

characterized by an impressive array of innovative methods and transformations, ranging from effective preparations of the amino acid building blocks to selective introduction and subsequent retention of the labile *N,O*-acetal. In combination with the high degree of convergence and modularity of the approach, this should also make it readily adaptable to the synthesis of tubulysin analogs. Along these lines, it will be of interest to increase the specificity of tubulysins for cancer cells, to improve their pharmacologic properties and to simplify

and stabilize the structure while retaining potency. Overall, the success of Peltier *et al.* is expected to greatly enhance the further development of these antimetabolic agents toward the development of a drug that can stop cancer cell propagation.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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An RNA transcriptional regulator templates its own regulatory RNA

Jennifer F Kugel & James A Goodrich

The bacterial transcriptional repressor 6S RNA mimics the DNA contained in a melted promoter and forms specific interactions with the active site of RNA polymerase. A new study shows that, surprisingly, 6S RNA acts as a template for the synthesis of small RNAs, which liberates the polymerase from 6S RNA. Hence, the transcriptional repressor 6S RNA serves as a template for the synthesis of its own de-repressive RNA.

6S RNA is a non-coding RNA that inhibits transcription in *Escherichia coli* during the stationary phase of growth by associating with RNA polymerase (RNAP)¹. In a recent report in *Science*, Wassarman and Saecker show that 6S RNA forms specific interactions with RNAP to prevent it from associating with its typical target, the promoter DNA². 6S RNA associates with the active site of RNAP and serves as a template for the synthesis of short RNA products (pRNAs) *in vitro* and in cells. pRNA synthesis destabilizes the complex between RNAP and the 6S RNA–pRNA hybrid². These new findings document a previously unobserved function for a non-coding RNA (ncRNA) and a new mechanism of transcriptional regulation.

Transcription by *E. coli* RNAP has long been used as a model system for studying the way DNA is copied to make RNA and how this process can be controlled. RNAP consists of a core enzyme and a dissociable σ subunit that together form the holoenzyme (holo RNAP) that is competent to bind the promoters of genes, melt the DNA to form open complexes,

and initiate transcription³ (Fig. 1a). The predominant σ factor in *E. coli* is σ^{70} , which contacts single-stranded DNA in the transcription bubble³.

In 2000, Wassarman and Stortz showed that 6S RNA binds the σ^{70} -containing holo RNAP and represses transcription in stationary phase¹. The secondary structure of 6S RNA contains a central single-stranded bulge that is suggestive of the melted conformation of promoter DNA in open complexes^{4, 5}. Consequently, it was predicted that 6S RNA repressed transcription by disrupting contacts between the promoter DNA and the polymerase^{4, 5}.

In new work, Wassarman and Saecker demonstrate a unique mechanism for the way that 6S RNA represses transcription and then actively participates in relieving that repression² (Fig. 1b). First, the authors establish that 6S RNA prevents holo RNAP from binding promoter DNA by mimicking the DNA contained in a melted promoter and forming specific interactions with the active site of RNAP. To determine the proximity of 6S RNA to the active site of RNAP, they incorporated iron into the active site, and they found that the resulting hydroxyl radicals cleaved 6S RNA at a cluster of specific positions. The authors then directly tested the ability of 6S RNA to serve as a template for RNA synthesis *in vitro*. They found that ncRNAs of 14–20 nucleotides—

pRNAs—were produced by holo RNAP when nucleotide concentrations were relatively high. Importantly, pRNAs were synthesized from a specific initiation site on the 6S RNA template that mapped to the location of the primary cleavage site identified by the hydroxyl radical proximity experiment. These results provide the first evidence that *E. coli* RNAP can use a biologically important RNA as a substrate for site-specific RNA synthesis.

The authors next assessed the consequence of pRNA synthesis for 6S RNA and holo RNAP. Upon pRNA synthesis *in vitro*, σ^{70} was released, and the resulting complexes between core RNAP and the 6S RNA–pRNA hybrid were unstable, perhaps owing to the inability of 6S RNA to anneal behind the moving RNAP (Fig. 1b). These data suggest that 6S RNA-directed pRNA synthesis releases RNAP, thereby de-repressing transcription.

The question then arises of how transcriptional repression occurs during stationary phase in bacterial cells. The authors detected 6S RNA–pRNA hybrids in extracts prepared from *E. coli* cells released from stationary phase, thus directly demonstrating that pRNAs are synthesized under specific conditions in cells. The authors propose the following biological model: in the stationary phase, 6S RNA is bound to holo RNAP and is able to repress transcription because nucleotide concentrations are insufficient for pRNA synthesis. The

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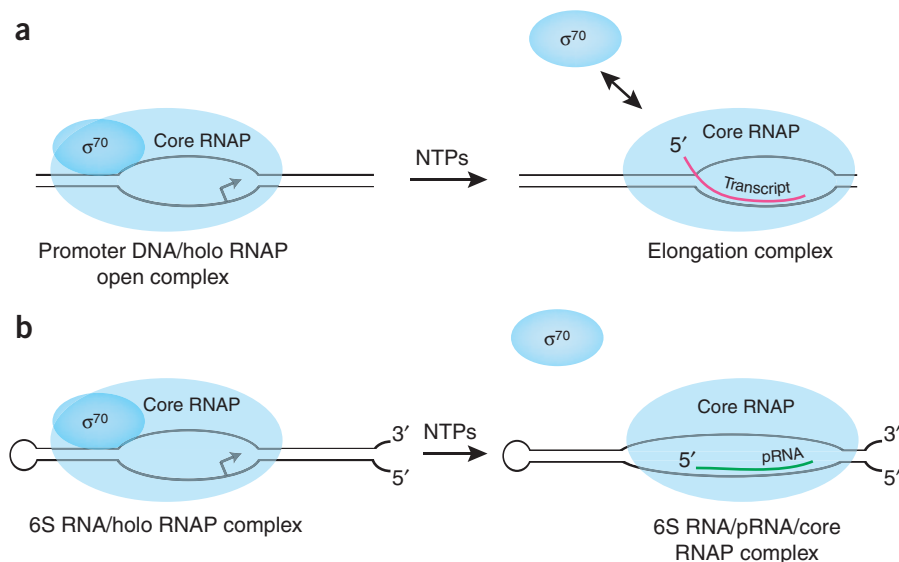


Figure 1 6S RNA mimics promoter DNA. **(a)** Transcription by *E. coli* RNAP. Open complexes contain holo RNAP and melted promoter DNA. Upon addition of NTPs, transcription begins, σ^{70} can dissociate and elongation complexes form. During this process, the upstream region of the bubble reanneals and the 5' end of the RNA transcript peels away from the template DNA. **(b)** Transcriptional regulation by 6S RNA. During the stationary phase, 6S RNA binds σ^{70} RNAP holoenzyme in a manner similar to DNA in an open complex, thereby repressing transcription. Repression is relieved as cells exit stationary phase because increased NTP concentrations allow RNAP to synthesize pRNAs using 6S RNA as a template. This results in the release of σ^{70} , and the remaining 6S RNA–pRNA–core RNAP complexes are unstable. This instability is proposed to occur because the 6S RNA bubble cannot anneal behind the RNAP.

increase in nucleotide pools that occurs upon exit from stationary phase⁶ allows 6S RNA–directed pRNA synthesis, thereby relieving repression by 6S RNA.

Before this study, it was unknown how transcriptional repression by 6S RNA was relieved

as cells exited from stationary phase. The work of Wassarman and Saeker explains this and demonstrates a unique and resourceful mechanism of biological regulation: 6S RNA simulates a specific conformation of promoter DNA and binds RNAP, thereby repressing

transcription. Remarkably, the 6S RNA–holo RNAP complex has a built-in means for de-repression—the 6S RNA serves as a template for synthesis of its own de-repressive RNA. Interestingly, 6S RNA–holo RNAP complexes were still detectable 1 h after cells were released from stationary phase²; the relevance of these complexes to transcriptional regulation remains to be determined.

This report raises many new and intriguing questions. Are there as-yet-unknown factors that regulate 6S RNA levels and the complex of 6S RNA and holo RNAP? Do other RNAs in prokaryotic or eukaryotic cells serve as templates for RNA synthesis? For example, mouse B2 RNA binds RNA polymerase II and represses transcription⁷; it might serve as a template for RNA synthesis in a biologically relevant manner. Lastly, do the pRNAs themselves have a function in cells? Answers to these questions will provide further insight into the role of 6S RNA and reveal whether this is a generalized mechanism of regulation.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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