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Bacterial Landlines: Contact-dependent Signaling in Bacterial Populations

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Summary

Bacterial populations utilize a variety of signaling strategies to exchange information, including the secretion of quorum-sensing molecules and contact-dependent signaling cascades. Although quorum sensing has received the bulk of attention for many years, contact-dependent signaling is forging a niche in the research world with the identification of novel systems and the emergence of more mechanistic data. Contact-dependent signaling is likely a common strategy by which bacteria in close contact, such as within biofilms, can modulate the growth and behavior of both siblings and competitors. Ongoing work with diverse bacterial systems, including *Myxococcus xanthus*, pathogenic *Escherichia coli* strains, *Bacillus subtilis*, and dissimilatory metal-reducing soil bacteria, is providing increasingly detailed insight into the dynamic mechanisms and potential of contact-dependent signaling processes.

Introduction

We live in an information rich, highly interconnected world, much of which lies beyond our unaided senses in the domain of microbes. As a means of coordinating responses to changing environmental settings and surrounding microbes, bacteria have evolved a number of often-complex communication systems [1]. These can be broadly described as contact-independent and contact-dependent signaling mechanisms. The former is known classically as quorum sensing and involves the transfer of secreted molecules called autoinducers. As autoinducer levels increase throughout a growing bacterial population, changes in gene transcription are triggered resulting in altered growth rates and group dynamics [2]. Under some conditions, the secretion of autoinducers may become too taxing on available resources or may even stimulate unwanted attention from neighbors or host cells. Contact-dependent signaling cascades offer a means for more direct, and possibly less costly, communication between bacteria [1]. Like quorum sensing, contact-dependent signaling is also prone to modulation by cell density, with high cell numbers increasing the likelihood of interbacterial contact and subsequent signaling. The capacity of contact-dependent signaling cascades to modulate the behavior of both pathogenic and non-pathogenic bacteria remains incompletely understood, although emerging data indicate that this mode of direct cell-to-cell signaling can have profound impact on bacterial populations [1,3,4]. Here we review several of the known types of contact-dependent

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Conflicts of Interest

The authors have no conflicts of interest with respect to this manuscript.

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signaling, including C-signaling in myxobacteria and contact dependent inhibition in strains of *Escherichia coli*, emphasizing recent findings from the past two years.

C-signaling by *Myxococcus xanthus*

Myxobacteria are social predatory Gram-negative microbes that roam through soil in large packs stalking and consuming bacterial prey [3]. When faced with starvation, myxobacteria undergo a remarkable multicellular developmental process resulting in the formation of macroscopic fruiting bodies containing thousands of spores [3,5]. Complex intercellular signaling cascades drive sporulation, which promotes bacterial resistance to harsh environmental conditions and thereby enhances colony survival. Myxobacteria move by gliding motility to create foci where fruiting body formation is initiated, a process that relies on two types of bacterial motors [6–8]. The S (social) motor consists of type IV pili that extend and retract at the leading poles of migrating myxobacteria, pulling the microbes forward. At the trailing poles, nozzle-like structures comprising the A (adventurous) motor extrude slime that push the bacteria ahead. Pole-to-pole oscillations of motor components and regulators like FrzS appear to modulate activities of the S and A motors and consequently alter the reversal frequency of gliding motility. Changes in reversal frequencies cause mycobacteria to move as a traveling wave (rippling) or to aggregate, forming nascent fruiting bodies. Work with *Myxococcus xanthus* has demonstrated that rippling, aggregation, and subsequent sporulation require direct cell-to-cell contact. In some cases, this may involve transient fusion of membranes between adjacent bacteria, allowing the transfer and equilibration of A and S motor components among siblings [9]. These brief fusion events may help fine-tune fruiting body development, which is also heavily reliant upon another contact-dependent process known as C-signaling.

C-signaling is mediated by a non-diffusible 17 kDa surface protein (p17) encoded by the *csgA* gene [3]. Contact between neighboring myxobacteria initiates a bifurcated p17-dependent signaling cascade resulting in expression of genes required for either 1) rippling and aggregation or for 2) sporulation and further *csgA* expression [3,10]. Low levels of p17-mediated C-signaling elevate the reversal frequencies of the A and S motors and thereby induce rippling, while high levels of C-signal reduce reversal frequencies and promote aggregation [6]. It is currently unclear how p17 promotes contact-dependent C-signaling and orchestrates the opposing processes of rippling and aggregation. However, recent work has delineated an intriguing mechanism by which C-signaling is initially activated [11]. The p17 C-signal protein is derived from proteolytic cleavage of an inactive precursor, p25, which accumulates at the surface of both vegetative and starving cells. During starvation, Rolbetzki et al. found that a subtilisin-like protease referred to as the *protease required for processing of C-signal precursor* (PopC) cleaves p25 to p17. PopC is retained within the cytoplasm of vegetative cells and is slowly secreted from myxobacteria only during starvation. Once secreted, PopC is rapidly degraded and is unable to act *in trans* on neighboring microbes. This system ensures the gradual build-up of C-signaling as nutrient levels are depleted, and represents the first example of regulated proteolysis by secretion of a protease to the extracellular environment. PopC has no recognizable signal peptide and the pathway by which it is secreted is not yet known. It will be interesting to learn what specifically triggers PopC secretion and its subsequent rapid degradation outside of the cell.

Crosstalk in *Bacillus subtilis*

Like *M. xanthus*, the Gram-positive soil bacteria *Bacillus subtilis* also undergoes a contact-dependent differentiation process as a means to produce dormant spores when faced with starvation [12]. Under nutrient poor conditions, a vegetative *B. subtilis* cell will divide asymmetrically, forming a large mother cell and a smaller daughter cell called a forespore [12,13]. Internalization of the forespore by the mother cell creates a two-chamber sporangium

in which the forespore matures into an endospore. Despite their intimate association within the sporangium, mother cell and forespore remain separated by two membrane bilayers and maintain distinct gene expression profiles [12,14,15]. Endospore formation is an energy intensive process that is coordinated by multiple signaling pathways involving several different sigma factors in both the forespore and mother cell. Consideration of one of these sigma factors, σ^G , highlights the degree of crosstalk between forespore and mother cell during sporulation.

Activation of σ^G stimulates late gene expression within the forespore and is dependent upon two other genes, *spoIIAH* and *spoIIQ*, expressed by the mother cell [16–18]. SpoIIAH is homologous to the YscJ/FliF family of proteins that form ring-shaped multimeric complexes as part of type III secretion and flagella systems. Recent work indicates that SpoIIQ anchors SpoIIAH in punctate foci within the membranes (septum) surrounding the forespore [17]. These complexes made up of SpoIIAH and SpoIIQ may then act as scaffolding for the assembly of export channels leading from mother cell to forespore [16,18]. Presumably, the delivery of one or more substrates into the forespore via SpoIIAH-dependent channels is required for σ^G activation and completion of endospore formation. Whether the required substrate is a protein, nutrients, or some other small molecule remains to be determined. The formation of channels within the septum of sporangium does not appear to be restricted to SpoIIAH/SpoIIQ, as evidence by recent results indicating that an ATPase known as SpoIIIE can assemble into linked membrane spanning channels involved in the translocation of DNA from mother cell to forespore [19].

Contact-Dependent Inhibition Systems in *E. coli*

In 2005, Low and coworkers discovered a novel contact-dependent inhibition (CDI) system while working with the wild-type *E. coli* isolate EC93 [20]. While in logarithmic growth phase, a single *E. coli* cell expressing the CDI system can inhibit the growth of hundreds of susceptible target cells in mixed cultures, forcing them to enter a viable but non-replicating state. CDI requires direct contact between effector and target cells and is mediated by expression of two contiguous genes, *cdiB* and *cdiA*, that encode members of the two-partner secretion (TPS) family. Based on similarities with other TPS members, it is likely that CdiB forms a β -barrel channel within the outer membrane of *E. coli* through which CdiA is exported. CdiA is an exceptionally large protein by bacterial standards, having an initial mass of about 303-kD. However, during maturation CdiA is proteolytically cleaved at two sites within its C-terminus, causing the release of soluble CdiA fragments and leaving two thirds of the protein bound to the outer membrane. Recent results indicate that inter-bacterial interactions between membrane-bound CdiA and the outer membrane protein BamA (β -barrel assembly machine protein A) likely initiate CDI in susceptible target cells [21]. BamA is a conserved member of the YaeT/Omp85 family of proteins required for the biogenesis of β -barrel outer membrane proteins (OMPs) in bacteria, mitochondria, and chloroplasts [22–24]. Anti-BamA antibodies, as well as reduced BamA expression, decrease the susceptibility of target *E. coli* cells to CDI [21]. Interestingly, mutation of BamA so that it no longer functions in the assembly of β -barrel OMPs does not protect against CDI. The same genetic screen that identified BamA as a putative receptor for CdiA also indicated a role for *acrB* in CDI susceptibility. The AcrB gene product is an inner membrane protein that associates with AcrA in the periplasm and TolC within the outer membrane to form an envelope-spanning multi-drug efflux pump [25]. Mutation of *acrB* renders *E. coli* cells resistant to CDI, while *tolC* and *acrA* mutants are still susceptible [21].

Natural resistance to CDI is afforded by expression of a small immunity gene, *cdiI*, which is physically linked with *cdiBA* in some *E. coli* isolates [20]. In the absence of CdiI, the expression of capsule and certain types of filamentous adhesive organelles known as S and P pili can also provide immunity [4,20]. The mechanisms by which these factors mediate immunity to CDI

are not yet clear. One potential argument that S and P pili block CDI by sterically hindering contact between CDI⁺ effector cells and target bacteria is countered by observations that expression of related type 1 pilus structures does not provide immunity [20]. By using an inducible *cdiI* construct, Low and colleagues recently generated an inducible CDI autoinhibition system, allowing them to monitor a single population of *E. coli* undergoing CDI [4]. Use of this system revealed that the onset of CDI correlates with substantial drops in proton motive force (Δp), ATP levels and aerobic respiration, although it is not yet clear if these metabolic effects are the cause or consequence of CDI. The CDI-induced decrease in Δp was found to also induce a phage shock response, but this did not appear to be necessary for CDI [4]. Induction of CdiI expression in the presence of a suitable carbon source (maltose, ribose, lactose, or glycerol) prompted CDI-arrested cells to resume growth, demonstrating that the effects of CDI are reversible.

A summary of our current understanding of CDI and CDI resistance is presented in Figure 1. One intriguing scenario for induction of CDI put forth by Aoki et al. involves delivery of a proteolytic fragment of CdiA into target cells via a BamA-dependent process [21]. Once internalized, this peptide may alter the antiporter activity of AcrB, resulting in ion leakage and subsequent breakdown of Δp . Alternately, AcrB may act to transport the CdiA fragment further into the cell where it modulates the activities of other factors. Determination of whether CdiA acts in such a direct fashion or functions more indirectly by triggering specific signaling cascades at the bacterial cell surface awaits further study. Similarly mysterious is the role of CDI within wild bacterial populations. Comparative bioinformatics studies have revealed that homologous CDI systems are encoded by many strains of uropathogenic *E. coli* as well as pathogens like *Yersinia pestis* and *Burkholderia pseudomallei* [20]. CDI may enable these pathogens to inhibit competing bacteria or may enhance their own resistance to oxidative stress and antibacterial peptides, most of which are ineffective when Δp is low [4]. Many of the bacteria that possess CDI systems can act as opportunistic intracellular pathogens, raising the possibility that CdiA may also target host components. Specifically, by interacting with the BamA homologues Sam50 and Toc75 expressed by mitochondria and chloroplasts, respectively, CdiA may allow some intracellular pathogens to manipulate vital host organelles during the course of an infection.

Nanowires

One of the first recognized instances of contact-dependent communication between bacteria is, arguably, conjugation mediated by sex (F) pili [26]. Bacteria encode a large variety of other pilus types and adhesive molecules, many of which have been studied primarily with respect to their abilities to modulate bacteria-host cell interactions. However, it is feasible that some of these organelles also function in inter-bacterial communication. For example, recent studies indicate that several types of soil bacteria known as dissimilatory metal reducing bacteria can express complex networks of electrically conductive pili known as nanowires [27–29]. It is proposed that nanowires function in the transfer of electrons from the environment to and between bacteria within a localized population, presumably as a means to augment more standard energy exchange mechanisms. It is conceivable that such a system may also be adopted to transmit signals between bacteria as a possible means to coordinate group bacterial behavior. Nanowires, like other pilus types, can also apparently promote biofilm formation independent of their ability to transfer electrons [30]. Emerging data suggests that nanowire expression is widespread among metabolically and taxonomically diverse groups [28,29], raising the possibility that these structures can impact many bacterial processes, including pathogenesis.

Conclusions

Since the 2005 discovery of CdiA and CdiB, two other CDI systems have been identified in *E. coli* [31,32]. In contrast to the original CDI system defined by Aoki et al. [20], these other systems are active only with stationary phase cultures and currently have no clear mechanism of action. These findings indicate that contact-dependent mechanisms like CDI may be more wide spread among *E. coli* and other bacteria than previously suspected. Defining the functional relevance of these and related systems within environmental and host settings is emerging as a major challenge. A greater understanding of contact-dependent bacterial communication promises to highlight novel anti-bacterial therapeutic targets in pathogens and may also help advance fields such as fuel cell biology, where increased inter-bacterial connectivity via nanowires or other structures can potentially enhance productivity [33]. Furthermore, continued analysis of diverse contact-dependent bacterial communication systems may shed light on the development of true multicellularity during evolution.

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Figure 1. Model of The Contact-Dependent Inhibition System

Contact with CdiBA-positive *E. coli* cells triggers decreased membrane potential ($\Delta\psi$), ATP levels, and respiration in susceptible target cells (sad face). This metabolic downregulation coincides with activation of the phage shock response (pspA). BamA acts as the receptor for CDI, with AcrB as a downstream target (close-up inset). Other targets may potentially include BamA homologues expressed by mitochondria and chloroplasts. Expression of capsule, P or S pili, or CdiI renders bacterial cells (happy faces) resistant to CDI. Growth arrest by CDI is reversible by induced expression of CdiI in the presence of suitable carbon sources.