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CarD: A new RNA polymerase modulator in mycobacteria

Christina L. Stallings
Washington University School of Medicine in St. Louis
Michael S. Glickman
Memorial Sloan-Kettering Cancer Center

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CarD

A new RNA polymerase modulator in mycobacteria

Christina L. Stallings[1,*] and Michael S. Glickman[2,3]

[1]Department of Molecular Microbiology; Washington University; St. Louis, MO USA; [2]Immunology Program; Sloan-Kettering Institute; [3]Division of Infectious Diseases; Memorial Sloan-Kettering Cancer Center; New York, NY USA

Mycobacteria CarD is an essential RNAP binding protein that regulates many transcripts including rRNA. This Point-of-View will review our present state of knowledge regarding CarD and compare the known functions of CarD with other RNAP binding proteins in *E. coli*, emphasizing how this information can guide future investigations.

Identification of CarD in Mycobacteria

Mycobacterium tuberculosis is an important global pathogen that continues to cause substantial suffering and death. Although the focus of most basic research on mycobacteria is drug or vaccine development, it has become clear that mycobacteria differ substantially from the model organisms *Escherichia coli* and *Bacillus subtilis* and thereby can expand paradigms established in these model systems. Our investigations into mycobacterial double strand break (DSB) repair have revealed the existence of a nonhomologous end-joining pathway, which repairs DSBs without the use of a homologous template, especially in late stationary phase.[1-3] As part of our efforts to understand the transcriptional response to DSBs in mycobacteria, we identified the transcript for a CarD family protein as highly induced after a homing endonuclease-generated double strand DNA break.[4] CarD represents a new class of RNAP binding proteins with unexpected functions in DNA damage responses, stringent control and mycobacterial viability.

Physiologic Roles of CarD in Mycobacteria

The CarD protein is highly expressed in *M. tuberculosis* and *Mycobacterium smegmatis* under basal conditions, but is also transcriptionally induced by multiple types of genotoxic stress and starvation.[4] Efforts to delete CarD from the *M. smegmatis* and *M. tubereulosis* chromosomes were unsuccessful, suggesting that CarD may be essential for viability, a suspicion that was confirmed by construction of depletion strains.[4] Transient depletion of CarD revealed several interesting phenotypes. Cells lacking CarD are sensitive to killing by hydrogen peroxide, ciprofloxacin and starvation, the same agents that induce CarD expression. Whole genome transcriptional profiling and quantitative real-time PCR revealed that depletion of CarD causes accumulation of mRNAs encoding ribosomal proteins as well as ribosomal RNA (rRNA). This finding provided critical insight into the function of CarD in mycobacteria, as the overexpression of ribosomal components was reminiscent of defective stringent control. Stringent transcriptional regulation of the translation machinery, as mediated by the *M. tuberculosis* RelA protein through production of (p)ppGpp, is critical for *M. tuberculosis* persistence during infection of mice, but is dispensable for *M. tuberculosis* viability in culture.[5] In vivo depletion of CarD in mice showed that CarD is also required for *M. tuberculosis* growth and persistence. These diverse phenotypes are illustrated in **Figure 1** and stimulated a detailed examination of the molecular function of the CarD protein.

Key words: CarD, mycobacteria, DksA, DNA damage, TRFC, stringent response, rRNA

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*Correspondence to: Christina L. Stallings; Email: stallings@borcim.wustl.edu

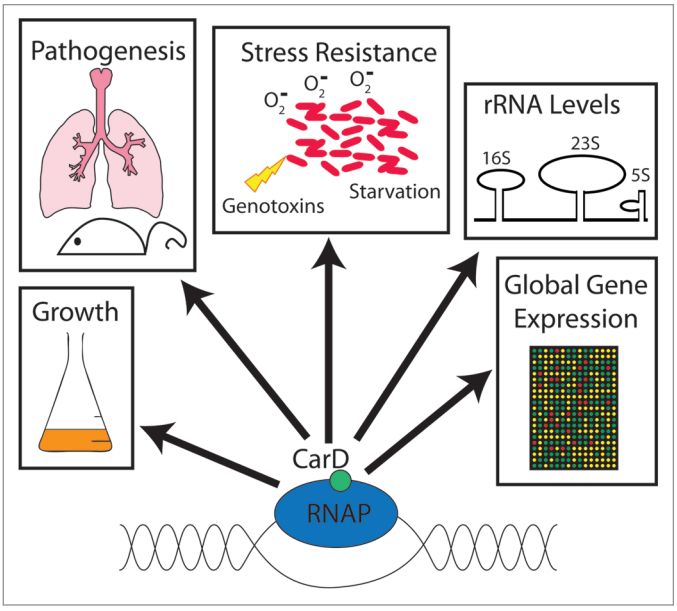

Figure 1. The diverse physiological effects of the RNAP binding protein CarD on mycobacteria.

Phylogenetic Distribution of CarD and Function in Other Bacteria

Although not conserved in eukaryotes, CarD proteins are widely distributed in bacteria, including *B. subtilis, Thermus thermophilus, Myxococcus xanthus* and *Borrelia burgdorferi*, and research in these organisms has provided additional information regarding CarD. Earlier work in *M. xanthus* had identified a paralog of CarD that contains an HMGA domain and is involved in fruiting body formation and carotenogenesis but which is nonessential.[6] More recently, the same research group examined the function of the *M. xanthus* ortholog of mycobacterial CarD (called CdnL in ref. 7 to distinguish this protein from the HMGA domain containing CarD). Studies in *B. burgdorferi* have also identified a CarD homolog, LtpA, that is induced at low temperatures.[8]

Molecular Features of CarD

The CarD protein (encoded by the *rv3583c* gene in *M. tuberculosis*) is 162 amino acids in primary sequence and contains an N terminal motif similar to the RNA polymerase (RNAP) interacting domain (RID) of transcription repair coupling factor (TRCF). Immunoprecipitation of CarD from *M. smegmatis* cell lysates identified three subunits (α, β, β') of the mycobacterial RNAP as associated with CarD, suggesting strongly that CarD directly binds RNAP. Further experiments using the bacterial two-hybrid system demonstrated that *T. thermophilus* CarD binds directly to the N-terminus of the RNAP β subunit (the β1 domain).[4] The same interaction has also been demonstrated between *M. tuberculosis* CarD and RNAP β (Stallings CL, Glickman MS and Nickels BE, unpublished data) as well as between *M. xanthus* CdnL and RNAP β.[7] The C terminal region of CarD is of unknown function but contains a putative leucine zipper. Deletion experiments revealed that the C terminus of CarD is essential for mycobacterial viability, implying that the C terminus is critical to the essential function of CarD.[4]

Similarities and Differences between CarD and DksA

The finding that Mycobacterial CarD is required for suppression of rRNA and ribosomal protein transcripts was reminiscent of the DksA protein in *E. coli*. Accordingly, heterologous expression of CarD in *E. coli* Δ*dksA* was able to rescue multiple phenotypes of the *E. coli dksA* null mutant, including amino acid auxotrophy and failure to suppress rRNA during starvation. Importantly, the RID domain of *E. coli* TRCF, but not TRCF RID L499R, which cannot bind RNAP β subunit,[9] was also able to suppress rRNA in the *E. coli dksA* mutant.[4] DksA is assumed to bind the secondary channel of RNAP, a site distinct from that of CarD, and is absolutely necessary for stringent regulation of rRNA transcription during nutrient limitation in *E. coli*.[10,11] The most biochemically defined action of the DksA protein is to decrease the open complex half-life during transcription initiation, an activity that strongly downregulates transcription from the P1 promoter of the *E. coli* rRNA *rrnB* operon.[11] Although much attention has focused on the role of DksA at the *rrnB* P1 promoter, deletion of *dksA* has pleiotropic effects on the cell, including DNA damage sensitivity,[12-15] which have not been clearly linked to its effects on transcription initiation. Thus, DksA and CarD are related insofar as CarD can functionally substitute for some roles of DksA and loss of DksA or CarD causes similar phenotypes despite the distinct interaction sites of the two proteins on RNAP.

RNAP Stability, DNA Replication and DNA Damage

Phenotypes of *E. coli* Δ*dksA* and mycobacteria CarD deficient cells both suggest a link between surviving DNA damage and global transcription regulation. DNA replication and RNA synthesis occur on the same DNA template and have an inherent potential to interfere with each other.[16] In vitro, DNA replication can be slowed significantly by encounters with the RNAP.[17,18] Regardless of whether the faster moving replisome hits a co-directional RNAP or collides with an RNAP head-on, the RNAP is generally dislodged by the replisome,[19-21] but replication is most affected and impeded by head-on transcription complexes.[18,21] If the replisome is unable to read through an RNAP roadblock and the replication fork arrests, the result is disruption of DNA replication, activation of DNA damage responses, loss of genome integrity and cell death.[22] Therefore, regulating transcription and replication on the same template is critical for genomic integrity and survival, implying specific mechanisms must exist to resolve conflicts between replication and transcription.

One mechanism of minimizing head on collisions between replication and transcription is structural. Many bacteria have evolved a genome-wide bias towards co-orientation of replication and transcription, with highly expressed and essential genes further enriched co-directionally.[23,24] It appears that this is particularly important at the rRNA operons, where ~95% of the cellular transcriptional activity is focused. Srivatsan et al.[22] showed that inversion of rRNA loci in *B. subtilis* strongly obstructed replication in nutrient rich media and the cells were more sensitive to the genotoxic agent mitomycin C. In contrast, *E. coli* viability was not affected by inverting large chromosomal regions that carry several rRNA operons, which required the three helicases DinG, Rep and UvrD to facilitate progression of the replication fork across transcribed sequences.[25-27] However, structural aspects of genome organization are not enough to eliminate RNAP as an impediment to replication in all circumstances and an increasingly recognized number of RNAP interacting proteins and small molecules play a role in this process.

RNAP Interacting Proteins that Resolve Conflicts between Transcription and Replication

The essentiality of CarD is surprising as CarD does not seem to be a component of the basal transcription apparatus and *dksA* in *E. coli* is not essential in nutrient rich media. In a recent paper, Tehranchi et al. show that, in addition to its role in regulating rRNA transcription initiation, DksA ensures progression of DNA replication in *E. coli* by removing transcription roadblocks during nutrient deprivation. Without DksA, DNA replication arrests, an effect that is dependent on transcription and is alleviated by stringent RNAP mutations that compensate for DksA loss. This replication arrest occurs independently of exogenous DNA damage but induces a DNA damage response and recruits the recombination protein RecA.[28] Importantly, this effect of DksA on replication was independent of the conserved aspartic acid residues in DksA that are required for regulation of initiation at the *rrnB* P1 promoter. DksA is not the only protein factor that has been shown to prevent or resolve conflicts between transcription, replication and DNA repair.[15,25,26] Specifically, other secondary channel interactors TraR and GreA/B also prevent replication arrest during nutrient stress.[15,28] Thus, there is substantial evidence that proteins that interact in the secondary channel help resolve conflicts between DNA replication and transcription both in vitro and in vivo. In addition to the secondary channel binding proteins, TRCF has also been demonstrated to promote direct restart of replication following a collision by displacing the stalled elongation complex.[20] Thus, multiple RNAP interacting proteins, interacting at distinct sites on RNAP, have a role in destabilizing RNAP to allow replication to proceed.

This background provides a conceptual model that allows us to speculate on the possibility of CarD playing a role in resolving conflicts between transcription and DNA replication, and that this function is required for viability of the mycobacterial cell. A defining feature of mycobacterial genomes is the presence of only one or two rRNA operons, in contrast to other bacteria like *E. coli* or *B. subtilis*, which contain seven and ten operons, respectively. The low numbers of rRNA operons correlates with the slow growth of mycobacteria, but also means increased RNAP occupancy focused on one or two loci on the chromosome instead of being distributed over seven or more rRNA operons, thus making coordination of RNAP activity particularly important. Incidentally, the organisms in which CarD is known or suspected to be essential (mycobacteria, *M. xanthus* and *B. burgdorferi*) all contain four or fewer rRNA operons.[4,7,8] Whereas, in *B. subtilis*, which contains ten rRNA operons, CarD is nonessential (J Dworkin, personal communication).[29]

Summary and Future Directions

The CarD protein of Mycobacteria represents a new class of RNAP modulators with functions in stringent control, genomic integrity and cell survival. The finding that CarD interacts with the RNAP β1 domain, the same site as TRCF, suggests that the β1 domain is yet another polyfunctional surface on RNAP to modulate its activity.[30] Many questions remain unanswered regarding CarD functions, including what makes this protein essential for viability of some bacteria, and not for others. Future investigations that focus on the overlap in roles between proteins with similar cellular functions as CarD (DksA) and proteins that interact with the same domain of the RNAP as CarD (TRCF) will provide critical insight into the control of transcription in organisms that encode CarD.

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