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Contact-dependent interbacterial toxins deliver a message

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Both Gram-negative and Gram-positive organisms harbor systems for delivering toxins to neighboring bacteria upon direct cell contact. These systems, typified by type VI secretion (T6S) and contact-dependent growth inhibition (CDI) systems, are defined by their ability to mediate interbacterial competition *in vitro*, while their biological roles have remained uncertain. Recent research into the mechanisms of toxin delivery and activity, as well as investigation of contact-dependent toxin function during relevant biological processes, has offered insight into how interbacterial competition might work outside of the laboratory. Furthermore, non-competitive roles for contact-dependent toxin delivery systems, including interbacterial signal transduction, have been described. This review suggests that contact-dependent toxin delivery systems that exhibit functions beyond interbacterial competition are probably more common than currently appreciated.

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Introduction

Whether in the soil rhizosphere or the mammalian gastrointestinal tract, bacteria encounter fierce competition for limited resources and must respond quickly and appropriately to changing conditions, including the presence of other bacteria. Diffusible toxic compounds produced by bacteria, such as bacteriocins and antibiotics, act at a distance to damage susceptible cells, providing the producing cell with a competitive edge over its neighbors [1]. Similarly, quorum sensing molecules released into the environment facilitate cooperation by allowing bacteria to sense and respond to other bacteria [2]. In addition to this network of diffusible signals and toxins, we are beginning to appreciate that bacteria harbor equally complex systems to sense and respond to direct contact with neighboring cells. Many contact-dependent mechanisms of bacterial interactions are defined by their ability to mediate interbacterial antagonism, but key differences exist among these systems. Moreover, recent research has begun to explore the biological consequences of contact-dependent toxin exchange between bacteria, raising questions about the physiological role of these interactions.

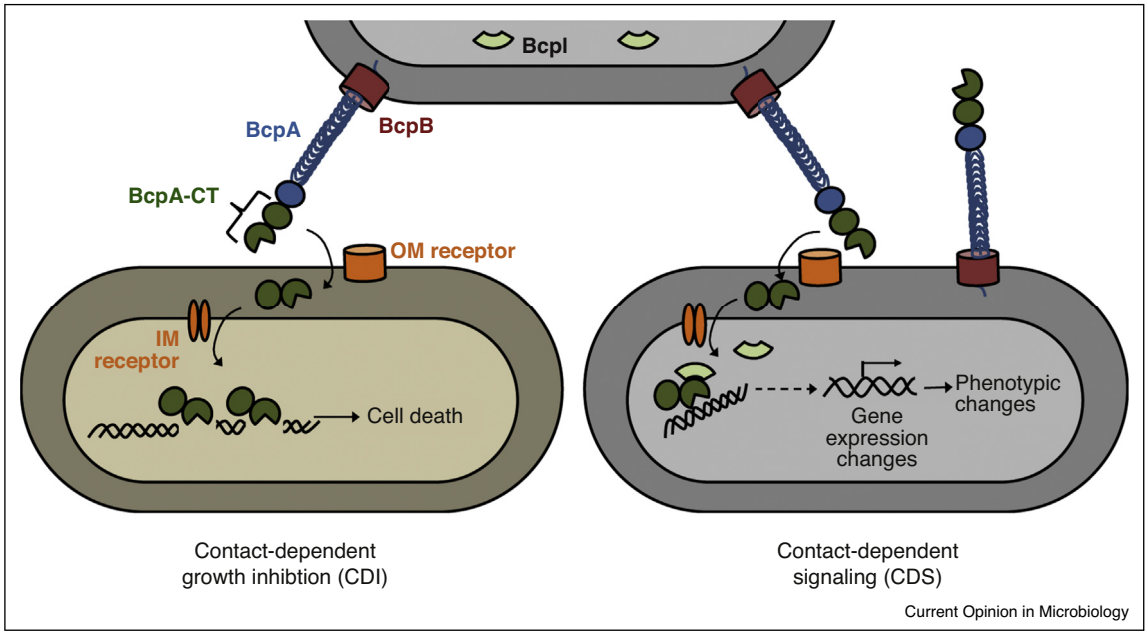
Mechanisms of bacterial contact-dependent toxin delivery

Contact-dependent growth inhibition

Contact-dependent growth inhibition (CDI) is a phenomenon in which Gram-negative bacteria deliver the toxic C-terminus of a polymorphic surface-exposed protein to the cytoplasm of neighboring cells upon cell-cell contact (Figure 1) [3,4]. CDI systems are composed of Two-Partner secretion pathway (type V secretion) proteins [5•]. CdiB (BcpB in *Burkholderia* and related species) is an outer membrane beta-barrel protein that facilitates the secretion of a large (>3000 aa) CdiA (BcpA) exoprotein to the bacterial cell surface. The majority of the CdiA N-terminus is predicted to adopt a beta-helical structure that extends several hundred angstroms from the cell surface [6]. While this N-terminal region is highly conserved across species, the CdiA C-terminal ~300 amino acids are variable. Termed the CdiA-CT (BcpA-CT), at least a portion of this region is translocated into the target bacterium, although the precise identity of the delivered molecule is unknown. Unlike type VI secreted effectors (see below), which target a wide range of conserved bacterial molecules [7], the majority of characterized CdiA-CT/BcpA-CT toxins have nuclease activity [4,8]. Bacteria producing CDI system proteins protect themselves from self-intoxication by producing small immunity proteins (CdiI/BcpI) that bind to and inactivate their cognate CdiA-CT/BcpA-CT toxins [4,9].

CDI systems were first described based on their ability to mediate interbacterial competition, and growth inhibition has defined this family of proteins [3,9]. CDI system-mediated interbacterial antagonism has been observed for systems found in *Escherichia coli* [3], *Burkholderia thailandensis* [9], *Neisseria meningitidis* [10], *Pseudomonas aeruginosa* [11], and *Acinetobacter baumannii* [12], and functional CdiA-CT toxins have been characterized from many other species [4,13]. However, the relevance of this competition is not clear, and evidence is increasing that CDI system proteins play other roles in bacterial biology.

Figure 1



Model for contact-dependent growth inhibition (CDI) and contact-dependent signaling (CDS). During CDI (left), a portion of the BcpA-CT (or CdiA-CT) is translocated into target cells via specific outer and inner membrane receptors. Once in the cytoplasm, BcpA-CT degrades target cell DNA or tRNA, causing growth inhibition and cell death. When BcpA-CT is delivered to another cell producing the same CDI system proteins (right), delivered BcpA-CT binds to its cognate BcpI immunity protein, preventing cell death. Additionally, interaction of the BcpA-CT/BcpI complex with target cell nucleic acids and/or proteins induces gene expression changes in a process termed CDS. OM, outer membrane; IM, inner membrane.

Type VI secretion

The type VI secretion system (T6SS) is a contractile nanomachine that delivers effector protein toxins directly from the cytoplasm of Gram-negative bacteria into neighboring cells [7]. With structural homology to contractile bacteriophages, the T6SS consists of a multi-protein core complex that spans the inner and outer membranes, a cytoplasmic tube, a puncturing tip, and an outer sheath that surrounds the tube. Upon cell-cell contact, the outer sheath contracts, forcing the inner tube and puncturing device into the neighboring cell, where effectors carried within the inner tube or attached to the puncturing tip are released. Although T6SSs were first recognized for injection of toxic effectors into eukaryotic host cells [14], these systems perhaps primarily target prokaryotic cells and deploy dedicated antibacterial effectors [15], including nucleases, peptidoglycan-degrading amidases, and membrane-targeting lipases [7]. As with CDI systems, bacteria elaborating T6SSs protect themselves from self-intoxication by producing immunity proteins specific to each deployed effector toxin. T6SS activity has been demonstrated to deliver toxins between numerous Gram-negative bacteria, mediating intra-species and inter-species competition [16,17].

Other mechanisms

In addition to type VI secretion and CDI, other mechanisms of contact-dependent toxin delivery have recently been described. A unique class of type IV secretion systems in *Xanthomonas* species delivers a peptidoglycan hydrolase to neighboring cells upon cell-cell contact, mediating interbacterial killing unless the toxin is bound by a specific immunity protein [18]. Additionally, cell-associated aggregates of bacteriocin-like glycine zipper proteins in *Caulobacter crescentus* mediate interbacterial killing though direct contact with neighboring bacteria [19•]. In Gram-positive organisms, the YD repeat-containing protein WapA and LXG toxin family proteins have been shown to mediate interbacterial antagonism in *Bacillus subtilis* and *Streptococcus intermedius*, respectively [20,21]. Similar to CDI systems, these surface proteins deliver polymorphic toxins derived from their C-termini to neighboring bacteria and specific immunity proteins prevent self-intoxication. Thus, both Gram-negative and Gram-positive organisms are capable of deploying interbacterial toxins upon cell-cell contact and many, if not most, species likely utilize several mechanisms of contact-dependent antagonism.

Specificity of toxin translocation into recipient cells

Specificity associated with toxin delivery, or lack thereof, may provide insights into the biological roles of bacterial contact-dependent toxin delivery systems. In general, T6SSs do not appear to require specific target