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Mitochondria teach ribosome assembly:

Ribosome assembly factors regulate structure formation to prevent misfolding

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Mitochondrial ribosomes translate messenger RNAs (mRNAs) encoded in mitochondrial DNA. Many of the resulting proteins are components of the electron transport chain, an essential mitochondrial pathway that produces the cellular energy source adenosine triphosphate (ATP). These mitoribosomes are assembled from mitochondrially encoded ribosomal RNAs (rRNAs) and from ribosomal proteins (RPs) mostly imported from the cytosol. In keeping with the evolutionary origin of mitochondria, mitoribosomes are more closely related to bacterial ribosomes than to their cytoplasmic counterparts, although often their rRNAs are smaller and additional RPs are recruited. An extreme example of this is found in trypanosomes, single-celled parasites that cause sleeping sickness. On page 1144 of this issue, Saurer *et al.* (1) report the identification and structure of three assembly intermediates of the *Trypanosoma brucei* mitoribosomal small subunit (SSU). Comparison to assembly intermediates from cytoplasmic ribosomes and to those from bacteria suggests universal and specific assembly problems and their solutions.

Saurer *et al.* purified mitochondrial SSU assembly intermediates from sucrose gradients and visualized them through single-particle cryo-electron microscopy (cryo-EM). This revealed three molecules of increasing complexity and size. Comparison to the mature mito-SSU (2) then allowed for the identification of one group of RPs and three groups of assembly factors (AFs) that are successively incorporated and dissociated from the intermediates. The earliest and most complex of these intermediates was also further enriched and visualized at atomic resolution, which allowed for the identification of 34 mitochondrial-SSU AFs (mSAFs). These data demonstrate (i) a surprisingly large assembly machinery for trypanosome mito-SSU, rivaling that of its cytosolic counterparts; (ii) that mSAFs are organized into localized clusters that appear to bind and dissociate together; and (iii) extensive remodeling of RNA structure and RNA-protein contacts between these assembly intermediates.

All SSUs feature three structural components—body, platform, and head—that can assemble independently. Trypanosomal SSU has a highly reduced rRNA, containing essentially only the conserved decoding site, the platform, and the base structure of the head, while recruiting trypanosome-specific RPs to the head and body. Saurer *et al.* demonstrate that the head is fully assembled in the earliest identified assembly intermediate, whereas incorporation of 10 RPs from the body and platform is delayed to the last intermediate (see

the figure). This is in surprising contrast to prior observations from bacteria and eukaryotes that have shown that the body generally assembles first, and head assembly occurs last (3–6). Although it is possible that this finding is specific to trypanosomes with their highly expanded RP content, only five of the late-assembling RPs are trypanosome-specific; four are conserved in other mitochondria, and one is conserved even in bacteria. Thus, it appears that the simplified head rRNA and/or the mitochondrial assembly machinery have allowed the head to assemble faster. Notably, studies of bacterial rRNA folding show that the head RNA folds slowly, likely as a result of misfolding (7). Thus, perhaps the streamlining of rRNA in the head avoids misfolding traps, thereby speeding its assembly and providing an evolutionary rationale for the reduced head rRNA.

In contrast to these differences, there are also marked similarities in the mechanism of SSU assembly in trypanosome mitochondria to that in bacteria and cytoplasmic eukaryotic ribosomes. For example, the formation of the central pseudoknot (CPK), which anchors the platform and head to the body, appears to be highly regulated in all cases. However, different solutions have been found in eukaryotes, bacteria, and mitochondria to prevent premature CPK formation. These include preventing the binding of the essential RP uS12, and steric block by the assembly machinery. Because CPK formation is expected to stabilize not just correctly folded but also misfolded structures (8), this likely reflects the importance of guiding rRNA away from misfolding traps (9). Another similarity between all studied assembly pathways is the regulated and late construction of the decoding site, formed by helices h18 and h44. For h18, this involves extensive secondary-structure changes observed in both mitochondrial and cytoplasmic eukaryotic ribosomes (1, 4–6), whereas h44 is dislocated by binding of AFs near its top (10–12). The different solutions to carefully orchestrate the formation of the decoding site in all SSUs may reflect either a difficult folding barrier and/or a necessity to protect this functionally critical area of the molecule from adopting its structure (and thus function) prematurely.

The platform is largely unchanged between bacterial and trypanosomal mito-SSU and also does not change much in cytoplasmic ribosomes. Nonetheless, comparison of the high-resolution structures of immature mitochondrial and cytoplasmic subunits demonstrates that the pathway to the mature platform structure is different in these intermediates, with different rRNA helices blocked from their native sites by AFs. Platform assembly without AFs is slow (7), possibly because of misfolded structures. It could be that the stepwise assembly of the platform, mediated by AFs in either order, is a mechanism to prevent the formation of stable misfolded structures.

The structures reported by Saurer *et al.* identify and structurally characterize the assembly landscape of the mito-SSU in trypanosomes. Comparison with assembly pathways of cytoplasmic ribosomes and in bacteria suggests that one of the roles of the differing assembly machineries is to prevent the premature adoption of tertiary structure, which can lock in not just native but also misfolded structures (8). In addition, AFs also block premature functionality. Nonetheless, the tiny, streamlined rRNA of trypanosomes requires an assembly machinery rivaling that of eukaryotes, suggesting that this machinery also enables proper RP binding. Additionally, given the co-regulation of translation in mitochondria and the cytoplasm (13), assembly of cytoplasmic and mitoribosomes may also

be co-regulated in eukaryotes, which could be achieved by the assembly machinery. Future experiments will be required to test these additional roles of the assembly machinery.

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Mitochondrial-ribosome small subunit assembly

The conversion between three assembly intermediates and the mature small subunit (SSU) involves the formation of the central pseudoknot (CPK) and the helix h18 after head, body, and platform have been largely preassembled. After late-binding ribosomal proteins (RPs) are incorporated into the body and platform, all but two assembly factors (AFs) dissociate. The remaining AFs block h44 of the decoding site and dissociate in the last step.