tion (*6*–*8*). In this case, the union in H_2 becomes polarized upon interaction with a positively charged or electron-poor (electrophilic) metal, resulting in splitting to a metal hydride and a proton (H^+) that can easily transfer to a ligand (see the figure, middle panel) or external substrate. H_2 can become more acidic than sulfuric acid when bound to an electrophilic metal center (*7*).

Heterolytic cleavage has been mainly observed in transition-metal complexes, most notably as part of the mechanism of Noyori's asymmetric hydrogenation systems (*9*) and hydrogen activation in hydrogenase enzymes (*10*). However, it can also occur on nonmetal centers. For example, metal-bound sulfide ligands are known to react with H_2 to form SH ligands (*11*), and metal-free hydrogenation of ketones on strong bases appears to proceed via base-assisted heterolysis of H_2 (*12*, *13*).

The phosphine-borane species used by Welch *et al.* combines a strongly electrophilic center (boron) with a nearby nucleophilic site (phosphorus) that can apparently accept the proton from heterolytic splitting of H_2. Several mechanisms are possible, but it is likely that H_2 initially interacts with the electrophilic boron center, followed by proton migration from an H_2-like complex to the basic phosphorus atom, which is separated from the boron center by a perfluorophenyl linker (see the figure, bottom panel). This migration could proceed stepwise via the linker, as proposed by Welch *et al.*, or could be assisted by the solvent.

Regardless of the mechanism, the discovery is important because of the reversible nature of the hydrogen activation. Materials for hydrogen storage are a vexing challenge, particularly for vehicles, because energetically favorable extrusion of hydrogen from materials is rare [a recent example is H_2 evolution from hydride-like organic compounds (*14*)]. It can also be very difficult to add hydrogen back. Furthermore, the materials must be lightweight, reducing the prospects for known, easily reversible systems such as dihydrogen or hydride binding to transition metals. Amine borane is a popular candidate and also combines both electrophilic (B) and nucleophilic (N) centers. However, these centers are directly bonded, whereas in the phosphine-borane described by Welch *et al*., they are separated by a linker, increasing the electrophilicity of B and the nucleophilicity of P.

The hunt is now on for compounds similar to that reported by Welch *et al.* for hydrogen storage and activation. Biomimetic hydrogen production by splitting of water, particularly in processes that use sunlight, is also a challenge and may take a cue from models of the iron-containing active site of hydrogenases (*15*). Here, the mechanistic reverse of heterolytic splitting of H_2 will be crucial, involving formation of hydrogen from protons and electrons, a highly reversible rapid process in hydrogenases. Clearly, there are now many new avenues for chemical bond splitting and transformations.

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BIOCHEMISTRY

RNA Polymerase, a Scrunching Machine

Jeffrey W. Roberts

RNA polymerase stores energy to break its initial bonds with DNA by scrunching the single strands of DNA that were unwound in the region where the polymerase started RNA synthesis.

RNA polymerase (RNAP) mediates the critical steps in gene expression and is thus an important target for mechanistic analysis by sophisticated biophysical techniques. A striking example is the subject of two reports in this issue of *Science*. On pages 1144 and 1139, Kapanidis *et al.* and Revyakin *et al.* (*1*, *2*) illuminate the initial steps of making an RNA chain by showing how the energy of nucleoside triphosphate hydrolysis is captured to break the enzyme loose from its tight contact with DNA at the beginning of the transcribed segment. The results reveal an unexpected structure of DNA in the transcribing complex that may well have an important role in regulating gene expression.

Attempts to explain a strange property of RNAP stimulated these experiments. Through the process of transcription, coiled and double-stranded DNA is unwound into single strands, and RNAP synthesizes RNA that is complementary to the templating strand of DNA. Instead of continuing every RNA chain that it starts, RNAP tends to falter badly, releasing most chains near the beginning of transcription—generally after 5 to 10 nucleotides have been assembled into RNA—and then starting over, a process called "abortive initiation" (*3*–*6*).

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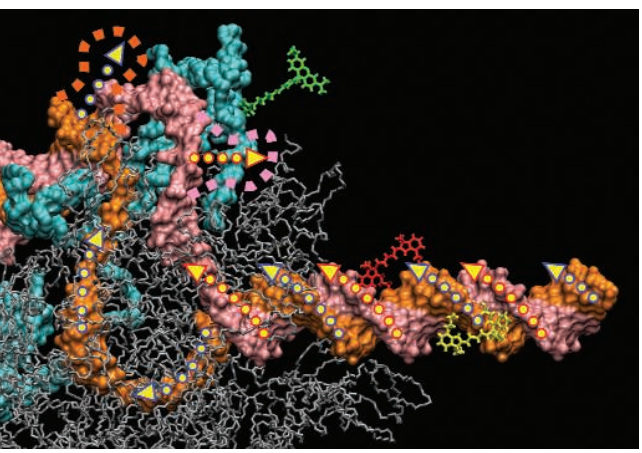
It has long been clear that the cause of abortive initiation is the failure of RNAP to break the bonds that bound it to DNA—specifically, to a region called the promoter—in the first place. For bacterial RNAP, these bonds connect sigma factor regions 2 and 4 (in the example of the σ^{70} RNAP) to the promoter –10 and –35 elements, respectively. Comparable but more complex networks of initiation factors presumably bind eukaryotic RNA polymerase II to its promoter. Nearly 20 years ago, Straney and Crothers (*7*) suggested that energy to break promoter contacts is stored in a "stressed intermediate" form of the RNAP-promoter complex during the first few nucleotide addition steps in RNA synthesis and that abortive cycling represents failed attempts to use this energy productively.

But how might the energy be stored? One structure-based proposal of Darst and associates (*8*) suggests that emerging RNA must actively force the protein linker between sigma domains 3 and 4 from the RNA exit channel of RNAP. This model is specific in detail to bacterial polymerase but possibly applicable to other RNAPs.

However, a more general answer comes from the two new reports, designed to answer a related question: How can the transcribing complex be flexible enough to allow synthesis of RNA 10 nucleotides long (or more) and at the same time keep its grip on the promoter? Clearly, something has to bend or move, and

the two reports consider three possibilities. First, RNAP itself might be flexible, allowing the active center to move downstream along DNA with the bubble of "melted," unwound DNA where templating of RNA occurs, whereas sigma (in the case of bacterial RNAP) stays bound to the promoter elements. In this scenario, energy is stored in the distorted protein. Second, promoter-sigma bonds might break transiently, allowing RNAP to move downstream during RNA synthesis but diffuse back to the promoter when the abortive RNA is released. Third, the DNA bubble might unwind downstream DNA without any movement at the upper end, meaning that sigma stays bound to the promoter and melted downstream single-stranded DNA is "scrunched" into a nearly rigid enzyme. Energy would then be stored as melted DNA. Scrunching of DNA in fact was detected originally in an initiation complex of the single subunit T7 RNA polymerase revealed through atomic crystallography by Cheetham and Steitz (*9*).



Ready to scrunch. The model shows an open promoter complex of bacterial RNA polymerase (RNAP) poised to begin RNA synthesis, with arrows designating motions of DNA segments that occur as the first nucleotides are combined to form an RNA chain. Downstream DNA (on the right) rotates inward and separates in the active site channel of the polymerase. Template (orange) and nontemplate (pink) DNA strands follow the indicated paths, moving as the RNA chain (not shown) is polymerized on the template DNA strand. Sites of extrusion of single-stranded DNA are shown by outlined arrows at the top. σ^{70} (blue); RNAP core chains (gray); a fluorescent tag on σ^{70} region 2 used for FRET (green); a fluorescent tag on downstream DNA (initial position in yellow; scrunched position in red).

Two methods of single-molecule analysis were used to distinguish these models, and these methods give complementary results. Single-molecule fluorescence resonance energy transfer (FRET) was used to measure relative movements of various reference points on the transcription complex during transcription, specifically sites on RNAP near its upstream and downstream edges, and sites in upstream and downstream DNA. The result is that downstream DNA moves closer to the other sites during abortive initiation, but upstream DNA and sites within RNAP do not move relative to each other. This result is predicted only by the scrunching model and rules out either protein flexibility or RNAP excursions from the promoter during abortive cycling.

In the second method, DNA unwinding induced by RNAP was measured by nanomanipulation of an extended template DNA on which melting can be measured, to 1-base pair resolution, as a change in supercoiling-induced contraction. The result showed the expected melting when the initial promoter bubble (open complex) formed, with further melting corresponding to the length of the abortive RNA that was made—as predicted by the scrunching model. Furthermore, the nanomanipulation method showed that promoter escape is preceded by scrunching in most if not all transcription events, considering the 1-s limit of detection. The authors conclude that scrunching is an obligatory step in promoter escape, which occurs as scrunched DNA rewinds and exits through the back of the enzyme, breaking the bonds to promoter sequences.

This discovery has implications for several basic processes of transcription and thus of gene expression. Most strikingly, the scrunching model implies a novel and unexpected structure. Scrunched single-stranded DNA eventually would be extruded from the enzyme channel at predictable sites (see the figure) and thus would be available for interaction with transcription regulatory proteins. No such interactions are yet known, but it seems unlikely that this opportunity has been wasted in evolution. These experiments also define the critical structure present when the choice between elongation of RNA and abortive loss of RNA is made—a mysterious process and potential point of regulation. Knowledge of the structure of the abortive complex and the stability of the scrunched state should guide studies of the mechanism of bacterial Gre proteins (and their eukaryotic counterpart TFIIS), which inhibit abortive initiation in vitro and are likely to be important modulatory elements of both initiation and elongation of RNA synthesis in the cell.

Scrunching also could explain a perplexing incongruity between transcription initiation in higher eukaryotes and the otherwise closely similar model, the yeast *Saccharomyces cerevisiae*. In the former, initiation occurs at a fixed distance from a dominant site of polymerase interaction in the promoter called the TATA box. In yeast, initiation occurs at variable and more distant sites. The latter could be explained if the melting function of the initiation factor TFIIH used adenosine triphosphate energy to pump DNA into a scrunched state as the enzyme searched for a suitable initiation site on the template DNA strand (*10*). In this case, many nucleotides of melted DNA could be extruded out the sides of the polymerase (see the figure).

Equally interesting is the implication of scrunching for the structure of transcription complexes paused during elongation. Paused transcription elongation complexes are important sites of regulation, and certain examples are likely to be similar to abortive complexes at the promoter. At such sites, RNAP constrained from upstream could continue transcription for some distance by drawing in downstream DNA. One example occurs in an antitermination regulatory circuit of the *Escherichia coli* bacteriophage lambda in which the sigma initiation factor rebinds a promoter-like sequence in downstream DNA, forming a specialized elongation complex that is substrate for an antitermination protein (*11*). This structure is almost certainly formed by scrunching, and a stable scrunched state could be the regulatory target. Promoter-proximal paused transcription complexes are an important feature of eukaryotic transcription, well characterized for the heat-shock promoters in *Drosophila melanogaster* and the human HIV promoters and conjectured to occur widely (*12*); DNA scrunching could well be an element of their formation and regulation. It is a good bet that scrunched DNA will appear in future detailed views of transcription regulatory complexes.

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