The ABcs of mitochondrial transcription

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Two forms of a transcription factor, similar in sequence to an rRNA modifying enzyme, have been identified as the missing components of the transcription initiation machinery for human mitochondrial DNA (mtDNA). Transcription from mtDNA promoters can now be reconstituted *in vitro* with three recombinant proteins: mitochondrial transcription factors A and B and the core mitochondrial RNA polymerase.

Human mtDNA is a small circular genome encoding a handful of proteins that are essential components of the enzyme complexes of oxidative phosphorylation. Mutations in mtDNA cause a wide spectrum of multisystem human disorders with varying degrees of tissue specificity and severity. Transcription of mtDNA-encoded genes is initiated at two promoters on the so-called heavy and light strands of the molecule, and the resultant polycistronic transcripts are processed to produce mature rRNAs, tRNAs and mRNAs[1]. A transcript generated from the light-strand promoter is also necessary to prime mtDNA replication, functionally coupling mitochondrial gene expression with genome maintenance[2].

This comparatively simple transcription system requires an activator, mitochondrial transcription factor A (TFAM) and a core RNA polymerase; however, specific transcription initiation cannot be reconstituted from these components alone, suggesting that additional factors are necessary. On page 289 of this issue, Maria Falkenberg and colleagues[3] report on the identification of the missing factor and the development of an *in vitro* system that can be used to investigate the control of mitochondrial transcription and its coupling to mtDNA replication.

Yeast versus man

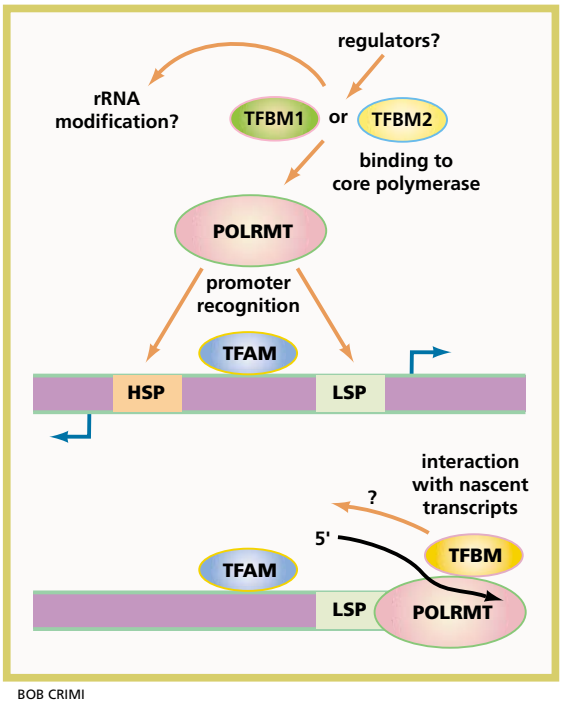
In the yeast *Saccharomyces cerevisiae*, specific mitochondrial transcription requires only two components[4]: a core mitochondrial RNA polymerase (Rpo41), which shares sequence similarity with bacteriophage RNA polymerases[5], and a single transcription factor, sc-mtTFB (Mtf1), which shares some sequence similarity with, but is functionally distinct from, bacterial σ factors[6]. The TFAM protein, which is essential for mammalian mtDNA transcription and genome maintenance[7], is a member of the high mobility group (HMG)–box family of DNA-binding proteins, and has no sequence similarity with the yeast transcription factor. The yeast ortholog of TFAM, Abf2p, has an essential role in mtDNA packaging[8], but does not seem to be important in mitochondrial transcription. This difference in function between the yeast and human proteins has been ascribed to a carboxy-terminal extension in mammalian proteins, which is absent in Abf2p[9].

Attempting to bridge the gap between the yeast and human studies, Bogenhagen and colleagues[10] carried out a series of studies of *Xenopus laevis* in which they identified a biochemical activity distinct from TFAM that, in combination with the core RNA polymerase, could support basal mitochondrial transcription. Transcription in this system could be further activated by the ortholog of human TFAM. These observations lent strong support to the notion that an ortholog of Mtf1 might exist in vertebrates.

The missing transcription factor

Using PSI-BLAST and the Mtf1 ortholog from the fission yeast *Schizosaccharomyces pombe* as the initial query sequence to search the database of the National Center for Biotechnology Information, Falkenberg *et al.*[3] identified two human orthologs of Mtf1, which they called TFBM1 and TFBM2 (ref. 3). Using a similar strategy, Shadel and colleagues[11] also identified a transcription factor they called h-mtTFB, which is identical to TFBM1.

To show that these proteins are *bona fide* mitochondrial transcription factors, Falkenberg *et al.*[3] purified recombinant versions of both TFBM proteins—TFAM and the mitochondrial RNA polymerase—and demonstrated specific transcription from both heavy- and light-strand human mtDNA promoters in the presence of either of the TFBM proteins, TFAM and the core polymerase. Interestingly, TFBM2 was an order of magnitude more active than TFBM1 in the transcription assay.



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Time to transcribe. Schematic drawing of transcription initiation in human mtDNA showing potential roles of the TFBM transcription factors. Transcription initiates from two promoters (LSP, HSP). TFAM promotes bi-directional transcription by binding DNA upstream of the promoters and unwinding the DNA template. TFB1M or 2 bind the core polymerase (POLRMT) in a 1:1 stoichiometry. Either of the TFBM factors, in combination with TFAM and POLRMT, is sufficient to initiate transcription from either promoter, although TFB2M is an order of magnitude more effective. The factors that might regulate this process are unknown. The TFBMs share strong sequence homology with bacterial rRNA dimethylases; however, it is not known whether the TFBMs also play a role in rRNA modification, or whether the RNA binding motif is important in interacting with or subsequent processing of nascent transcripts.

TFBMs: bifunctional proteins?

Remarkably, the TFBM transcription factors share a striking sequence similarity with a family of RNA modifying enzymes, the rRNA dimethyl transferases. While the studies of the human TFBMs were in progress, the crystal structure of sc-mtTFB was solved[12], and a search of the Protein Databank determined that it is part of a large family of DNA/RNA methyltransferases, and not structurally related to bacterial σ factors, as had initially been thought. In particular, a C-terminal helix motif in sc-mtTFB has so far only been observed in the rRNA methyltransferase ErmC′ from *Bacillus subtilis*[12].

These observations raise the possibility that the TFBM family members are bifunctional proteins that have roles both in mitochondrial transcription initiation and RNA modification. The methyl donor of the rRNA dimethyl transferases is *S*-adenosyl methionine (SAM). The crystal stucture of sc-mtTFB shows that many, but not all, of the amino-acid residues that bind SAM have been conserved[12], and human TFBM1 has been shown to bind SAM *in vitro*[11]. Specific methyl transferase activity has, however, not been demonstrated[3], and SAM is not required to initiate transcription in yeast. So, although the jury is still out on this issue, it seems probable that a methyl transferase protein has been co-opted for a different role.

This may turn out to be a common theme in mitochondrial nucleic acid metabolism. The small accessory subunit of the mitochondrial γDNA polymerase shares sequence similarity with another family of RNA-modifying enzymes, the aminoacyl tRNA synthetases, and this is thought to relate to its function in binding to a tRNA-like structure near the origin of heavy-strand replication[13,14].

What, then, is the function of TFBM, and why has an RNA modifying protein been co-opted as a transcription factor? It seems probable that TFBM has a role not in promoter recognition, but rather in activating the core RNA polymerase with which it heterodimerizes. It is possible that the RNA binding function of the methyl transferase protein domain is useful in this context, not in modifying RNA *per se*, but in interacting with or stabilizing nascent transcripts.

The TFBM1 protein of mouse and human share significantly more sequence identity than do human TFBM1 and TFBM2, suggesting that the two proteins are the result of a gene duplication event early in mammalian evolution. Why do mammals have two factors that differ by about an order of magnitude in the strength of their transcriptional activation? One obvious possibility is that the two factors initiate transcription from specific promoters, but the available data do not support this suggestion. Another possibility is that regulation of mitochondrial gene expression in mammals requires the elaboration of different tissue-specific pathways, reflecting different metabolic demands. If, however, tissue-specific differences in mitochondrial transcription or replication are part of this equation, then this does not appear in the patterns of transcription of the two TFBM genes, which seem to be virtually identical by northern-blot analysis. It seems more likely that at least partially non-overlapping functions of both factors are important in all tissues in which they are expressed. This might involve the decision to process the nascent transcripts for DNA replication primers, or continue productive transcription of the light strand.

The future

The basic components of the mitochondrial transcriptional machinery in mammals now seem to be in hand, but this only marks the end of the beginning. The really crucial questions concerning the control of mitochondrial transcription and DNA replication, and the regulation of mtDNA copy number, remain to be answered. The mitochondrial genome has been pared down during evolution to the barest of essentials, and the investigation of its fundamental molecular biology continues to uncover surprises and evolutionary insights. It is to be hoped that some of the biological insights generated during the course of these studies will find application in the treatment of patients with mitochondrial-DNA diseases. □

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Saving sulfur

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Cadmium, like many heavy metals, is non-essential and toxic to all organisms. A new study shows that yeast exposed to cadmium cleverly reprogram the transcriptome and proteome to protect themselves from its toxic effects.

The mechanisms of cadmium toxicity and the response of the budding yeast *Saccharomyces cerevisiae* to cadmium exposure have been the focus a great deal of work. The focus on yeast is owing to its well-characterized transcriptome and proteome and to the availability of extremely powerful and facile genetic techniques. Although the precise mechanism of cadmium toxicity is not yet understood, it is known to bind to thiol groups on proteins, to deplete cellular glutathione and to generate oxidative stress. We have also learned a great deal about the mechanisms yeast use to protect themselves from this