The Dynamic Range of Transcription

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In a recent issue of *Molecular Cell*, Gorski et al. (2008) demonstrate directly that polymerase assembly kinetics regulate Pol I transcription.

Within the last 5 years, tools have become available to measure dynamic rates for many biological processes in living cells. One of the processes most amenable to this approach is transcription since it takes place at a defined location that can be interrogated by fluorescence recovery after photobleaching (FRAP) (Darzacq et al., 2005, 2007 Dundr et al., 2002; Yao et al., 2007). In this experimental design, the transcription site, containing many fluorescently labeled RNA polymerases labeled by a fused fluorescent protein, is bleached. Replacement by unbleached polymerases is a direct measure of the combination of events, including assembly at the promoter, initiation, and elongation (Darzacq et al., 2007; Dundr et al., 2002). By fitting curves to these measurements, it is possible to dissect these various kinetic processes and model their rate constants. Thus, the mechanisms of transcriptional regulation can be more fully understood.

Applied to both Pol I and Pol II, these approaches have shown that transcription is inefficient (Darzacq et al., 2007; Dundr et al., 2002; Yao et al., 2007). Only a minor fraction of the polymerases assembling at the promoter ultimately make a transcript. This inefficiency could have its foundation in the assembly of a polymerase at the gene. Indeed, in such a "neoassembly" model, polymerase subunits transiently interact and constantly assemble and disassemble on the promoter (Figure 1, top). This scenario has two possible outcomes: factor dissociation or complete polymerase assembly, leading to promoter escape. Different components of the Pol I enzyme were found to interact with rDNA with different residence times, strongly supporting the neoassembly model (Dundr et al., 2002). While this hypothesis challenges our views of a more stable assembly, it can explain the accessibility of the transcriptional machinery to compact chromatin regions (Chen et al., 2005). Moreover, findings in *Drosophila* showed that during the heat shock transcriptional response, the efficiency of polymerase recruitment can evolve from an inefficient mode toward an efficient mode where enzymes may be recycled internally among thousands of amplified genes (Yao et al., 2007).

Knowing whether polymerases engage DNA as preassembled, stable complexes or if polymerase assembly occurs on DNA is critical because how polymerases assemble and engage DNA has implications for how transcription is regulated. In the "preassembly" model, transcription may be regulated at the level of initiation and elongation of preassembled polymerases. Alternatively, in the "neoassembly" model, sequential steps in assembly may be regulated, allowing transcription to occur rapidly with a large dynamic range. This is because in this scenario, an increase in the efficiency of any assembly step could amplify transcriptional output accordingly.

In a recent issue of *Molecular Cell*, Gorski et al. (2008) have demonstrated directly that polymerase assembly kinetics regulate Pol I transcription. Transcription of the ribosomal RNAs by Pol I is greatly increased in S phase, and Gorski et al. have tested the kinetics leading to this increase using not only FRAP, but a biochemical approach, the ChIP assay. The results clearly show that the residence times of the components of the

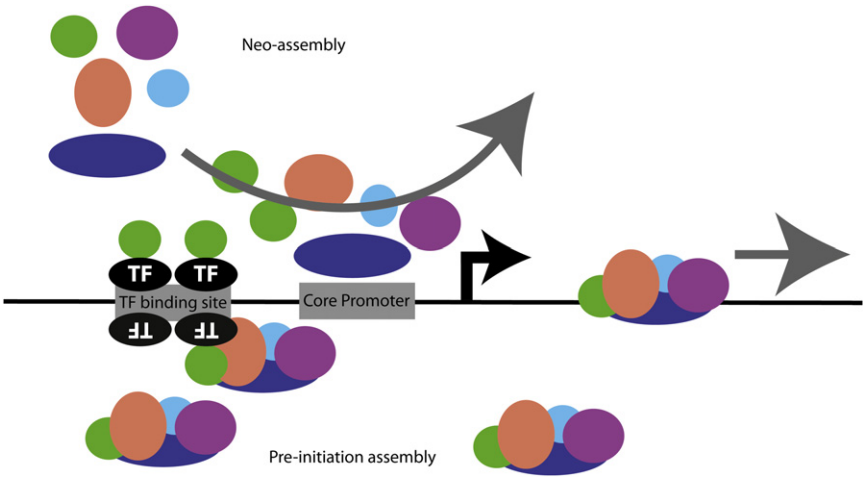

Figure 1. Two Possible Modes of Polymerase Binding to DNA: Preassembly versus Neoassembly
Two models for polymerase transcriptional recruitment to genes. In the upper half, the polymerase subunits are recruited to the promoter where they assemble there into a functional enzyme. In the lower half, the polymerases are preassembled and recruited as whole units helped by the interaction with transcription factors. While these two models seem very similar, the upper one offers more opportunity for regulation since the stoichiometry of the subunits can influence the initiation process.

transcription complex are increased upon transcriptional upregulation, thereby affecting the rate of entry into elongation. The factors, TIF-1A and PAF53, which mediate the interaction between the preinitiation complex and the core polymerase, showed slower recovery in S phase cells. Although these measurements translate into only about 2-fold increases in residence time, the modeling demonstrates that this increase can have profound effects on the assembly rate and significantly improve the probability of entering into processive elongation and RNA production. Conversely, mutants of these transcription factors that are unable to promote initiation slow down this process, specifically affecting the kinetics of the particular subunit with which they interact. This demonstrates that the mode of action of these transcription factors is mediated by generating a local DNA-bound concentration of subunits pushing the reaction toward assembly by mass action. Regulation of these factors during S phase appears to be due to the usual suspects, namely various kinases in the cell-cycle pathway.

All of this strongly supports a model of sequential regulation of each of the kinetic steps of transcription machinery assembly rather than a model that would provide a greater supply of assembled complexes available to access the gene. This likely occurs in Pol II transcription as well, although unlike Pol I, these polymerases can pause during elongation of the whole transcription unit, perhaps reflecting a continuous exchange of elongation factors not present in Pol I. While initiation and transcriptional proximal regulation events are well understood biochemically and the sequential events of promoter binding, preinitiation complex formation, initiation, promoter escape, and proximal pausing are well characterized (Core and Lis, 2008), none of the kinetic rates observed in vivo can unambiguously be attributed to one or the other of these molecularly defined events. More specific assays will enable us identifying these steps in live cells. The biggest challenge will be to unify molecular interpretations with kinetic live-cell approaches.

Improvements in the sampling rate with higher sensitivity cameras, better optics, and fluors will allow ever more temporal detail to be delineated. This will provide more exact rate constants and perhaps reveal even more components in the transcriptional cascade.

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