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Going Nuclear: Transcribers in Transit

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Abstract

Recent experiments have identified novel RNA polymerase-associated proteins with roles in assembly and nuclear transport of multisubunit eukaryotic RNA polymerases. In this issue of *Molecular Cell*, Czeko et al. (2011) characterize a novel Pol II transport factor that is conserved from yeast to humans.

While the mechanisms of transcription by eukaryotic RNA polymerases are now well understood at the atomic scale (Cramer et al., 2008), much less is known about the biosynthesis of these multisubunit enzymes. RNA polymerases I, II, and III each contain two large subunits that are unique to each form and constitute the active center of the enzyme. In addition, each polymerase has 10–15 smaller subunits, of which five are common to all three enzymes. The mechanism by which each of these 33 subunits is assembled into three distinct polymerases is not understood. Are the subunits transported to the nucleus individually, as partial assemblies, or as a completed enzyme? Is the biosynthetic pathway the same for all three polymerases? RNA polymerase II (Pol II) assembly has been recently shown to occur in the cytoplasm (Boulon et al., 2010), necessitating transport to the nucleus.

Insight into nuclear import of multisubunit RNA polymerases has come from recent analysis of assembly intermediates. Mass spectrometry of affinity-purified complexes containing tagged RNA polymerase subunits has led to the discovery of many proteins that interact with RNA polymerases (Gavin et al., 2002; Jeronimo et al., 2007; Krogan et al., 2006). Two recent studies have focused directly on complexes that may be intermediates in Pol II assembly (Boulon et al., 2010; Forget et al., 2010). The emerging picture from these studies is the presence of two subcomplexes, each containing one of the two largest subunits, one or more small subunits, and a set of putative assembly factors, which include HSP90, the R2TP/Prefoldin complex, hSpagh (RPAP3), and RPAP4/GPN1 (Boulon et al., 2010; Forget et al., 2010).

Unassembled Rpb1 is found together with Rpb8 in a complex associated with the R2TP/Prefoldin-like complex. Interestingly, the R2TP/Prefoldin-like complex also contains Rpb5, one of the subunits shared by all three polymerases. Rpb2 is part of a complex containing Rpb3, Rpb10, Rpb11, and Rpb12. Both of these subassemblies contain a number of other proteins with potential roles in assembly of multiprotein complexes. While it is unclear whether these subassemblies are uniquely cytoplasmic, depletion of any of the Pol II subunits by siRNA leads to accumulation of the other subunits in the cytoplasm, strongly suggesting cytoplasmic assembly (Boulon et al., 2010). The data are not yet sufficient to

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define a precise pathway, but the framework is consistent with two “halves” assembled in the cytoplasm to produce the complete enzyme (Figure 1).

How is the assembled polymerase transported to the nucleus? The answer again comes from examination of proteins associated with polymerase. Yeast Iwr1 was first identified in high-throughput mass spectrometry screens as a protein that interacts with Pol II (Gavin et al., 2002; Krogan et al., 2006). In this issue, Czeko et al. describe a range of experiments that demonstrate that Iwr1 is a Pol II transport factor. Deletion of *IWR1* is not lethal, but does result in slow growth, altered cell morphology, and accumulation of Pol II subunits in the cytoplasm. These phenotypes can be partially reversed by expression of the human homolog, suggesting that Iwr1 function is conserved through evolution.

Iwr1 is a 40 kDa protein with a bipartite NLS, and Czeko et al. show that recombinant Iwr1 binds directly to Pol II to form a 1:1 complex. Mutations in the NLS do not prevent Pol II binding but do result in cytoplasmic accumulation of Pol II subunits. These mutants are more severe than the *iwr1* deletion, indicating a dominant-negative effect, perhaps through interfering with alternative modes of transport. Using cryo-electron microscopy (cryo-EM), the authors mapped the position of Iwr1 on Pol II, showing that the transporter binds in the active center cleft contacting both of the largest subunits. Notably, this binding pocket occurs only on the mature poly-merase, suggesting that Iwr1 senses the completely assembled polymerase. Finally, Czeko et al. show that the Pol II-Iwr1 complex interacts with karyo-pherin α, a transport factor that recognizes the NLS. The authors present a model in which interaction of Iwr1 with the mature Pol II unveils the NLS, leading to nuclear transport. Once in the nucleus, Iwr1 can be displaced by general transcription factors and promoter DNA, leading to exposure of a nuclear export signal and a return of Iwr1 to the cytoplasm to pick up another Pol II.

Despite the prominent role Iwr1 plays in Pol II transport, the *IWR1* gene is not essential, suggesting that Pol II has an alternative route to the nucleus. Here the work on cytoplasmic assembly intermediates may offer some clues to alternative transport pathways. One protein found in two studies (Boulon et al., 2010; Forget et al., 2010) is GPN1 (RPAP4), a novel GTPase that binds Pol II (Jeronimo et al., 2007). Downregulation of *GPN1* or mutation of its yeast homolog *NPA3* results in cytoplasmic accumulation of RNA poly-merase subunits, suggesting a role for this protein in transporting Pol II to the nucleus (Forget et al., 2010). It is interesting to note that benomyl, a drug that promotes the depolarization of microtubules, leads to the accumulation of Rpb1 in the cytoplasm. In addition, *npa3* mutants display increased sensitivity to benomyl. Together, these results indicate a role for GPN1/Npa3 in coupling microtubule integrity to Pol II transport. Another possible transport factor is the RPAP2 (yeast *RTR1*) gene. This Pol II car-boxy-terminal domain (CTD) phosphatase (Mosley et al., 2009) was identified in Pol II cytoplasmic subassemblies and also binds to the mature Pol II (Jeronimo et al., 2007). Importantly, RPAP2 shuttles between the nucleus and cytoplasm (Boulon et al., 2010). Perhaps this factor acts to keep the CTD in an unphosphorylated state so that premature interactions with RNA processing factors are avoided. The possible involvement of multiple transport factors in shuttling large macro-molecular complexes from the cytoplasm to the nucleus has been likened to tugboats guiding an ocean liner to harbor (Wente and Rout, 2010). In this analogy several tugs yield more precise positioning and perhaps regulation of Pol II nuclear transport.

What about the assembly and transport of the other multisubunit RNA polymerases? The dynamics of the reappearance of labeled Pol I subunits in fluorescence bleaching experiments on Pol I transcription sites in the nucleolus has argued for assembly of Pol I at the rDNA promoter (Dundr et al., 2002). Whether similar promoter-specific assembly takes place at some Pol II promoters is not known, but recent evidence makes this seem unlikely

(Boulon et al., 2010). Regardless, assembly in the nucleus does not preclude assembly in the cytoplasm. The fact that five subunits are shared by all three polymerases suggests that the assembly pathways may be interrelated. Indeed, several of the chaperonins identified in Pol II subassembly complexes also interact with Pol I subunits (Boulon et al., 2010; Forget et al., 2010). If Pol I and Pol III are assembled in the cytoplasm, how are they transported to the nucleus? Mutants in *iwr1* do not accumulate Pol I or Pol III subunits in the cytoplasm (Czeko et al., 2011), indicating that this factor is Pol II specific and suggesting that Pol I and Pol III transporters are yet to be discovered.

Our understanding of RNA polymerase biosynthesis is bound to increase rapidly in the next few years. Of particular interest will be understanding the stepwise assembly process and determining what quality control is exerted during assembly. To what degree these processes are physiologically regulated is also of particular importance. Finally, a more thorough understanding of the assembly process may allow for the in vitro assembly of eukaryotic multisubunit RNA polymerases, allowing structural studies of a wide range of polymerase variants.

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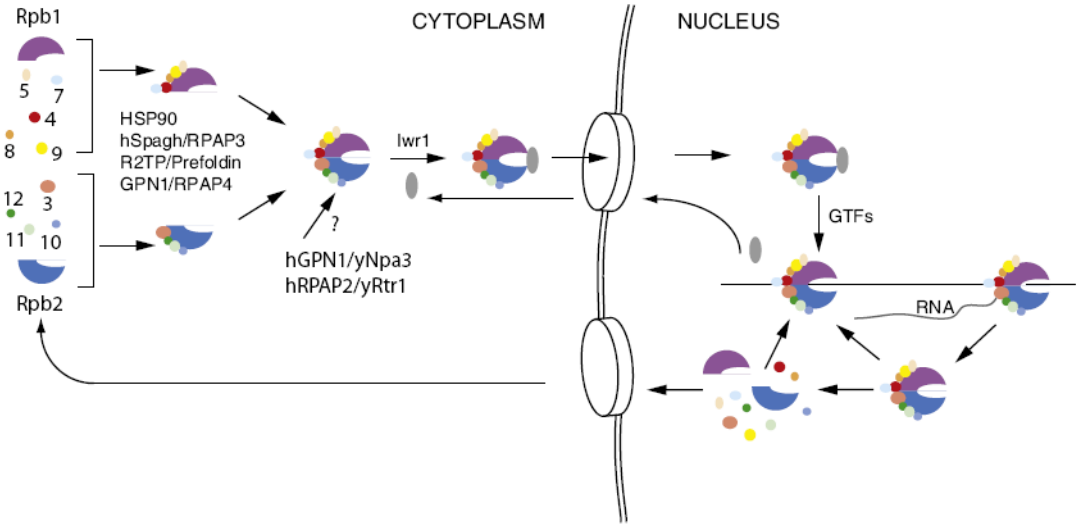

Figure 1. Assembly and Transport of RNA Polymerase II

The individual subunits of Pol II are indicated as colored shapes. Potential assembly intermediates contain one or the other large subunits and a set of smaller subunits. These complexes also contain the putative assembly factors indicated by name. Pol II transport factor Isw1 is indicated by a gray oval. The right side of the figure indicates the possible fates of terminated Pol II. Either the enzyme relocalizes to a promoter or breaks down into individual subunits. These subunits may either be transported back to the cytoplasm for reassembly or may reassemble at the promoter as has been shown for Pol I.