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Cell wall stress in bacteria increases a nucleotide second messenger to reduce turgor

Cyclic-di-AMP is a broadly conserved nucleotide second messenger that is critical for growth and virulence in Gram-positive bacteria. Genetic, cell biological and biophysical analyses reveal that cyclic-di-AMP functions to control cytoplasmic turgor pressure in response to cell wall stress. The reduction in turgor enables cells to withstand lysis.

This is a summary of:
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The question

Cyclic-di-AMP (c-di-AMP) is a nucleotide second messenger that is broadly conserved among Gram-positive bacteria. Research over the past decade indicates that c-di-AMP functions to modulate the activities of ion and osmolyte transporters[1,2]. Yet, how cells use c-di-AMP to control their physiology remains an open question. Very few stimuli that alter the levels of this second messenger have been identified. Furthermore, the molecular mechanisms that modulate c-di-AMP levels are undiscovered. One clue comes from clinical findings: mutations that elevate intracellular c-di-AMP levels confer resistance to antibiotics that target synthesis of the bacterial peptidoglycan cell wall, an essential barrier against osmotic lysis[3]. Specifically, high c-di-AMP levels are a common mechanism of β-lactam resistance in clinical isolates of *Staphylococcus aureus*, including methicillin-resistant *S. aureus*[4]. These findings suggest a potential coordination between c-di-AMP levels and the cell envelope.

The discovery

We set out to study the relationship between c-di-AMP and the cell wall in the model Gram-positive bacterial species, *Bacillus subtilis*. First, we replicated reports from the literature and demonstrated that c-di-AMP levels correlate with resistance to cell-wall-targeting antibiotics and to genetic perturbations affecting the cell envelope. This relationship led us to hypothesize that a cell might actively modulate c-di-AMP levels in response to the integrity of its cell wall. In support of this hypothesis, we observed that levels of the second messenger increased in response to deletion or chemical inhibition of a major cell wall synthesis enzyme. We found that this increase was dependent on the c-di-AMP synthase CdaA and its regulator CdaR.

Examination of the predicted structure of CdaR revealed a large intrinsically disordered region at its extreme C terminus, facing outwards from the cell. This region is similar in amino acid content to extracellular-facing disordered regions of two other membrane proteins: the cell wall synthase PBP1 and the cell envelope stress-response sensor–transducer RsgI. Of note, both these unstructured regions were recently shown to function as cell wall integrity probes in *B. subtilis*[5]. We found that this region on CdaR is required to stimulate CdaA-dependent synthesis of c-di-AMP when cell wall synthesis is impaired. Furthermore, swapping disordered regions from CdaR homologues indicates that the function of this region is likely to be conserved across CdaR homologues and across multiple signalling pathways.

Knowing that c-di-AMP levels increase during cell wall stress, we investigated whether c-di-AMP functions to control cytoplasmic turgor pressure (the pressure exerted by the cytoplasm on the cell envelope). We found that manipulation of c-di-AMP levels causes morphological changes consistent with alterations in turgor. Using biophysical approaches, we discovered that low intracellular levels of c-di-AMP cause increased turgor pressure and high levels decrease turgor pressure. To our knowledge, these results provide the first experimental evidence that c-di-AMP functions to control turgor. Finally, we established that the elevated c-di-AMP levels triggered by cell wall stress protect the cell from lysis via a reduction in its turgor pressure.

The interpretation

Taken together, our findings suggest that the unstructured region in CdaR senses defects in the cell wall and activates CdaA-dependent c-di-AMP production. The increase in c-di-AMP reduces cellular turgor pressure and thus prevents osmotic lysis (Fig. 1). Our results clarify the role of c-di-AMP in bacterial physiology and define, to our knowledge, the first signalling pathway that modulates c-di-AMP levels. Moreover, we established that bacteria manage envelope stress by reducing turgor. Importantly, we provide evidence that a reduction in turgor pressure might be a mechanism for the increased β-lactam resistance of clinical isolates of *S. aureus* with high c-di-AMP levels. As this work was conducted in *B. subtilis*, further experiments in *S. aureus* are needed to solidify this model.

Cell wall stress is one signal that modulates c-di-AMP production to decrease turgor. We hypothesize that other signals and signalling pathways exist to modulate this essential force. *B. subtilis* has two additional c-di-AMP synthases and two enzymes that degrade the second messenger. We predict that each of these proteins is regulated by distinct inputs. Defining these signalling pathways will establish the suite of stimuli that regulate cellular turgor pressure.

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EXPERT OPINION

"Overall, this is a really well conducted study with excellent and careful bacterial genetics, bacterial cell biology and quantitative microscopy data. In my opinion the study is of broad interest, as the regulation of cellular turgor is a general problem in microbiology that is not nearly as well understood as it ought to be, and these findings are likely to be of broad relevance across many Gram-positive bacteria." **Séamus Holden, University of Warwick, Coventry, UK.**

FIGURE

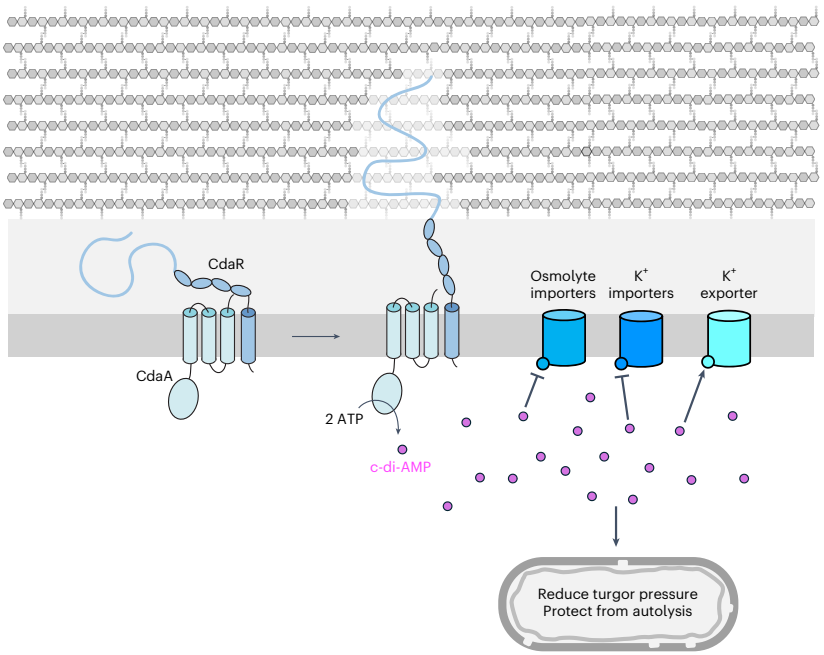

Fig. 1 | CdaA and CdaR function to reduce cytoplasmic turgor pressure during cell wall stress. The cyclase regulator CdaR (a membrane protein) senses defects in cell wall synthesis using its extracellular-facing intrinsically disordered region. In response, CdaA is stimulated to increase synthesis of c-di-AMP. C-di-AMP modulates the activities of ion and osmolyte transporters to reduce osmolarity and cellular turgor pressure. The decrease in the turgor reduces strain on the cell wall and prevents lysis. © 2025, Brogan, A. P. et al.

BEHIND THE PAPER

Over the past decade, our laboratory has focused on mechanisms of cell envelope homeostasis in Gram-positive bacteria. Genes involved in c-di-AMP synthesis and degradation were common hits in our genetic screens related to cell envelope maintenance. Upon diving into the c-di-AMP literature, we came to appreciate the strong evidence that c-di-AMP controls intracellular osmolyte concentrations and thus could function to modulate cytoplasmic turgor pressure. This evidence base led us to hypothesize that the cell maintains envelope integrity by modulating its turgor. We found it exciting to see that this regulation does in fact exist. Once we had characterized the signalling pathway, we were eager to rigorously test the hypothesis that c-di-AMP controls turgor pressure. This work would not have been possible without our collaboration with Rico Rojas and Paola Bardetti (New York University, NY, USA). Their expertise in measuring turgor pressure enabled a more complete understanding of the role of c-di-AMP in bacterial physiology. **A.P.B. & D.Z.R.**

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   This paper reports the discovery that intrinsically disordered regions serve as cell wall integrity probes in *B. subtilis*.

FROM THE EDITOR

"This study demonstrates a link between intracellular levels of c-di-AMP, cell wall peptidoglycan maintenance and the modulation of cytoplasmic turgor pressure. These findings extend our understanding of how bacteria respond to stress and help us understand bacterial tolerance or resistance to cell-wall-targeting antibiotics." **Editorial Team, *Nature Microbiology*.**