A little less leads to lots more

Jonathan R Warner

Mammalian cells have both cytoplasmic and mitochondrial ribosomes, which have long been considered to operate completely independently. However, a new report shows that after heat shock, MRPL18, a human mitochondrial ribosomal protein, binds to cytoplasmic ribosomes to influence translation of heat-shock mRNAs.

Approximately 160 human genes encode ribosomal proteins (RPs). Although these are destined to form part of either cytoplasmic or mitochondrial ribosomes, all are translated in the cytoplasm. Thus, sorting to the right destination is a potential problem. Nuclear-localization signals and chaperones[1] target the RPs intended for cytoplasmic ribosomes to the nucleus and then to the nucleolus, where they are assembled onto the nucleolar rRNA transcripts. Most of the mitochondrial RPs (mRPs) are targeted by mitochondrial-localization sequences[2], which are cleaved during their assembly with the rRNA transcripts of the mitochondrial genome to form the mitochondrial ribosomes, which are responsible for the translation of just 13 mitochondrial gene products (reviewed in ref. 3).

Many stresses, such as heat shock, lead to a rapid reduction in the translation of new proteins[4]. Yet it is important for the cell that during stress certain classes of proteins, such as the heat-shock proteins, accumulate. This is partly accomplished through the induction of transcription of new mRNAs encoding such proteins, but it is now apparent that the regulation of translation of these mRNAs plays an important part as well.

The exciting discovery in a new report by Zhang *et al.*[5] in this issue is that in response to heat shock, the phosphorylation of translational initiation factor eIF2α causes cytoplasmic ribosomes of HeLa cells to initiate translation of the mRNA encoding the mitochondrial ribosomal protein MRPL18 at an unusual CUG codon to generate the protein MRPL18(cyto), which lacks the mitochondrial signal sequence and remains in the cytoplasm. This truncated MRPL18(cyto) is itself phosphorylated by the heat shock–activated Lyn kinase, thus leading to its association with cytoplasmic ribosomes. These hybrid ribosomes are activated for cap-independent translation of mRNAs encoding heat-shock proteins such as HSP70 and HSP40 (**Fig. 1**). The physiological importance of this phenomenon is established by the observation that lack of MRPL18(cyto) prevents the thermotolerance that cells develop when initially exposed to a mild heat shock.

What is particularly appealing about the story developed by Zhang *et al.*[5] is that, in order to stimulate the translation of HSP70-encoding mRNAs under conditions in which translation of most mRNAs is reduced, the cell integrates, in a quite novel way, at least three types of translational controls that have been described in the past few decades[6]:

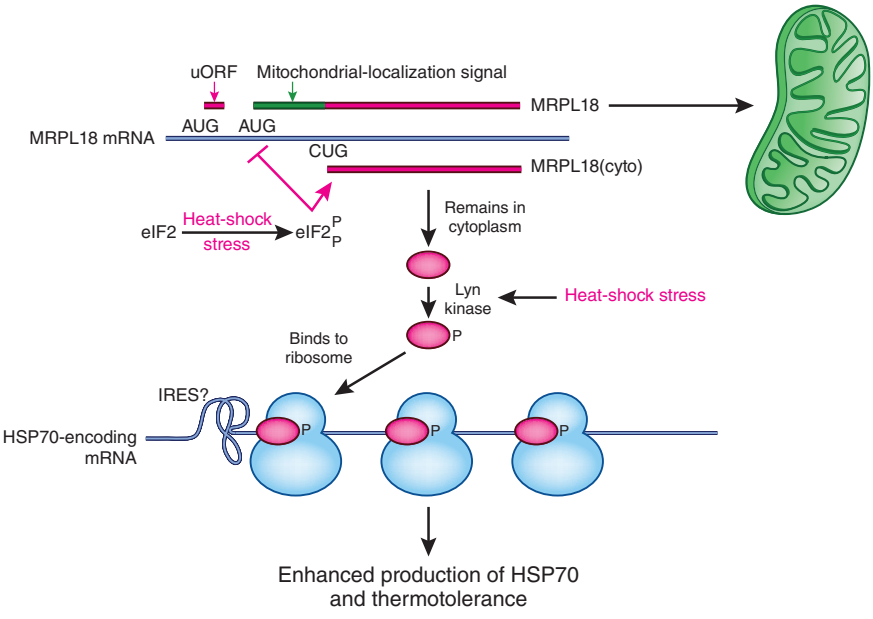

Figure 1 A mitochondrial ribosomal protein is hijacked to control translation of HSP70 by cytoplasmic ribosomes. The mRNA encoding human MRPL18 has a short upstream ORF (uORF) but is usually translated from its second AUG to form MRPL18, which is translocated to the mitochondrion and incorporated into mitochondrial ribosomes. As a result of heat shock, and probably other types of stress, eIF2 is phosphorylated, thus reducing normal translation initiation. Paradoxically this leads to increased translation initiation at the indicated CUG, yielding MRPL18(cyto), which lacks the mitochondrial-targeting sequence. MRPL18(cyto) remains in the cytoplasm, where it is phosphorylated by Lyn kinase, which is itself activated by heat shock. The phosphorylated MRPI18(cyto) binds to 80S ribosomes, at a yet-unknown location. The resulting hybrid ribosomes are competent to translate HSP70 in a cap-independent manner, perhaps through a specialized IRES located in the 5′ UTR.

Jonathan R. Warner is at the Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York, USA.
e-mail: jon.warner@einstein.yu.edu

(i) The mRNA encoding MRPL18 has a short upstream ORF (uORF) of nine codons, which is followed by the 'authentic' AUG and then by the CUG identified by the authors as the initiator of MRPL18(cyto) (**Fig. 1**). This arrangement is conserved in mice. The role of such uORFs in regulating translation of downstream sequences was initially identified in the classic experiments on translation of GCN4 in yeast[7]. Although reinitiation can occur downstream of the uORF, the site of reinitiation is influenced by the activity of translation initiation factors. Such a mechanism has also been established in mammalian cells, for mRNAs encoding proteins such as ATF4 (ref. 8). In the case of the *MRPL18* mRNA, phosphorylation of eIF2α slows translational initiation, thus leading to skipping of reinitiation at the second AUG and permitting some reinitiation at the subsequent CUG to synthesize MRPL18(cyto). A key point is that eIF2 can be phosphorylated by several kinases, each responding to a different type of stress[9]. Thus MRPL18(cyto) may be generated in response to many types of stress beyond heat shock. The relationship between stress, slowed translation initiation and the selective initiation of translation is evident from the observation that hyperactivation of translation, by means of a constitutively activated TORC1 complex, suppresses translation of heat-shock proteins[10], possibly owing to reduced production of MRPL18(cyto).

(ii) The phosphorylated MRPL18(cyto) associates with 80S ribosomes to yield a 'hybrid' ribosome that is essential for translation of the HSP70-encoding mRNA. The relatively new concept of 'specialized' ribosomes, tailored for the translation of specific mRNAs, has now been demonstrated in several instances[11] and may prove to be an important element in the overall regulation of translation.

(iii) It has been known for some time that HSP70-encoding mRNA is translated in a manner that is cap independent but dependent on the 5′ untranslated region (5′ UTR), perhaps through an internal ribosome entry site (IRES)[12], although that has been disputed[10]. It now seems likely that the presence of MRPL18(cyto) permits the hybrid ribosome to bypass normal cap-dependent initiation, presumably by interacting with some structure in the 5′ UTR to effect translation. Future studies determining the location of the MRPL18(cyto) on the cytoplasmic ribosome may suggest the mechanism by which this occurs.

The conceptual novelty of this story is the realization that any of the mRPs might be co-opted for regulatory functions in the cytoplasm or in the nucleus. Although the unusual structure of mammalian mitochondrial ribosomes involves far more protein-protein interactions than occur in cytoplasmic ribosomes, the mRPs are still predominantly RNA-binding proteins. Thus there should be many opportunities to adapt them as regulatory elements at a variety of levels in the nuclear and cytoplasmic RNAome. Need we remind the reader that plants have an additional source of such regulators in the form of the proteins making up the chloroplast ribosome?

MRPL18 is closely related to RPs whose ancestry can be traced over three billion years and which are present in all ribosomes; they have recently been given the structural name uL18 (ref. 13). uL18 is one of the two proteins that associate with 5S rRNA in the 'crown' of the large ribosomal subunit. However, this does not hold true for human mitochondria. The recent high-resolution structure of the large subunit of the human mitochondrial ribosome[14] has revealed that MRPL18 associates with a mitochondrially encoded $tRNA^{Val}$, which substitutes for 5S rRNA. Perhaps MRPL18 was selected for the regulatory role unveiled by Zhang *et al.*[5] because of its unique way of interacting with a tRNA.

In summary, Zhang *et al.*[5] have shown that the regulated mistranslation of a mitochondrial ribosomal protein can lead to the formation of a new hybrid ribosome that permits the enhanced translation of one class of mRNAs even when the translation of most mRNAs is repressed. Perhaps more importantly, it is now apparent that the mitochondrial ribosomal proteins may serve two masters.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Jäkel, S. & Gorlich, D. *EMBO J.* **17**, 4491–4502 (1998).
2. Fukasawa, Y. *et al. Mol. Cell. Proteomics* **14**, 1113–1126 (2015).
3. Hällberg, B.M. & Larsson, N.G. *Cell Metab.* **20**, 226–240 (2014).
4. Richter, K., Haslbeck, M. & Buchner, J. *Mol. Cell* **40**, 253–266 (2010).
5. Zhang, X. *et al. Nat. Struct. Mol. Biol.* **22**, 404–410 (2015).
6. Sonenberg, N. & Hinnebusch, A.G. *Cell* **136**, 731–745 (2009).
7. Hinnebusch, A.G. *Microbiol. Rev.* **52**, 248–273 (1988).
8. Lu, P.D., Harding, H.P. & Ron, D. *J. Cell Biol.* **167**, 27–33 (2004).
9. Wek, R.C., Jiang, H.Y. & Anthony, T.G. *Biochem. Soc. Trans.* **34**, 7–11 (2006).
10. Sun, J., Conn, C.S., Han, Y., Yeung, V. & Qian, S.B. *J. Biol. Chem.* **286**, 6791–6800 (2011).
11. Xue, S. & Barna, M. *Nat. Rev. Mol. Cell Biol.* **13**, 355–369 (2012).
12. Rubtsova, M.P. *et al. J. Biol. Chem.* **278**, 22350–22356 (2003).
13. Ban, N. *et al. Curr. Opin. Struct. Biol.* **24**, 165–169 (2014).
14. Brown, A. *et al. Science* **346**, 718–722 (2014).

Light-driven Na^+ pumps as next-generation inhibitory optogenetic tools

Przemyslaw Nogly & Jörg Standfuss

The first structures of a light-driven sodium pump provide insight into the mechanism of ion transport and selectivity. Genetic manipulation of rat neuronal cells and of *Caenorhabditis elegans* worms demonstrates the utility of such pumps for optogenetic applications.

Retinal-binding proteins have revolutionized neurobiology, serving as tools to analyze nerve action and to control animal behavior by light[1]. Key for the development of the modern optogenetics field were the discovery and characterization of light-gated channelrhodopsins[2,3] and the light-driven chloride pump halorhodopsin[4]. There has been much debate as to whether a light-driven sodium pump that could serve as a tool for silencing nerve action might exist. Following the discovery of the sodium pump Kr2 in the marine bacterium *Krokinobacter eikastus*[5], the article by Gushchin *et al.*[6] in this issue of *Nature Structural & Molecular Biology* and the concurrently published article by Kato *et al.*[7] in *Nature*

Przemyslaw Nogly and Jörg Standfuss are at the Laboratory of Biomolecular Research, Paul Scherrer Institute, Villigen, Switzerland.
e-mail: joerg.standfuss@psi.ch