylases and methylases, some of which are implicated in cancer.

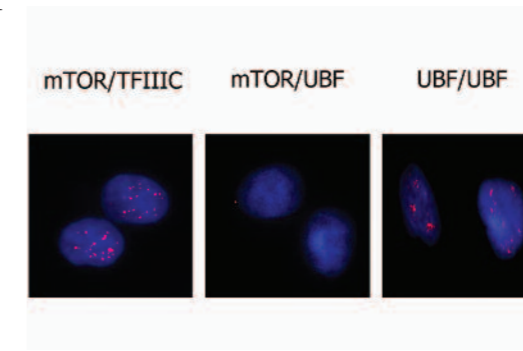
We were surprised to find pol II and its associated machinery just upstream of many pol III-transcribed genes, even in the absence of recognisable pol II target genes; the pol II at these sites does not seem to be transcribing but its presence correlates strongly with activity of the downstream pol III-transcribed gene. The significance of these intriguing discoveries remains unclear.

Dr1 and Maf1 are repressors of pol III transcription

In healthy cells, pol III transcription is inhibited by several tumour suppressors including p53, Arf, RB and PTEN (Marshall & White, Nature Rev. Cancer 2008; 8: 911). 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 pols I and III to rise.

Dr1 is a transcription factor with a well-characterised role in regulating pol II transcription of genes encoding protein. Using a biochemical approach, I found some years ago that Dr1 can also repress transcription by pol III but not by pol I (White *et al.*, Science 1994; 266: 448). Like p53 and RB, Dr1 binds and inhibits TFIIB, a factor required to recruit pol III to its target genes. The mechanism of control now seems to be more complicated than we previously appreciated on the basis of biochemical assays. We can detect Dr1 *in vivo* at all the pol III-transcribed genes we have tested but only some of them appear to be responsive to it (Kantidakis & White, Nucleic Acids Res. 2010; 38: 1228). For example, Dr1 represses tRNA but not 5S rRNA expression, although it binds to both sets of genes. We do not yet understand the basis of this selectivity. Around half of all neuroblastomas have deleted Dr1, whereas other cases find alternative ways of reducing Dr1 levels. Our data suggest that this will result in elevated tRNA expression, which may contribute to aberrant growth and other features of neuroblastoma pathology.

Figure 1
Proximity ligation assay, in which fluorescent spots can be detected if two proteins are close together in fixed cells. Endogenous mTOR is found in proximity to endogenous TFIIC but not to the abundant pol I-specific transcription factor UBF. Co-staining of DNA with DAPI (blue) demonstrates that the mTOR/TFIIC interaction occurs within the nucleus. A control experiment confirms that UBF can be detected by this approach.



Like Dr1, Maf1 is a repressor of pol III but not pol I transcription. It was discovered in yeast, where it inhibits pol III output in response to a broad range of stresses including DNA damage and nutrient starvation. Mammals contain an 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 suppress anchorage-independent growth of tumour cells. We and several other groups established that mammalian Maf1 binds tRNA and 5S rRNA genes and suppresses their expression (Goodfellow *et al.*, J. Mol. Biol. 2008; 378: 481). This reflects direct interactions of Maf1 with both pol III and TFIIB.

mTOR targets tRNA and 5S rRNA genes and releases them from repression

We found that Maf1 is phosphorylated directly by the kinase mTOR (Kantidakis *et al.*, (2010) Proc. Natl. Acad. Sci. USA 2010; 107: 11823). This inactivates Maf1 and thereby releases pol III and TFIIB from repression, allowing elevated synthesis of tRNA and 5S rRNA. Endogenous mTOR is found at pol III-transcribed genes *in vivo*. We believe that it is recruited to these sites through interaction with TFIIC, the DNA-binding

factor that binds directly to most pol III promoters. We used proximity ligation assays to show that TFIIC associates with mTOR in cell nuclei (Fig. 1). Raptor is a substrate recognition polypeptide that binds to mTOR and directs it to target proteins with TOR-signalling motifs. We found such a motif in TFIIC and showed that it is required for the interaction with mTOR. We therefore believe that mTOR is recruited to pol III-transcribed genes through raptor, which binds to TFIIC at promoters; this allows mTOR to stimulate tRNA and 5S rRNA synthesis by phosphorylating and inactivating the repressor Maf1 (Fig. 2).

The best-characterised role of mTOR is in the control of protein production by the translation machinery. A role in regulating pol III transcription is consistent with this, since tRNA and 5S rRNA are essential for protein synthesis. Furthermore, signalling by mTOR is sensitive to amino acid availability and so it seems logical that this controls levels of tRNA, which serves to deliver amino acids to ribosomes. Growth factors activate mTOR via a signalling pathway that includes Ras, PI3 kinases and Akt, all of which are products of proto-oncogenes. The tumour suppressor PTEN antagonises these signals. Many cancers carry mutations that cause inappropriate activation of this pathway, so that it remains active regardless of conditions that would normally switch it off. Accordingly, mTOR inhibitors are in clinical trials to test for anti-cancer efficacy. Such inhibitors can suppress pol III transcription by maintaining Maf1 in its dephosphorylated, functional state.

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Figure 2
Transcription of tRNA and 5S rRNA genes by pol III is activated by mTOR in response to signalling from growth factors via Ras, PI3 kinase and Akt; the tumour suppressor PTEN antagonises this pathway. TFIIC binds promoter sequences within pol III-transcribed genes and recruits raptor and hence mTOR, which then phosphorylates and inactivates the pol III repressor Maf1.

