Regulation of ribosome biogenesis: Where is TOR?

Li et al. (2006) have shown that TOR complex 1 in yeast binds directly to the rDNA promoter and thereby activates Pol I-dependent synthesis of 35S RNA. This is an important advance in the understanding of how ribosome biogenesis is regulated in response to environmental conditions.

The regulation of ribosome biogenesis is a key aspect of cell growth control. In a robustly growing cell, ribosome biogenesis is a major consumer of cellular energy and building blocks. Thus, as growth conditions change, cells must accurately and rapidly rebalance ribosome production with the availability of resources. The regulation of ribosome biogenesis occurs primarily at the transcriptional level and involves all three nuclear RNA polymerases (Pol I thru III) (see Figure 1). Pol I transcribes rDNA encoding the 35S rRNA precursor, Pol II (Pol II) transcribes the ribosomal protein (RP) genes, and Pol III produces 5S rRNA (and tRNA). It has been estimated that the transcription of these genes encoding structural components of the ribosome accounts for up to 90% of total cellular transcription in a rapidly growing cell (Warner et al., 2001). The growth conditions that impinge on ribosome biogenesis include nutrients and stress.

A central regulator of cell growth and metabolism in all eukaryotes is the Ser/Thr kinase TOR (target of rapamycin) and its namesake signaling network (for review see Wullschleger et al., 2006). TOR controls cell growth in response to nutrients and stress and exists in two structurally and functionally distinct protein complexes termed TORC1 (TOR complex 1) and TORC2. In yeast, TORC1 contains either one of the two TORs TOR1 and TOR2, whereas TORC2 contains TOR2 but not TOR1. The two TORCs control different sets of growth-related processes. TORC1, but not TORC2, controls translation and ribosome biogenesis. Only TORC1 is inhibited by rapamycin, an immunosuppressive and anticancer drug. In yeast, inactivation of TORC1 by rapamycin treatment (or nutrient deprivation) leads to a fast and strong downregulation of essentially all genes involved in ribosome biogenesis (see Figure 1). This downregulation is due to a general inhibition of Pol I and Pol III activities and reduced Pol II activity at RP gene promoters (Powers and Walter, 1999). Rapamycin treatment also leads to a strong repression of Pol II genes encoding nonribosomal proteins involved in ribosome synthesis and maturation, collectively termed the Ribi (*ri*bosome *bi*ogenesis) regulon (Jorgensen et al., 2004). The Ribi regulon is the largest set of coordinately expressed genes in yeast, again illustrating the magnitude of ribosome biogenesis and its regulation by TOR. How all three RNA polymerases are regulated by the TORC1 signaling pathway is largely unknown. In general, TORC1 controls gene expression by regulating the subcellular localization of a variety of specific transcription factors. For example, cytoplasmic TORC1 controls nuclear localization of the two transcription factors SFP1 and CRF1 that act at Pol II-dependent RP gene promoters (Jorgensen et al., 2004; Martin and Hall, 2005).

Zheng and coworkers (Li et al., 2006) have now presented evidence that TORC1 in yeast controls Pol I more intimately than previously anticipated. This study shows that a significant fraction of TOR1 (i.e., TORC1) is localized to the nucleus and binds directly to the 35S rDNA promoter. Upon rapamycin treatment (inhibition of TOR1 activity), TOR1 is exported from the nucleus by the exportin CRM1 and a newly characterized NES (nuclear export signal) in TOR1. Upon favorable growth conditions (TOR1 is active), TOR1 is imported into the nucleus by the importin SRP1 and an also newly characterized NLS (nuclear localization signal) near the kinase domain in TOR1. Furthermore, using a genetic trick to create ''conditional'' mutations in the NES and NLS, they show that nuclear import of TOR1 is necessary for 35S rRNA synthesis but of no consequence for expression of TOR1-regulated Pol II target genes. TOR1 binds directly to the 35S rDNA promoter via an also heretofore uncharacterized HTH (helix turn helix) motif. Deletion of the HTH motif specifically affects 35S rRNA synthesis by Pol I but not the expression of known TOR1-regulated Pol II genes. Thus, TOR1 appears to activate Pol I directly at the promoter whereas, as shown previously, it activates Pol II indirectly from the cytoplasm. Interestingly, TORC1 can still perform its cytoplasmic function when stuck in the nucleus due to an NES mutation in TOR1.

A remaining important issue is whether TOR1 kinase activity, which is necessary for TOR1 nuclear import, is also required to activate Pol I at the rDNA promoter. The findings presented by Zheng and coworkers raise the possibility that TORC1 directly phosphorylates one of the Pol I subunits or its associated transcription factors. One such candidate factor could be RRN3 (TIF1-A in human cells), a key activator of Pol I in yeast and mammalian cells (for a review see Moss, 2004). In growing yeast cells, RRN3 associates with the RPA43 subunit of Pol I in a TORC1-dependent manner and thereby activates Pol I. The histone deacetylase RPD3 has also been proposed to act at rDNA promoters in a TORC1-sensitive manner, although there is disagreement on this finding. Nuclear TORC1 may have several targets involved in rDNA transcription, maintenance of nucleolar structure, and nuclear import/export. The elucidation of these potentially direct nuclear substrates and the exact mechanisms of transcriptional regulation will significantly enhance the understanding of cell growth control.

The observations of Li et al. (2006) provide new insights into the role of TORC1 in ribosome biogenesis but are also quite surprising. One intriguing finding of this report is the nuclear localization of TOR1. This is in apparent contrast to previous reports that showed by

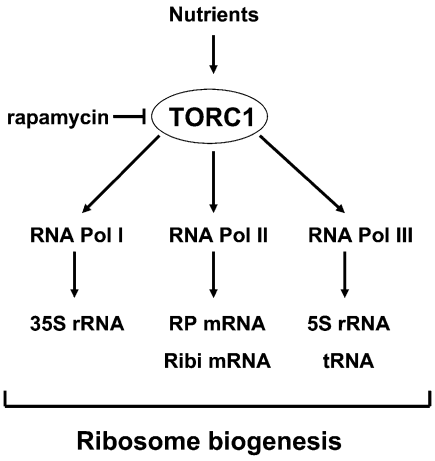

Figure 1. Transcriptional control of ribosome biogenesis by TORC1 in yeast

several techniques, including subcellular fractionation, indirect immunofluorescence (IF), and immunoelectron microscopy (IEM), that TOR in yeast is associated with internal membranes, mainly at or near the plasma membrane (Kunz et al., 2000; Wedaman et al., 2003). Several other yeast TORC1 components have also been localized to discrete intracellular locations but not within the nucleus (e.g., Wedaman et al., 2003). Moreover, the localization pattern of TOR1 and TOR2 was reported previously to be insensitive to rapamycin treatment. Thus, additional studies are necessary to substantiate the functional significance of TOR1's nuclear localization. For example, it will be important to determine whether the same holds true for TOR2, which can functionally replace TOR1 within TORC1. However, in line with Li et al. (2006), signaling kinases are often found bound to genes (Pokholok et al., 2006), and mTOR in mammalian cells has been shown to shuttle in and out of the nucleus and this shuttling is important for mTOR signaling (Bachmann et al., 2006). It will be of interest to determine whether mTOR binds directly to promoters in mammalian cells.

TOR coordinates the relative activity of all three RNA polymerases to achieve the proper stoichiometry of ribosomal components. Do Li et al. (2006) provide insight into this aspect of TOR-mediated regulation of ribosome biogenesis? A recent report by Laferte et al. (2006) suggests that rRNA synthesis by Pol I is a key determinant for transcriptional regulation of all ribosomal components, including ribosomal proteins and 5S rRNA. Laferte et al. (2006) constructed a functional, rapamycin-insensitive RNA Pol I by fusing the Pol I-specific transcription factor RRN3 to the Pol I subunit RPA43. This RRN3-Pol I hybrid functioned normally under good growth conditions. However, when cells harboring RRN3-Pol I were treated with rapamycin, or starved for glucose, 35S rRNA synthesis remained at an elevated level. Surprisingly, these cells also failed to properly attenuate expression of Pol II-dependent RP genes and Pol III-dependent 5S rRNA synthesis. These results suggest the appealing possibility that TOR may coordinate the three RNA polymerases via Pol I. However, Li et al. (2006) observed that mutations that alter TOR1-mediated regulation of Pol I have no effect on expression of RP genes. Thus, the mechanism of coordinated control of ribosomal components by TOR remains to be elucidated.

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Treating obesity: Does antagonism of NPY fit the bill?

In this issue of *Cell Metabolism*, Erondu et al. (2006) identify a selective neuropeptide Y5 receptor antagonist that, as predicted from rodent studies, results in weight loss when administered to overweight and obese human subjects. In a one-year randomized placebo-controlled clinical trial, the weight loss was modest; the results support the emerging concept that NPY acts via overlapping and redundant energy homeostasis pathways.

Given the disease burden and health costs associated with the rising prevalence of obesity and associated comorbidities such as type 2 diabetes, there is a growing need to find effective, safe, and well-tolerated therapies to treat