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GUEST COMMENTARY

Gene-Specific Regulation by a Transcript Cleavage Factor: Facilitating Promoter Escape[▽]

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The transcript cleavage factor GreA can play a significant role in stimulating the escape of RNA polymerase from native promoters in *Escherichia coli*.

The bacterial Gre factors associate with RNA polymerase (RNAP) during transcription and induce cleavage of the nascent transcript at the active site of the enzyme (7, 17). Analysis with purified components in vitro has indicated that Gre factors can mediate effects at multiple stages of the transcription cycle. During the elongation phase of transcription, Gre factors can rescue stalled elongation complexes through their ability to stimulate endonucleolytic cleavage of the nascent RNA (1, 2). In addition, during the early stages of transcription, they can facilitate the process by which RNAP escapes the promoter and makes the transition to the elongation phase of transcription (6, 9, 28). While the Gre factors have been extensively studied in vitro, their in vivo roles are less well understood. In this issue of the Journal of Bacteriology, Borukhov and colleagues (27) provide new insight into Gre factor function by identifying in vivo targets of the *E. coli* cleavage factor GreA. Their findings indicate that GreA can play a significant role in stimulating promoter escape at native *E. coli* promoters and, furthermore, that this function likely depends on the ability of GreA to stimulate transcript cleavage.

GRE FACTOR MECHANISM OF ACTION

The Gre factors are members of a diverse family of structurally related proteins that modulate the function of RNAP through a direct binding mechanism (17). The bacterial RNAP core enzyme (subunit structure $\alpha_2\beta\beta'\omega$), like all multisubunit RNAPs, adopts a conserved crab claw structure (3, 5). The large β and β′ subunits form the pincers of the claw and encompass the main channel of the enzyme, which accommodates the template DNA and the RNA-DNA hybrid that forms during transcription. Deep within this channel is the catalytic center of the enzyme, which is marked by a stably bound Mg^{2+} ion that participates in catalysis. Another, smaller channel, the so-called secondary channel, also links the external milieu to the catalytic center of the enzyme and is thought to serve as the entry port for substrate nucleotides (13). The Gre factors can directly modify the catalytic activities of RNAP by gaining access to the catalytic center of the enzyme via the secondary channel (15, 18, 23). (In eukaryotic cells, the structurally unrelated transcription factor TFIIS functions analogously to the bacterial Gre factors, gaining access to the catalytic center of PolII via the secondary channel [11]).

Members of the extended Gre family (including DksA [20] and Gfh1 [12, 14, 29; reviewed in reference 4]) share a conserved two-domain structure, consisting of an elongated N-terminal domain that forms a characteristic antiparallel coiled-coil and a globular C-terminal domain (25). These family members use the C-terminal domain to bind RNAP near the opening of the secondary channel and extend the coil-coil motif into the secondary channel such that its tip approaches the catalytic center of the enzyme and, in the case of the Gre factors, contributes directly to active site function (15, 18, 23). Specifically, Gre factors stimulate an endogenous endonucleolytic cleavage reaction that reactivates stalled elongation complexes formed as a result of backtracking, a process associated with transcription pausing. During backtracking, the RNAP catalytic center slides back relative to the 3′ end of the nascent transcript with displacement of the 3′ tail of the RNA into the secondary channel (7). The endonucleolytic cleavage reaction stimulated by the Gre factors creates a new RNA 3′-OH in the active site, now correctly aligned for the addition of the next nucleotide. This stimulatory effect of the Gre factors depends on a conserved pair of acidic residues at the tip of the coiled-coil motif that helps to coordinate the binding of one of two Mg^{2+} ions that are both required for all the catalytic activities of RNAP, including endonucleolytic cleavage of the nascent RNA (15, 18, 23, 24, 26).

As well as stimulating transcription elongation by reactivating backtracked transcription complexes, the Gre factors can also influence the early stages of transcription. In particular, in vitro analysis has revealed that Gre factors can facilitate the process by which RNAP escapes the promoter to enter the elongation phase of transcription (promoter escape) (6, 9, 28). Promoter escape is preceded by a phase of transcription known as the abortive phase, during which the initial transcribing complex produces short (typically 2 to ~11 nucleotides in length) abortive RNAs that are repetitively synthesized and released while RNAP maintains its contacts with the promoter (reviewed in reference 8). The Gre factors have been shown to inhibit the production of abortive RNAs by increasing the efficiency of promoter escape (6, 9, 28). Whereas it has been clearly established that their ability to stimulate endonucleolytic cleavage of the nascent RNA is critical for the rescue of

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stalled elongation complexes, whether this ability is required for the effect of the Gre factors on promoter escape is less clear.

ACTIVITIES OF THE GRE FACTORS IN VIVO

Compared with what is known about the in vitro activities of the Gre factors, relatively little is known about their in vivo functions. Nevertheless, most bacteria contain *gre* genes, suggesting important physiological roles for the Gre proteins. *E. coli* contains two *gre* genes, *greA* and *greB*. Although neither is essential for viability, several phenotypes have been associated with *gre* mutants, including sensitivity to divalent metal ions, salt, and temperature (27, 28). In addition, *greA* is a member of the σ^E regulon, which encodes functions involved in the extracytoplasmic stress response (22), consistent with a regulatory role for GreA in the cellular response to various forms of stress.

In principle, Gre factors might serve primarily as general transcription factors, rescuing stalled elongation complexes (30). However, several lines of evidence indicate that Gre factors can also exert gene-specific effects on transcription (9, 16, 28). Although the most well-known regulators that exert gene-specific effects on transcription are sequence-specific DNA-binding proteins that facilitate transcription initiation at specific target promoters, transcription factors that are not sequence-specific DNA-binding proteins can exert gene-specific effects based on the presence of specific kinetic barriers that limit the expression of particular transcription units. One member of the extended Gre family of secondary channel regulators, DksA, provides a particularly well-characterized example of such a mechanism (19).

Although several previous studies have provided evidence correlating the in vitro activities of the Gre factors with in vivo activities (9, 16, 28, 30), the study of Borukhov and colleagues (Stepanova et al. [27]) in this issue of the Journal of Bacteriology provides the most comprehensive body of data to date in support of an important role for Gre factors in gene regulation. For the purpose of identifying in vivo targets of the Gre factors, they performed a set of microarray experiments, initially comparing otherwise isogenic *greA*$^+$ *greB* mutants and *greA greB* mutants (based on previous evidence suggesting that more phenotypes are associated with *greA* mutants than with *greB* mutants). Relatively few genes (19) were identified that were more highly expressed in the presence of GreA. Hypothesizing that the expression of *greA*, known to be induced under various stress conditions, might be limiting under the conditions of the microarray analysis, the researchers attempted to identify additional Gre-responsive genes by comparing the expression profiles of *greA greB* mutant cells containing a plasmid that overproduced either a wild-type GreA or a GreA mutant bearing a single amino acid substitution that specifically impairs its transcript cleavage activity (15). In this way, the researchers hoped, moreover, to identify genes whose expression is affected specifically by the transcript cleavage activity of GreA. This protocol resulted in the identification of 126 genes whose expression increased specifically in the presence of wild-type GreA (including the 19 originally identified). Furthermore, operon analysis of the identified genes suggested that GreA exerts its effects at the early stages of transcription (e.g., initiation, promoter escape, or early elongation pausing), an observation that would not necessarily have been anticipated.

After confirming the microarray results for several randomly chosen transcription units by primer extension analysis, Stepanova et al. (27) examined the effect of GreA (wild-type or mutant) on transcription from a subset of the corresponding promoters in vitro. In general, they observed a decrease in the amount of abortive RNAs relative to full-length (runoff) transcription product specifically in the presence of wild-type GreA. From these results, they were able to infer that the stimulatory effect of GreA on the expression of specific transcription units (at least under the conditions of the microarray analysis) typically affects promoter escape and, moreover, that this effect likely depends on the ability of GreA to stimulate transcript cleavage.

Somewhat surprisingly, the microarray analysis also identified 82 genes whose expression was decreased in the presence of either chromosomally encoded GreA or plasmid encoded wild-type GreA. Primer extension analysis to quantify RNA levels confirmed the inhibitory effect of overexpressed wild-type GreA in several cases, suggesting that the observed down-regulation also affects the early stages of transcription. However, the failure to observe corresponding effects of GreA on the same selected promoters in vitro indicates either that these are indirect effects or that GreA is functioning in conjunction with other factors to mediate these effects.

MECHANISTIC IMPLICATIONS

How might GreA suppress the production of abortive RNAs and stimulate promoter escape? In principle, this could occur without transcript cleavage. Recent studies have established that during the abortive phase of transcription, nucleotide addition occurs without forward translocation of the enzyme with respect to the core promoter elements, requiring the downstream DNA to be reeled into the main channel of the enzyme in a process that has been referred to as DNA scrunching (10, 21). The transcription bubble thus expands in a stepwise fashion as each successive nucleotide is added. The release of an abortive product is accompanied by bubble contraction, and the process repeats itself. Alternatively, the bubble contraction can be accompanied by forward translocation of the enzyme and escape into productive elongation. Based on this model, a factor could facilitate promoter escape by affecting the relative probabilities of these competing events (nucleotide addition with bubble expansion, the release of an abortive product, or nucleotide addition with promoter escape). It is easy to imagine that a factor that binds within the secondary channel of RNAP could influence these events either by an allosteric mechanism or by altering the chemical environment of the active center (for an example, see reference 19).

However, it is possible that the situation is somewhat more complicated and that scrunched complexes containing nascent RNAs of a certain minimal length may be susceptible to backtracking, with partial contraction of the bubble. If this occurs and if the release of the nascent RNA from such a complex is rate imiting, then the action of a transcript cleavage factor could allow for resynthesis without release of the RNA, providing the complex with another opportunity for promoter escape (6, 9). By using a GreA mutant specifically defective for

transcript cleavage (i.e., fully functional in RNAP binding), Stepanova et al. (27) provide support for such a scenario. Nevertheless, the possibility that this mutant, which bears an amino acid substitution at the tip of the coiled-coil domain, might differ from wild-type GreA in other relevant characteristics cannot be excluded.

The use of the GreA cleavage-defective mutant was important in another respect, as well. As mentioned above, the Gre factors are members of a larger family of secondary channel regulators, including the regulator DksA, which plays a critical role in mediating the effects of ppGpp on gene expression from the rRNA promoters, as well as others (19). The existence of multiple secondary channel regulators in the cell implies competition for access to the secondary channel. The intracellular concentrations of the relevant proteins as well as their intrinsic binding affinities will necessarily influence this competition. Accordingly, changes in the levels of any particular factor can be expected to produce both direct and indirect effects on cellular physiology. By comparing cells containing excess wild-type or mutant GreA, Stepanova et al. (27) elegantly controlled for such indirect effects.

Whereas the current study uncovered genes that are upregulated by GreA due to its ability to stimulate promoter escape, this does not exclude the possibility that GreA and/or GreB also function to regulate elongation in vivo. In one well-characterized example involving the λ late promoter, the Gre factors have been shown to influence the lifetime of an early elongation pause both in vitro and in vivo, under circumstances where the paused elongation complexes are known to undergo backtracking (16). Other types of gene-specific blocks to elongation may occur under particular growth conditions or in the presence of particular forms of stress and the Gre factors may facilitate the expression of specific genes under these circumstances. In addition, the Gre factors might function to rescue stalled elongation complexes that form independently of any gene-specific signals, due to DNA damage or other general circumstances that can produce elongation barriers (30). Further investigation is likely to expand the observed repertoire of in vivo functions for this class of proteins.

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