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Transcription by Moonlight: Structural Basis of an Extraribosomal Activity of Ribosomal Protein S10

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Abstract

In this issue of *Molecular Cell*, Luo et al. (2008) show that S10 protein can function in the ribosome or the transcript elongation complex with minimal structural change, providing new insights into the roles of S10 and NusB in transcript elongation.

Some proteins have multiple, apparently unrelated jobs in the economy of the cell, a phenomenon known as “gene sharing” or, more colorfully, “moonlighting” (Piatigorsky et al., 1988; Jeffery, 2003). Moon-lighting is particularly widespread among ribosomal proteins, many of which have extraribosomal “employment” in addition to their daytime jobs as components of the translation machinery. Indeed, one of the earliest examples of moonlighting was discovered when Friedman et al. (1981) showed that a mutation that altered ribosomal protein S10 affected transcript elongation in phage-infected *E. coli*. The work of Luo et al. (2008) has now revealed how S10 acts in two such disparate roles, and provides guidance to help us understand how other moonlighting proteins increase the functional diversity of proteomes.

The dual role of S10 is intriguing because the transcription and translation machines share few common components. In its transcriptional role, S10 forms a complex with the *E. coli* NusB protein, and the complex specifically binds transcripts containing the BoxA sequence. BoxA was discovered as a sequence required for antitermination of phage λ early transcription and subsequently was shown to be required for antitermination of transcription in the *E. coli* ribosomal RNA (rRNA) operons and for termination of transcription mediated by the Nun protein of phage HK022 (Olson et al., 1982; Li et al., 1984; Robert et al., 1987). In λ-infected cells, many phage genes are not transcribed until the transcription elongation complex (TEC) is modified so that it can suppress transcription termination signals located upstream of these genes. Only those TECs that have transcribed the phage *nut* sites are modified. *Nut* transcripts contain two sequences that bind proteins needed for antitermination, BoxA and BoxB. BoxA, as already noted, binds NusB-S10, and BoxB binds the λ N protein (Chattopadhyay et al., 1995; Mogridge et al., 1995). After binding *nut* RNA, NusB-S10 and N, together with other host proteins, associate stably with the TEC that transcribed *nut* (Figure 1A) and, in some way that remains poorly understood, increase the transcript elongation rate and suppress termination. It is likely that S10 and NusB act

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similarly in antiterminating rRNA transcription, although neither N nor BoxB is involved in this case. The λ *nut* transcripts are also targets of Nun protein, a transcription termination factor that is encoded by a λ relative, phage HK022. This opposite activity, *mirabile dictu*, also requires S10 and NusB as well as the same *nut* sequences needed for antitermination.

Analysis of ribosome structure reveals that S10 has a globular surface domain and an extended loop that penetrates into the interior to interact with other ribosomal proteins as well as with 16S rRNA. Does S10 retain this structure when moonlighting as a transcription factor? Can it moonlight while remaining a component of the ribosome? What is its role in transcript elongation? Luo et al. (2008) provide answers to all of these questions. They started by showing that an S10 mutant that lacks the extended ribosome-binding loop ($S10^{\Delta loop}$) was unable to support cell growth but retained its transcriptional functions: it supported λ antitermination and Nun termination, and formed a complex with NusB that bound BoxA RNA. Thus, it is likely that deletion of the loop is lethal because the mutant protein is defective in translation. By contrast, an antitermination-defective S10 mutant (*nusE71*) alters the globular domain, and this mutant is viable. $S10^{\Delta loop}$-NusB complexes, unlike S10 or NusB-S10, formed well-ordered crystals that allowed structure determination. The overall conformation of the S10 globular domain was similar in the ribosome and in the NusB complex, arguing strongly that a gross alteration in structure is not needed for S10 to perform different functions. Moreover, the surface of S10 that binds NusB is occluded in the ribosome, so an intact ribosome cannot provide the transcriptional activity of S10.

Analysis of UV-induced protein-RNA crosslinks in NusB-S10-BoxA and NusB-BoxA complexes allowed modeling of the structure of the complexes and furnished an unexpected insight into the roles of S10 and NusB in antitermination. According to the model, NusB-S10 uses a continuous surface involving both proteins to bind BoxA RNA (Figure 1B). NusB by itself binds BoxA in a similar way, as does NusB-S10, albeit with lower affinity because it makes fewer RNA contacts. However, S10 by itself binds RNA largely nonspecifically. These observations led Luo et al. (2008) to suspect that the only essential function of NusB in antitermination is that of an adaptor: it recruits S10 to the appropriate position in BoxA, thereby facilitating the interaction between S10 and the TEC. In support of this hypothesis, they showed that overproduction of S10 obviated the NusB requirement for λ antitermination and Nun termination, possibly because the excess promotes occupancy of BoxA without a NusB adaptor. It had previously been thought that NusB is a critical component of the antitermination complex and that S10 recruits NusB. It is now clear that the opposite is true.

When the number of intracellular S10 molecules exceeds that of ribosomes, the excess protein should promote rRNA transcription, thus allowing feedback control of ribosome synthesis. This is important for the metabolic efficiency of the cell, because ribosomes constitute a major fraction of the cell mass, especially at high growth rates. At first view, it might seem that suppressing terminators is key to increasing rRNA synthesis rates. However, Klumpp and Hwa(2008) recently argued that the S10- and BoxA-dependent modification of TECs promotes increased rRNA synthesis primarily by increasing the transcript elongation rate. They propose that the elongation rate increases because a modified TEC spends less time at transcriptional pause sites. It is unclear if antipausing and

antitermination are independent consequences of TEC modification or if suppression of pausing causes suppression of termination. Indeed, the molecular mechanisms of antipausing and antitermination in rRNA genes as well as in phage λ remain open questions and await further experimental testing.

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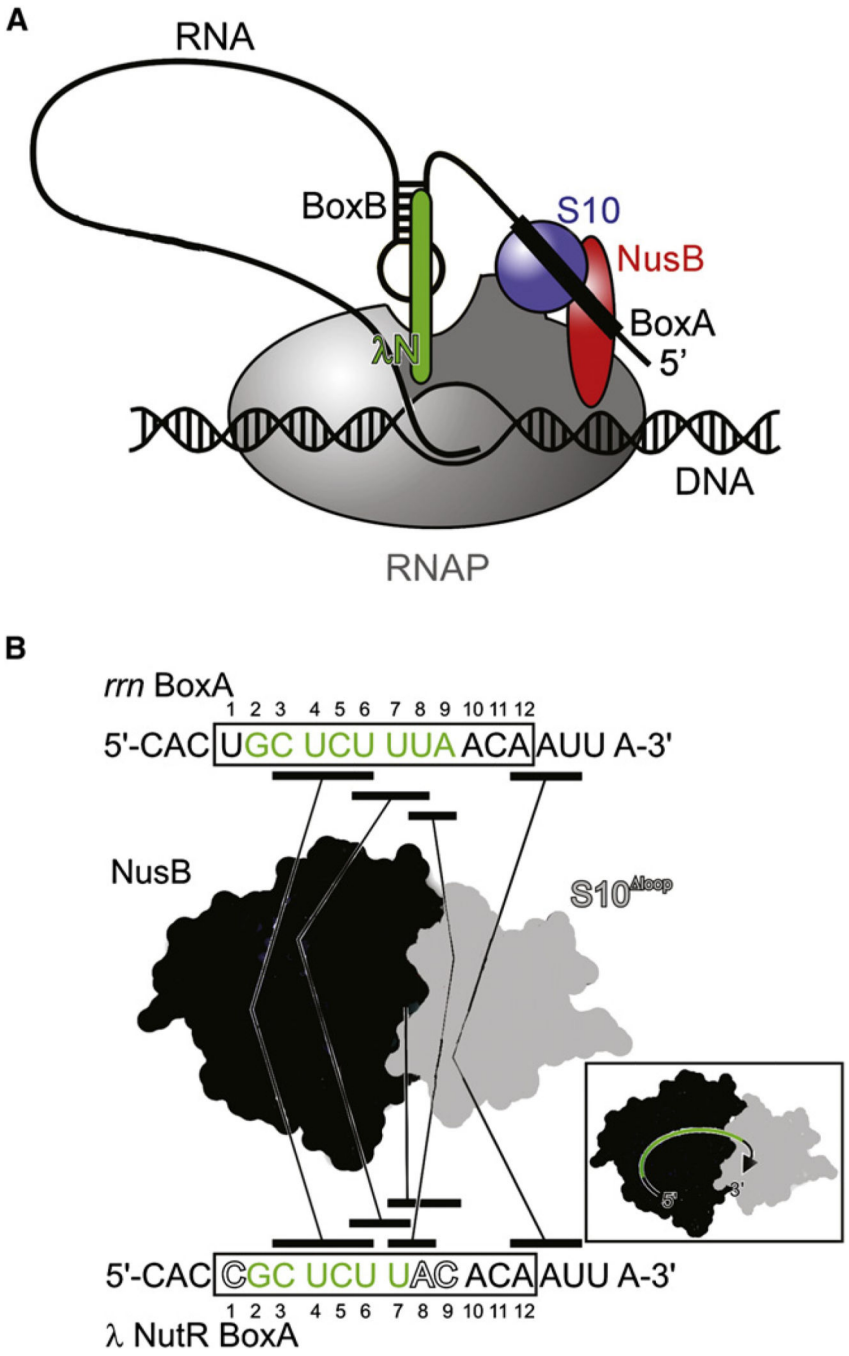

Figure 1. Models of the Phage λ Antitermination Complex and of the RNA-Binding Surface of λ NusB-S10

(A) A cartoon of a TEC that has been modified by antitermination factors bound to nascent BoxA and BoxB RNA (solid rectangle and folded hairpin, respectively) after traversing a λ *nut* site. The gray oval represents RNA polymerase (RNAP). Elongation factors NusA and NusG, known to be part of the complex, were omitted for clarity.

(B) The NusB-S10-BoxA complex. A NusB-S10$^{\Delta loop}$-BoxA complex was subjected to UV-induced protein-RNA crosslinking and the locations of the crosslinks determined. Two different BoxA sequences were used, one from the λ *nutR* site and the other from an *E. coli*

rRNA gene. The lines connecting regions in the proteins to segments of the two BoxA sequences indicate the approximate locations of peptide-RNA crosslinks. The inset illustrates the deduced topology. Adapted from Luo et al. (2008).