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MEETING REVIEW

D-Fining DarR, a LysR-Type Transcriptional Regulator That Responds to D-Aspartate

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ABSTRACT Work by Jones et al. (R. M. Jones, Jr., D. L. Popham, A. L. Schmidt, E. L. Neidle, and E. V. Stabb, J Bacteriol 200:e00773-17, 2018, https://doi.org/10.1128/JB.00773-17) describes a D-aspartate-sensing system in proteobacteria. D-Amino acids are critical components of peptidoglycan and other structures. The new study identifies the LysR-type transcriptional regulator DarR, which activates the aspartate racemase RacD. The overexpression of RacD enables it to synthesize D-glutamate and restore normal peptidoglycan in a *Vibrio fischeri murI* mutant. This study contributes to the understanding of emerging roles for D-amino acids and how they are synthesized under distinct conditions.

KEYWORDS D-amino acids, aspartate, LysR-type transcription factor, peptidoglycan, *Vibrio fischeri*, *Aliivibrio fischeri*

Ribosomes across all domains of life synthesize proteins containing L-amino acids; yet, there is increasing appreciation for significant biological roles for D-amino acids (1). D-Amino acids are synthesized by racemization of the L-form to the D-form, though there are additional enzymes (e.g., D-amino acid transferases) that form D-amino acids from α-keto acids (2). D-Amino acids can be incorporated into proteins by nonribosomal peptide synthetases, or by posttranslational modification of residues in ribosome-synthesized proteins by racemization from the L- to the D-isomer (1, 3). A major function for D-amino acids is as constituents of the peptide stem of peptidoglycan, which contains D-alanine and D-glutamate (4, 5). The recent use of fluorescently labeled analogs of these compounds has enabled the visualization of peptidoglycan synthesis in a wide range of bacteria (6, 7).

Despite our limited knowledge of the mechanisms by which D-amino acids influence biological processes, there has been increasing awareness that they play important functions. L-Alanine stimulates *Bacillus subtilis* spore germination, yet D-alanine strongly inhibits the process, with the L-isomer likely serving as a signal for nutrient availability (8). Additionally, D-serine is highly abundant in human urine. Uropathogenic *Escherichia coli* strains are enriched for the presence of a D-serine deaminase, yet its role during host colonization is unclear (9, 10). Additionally, there may be effects of D-amino acids on biofilm formation, although there has not been clear replication of these effects (11–14).

A new study from the laboratory of Eric Stabb and collaborators presents the discovery of DarR, a D-aspartate-responsive transcription factor in *Vibrio fischeri* that is likely to exhibit a conserved function across much of the *Proteobacteria*. A model summarizing the key results from the study is shown in Fig. 1. This work was presented by R. Mark Jones at the ASM Conference on *Vibrio* in November 2017, in Chicago, IL, and is published in this issue (15).

The group previously undertook a clever genetic screen to identify mutants that failed to grow on standard media but could grow upon supplementation with other compounds. Lyell et al. (16) identified 22 independent transposon insertions that mapped to 13 genes.

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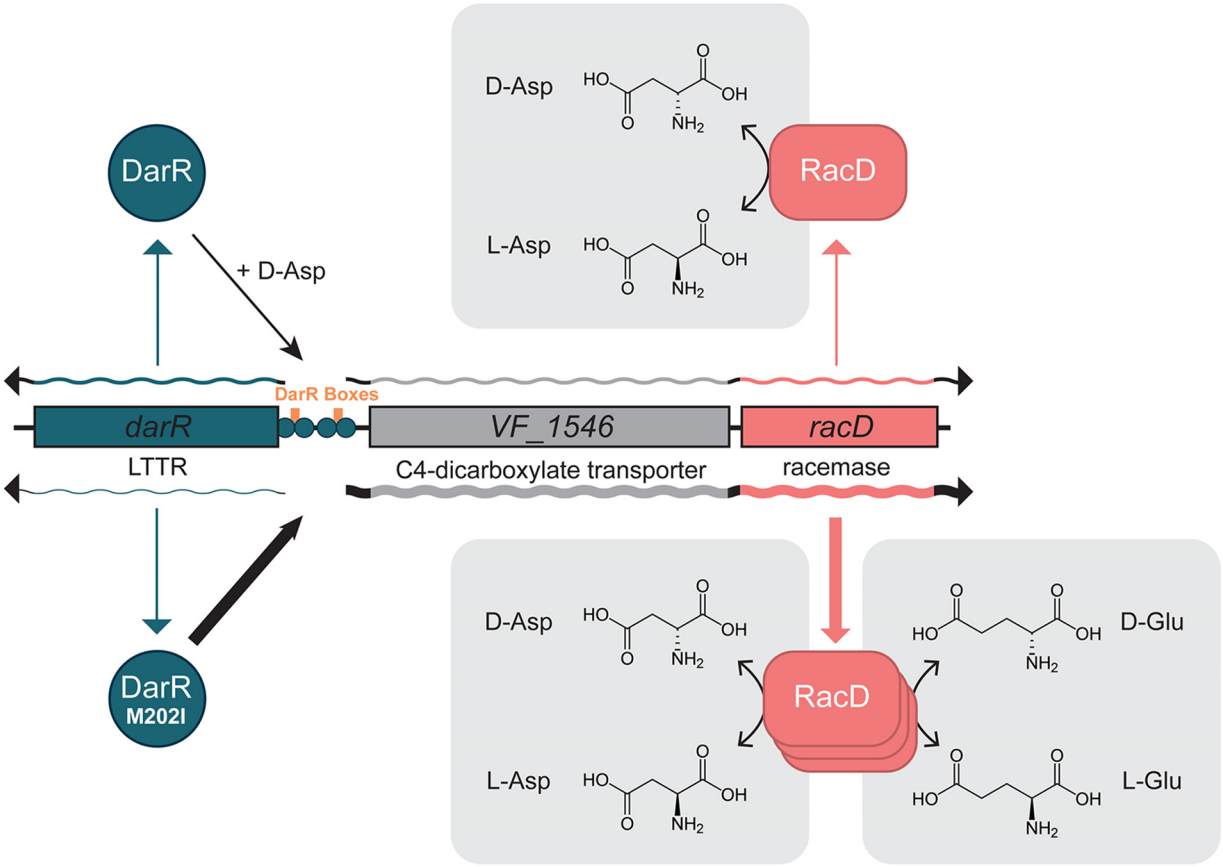

FIG 1 Key results from Jones et al. (15). DarR is a LysR-type transcription factor (LTTR) that regulates transcription of the *VF_1546-racD* operon in response to environmental D-aspartate. RacD is an aspartate racemase and likely can interconvert L-Asp and D-Asp, as both DarR and RacD are required for D-aspartate utilization. A DarR(M202I) allele leads to overexpression of the *VF_1546-racD* transcript and suppression of a D-glutamate auxotrophy. Overexpression of RacD is sufficient to suppress the D-glutamate auxotrophy. There is also evidence for DarR autorepression.

In each case, the mutant could not grow on buffered rich medium but grew upon supplementation with 17 diverse compounds, including hemin, *N*-acetylglucosamine, D-glutamate, D-alanine, $MgSO_4$, and other biologically relevant compounds. Three of these mutants contained transposon insertions in *murI*, which encodes a racemase required to generate D-glutamate from L-glutamate for peptidoglycan synthesis. Supplementation with D-glutamate alone enabled growth of the *murI* mutants.

In their current work, Jones et al. (15) then conducted suppressor analysis, identifying spontaneous suppressors of the *murI* transposon insertions that were no longer D-glutamate auxotrophs. Three isolated suppressors all exhibited the same amino acid replacement (M202I) in a LysR-type transcriptional regulator (LTTR), which they named DarR (D-aspartate-responsive regulator). DarR(M202I) exhibited suppression of the D-glutamate auxotrophy when expressed in *trans*, supporting a functional and dominant role for the *darR* mutation in enabling growth on unsupplemented rich medium.

LTTRs are DNA-binding transcription factors and are typically transcribed divergently from the genes they activate (17). The chromosomal organization of DarR suggested putative target genes, as follows: the divergent two-gene operon included an aspartate racemase that they named RacD (racemase for aspartate, amino acid symbol D). The authors proceeded to demonstrate that RacD (removal or overexpression) is epistatic to the suppressing DarR allele. This set of results supports a hypothesis that the DarR point mutation leads to the upregulation of RacD, and that RacD activity is responsible for the suppression.

How does RacD overexpression suppress a D-glutamate auxotrophy? Given its annotation as an aspartate racemase, the authors posit that at high RacD levels, the

racemase is promiscuous and can generate L-glutamate from D-glutamate. This is supported by biochemical analysis of the peptidoglycan; the suppressor's peptidoglycan is indistinguishable from that of the wild type, arguing against the replacement of D-glutamate with another amino acid.

The authors proceed to ask how the DarR-RacD system is regulated. D-Aspartate induces *racD* in a DarR-dependent manner, and DarR autorepresses independent of D-aspartate. There are two putative LTTR binding sites upstream of the *racD* operon and a mutation of one abolished induction by D-aspartate. Furthermore, the system is required for full utilization of D-aspartate as a nutrient source. DarR homologs were identified across numerous proteobacterial species, and functional studies in another organism (*Acinetobacter baylyi*) support a conserved function. In many of the taxa, DarR is transcribed opposite genes connected to racemase activity or aspartate metabolism. A likely interpretation of this observation is that DarR is responsive to D-aspartate in diverse organisms, and there has been evolutionary flexibility in the target gene transcribed opposite the regulator. Whether there are additional DarR targets in *V. fischeri* or other organisms remains to be elucidated.

The study provides strong support for the use of the model organism *V. fischeri* to dissect novel biological processes. *V. fischeri* peptidoglycan has been studied extensively, as it is involved in the specific mutualism with the Hawaiian bobtail squid host, *Euprymna scolopes*. Peptidoglycan generally is required for stimulating ciliated host appendages that recruit the symbiont to produce mucus, which functions to entrap bacteria from seawater (18). Furthermore, the specific peptidoglycan fragment tracheal cytotoxin signals successful colonization to the host, stimulating host development, including apoptosis and regression of the appendages (19). There are other intriguing responses of the squid host to peptidoglycan (20–22). In previous work, the Stabb laboratory has elucidated peptidoglycan remodeling and recycling pathways that can influence such interactions with the host (23).

Enzymes involved in the construction and degradation of peptidoglycan remain appealing targets for antibiotic development, and MurI inhibitors in particular have been identified (24, 25). Applying a similar approach to identify suppressors of *murI* in *Mycobacterium smegmatis*, mutations were identified that activated a D-amino acid transaminase (26). Therefore, these two studies describe distinct mechanisms by which resistance can arise in response to deletion of an essential peptidoglycan biosynthetic enzyme that is a target for antibacterial compounds.

This discovery adds DarR to *E. coli* DsdC and *Pseudomonas aeruginosa* DguR as transcription factors that are activated by D-amino acids (D-serine and D-glutamate, respectively). With D-amino acids observed in marine environments, it seems likely that the DarR-RacD system may be useful for the utilization of nutrient sources that *V. fischeri* encounters (27, 28). Furthermore, in the earlier paper (16), Lyell et al. identified other mutants that could grow on rich medium only with supplementation by peptidoglycan precursors, suggesting that the approach presented here may prove useful for further characterization of D-amino acid regulatory mechanisms in bacteria.

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