CarD Tricks and Magic Spots: Mechanisms of Stringent Control in Mycobacteria

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Global reprogramming of bacterial gene expression in response to nutritional stress, the stringent response, is well studied in *E. coli*. Now Stallings et al. report that *Mycobacterium tuberculosis* employs a different strategy involving the general transcription factor CarD for growth control and persistence in response to stresses encountered during infection.

Tuberculosis (TB) continues to be a major global health problem, with 8.9 million new cases and 1.7 million deaths per year (Dye, 2006). A hallmark of *Mycobacterium tuberculosis* pathogenesis is the ability of bacilli to enter a slow-growing or nonreplicative state, leading to a latent infection lasting the lifetime of the host. Lack of vital nutrients, oxygen, and iron is thought to restrict bacterial replication during infection (Boshoff and Barry, 2005). For example, *M. tuberculosis* bacilli have the exceptional ability to persist for decades in vitro under starvation conditions and can revive upon addition of nutrients. This remarkable ability of *M. tuberculosis* to persist in the face of nutritional and immune stresses renders tuberculosis difficult to treat with current antibiotics, which generally require some bacterial growth to exert their killing effects. Thus, elucidating the mechanisms of persistence is of great interest in the field and critically important to the global control of this deadly disease.

Our understanding of bacterial responses to starvation stress has largely been informed by studies in *Escherichia coli*. In response to amino acid deprivation, *E. coli* cells inhibit expression of stable RNAs required for protein synthesis, rRNA and tRNA, and stimulate transcription of other operons, including those required for the biosynthesis and transport of certain amino acids (Srivatsan and Wang, 2008). At the heart of this regulatory cascade are the "magic spot" starvation signals ppGpp and pppGpp, referred to collectively as (p)ppGpp. In response to amino acid starvation, (p)ppGpp synthesis is catalyzed by the ribosome-associated RelA protein. Importantly, the transcriptional changes in response to increased (p)ppGpp are not mediated by DNA-binding transcription factors but, rather, by direct alteration of the stability of the RNA polymerase (RNAP) complex at regulated promoters. (p)ppGpp alone is not sufficient to mete out these changes but works along with the general transcription factor DksA, which interacts directly with RNAP to exert its effects on gene expression. Intrinsic kinetic differences between rRNA promoters and amino acid biosynthetic promoters allow for DksA-modified RNAP to favor one over the other. Although the stringent response has been studied primarily in regards to starvation, a diverse array of stresses activate (p)ppGpp synthesis, including deprivation of phosphorous, iron, and fatty acids (Srivatsan and Wang, 2008).

In a recent study published in *Cell*, Stallings et al. set out to identify genes whose transcription was induced in response to double-strand DNA breaks in the chromosome of *Mycobacterium smegmatis*, a nonpathogenic mycobacterium distantly related to *M. tuberculosis*. A general transcription factor *carD* was among the most highly induced genes under these conditions, and the authors show that *carD* is upregulated in response not only to agents that induce DNA damage, but also in response to oxidative damage and nutritional limitation. The authors show that *carD* is an essential gene and thus use conditional alleles under the control of regulated promoters for their studies. As expected, CarD depletion renders the bacteria sensitive to DNA damage, oxidative stress, and starvation. Together, these findings suggest that CarD is a key player in the general stress response.

The clue that CarD is involved in stringent control arose from microarray studies of cells depleted of the protein. Stallings et al. show that levels of stable rRNA and mRNAs encoding ribosomal protein subunits are strongly induced in the CarD-depleted strain compared to the wild-type, even under normal growth conditions. As in *E. coli*, rRNA transcription is repressed under starvation conditions in wild-type *M. smegmatis*, but not in the CarD depletion strain, even though (p)ppGpp still accumulated in the mutant. Therefore, CarD appears to control the stringent response in *M. smegmatis* in a fashion analogous to that of DksA in *E. coli*, even though these two proteins share no sequence similarity. Astonishingly, despite the apparent differences in these proteins, the *carD* gene from *M. tuberculosis* can functionally complement an *E. coli dksA* mutant.

Despite the similarity in their function, the molecular mechanism of CarD likely differs from that of DksA. Structural and biochemical studies suggest that DksA interacts with RNAP in the secondary channel, the same portal used by the structurally similar transcriptional elongation factors GreA/B (Haugen et al., 2008). DksA is thought to act primarily by affecting specific kinetic steps during initiation of transcription. CarD, however, likely binds to a different region of RNAP via a domain similar to the RNAP-binding

domain of the transcription repair coupling factor (TRCF). TRCF functions to remove stalled RNAP at sites of lesions in the DNA template, but how CarD binding influences RNAP activity is unknown. Given the differences in the mode of interaction with RNAP between these two proteins, it seems likely that CarD will alter RNAP activity via a mechanism distinct from that of DksA.

The authors show that, in *M. tuberculosis*, depletion of CarD leads to bacterial killing in a mouse model of infection. Although there is growing evidence that at least limited replication occurs during chronic infection and thus any essential gene may be required for persistence (Gandotra et al., 2007; Gill et al., 2009), this result underscores the importance of stringent control of bacterial growth in the host (Dahl et al., 2003). In addition, CarD is vital for bacterial resistance to oxidative stress and DNA damage, two further stresses likely encountered during infection. Although these results may not be surprising, they highlight the importance of the general bacterial stress response during chronic infection and support the notion that the CarD-RNAP interaction may be a viable target for therapeutic intervention.

Why is CarD essential in mycobacteria? *E. coli* mutants deficient for DksA, (p)ppGpp synthesis, or both are viable in rich media, as are *relA* mutants of *M. tuberculosis* and *M. smegmatis* (Dahl et al., 2003; Stallings et al., 2009). One possible explanation is that the stringent response is not essential for growth, but CarD performs an additional, (p)ppGpp-independent role in mycobacterial cells. Alternatively, the stringent response itself may be essential, but mycobacterial (p)ppGpp synthases other than RelA are able to support low-level concentrations of the nucleotide that are sufficient to allow survival. Interestingly, in *Bacillus subtilis*, which also has CarD, but not DksA, the *carD* gene is dispensable for growth (Kobayashi et al., 2003). This suggests that mycobacteria may be uniquely susceptible to stresses countered by CarD. For example, in contrast to *B. subtilis*, which encodes 10 rRNA operons scattered throughout the genome, most mycobacteria encode only one or two such operons. The authors suggest that, in the absence of CarD, stalled RNAP complexes may accumulate at these few sites in the genome, effectively blocking DNA replication and repair. Indeed, this work underscores a relatively underappreciated link between the stringent response and DNA damage, which posits that one of the critical roles of RNAP modulators such as DksA and CarD is to remove stalled transcription complexes that arise as a result of DNA damage (Trautinger et al., 2005).

Homology searches of sequenced prokaryotic genomes show that both *dksA* and *carD* homologs are found across diverse bacterial taxa. Indeed, some genomes encode both *dksA* and *carD*, supporting the notion that, despite their functional similarities, the two factors are not redundant. Organisms ranging from Actinomycetes to Cyanobacteria encode only *carD* homologs, indicating that CarD-mediated regulation of the stringent response is likely widespread in the microbial world. Finally, diverse bacteria, including important human pathogens, lack both *dksA* and *carD* homologs despite encoding (p)ppGpp synthases. Therefore, other mechanisms to read out (p)ppGpp await discovery.

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