COMMENTARIES

Regulating the Shuttling of Eukaryotic RNA Polymerase II*†

Luciano Di Croce*

Centre de Regulació Genòmica, UPF, and Institució Catalana de Recerca i Estudis Avançats, 08003 Barcelona, Spain

The eukaryotic RNA polymerase II (RNAPII) transcribes most protein-coding RNAs (mRNAs) and the capped noncoding RNAs (ncRNAs), including microRNAs (miRNAs), small nuclear RNAs (snRNAs), and small nucleolar RNAs (snoRNAs) (10). In the past 3 decades, our knowledge about the functional role of RNAPII during gene transcription has dramatically advanced (8). However, there are still several open questions regarding the regulatory mechanisms involved in the steps before and after transcription. In particular, how RNAPII is imported into the nucleus remains elusive. In this issue, Carré and Shiekhattar (3) report that the evolutionarily conserved GTPases GPN1 and GPN3 stably associate with and regulate the nuclear import of the human RNAPII. These exciting results now make it possible to begin to dissect the regulatory pathways of the intracellular localization and nuclear import of RNAPII.

Recently, the structures and subunit compositions of the three RNA polymerases (Table 1) have been characterized (4). RNAPII is a highly conserved enzyme composed of 12 subunits, 10 of which form the central core. The other two subunits (Rpb4 and Rpb7) form a peripheral heterodimer that protrudes out from the core enzyme structure. This stabilizes the RNAPII complex in a clamp-closed conformation, favoring efficient transcription initiation. Rpb10 and Rpb12 are common subunits for all of the eukaryotic RNA polymerases and are implicated in the interaction with the basal transcription factors, such as the TATA box-binding protein (TBP). These interactions lead to the formation of the preinitiation complex (PIC), which contains RNAPII as well as the transcription factors TFIIA, TFIIIA, TFIIID, TFIIE, TFIIF, and TFIIH. In the past years, numerous coactivators that both facilitate the access of PIC to its target promoters and increase the stability of the complex have been functionally characterized. Recent data (1) indicate that the RNAPII assembly pathway occurs in the cytoplasm and that assembly is coordinated by the consecutive actions of the cochaperone hSpagh and the chaperone Hsp90. The two largest subunits, Rpb1 and Rpb2, are initially preassembled into two separated subcomplexes, together with several other subunits that are either common to all three RNA polymerases or are specific for RNAPII (Fig. 1). It is likely that the two subcomplexes are then assembled together before they enter the nucleus through the nuclear pore complex (NPC). Interestingly, it now seems that access to the nucleus is restricted exclusively to the assembled RNAPII, thus preventing any premature binding of the independent subunits at promoter regions.

But the question of how the assembled RNAPII is imported into the nucleus remains. How is this step regulated? Carré and Shiekhattar tackled this question using a combination of cell biology approaches and mass spectrometry of affinity-purified complexes containing tagged cytoplasmic and nuclear RNAPII (3). They identified GPN1 and GPN3 as novel interactors of the RNAPII, which remained associated with the complex even after it was subjected to gel filtration columns using highly stringent conditions. These interactions are mediated by the Rpb4/Rpb7 heterodimer and by the carboxy-terminal domain (CTD) of RNAPII, and they were found to occur in both the cytoplasm and the nucleus.

The human GPN1 and GPN3 proteins are members of a newly identified family of small GTPases that contains only three members to date (GPN2 in addition to GPN1 and GPN3) (7). This GTPase family has been highly conserved during evolution and is also present in yeast. A main characteristic of this family is the presence of a conserved GPN (glycine, proline, and asparagine residues) structural motif, which forms a loop near the GTP-binding site.

Interactions of the GPN proteins with RNAPII have been documented in two other recent studies (2, 6). Interestingly, GPN proteins have also been demonstrated to interact with the CCT complex, which controls the polymerization of tubulins into microtubules (9). Since the nuclear import of several nuclear proteins has been previously reported to be regulated by the CCT complex, these findings let the authors speculate that there is a possible physiological link between the GPN1 and GPN3 interactions and the import of RNAPII into the nucleus. Indeed, they find that deleting GPN1 and GPN3 by using small interference RNAs (siRNAs) blocked the import of RNAPII into the nucleus and led to its concomitant accumulation in the cytoplasm. Forget and colleagues have recently demonstrated that the deletion of NPA3, the yeast homolog of GPN1, also affected the nucleocytoplasmic shuttling of RNApolyII (6), which argues that the mechanism involved in the subcellular localization of RNAPII is conserved from yeast to mammals. More importantly, the catalytic function of GPN proteins is required for the correct nuclear localization of RNAPII. When the GPN1 catalytic activity was abrogated by mutating the GTP-binding domain, RNAPII was inappropriately localized in the cytoplasm, as documented by immunofluorescence microscopy and cellular fractionation experiments (3). These data thus identify the key proteins involved in controlling the intracellular localization of the RNAPII and their role in regulating transcription initiation.
reinforce previous findings that have indicated that RNAPII assembly is a cytoplasmic event (1).

Where is the nuclear localization signal (NLS) for RNAPII located? Bioinformatic analyses of all RNAPII subunits clearly excludes the presence of an NLS among them. Recent data indicate that the NLS is provided by a specific interactor, Iwr1, which was identified using a strategy similar to that described above. Indeed, Czeko and colleagues (5) recently reported that the yeast protein Iwr1 (which is also conserved in humans) contains a bipartite NLS and that it associates with the RNA-PII in a 1:1 ratio. Binding of Iwr1 to RNAPII is stimulated by the assembly of the two large subcomplexes containing Rpb1 and Rpb2. This is turn allows for the NLS to be unmasked and for both the cargo and the receptor to be transported into the nucleus. Once RNAPII engages with the promoter DNA, Iwr1 is released and recycled to the cytoplasm, ready to initiate a new cycle.

Together with a few recent publications, these new results from the Shiekhattar lab shed light on the steps necessary for RNAPII to assemble in the cytoplasm and to move across the nuclear envelope. Based on these findings, we can now begin to address several key questions. For example, how is the interaction between RNAPII and the GPN family members regulated? How is the GTPase activity of GPN controlled? How are the GPN proteins released from RNAPII once the cargo-receptor reaches the nucleus? And, since all three members of the GPN family have been shown to interact with all three RNA polymerases, how is any specificity achieved? Are the GPN proteins also directly involved in regulating RNAPII assembly, in addition to their role in transport? Finally, how are the assembly and shuttling of the other polymerases regulated? We have much less information about the mechanisms involved in the assembly and intracellular localization of RNA polymerases I and III. The fact that several subunits are shared among all three polymerase complexes might indeed indicate that mechanisms similar to those identified for RNAPII might

<table>
<thead>
<tr>
<th>RNA polymerase and subunit type</th>
<th>Subunits</th>
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<tbody>
<tr>
<td>RNAPI II</td>
<td>Rbp1, Rbp2, Rbp3, Rbp11, Rbp9, Rbp5, Rbp6, Rbp8, Rpb10, Rpb12</td>
</tr>
<tr>
<td>Other</td>
<td>TFIIFα (RAP74), TFIIFβ (RAP30), TFIIEα, TFIIEβ</td>
</tr>
<tr>
<td>RNAPIII</td>
<td>C160, C128, AC40, AC19, C11, Rpb5, Rpb6, Rpb8, Rpb10, Rpb12</td>
</tr>
<tr>
<td>Other</td>
<td>C37, C53, C82, C34, C31</td>
</tr>
</tbody>
</table>

* The common subunits are in boldface.

![FIG. 1. The life cycle of RNA polymerase II: its cytoplasmic assembly, nuclear import, and binding to promoters.](http://mcb.asm.org/)
apply to the other two and that these processes might be interconnected. These points need to be addressed to fully understand the physiological mechanisms that regulate the biogenesis and nuclear import of these complex molecular machines.

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REFERENCES


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