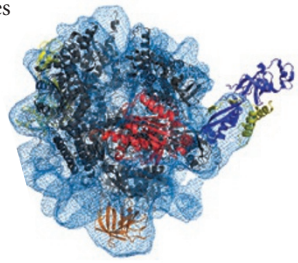
Deconstructing RNA Pol I



RNA polymerase I (Pol I) transcribes ribosomal RNA, which accounts for up to 80% of cellular RNA. Pol I is formed by 14 subunits, some of which are common or homologous to components in the other RNA Pol complexes. Past EM studies have uncovered the overall shape of Pol I. Now work from Cramer and colleagues combines cryo-EM analysis, crystal structure determination and modeling to reveal the details of domain organization of yeast Pol I at 12-Å resolution. Fitting the Pol II core crystal structure into the Pol I EM map showed differences between the two complexes that the authors explored by further structural and functional studies, shedding light on several particularities of Pol I. For example, comparison of the cryo-EM structure of complexes depleted of Pol I–specific subunits A49 and A34.5 with the full-complex structure allowed mapping of these subunits to a density near the enzyme funnel, a location similar to the one where transcription factor TFIIF binds Pol II. They showed that the A49 and A34.5 subunits also have sequence and functional similarities to TFIIF subunits, stimulating transcription elongation by Pol I *in vitro* and *in vivo*. Having such a 'built-in' elongation factor might be important for Pol I's high processivity. The authors also demonstrated an intrinsic 3′ RNA cleavage activity in the Pol I complex, linked to subunit A12.2, known to be required for transcription termination. Thus, the RNA cleavage activity could trim the 3′ end of rRNAs and contribute to proofreading, increasing transcription fidelity and preventing the biogenesis of defective ribosomes. This work opens the way for further analysis and a deeper understanding of rRNA transcription. (*Cell* **131**,1260–1272, 2007) *IC*

More tales of the tail

RNA polymerase II (Pol II) transcribes mRNAs and small nuclear RNAs (snRNAs). The activity is regulated through the conserved C-terminal tail, or CTD, of the large subunit. The CTD consists of a tandem, seven-amino-acid repeating unit with Ser residues at positions 2, 5 and 7. Phosphorylation of Ser5 and Ser2 affects transcription initiation and elongation, respectively, but no role was known for modification of Ser7. Two recent studies now confirm that Ser7 phosphorylation affects gene expression. Egloff and colleagues mutated Ser7 to an alanine. The mutant Pol II was unable to efficiently transcribe an snRNA-gene reporter *in vitro*, whereas expression of a protein-coding gene was unaffected. Recruitment of Pol II or of an snRNA gene–specific transcription factor to the promoter was not affected, but the mutant enzyme was unable to recruit Integrator complex, which functions in 3′ end processing of snRNAs. In contrast, Chapman and colleagues found by ChIP that Ser7-phosphorylated Pol II was associated with protein-coding genes *in vivo* and that the length of the CTD heptad repeat affected the ability of Ser7 to be phosphorylated. They concluded that phosphorylation of Ser7 facilitates a stable interaction of the polymerase with genes. Given the different results from the *in vitro* and *in vivo* studies, it is not yet clear how Ser7 phosphorylation specifically affects transcription by Pol II. Regardless of how this question is resolved, these studies have illuminated a new aspect of regulation of Pol II activity. (Egloff *et al. Science* **318**, 1777–1779, 2007; Chapman *et al. Science* **318**, 1780-1782, 2007) *AKE*

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Activating microRNAs

MicroRNAs (miRNAs) are small post-transcriptional regulators of gene expression. They are known to mediate repression, but work from Steitz and colleagues now indicates that miRNA-mediated regulation may be more subtle and complex than previously thought. The authors had formerly observed that, under growth arrest triggered by serum starvation, AGO and FXR-1, proteins found in the complex that mediates small RNA function, can activate translation through an AU-rich element in the TNF-α 3′ UTR. The new work implicates a small RNA, miR369-3, in this regulation. Synthetic miRNAs carrying compensatory mutations rescued target-site mutations that cause loss of regulation, implying direct base pairing with the target. Next, the authors examined what happened to translation of a reporter carrying TNF-α 3′-UTR sequences when the miRNA itself was knocked down. They also monitored target association of the miRNA itself, as well as of the AGO and FXR-1 proteins, under similar sets of experimental conditions. The results indicate that miR369-3 can activate translation, and the authors extend this to two previously validated miRNA-target interactions, arguing that under serum starvation the miRcxcr4 and let-7 miRNAs activate translation through the 3′ UTRs of CX and HMGA2, respectively. Finally, on the basis of results from synchronized cells or cells arrested at different phases of the cell cycle, the authors propose that the regulatory outcome—repression or activation—is cell cycle–dependent. Whether these observations hold true for other miRNAs, organisms, cell and tissue contexts, or developmental stages is now a set of open and intriguing questions to be resolved, as is the mechanism by which such positive regulation might occur. (*Science*, published online 29 November 2007, doi:10.1126/science.1149460) *SL*

How dynamin may destabilize

The GTPase dynamin is recruited to clathrin-coated pits during the late stages of endocytic vesicle formation through interactions with binding partners, and then associates directly with the negatively charged membrane using its PH domain. Dynamin self-assembles around the neck of clathrin-coated vesicles, where its GTP-hydrolyzing activity promotes fission of the neck. How dynamin exerts these changes at the molecular level is not well understood. Using real-time *in vitro* fluorescence assays on NBD- and BODIPY-labeled dynamin, Ramachandran and Schmid find that dynamin preferentially associates with negatively charged PIP_2-containing liposomes of high curvature, consistent with previous work and its role in fission events. Membrane association involves partial membrane insertion of dynamin's PH domain, which the authors suggest could cause spreading of one leaflet of the bilayer, leading to membrane bending. GTP binding and hydrolysis promotes membrane association and dissociation, respectively. Furthermore, GTP hydrolysis also triggers conformational rearrangements in the dynamin assemblies before membrane dissociation, which may contribute to membrane destabilization via their PH domains and lead to fission. Further data indicate that sorting nexin 9 (SNX9), known to stimulate dynamin's self-assembly, may aid in this process by promoting dynamin-membrane interactions and delaying dynamin dissociation from the membrane after GTP hydrolysis. Altogether, the findings indicate that dynamin's GTPase activity, when localized at the membrane by binding partners such as SNX9, may cause structural changes to the protein that would perturb the membrane and facilitate fission events. (*EMBO J*, published online 13 December 2007, doi:10.1038/sj.emboj.7601961) *MM*