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Small Molecules, Big Effects on Gene Expression: Control of Transcription Initiation by Molecules that Occupy the Secondary Channel of RNA Polymerase

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We study transcription initiation in *E. coli*, with a focus on the mechanistic basis for the extraordinary strength and complex regulation of rRNA promoters. These studies have important implications not only for ribosome synthesis, but also for the mechanisms that underlie stress responses, the regulation of multicomponent gene networks, and promoter recognition in general.

Several hundred transcription factors play central roles in regulation of *E. coli* gene expression. A common theme in the mechanisms of most is that promoter-specificity derives from binding of the factors to specific DNA sequences near or overlapping the RNA polymerase (RNAP) binding site. Most repressors compete with RNAP for binding to the promoter, and most activators make relatively arbitrary protein-protein contacts to appropriately positioned surfaces on RNAP. These interactions recruit RNAP to the promoter and/or stabilize/destabilize intermediates on the pathway to transcription initiation.

There are some trans-acting factors, however, that exert promoter-specific effects by interacting only with RNAP and not with DNA. This lecture will focus on mechanisms of regulation by these nontraditional transcription initiation factors. The effects of two such factors, ppGpp and the concentration of the first NTP in the transcript, derive from the ability of some promoters to exploit the kinetic variation resulting from differential interactions with modules of RNAP in addition to those parts that bind to the -10 and -35 elements. The effects of ppGpp and NTPs on transcription are made possible by DksA, a co-factor that binds to RNAP and adapts it for regulation.

We have investigated the mechanism(s) by which ppGpp/NTPs/DksA control transcription. There are at least three kinetically-significant steps on the pathway to transcription initiation. The first step is the formation of a closed complex (RP_C), and the last is the formation of the open complex (RP_O). There is at least one intermediate complex between RP_C and RP_O in this pathway, RP_I. Unlike the situation at most promoters, at rRNA promoters RP_O is very short-lived, and RP_I is highly populated. DksA targets this intermediate by binding in the secondary channel of RNAP, the part of the enzyme through which

NTPs are thought to gain access to the active site.

Widespread effects of ppGpp/DksA on gene expression are induced by limitation for amino acids or other nutrients. Effects of DksA have been reported not only on gene expression, but also on chaperone function, cell division, DNA repair, amino acid requirements, quorum sensing, phage sensitivity, and other cellular processes. While some of these phenotypes likely result from indirect effects of the direct effects of ppGpp/DksA on rRNA transcription, ribosome synthesis, and overall protein synthesis, others could result from direct effects of ppGpp/DksA at specific promoters. For example, we have shown recently that ppGpp/DksA directly regulates promoters for flagella synthesis and for the transcription factor Fis.

Surprisingly, the effects of ppGpp/DksA are not limited to negative regulation. ppGpp/DksA also directly activates some amino acid biosynthesis promoters, a promoter for the global transcription factor Hfq, and some promoters for pathogenesis-related functions. How ppGpp/DksA functions as a direct positive regulator will also be discussed. In any case, it has long been known that amino acid starvation (and the resulting "stringent response") has a broad impact on overall gene expression and cell physiology. It is now clear that much of this impact results from direct effects of ppGpp/DksA on RNAP, negative or positive depending on the individual promoter's intrinsic kinetic properties.

E. coli's rRNA operons can account for as much as two-thirds of all transcription activity in the cell under favorable growth conditions, implying that RNAP has evolved to a major extent to recognize these promoters. Thus, perhaps it is not surprising that the study of these promoters has provided us with insights into basic mechanisms of transcription initiation. The discovery of the importance of the RNAP secondary channel in control of transcription initiation adds to the list of discoveries that have resulted from studying rRNA promoters. These include the findings that changing concentrations of NTPs and ppGpp can serve as regulators of transcription (at different times) and that there are additional promoter elements (i.e. other than the -10 and -35 hexamers) that are recognized by RNAP and play important roles in transcription regulation.