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Previews

Something Old, Something New, Something Borrowed ...

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Two mammalian mitochondrial transcription factors (TFB1M and TFB2M) share homology with universally expressed dimethyltransferases that modify conserved adenines in the small ribosomal subunit rRNA. Work in this issue (Metodiev et al., 2009) shows that loss of TFB1M abolishes mitochondrial ribosome assembly without affecting mitochondrial transcription.

Mammalian mitochondria have retained a small genome, a remnant from their α-proteobacterial ancestor, that codes for a handful of essential proteins in the respiratory chain, and for the tRNAs and rRNAs necessary for their translation. The factors necessary for the maintenance and expression of these mitochondrially encoded genes are encoded in the nucleus and must be imported into the mitochondria. Belying their bacterial origins, some of these proteins, including the RNA polymerase (POLRMT) responsible for transcribing the mitochondrially encoded genes, clearly trace back to bacteriophage ancestors (Shutt and Gray, 2006).

But while the phage enzyme can work on its own, this is not so for the mitochondrial polymerase. It requires a couple of additional transcription factors. The first of these to be identified, called TFAM, belongs to the family of high-mobility group box proteins and works by binding to specific sequences upstream of the mitochondrial promoters, presumably altering DNA topology (Fisher and Clayton, 1985). The other factors, called TFB1M and TFB2M, belong to a completely different protein family and were identified by homology to the only known mitochondrial transcription factor in yeast (Falkenberg et al., 2002). They heterodimerize with POLRMT, and faithful transcription from mitochondrial promoters can be reconstituted in vitro with only POLRMT, TFAM, and either TFBM factor (Falkenberg et al., 2002). What was unexpected was the discovery that both TFB1M and TFB2M share significant homology with a dimethyltransferase that is highly conserved in all three domains of life (archea, eubacteria, and eukaryotes) and that is responsible for dimethylating two conserved adenine residues at the extreme 3′ end of the 12S rRNA in the small ribosomal subunit. This observation suggested that the mammalian proteins could be bifunctional, with roles in both mitochondrial transcription and translation (Shadel, 2008). If so, it might provide a mechanism to ensure a balance between the synthesis of mitochondrial mRNAs on the one hand and the availability of the translational machinery on the other.

The activity of one the factors,TFB1M, has also been suggested to modify the expression of a maternally inherited form of nonsyndromic or aminoglycoside-induced deafness caused by an A1555G mutation in the 12S rRNA, as a polymorphism that maps near the TF1BM gene is associated with reduced penetrance of the deafness phenotype (Bykhovskaya et al., 2004).

But why have two of these proteins? Does one play a role in transcription and the other in translation, or do they have overlapping functions? To resolve this issue, Larsson and coworkers knocked out the TFB1M gene in the mouse (Metodiev et al., 2009). Because a germline knockout was embryonic lethal, to study the gene's physiological function they used Cre-lox technology to create a mouse with a heart- and skeletal muscle-specific TFB1M deletion. These animals survived, but all developed a cardiomyopathy and died before 24 weeks of age. There was a progressive decline in respiratory chain activity and assembly of the respiratory chain complexes in their hearts and an increase in mitochondrial mass, a typical response to respiratory chain dysfunction in patients with mitochondrial disease. Surprisingly, the complete loss of TFB1M had little effect on the steady-state levels of mitochondrial transcripts, and in fact a substantial increase in de novo transcription could be detected in an in vitro assay in isolated mitochondria. Further in vitro experiments supported the lack of transcriptional initiation activity of TFB1M, and confirmed the previous finding that TFB2M is several orders of magnitude more active in promoting transcription from mitochondrial promoters (Falkenberg et al., 2002). In contrast, the loss of TFB1M abolished mitochondrial translation in heart mitochondria, and this correlated with a complete loss of dimethylation of the adenines in the 12s rRNA, a decrease in the steady-state level of small subunit ribosomal proteins, and impaired stability of the small ribosomal subunit. These results quite clearly demonstrate that the TFBM factors have independent, nonoverlapping functions in vivo: TFB1M acts as a nonredundant dimethyltransferase to modify the 12s rRNA, which leaves TFB2M with an essential role as a transcriptional activator.

While this study clarifies the independent functions of TFB1M and TFB2M, it leaves open the question of why dimethylation of the adenine residues on the 12S rRNA by TFB1M is so important to mammalian mitochondrial ribosome biogenesis, while the deletion of the bacterial homologue (KsgA), which prevents modification of the diadenine residues on the 16S rRNA, is almost without effect (Xu et al., 2008). It also does not address the mechanism for destabilization of the small ribosomal subunit. In bacteria, translation initiation starts with the recruitment of the initiation factors, mRNA, and the f-met initiator tRNA on the small ribosomal subunit; and ends with recruitment of the large

ribosomal subunit. It has been speculated that modification of the bacterial 16S rRNA acts as a checkpoint, controlling recruitment of mature, fully assembled small ribosomal subunits into the translation cycle (Xu et al., 2008).

Although the translational apparatus in mitochondria resembles that in prokaryotes, one crucial difference between bacterial and mammalian mitochondria is the lack of a significant 5′ untranslated regions in mitochondrial mRNAs (analogous to Shine-Dalgarno sequences in bacteria) that would permit registration of the start codon with the initiator tRNA on the small ribosomal subunit. Mitochondrial ribosomes are also unique from both bacterial and eukaryotic cytosolic ribosomes in that they have a much higher protein to rRNA ratio, and the rRNAs have been shortened by the loss of specific domains (Shoubridge and Sasarman, 2007). Structural studies reveal a unique gate-like structure at the mRNA entry site that has been suggested to be important for recruitment of the leaderless mRNAs (Sharma et al., 2003), and there is some evidence to suggest that, like some leaderless bacterial mRNAs, mammalian mRNAs may initiate translation on fully assembled 55S ribosomes (Shoubridge and Sasarman, 2007). One possibility is that the dimethylation of the small 12S rRNA has taken on a new importance in mammals to regulate the interaction between the small and large ribosomal subunits to ensure a supply of fully assembled ribosomes for the initiation of mitochondrial translation (Figure 1).

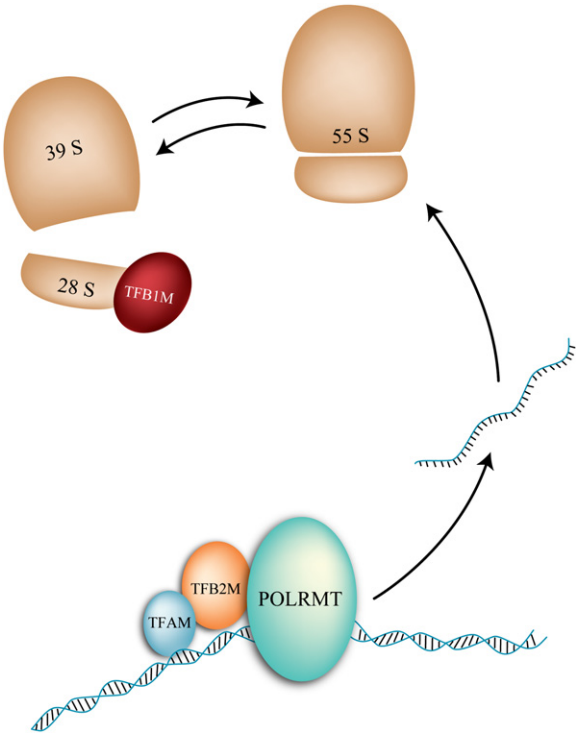

Figure 1. TFB1M Dimethylates Adenine Residues in the 12S rRNA in the Small Ribosomal Subunit, Possibly Regulating the Assembly of the 55S Mitochondrial Ribosome

TFB2M functions as a mitochondrial DNA transcriptional activator in a complex with TFAM and POLRMT. The concerted action of both TFBM factors functionally couples mitochondrial transcription and translation.

However, as the present study indicates, old proteins can evolve new functions or borrow activities for other functions that may not be immediately obvious. For instance, KsgA has recently been shown to possess glycosylase/AP lyase activity (Zhang-Akiyama et al., 2009), and the yeast orthologue Dim1 is essential for cell growth, not because of its methyltransferase activity, but because of its pre-18S rRNA processing activity (Lafontaine et al., 1998). Could TBF1M have an essential function in mammalian cells other than as a mitochondrial dimethyltransferase?

REFERENCES

Bykhovskaya, Y., Mengesha, E., Wang, D., Yang, H., Estivill, X., Shohat, M., and Fischel-Ghodsian, N. (2004). Mol. Genet. Metab. *82*, 27–32.

Falkenberg, M., Gaspari, M., Rantanen, A., Trifunovic, A., Larsson, N.G., and Gustafsson, C.M. (2002). Nat. Genet. *31*, 289–294.

Fisher, R.P., and Clayton, D.A. (1985). J. Biol. Chem. *260*, 11330–11338.

Lafontaine, D.L., Preiss, T., and Tollervey, D. (1998). Mol. Cell. Biol. *18*, 2360–2370.

Metodiev, M.D., Lesko, N., Park, C.B., Cámara, Y., Shi, Y., Wibom, R., Hultenby, K., Gustafsson, C.M., and Larsson, N.G. (2009). Cell Metab. *9*, this issue, 386–397.

Shadel, G.S. (2008). Am. J. Pathol. *172*, 1445–1456.

Sharma, M.R., Koc, E.C., Datta, P.P., Booth, T.M., Spremulli, L.L., and Agrawal, R.K. (2003). Cell *115*, 97–108.

Shoubridge, E.A., and Sasarman, F. (2007). In Translational control in biology and medicine, M.B. Mathews, N. Sonenberg, and J.W.B. Hershey, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 775–802.

Shutt, T.E., and Gray, M.W. (2006). Trends Genet. *22*, 90–95.

Xu, Z., O'Farrell, H.C., Rife, J.P., and Culver, G.M. (2008). Nat. Struct. Mol. Biol. *15*, 534–536.

Zhang-Akiyama, Q.M., Morinaga, H., Kikuchi, M., Yonekura, S.I., Sugiyama, H., Yamamoto, K., and Yonei, S. (2009). Nucleic Acids Res. Published online February 17, 2009. 10.1093/nar/gkp057.