Cell

Hashimoto, Y., Murakami, Y., Uemura, K., Hayashidani, Y., Sudo, T., Ohge, H., Fukuda, E., Shimamoto, F., Sueda, T., and Hiyama, E. (2008). J. Gastrointest. Surg. *12*, 17–28.

Hu, J., Hwang, S.S., Liesa, M., Gan, B., Sahin, E., Jaskelioff, M., Ding, Z., Ying, H., Boutin, A.T., Zhang, H., et al. (2012). Cell *148*, this issue, 651–663.

Lee, H.W., Blasco, M.A., Gottlieb, G.J., Horner, J.W., II, Greider, C.W., and DePinho, R.A. (1998). Nature *392*, 569–574.

Maser, R.S., Choudhury, B., Campbell, P.J., Feng, B., Wong, K.K., Protopopov, A., O'Neil, J., Gutierrez, A., Ivanova, E., Perna, I., et al. (2007). Nature *447*, 966–971.

O'Hagan, R.C., Chang, S., Maser, R.S., Mohan, R., Artandi, S.E., Chin, L., and DePinho, R.A. (2002). Cancer Cell *2*, 149–155.

CoSMoS Unravels Mysteries of Transcription Initiation

Richard L. Gourse[1,*] and Robert Landick[1,2,*]
[1]Department of Bacteriology
[2]Department of Biochemistry
University of Wisconsin-Madison, Madison, WI 53706, USA
*Correspondence: rgourse@bact.wisc.edu (R.L.G.), landick@biochem.wisc.edu (R.L.)
DOI 10.1016/j.cell.2012.01.042

Using a fluorescence method called colocalization single-molecule spectroscopy (CoSMoS), Friedman and Gelles dissect the kinetics of transcription initiation at a bacterial promoter. Ultimately, CoSMoS could greatly aid the study of the effects of DNA sequence and transcription factors on both prokaryotic and eukaryotic promoters.

The first step in gene expression is the recognition of promoter DNA by the basal transcription machinery, that is, RNA polymerase (RNAP) and a σ factor in bacteria or the basal transcription factors in eukaryotes. The initiation machinery then unwinds the DNA in the region of the transcription start site to form an open complex, and RNA synthesis commences. It has long been recognized that the initiation complex passes through multiple intermediates on the path to formation of an open complex, with the efficiency of each step traceable in principle to the promoter DNA sequence (Record et al., 1996). Remarkable diversity exists in the efficiency with which different promoters produce RNA. Estimates of this diversity range over several orders of magnitude.

Predicting transcription output from promoter sequence is an important goal of genome-scale biology, but making accurate predictions from sequence alone has been difficult. Multiple factors have contributed to the prediction problem, including the extraordinary difficulty of identifying the intermediates in the transcription initiation mechanism, the challenge of measuring the forward and reverse rates associated with their formation and decay, and the need to assign the promoter sequences responsible for each transition. Elucidating even a subset of these intermediates and rates for a few model bacterial promoters has occupied transcription researchers for decades. CoSMoS, as described in this issue of *Cell* (Friedman and Gelles, 2012), has the potential to greatly facilitate the kinetic analysis of transcription initiation.

Classic experiments by McClure and coworkers in the late 1970s and early 1980s (for instance, McClure, 1980) led to a paradigm for the mechanism of transcription initiation in which the formation of open complexes could be described as the product of two parameters, an RNAP concentration-dependent equilibrium constant for promoter DNA binding (sometimes referred to as K_B) and a composite second-order rate constant for the DNA unwinding steps (sometimes called k_f). However, as our understanding of the initiation reaction has grown to include multiple steps (Saecker et al., 2011), actually determining the rates of formation and decay of the individual intermediates has required considerable ingenuity, including the use of changes in temperature, a range of different solutes and perturbants, and rapid timescale chemical probing of DNA conformation.

The introduction of transcription factors into the equation adds further challenges for analysis. The identities of the transcription factors sometimes are known but sometimes are not. Even when the identities of the factors are known, it has not always been possible to purify them to homogeneity without loss of activity. Although the promoter targets of these factors are usually determined by DNA-binding sites near the specific promoter (Browning and Busby, 2004), sometimes transcription factors act simply by binding to the RNAP itself without binding to DNA. In this case, their specificities are

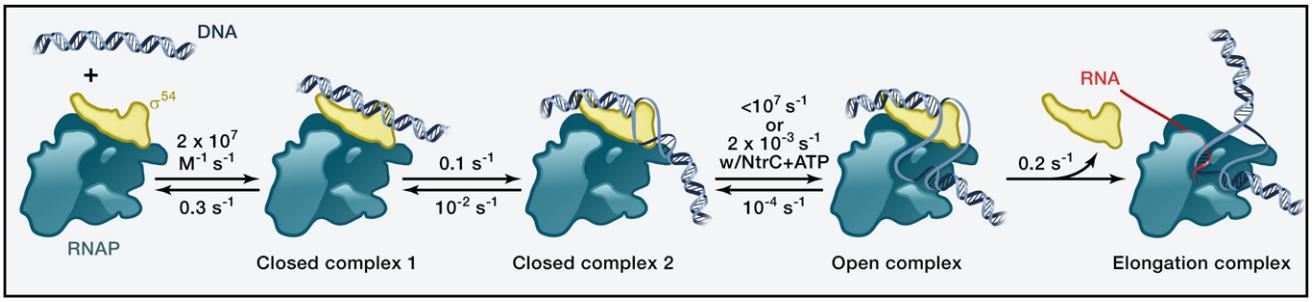

Figure 1. Transcription Initiation at a σ^{54}-Dependent Promoter

Rates are shown for individual steps as deduced by CoSMoS analysis (Friedman and Gelles, 2012). Promoter DNA and RNA polymerase (blue, with σ in yellow) come together to form two sequential closed complexes. Closed complex 2 then isomerizes into an open complex in which the DNA bends toward the active site and forms a single-stranded bubble surrounding the transcription start site. Finally, as the enzyme escapes the promoter, an RNA chain (red) is synthesized, σ is released, and the elongation complex forms.

determined simply by the kinetic properties of the promoter itself; that is, the transcription factors bind to all promoter complexes and alter a specific kinetic step in the mechanism, but they have consequences on the output from only the subset of promoters whose activities are rate limited at the step affected by the transcription factor (Haugen et al., 2008).

In CoSMoS, spectrally distinguishable dye labels located on different proteins and nucleic acids that participate in the transcription initiation reaction (such as DNA, core RNAP, σ factor, transcription factor) can be imaged using time-resolved total internal reflection fluorescence (TIRF) microscopy. This capability allows direct measurement of the rates at which initiation complexes assemble or dissociate under different conditions, from which both forward and reverse rate constants for multiple intermediates can be deduced.

Friedman and Gelles analyze the mechanism of transcription initiation of the *E. coli* promoter, $glnA_{P2}$, by RNAP holoenzyme containing the alternative σ factor, σ^{54}. Although initiation by the holoenzyme containing the major σ factor, σ^{70}, can occur without the aid of other transcription factors, σ^{54} holoenzyme more resembles the behavior of eukaryotic RNAP II in that unwinding the promoter DNA to form an open complex requires ATP hydrolysis by a transcription factor (NtrC for the promoter studied by Friedman and Gelles) (Wigneshweraraj et al., 2008). RNAP-σ^{54} binds promoters with conserved sequences centered at about −12 and −24 to form closed complexes, rather than at the elements centered at about −10 and −35, which are typical for σ^{70}-dependent promoters. Indeed, unlike σ^{70} but more like the basal factors in eukaryotes, σ^{54} can bind to some promoters even when not complexed with core RNAP.

Using dye labels on promoter DNA, RNAP, σ^{54}, and short oligos that hybridize to newly synthesized RNA, Friedman and Gelles visualize all of the major steps in initiation directly, including RNAP binding, open complex formation, transcript production, and σ factor release from the complex (Figure 1). Careful inspection of the rates of promoter association and dissociation reveals two sequentially formed closed complexes. Both closed intermediates are kinetically unstable, but the second intermediate is longer-lived than the first (Figure 1). NtrC acts on the second, more long-lived, closed intermediate, converting it to the open complex. However, formation of the open complex is rare; more often, the complex reverts to the first closed complex and dissociates without ever reaching the stable open complex state. Once open complex formation is achieved, the RNAP is committed to initiation, and σ^{54} is ejected from the complex. The authors conclude that the conformational change characterizing open complex formation is always the rate-limiting step in the reaction, even when activation by NtrC is maximal.

The analysis of the RNAP- σ^{54} mechanism by Friedman and Gelles suggests that, in some ways, it may be more similar to that of RNAP-σ^{70} than initially suspected. For example, partial melting in intermediate complexes starts at ~−11 in both cases, and tight binding of the resultant fork junction occurs in both the σ^{54} and σ^{70} pathways (Saecker et al., 2011; Wigneshweraraj et al., 2008; Friedman and Gelles, 2012). Once formed, the σ^{54} open complex is long-lived, similar to most σ^{70} open complexes. Unlike σ^{70} promoters, however, σ^{54} promoters exhibit no sequence conservation downstream of this initially melted region. A σ^{70}-DNA structure recently published in *Cell* (Feklistov and Darst, 2011) reveals that spontaneous unwinding of a σ^{70}-dependent promoter relies on the trapping of bases in tight-fitting pockets in the protein at not only the −11 position, the most highly conserved position in the −10 element, but also at the second most highly conserved position, the last (most-downstream) position in the −10 element. Perhaps σ^{54}-dependent promoters require ATP hydrolysis by NtrC-like factors in some part because of the absence of such base-specific contacts downstream of the −11 binding pocket.

Possible extensions of the CoSMoS method have the potential to allow rapid advances in our understanding of both prokaryotic and eukaryotic transcription. Instrumentation with similar capabilities is becoming commercially available. For example, zero-mode waveguide nanostructure arrays developed by Pacific Biosystems for single-molecule sequencing (Eid et al., 2009) allow similar measurements on many thousands of molecules simultaneously. One might envisage simultaneous characterization of the kinetic parameters of many promoter sequences, perhaps deconvoluted by sequence tags. Application to promoters recognized not only by σ^{70} and σ^{54}, but also by other alternative σ factors, could

allow accumulation of sufficient information to make genome-scale predictions of promoter strengths achievable. Furthermore, the CoSMoS method can be used in crude extracts (Hoskins et al., 2011). This feature might facilitate the study of the initiation mechanism of eukaryotic RNAP II, which requires multiple accessory factors whose activities can only be fully recapitulated in complex mixtures.

REFERENCES

Browning, D.F., and Busby, S.J. (2004). Nat. Rev. Microbiol. *2*, 57–65.

Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., Peluso, P., Rank, D., Baybayan, P., Bettman, B., et al. (2009). Science *323*, 133–138.

Feklistov, A., and Darst, S.A. (2011). Cell *147*, 1257–1269.

Friedman, L.J., and Gelles, J. (2012). Cell *148*, this issue, 679–689.

Haugen, S.P., Ross, W., and Gourse, R.L. (2008). Nat. Rev. Microbiol. *6*, 507–519.

Hoskins, A.A., Friedman, L.J., Gallagher, S.S., Crawford, D.J., Anderson, E.G., Wombacher, R., Ramirez, N., Cornish, V.W., Gelles, J., and Moore, M.J. (2011). Science *331*, 1289–1295.

McClure, W.R. (1980). Proc. Natl. Acad. Sci. USA *77*, 5634–5638.

Record, M., Jr., Reznikoff, W., Craig, M., McQuade, K., and Schlax, P. (1996). In Escherichia coli and Salmonella: Cellular and Molecular Biology, 2nd Edition, F. Neidhardt, R. Curtiss, III, J. Ingraham, E. Lin, K. Low, B. Magasanik, W. Reznikoff, M. Riley, M. Schaechter, and H. Umbarger, eds. (Washington, DC: ASM Press), pp. 792–821.

Saecker, R.M., Record, M.T., Jr., and Dehaseth, P.L. (2011). J. Mol. Biol. *412*, 754–771.

Wigneshweraraj, S., Bose, D., Burrows, P.C., Joly, N., Schumacher, J., Rappas, M., Pape, T., Zhang, X., Stockley, P., Severinov, K., and Buck, M. (2008). Mol. Microbiol. *68*, 538–546.