the patients with spermatogenic failure, Y chromosome anomalies, or sex reversal examined by Lange et al.), it is a clear drawback to the Y chromosome's mechanism of self-preservation. Indeed, every form of refuge really does have its price.

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The Rea1 Tadpole Loses Its Tail

Jason Talkish[1] and John L. Woolford, Jr.[1,*]
[1]Department of Biological Science, Carnegie Mellon University, Pittsburgh, PA 15213, USA
*Correspondence: jw17@andrew.cmu.edu
DOI 10.1016/j.cell.2009.08.019

More than 170 assembly factors aid the construction and maturation of yeast ribosomes. After these factors' functions are completed, they must be released from preribosomes. In this issue, Ulbrich et al. (2009) describe a mechanochemical process through which the AAA ATPase Rea1 induces release of an assembly protein complex from preribosomes.

Ribosomes, the ubiquitous factories that produce proteins from mRNAs, are essential for growth, proliferation, and adaptation of cells. In eukaryotes, assembly of these complex ribonucleoprotein particles (RNPs) begins in the nucleolus with the association of a subset of ribosomal proteins (r-proteins) and *trans*-acting assembly factors with the nascent ribosomal RNA (rRNA) to form the 90S pre-rRNP, the single precursor to both the 40S and 60S mature subunits. The assembly factors are transient actors—they are released once their role is completed. But do they just know when to let go or are they actively removed from the maturing subunits? In this issue, Ulbrich et al. (2009) provide the most detailed study to date to answer this question. They discover a mechanochemical process for release of assembly factors and suggest that release is an integral part of ribosomal subunit maturation.

Beginning with the 90S precursor, the pre-rRNP undergoes a series of pre-rRNA processing and assembly steps while transiting from the nucleolus through the nucleoplasm to the cytoplasm to form mature 40S and 60S functional subunits (Henras et al., 2008). The two ribosomal subunits contain intricate structural cores of rRNA decorated on their surfaces with r-proteins. Studies of bacterial ribosome assembly in vitro revealed that ribosomal subunit assembly is cooperative and hierarchical. Ribosomal RNA and bound r-proteins undergo multiple structural rearrangements induced by binding of additional r-proteins to enable successive assembly steps (reviewed in Nomura, 1990). However, binding of r-proteins to rRNA is not sufficient to drive assembly forward.

Genetic and proteomic analysis in yeast has identified >170 proteins present in pre-rRNPs, but not mature ribosomes, that are required for ribosome assembly in vivo. These assembly factors, largely conserved from yeast to humans, include AAA ATPases, GTPases, RNA-dependent ATPases/helicases, kinases, nucleases, scaffolding proteins, and RNA-binding proteins. At least nine of these proteins, including GTPases and ATPases, release other factors, reduce the complexity of pre-rRNPs, and power the progression of subunit maturation (Figure 1; reviewed in Henras et al., 2008; Zemp and Kutay, 2007).

Although we now have a great deal of insight into what mature ribosomal subunits look like, understanding the mechanism of ribosome assembly in detail requires learning the precise functions of each assembly factor. Several key questions immediately come to mind: At which point in the assembly pathway does each factor associate with pre-rRNPs? Where is each factor located in preribosomes? When does each factor function? Upon which molecules does each factor act? When, how, and why do assembly factors exit from pre-rRNPs?

In their new study, Ulbrich and coworkers use an elegant combination of electron microscopy, site-directed mutagenesis, and assays of factor release from preribosomes to answer these questions about the AAA ATPase Rea1. These analyses enable them to work out how Rea1 operates in ribosome biogenesis.

Rea1 is the largest protein in yeast, 550 kDa, and is related to the motor protein dynein. It contains six AAA ATPase protomers at its N terminus, followed by a long linker region, a negatively charged domain, and a C-terminal MIDAS (metal ion-dependent adhesion site) protein-protein interaction motif. Rea1 has been found in late, nucleoplasmic pre-60S particles. Rea1 can also be isolated from preribosomes in a salt-stable complex with the assembly factors Rix1, Ipi1, and Ipi3, indicating intimate interactions among these molecules (Galani et al., 2004). Importantly, the ability to purify preribosomes containing the Rea1 protein enabled demonstration of ATP-dependent dissociation of Rea1 and the Rix1-Ipi1-Ipi3 subcomplex from preribosomes (Nissan et al., 2004).

To build a more detailed mechanism for how Rea1 functions, Ulbrich et al. used negative staining and cryoelectron microscopy of the purified protein to investigate the structure of Rea1. Rea1 contains a ring (presumably the six ATPase protomers) connected to a flexibly hinged tail. Analysis of preribosomal particles by immunoelectron microscopy revealed a "tadpole"-like structure. The "head" of the tadpole contains r-proteins, and Rea1 comprises the tail. The AAA domains of Rea1 are located near the central protuberance of the preribosome body, and the C-terminal MIDAS domain is located at the end of Rea1's tail. The Rix1-Ip1-Ipi3 subcomplex is sandwiched between Rea1 and the pre-60S particle.

Whereas the ATPase domain is anchored to the preribosomal particle, Rea1's tail shows more heterogeneity in its localization. In some pre-60S particles, the tip of Rea1's tail appears to contact the body of the pre-60S particle, which is suggestive of a second, perhaps more transient interaction. Ulbrich et al. used structural and biochemical assays to identify an assembly factor, Rsa4, that may be a potential Rea1 interactor located on the pre-rRNP surface. Importantly, the authors show that the interaction of Rsa4 with Rea1 is needed for ribosome biogenesis and that it requires the MIDAS domain of Rea1 both in vitro and in vivo. Disrupting the Rea1-Rsa4 interaction prevents removal of Rea1 and the Rix1-Ipi1-Ipi3 complex from preribosomes. All of their data to date suggest a scenario in which the tail of Rea1 contacts Rsa4 and, upon ATP hydrolysis, peels it off of the pre-60S subunit. This is coupled to the release of Rea1 itself and the Rix1 subcomplex.

There are several reasons why assembly factors must be disassembled from preribosomes. Certainly recycling of these proteins is an efficient plan. Yeast produce just enough of each factor to feed the ribosome assembly pipeline for a few minutes under conditions of rapid growth. Also, there is a limit to the size of a particle that can squeeze through nuclear pores, so most factors must be removed prior to export of the largely mature ribosomal subunits. Release of assembly factors may be coupled with rearrangements of pre-rRNP structures necessary for subsequent steps in the biogenesis of ribosomal subunits. Finally, factor release may serve as a timing/quality control mechanism, signaling successful completion of a maturation step. Prior to their release, bound assembly factors may prevent premature binding of export or translation factors. For example, the release of Rea1 and the Rix1 subcomplex from preribosomes, just before nuclear export of the particles, may enable subsequent binding of the Mex67 export factor to 5S rRNA in the central protuberance of the ribosome (Yao et al., 2007). In *rea1* or *rsa4* yeast mutants where this release is blocked, there is no nuclear export of nascent ribosomes.

The authors present several models for how Rea1 could displace Rsa4 and the Rix1 complex. The "spring-like tension" model posits that binding of both the AAA domain and the tip of the flexible Rea1 tail to two different sites on the preribosome, followed by ATP hydrolysis, creates tension that translates into displacement of proteins bound to Rea1. Alternatively, the "long-range cooperative communication" model proposes that one or more conformational changes in Rea1 are transmitted from one end of the molecule to the other. For example, binding of the Rea1 tail to the preribosome might alter the conformation of an α helix in the MIDAS domain, which then is somehow coupled with forces created by the AAA motor domain, to initiate changes in the preribosome. The challenge now is to design appropriate experiments to test these models, perhaps by obtaining higher-resolution structures of the Rea1 motor protein, which will inform yeast mutagenesis studies. Clearly ribosome

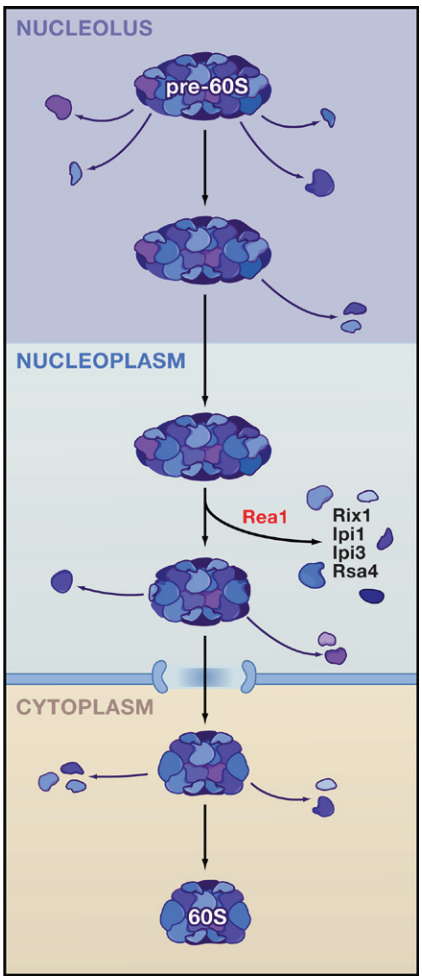

Figure 1. Giving Assembly Factors the Old "Heave Ho"

Proteins associated with preribosomal particles that are required for ribosome assembly are released by other factors as the preribosomes become mature ribosomal subunits during transit from the nucleolus through the nucleoplasm to the cytoplasm. Release of assembly factors is essential for the ribosome biogenesis pathway and may help to drive the maturation of ribosomal subunits. Rea1, a ribosome-associated factor related to dynein, removes the Rix1-Ipi1-Ipi3 assembly complex in the nucleoplasm. Rea1 associates with pre-60S particles through an interaction with the Rix1 complex. It also makes a second contact to the particle through an interaction between its C-terminal MIDAS domain and the Rsa4 assembly factor. Rea1 translates this two-point attachment and the hydrolysis of ATP into a mechanochemical process that removes Rsa4, the Rix1 complex, and itself from pre-60S particles.

biogenesis proceeds by means more complex than changes in conformation induced by r-protein binding. The process requires large inputs of energy generated by ATPases and GTPases.

It seems unlikely that the immense, energy-consuming enzyme Rea1 functions only to release a handful of assembly factors. It will be exciting to discover how Rea1-mediated remodeling is coupled with pre-rRNA processing. One will also want to discover whether and how release is regulated. Does one of the GTPases in the Rix1 particle activate Rea1? Does Rea1 contact any other preribosomal molecules? Are other assembly factors, in addition to Rsa4 and the Rix subcomplex, released by Rea1? Of course, once these questions are answered, the when, how, and why of the release of the remaining 150+ assembly factors from preribosomes await discovery.

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IKKε: A Bridge between Obesity and Inflammation

Jerrold M. Olefsky[1,*]

[1]Department of Medicine, University of California, San Diego, La Jolla, CA 92093, USA

*Correspondence: jolefsky@ucsd.edu

DOI 10.1016/j.cell.2009.08.018

Obesity leads to tissue inflammation and insulin resistance, which are features of metabolic diseases such as type 2 diabetes. Chiang et al. (2009) now show that the IκB kinase IKKε is an important link between obesity and inflammation and may be a new therapeutic target for treating obesity-related metabolic diseases.

Insulin resistance involves a decreased ability of tissues to respond to insulin and is a key metabolic abnormality in most patients with type 2 diabetes (Olefsky and Courtney, 2005). Although there can be genetic or other causes of insulin resistance, the predominant cause is obesity. The prevalence of obesity is increasing at an alarming rate in all age groups worldwide, and the obesity epidemic is driving the increased incidence of type 2 diabetes. Obesity leads to an increase in tissue inflammation, particularly within adipose (fat) tissue (Figure 1) (Schenk et al., 2008; Shoelson et al., 2007; Hotamsiligil and Erbay, 2008). It was Xu et al. (2003) and Weisberg et al. (2003) who first reported that large numbers of macrophages accumulate in fat depots in obese mice and humans. Tissue macrophages recruited to adipose tissue in obese animals exhibit increased expression of a broad array of genes encoding inflammatory pathway components. These macrophages secrete proinflammatory cytokines (TNF-α, IL1-β, IL-6, etc.) that work in a paracrine, and possibly endocrine, fashion leading to defects in insulin signaling and systemic insulin resistance. Blocking the function of these macrophages results in a glucose-tolerant, insulin-sensitive phenotype (Schenk et al., 2008; Shoelson et al., 2007; Solinas et al., 2007; Hotamsiligil and Erbay, 2008). Other insulin target tissues can also participate in the chronic inflammatory state. In the liver, the specialized resident macrophages or Kupffer cells show activation of proinflammatory pathways in response to obesity. In skeletal muscle, there is increased accumulation of proinflammatory macrophages within the intramuscular adipose depots that develop in obesity (Varma et al., 2009). Changes in the mixture of adipokines released from adipose tissue, as well as ectopic lipid deposition in liver and muscle, can also contribute to decreased insulin sensitivity. In this issue of *Cell*, Chiang et al. (2009) now show that the protein kinase IKKε is a crucial bridge between obesity and inflammation.

IKKε is a member of the IκB kinase family, and its expression is induced by activation of the inflammatory NF-κB signaling pathway. The function of IKKε is incompletely understood, although it