Review

Bacterial contact-dependent growth inhibition

Zachary C. Ruhe[1], David A. Low[1,2], and Christopher S. Hayes[1,2]

[1] Department of Molecular, Cellular, and Developmental Biology, University of California Santa Barbara, Santa Barbara, CA 93106-9625, USA
[2] Biomolecular Science and Engineering Program, University of California Santa Barbara, Santa Barbara, CA 93106-9625, USA

Bacteria cooperate to form multicellular communities and compete against one another for environmental resources. Here, we review recent advances in the understanding of bacterial competition mediated by contact-dependent growth inhibition (CDI) systems. Different CDI$^+$ bacteria deploy a variety of toxins to inhibit neighboring cells and protect themselves from autoinhibition by producing specific immunity proteins. The genes encoding CDI toxin–immunity protein pairs appear to be exchanged between *cdi* loci and are often associated with other toxin-delivery systems in diverse bacterial species. CDI also appears to facilitate cooperative behavior between kin, suggesting that these systems may have other roles beyond competition.

Bacterial competition

Bacterial cells are often regarded as isolated and autonomous entities, yet they exhibit a number of cooperative and competitive behaviors. Bacteria collaborate to assemble multicellular biofilm communities and secrete small diffusible signaling molecules to coordinate activities [1,2]. Soluble factors are also used for intercellular competition, with some bacteria releasing microcins and bacteriocins to inhibit the growth of competitors [3,4]. Other inhibitory systems require direct cell-to-cell contact between competing bacteria. Contact-dependent growth inhibition (CDI) was first observed in *Escherichia coli* isolate EC93, which deploys a two-partner (type V) secretion system to inhibit other *E. coli* strains [5]. Subsequently, type VI secretion systems were also found to mediate interbacterial competition in a contact-dependent manner [6–8]. Thus, Gram-negative bacteria possess at least two general mechanisms to inhibit neighboring cells. Both systems confer a substantial competitive growth advantage, suggesting that contact-dependent inhibition plays a significant role in shaping bacterial communities. In this review, we outline recent advances in our understanding of CDI mediated by the CdiAB family of two-partner secretion proteins. Readers are referred to a recent comprehensive review of type VI secretion for more information about its function in interbacterial competition [9].

Corresponding author: Hayes, C.S. (chayes@lifesci.ucsb.edu).
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CDI in *E. coli* EC93

CDI was discovered in *E. coli* EC93, an isolate from rat intestine that inhibits the growth of laboratory *E. coli* K-12 strains [5]. Enteric bacteria commonly produce soluble antibacterial toxins, but *E. coli* EC93 requires direct contact with target cells to inhibit growth. CDI is mediated by the *cdiBAI* gene cluster, which is sufficient to confer the CDI$^+$ inhibitor phenotype to *E. coli* K-12 cells. The *cdiB* and *cdiA* genes encode a two-partner secretion system [10,11]. CdiB is a β-barrel protein that exports CdiA across the outer membrane. CdiA is a very large (~319 kDa) hemagglutinin-repeat protein that contains the CDI growth inhibition activity. Based on its similarity to filamentous hemagglutinin from *Bordetella* species [12], CdiA is predicted to extend several hundred Å from the surface of CDI$^+$ cells to bind receptors on target bacteria (Figure 1). Upon contact with target cells CdiA appears to be cleaved to release a C-terminal toxin domain (CdiA-CT) for translocation into target cells. Expression of CdiA-CT inside *E. coli* K-12 cells results in dissipation of the proton motive force [13], suggesting that the toxin forms a pore in the inner membrane. The CdiA receptor, BamA, was identified in genetic selections for *E. coli* K-12 mutants that are resistant to CDI [14]. BamA is a highly conserved, outer membrane, β-barrel protein that is required for the assembly of other β-barrel proteins [15–17]. BamA is present in all Gram-negative bacteria, raising the possibility that *E. coli* EC93 uses CDI to inhibit other bacterial species. However, the predicted extracellular loops of BamA are highly variable between species [18], suggesting that unrelated bacteria are resistant to *E. coli* EC93 (Figure 1). The *cdiI* gene is tightly linked to *cdiA* and encodes an immunity protein that protects *E. coli* EC93 from autoinhibition [5]. CdiI expression is also sufficient to protect *E. coli* K-12 from CDI. CdiI is small (8.9 kDa) and contains two predicted transmembrane regions suggesting that it is localized to the inner membrane, where it could potentially block the assembly or opening of the CdiA-CT pore. Thus, the *E. coli* EC93 *cdi* locus encodes a toxin–immunity protein pair that confers a competitive growth advantage over other *E. coli* strains.

CDI diversity in other bacteria

Genes encoding CDI systems are found in many different α-, β-, and γ-proteobacteria [19]. Most *cdi* loci are organized in the same *cdiBAI* gene order as *E. coli* EC93, but the

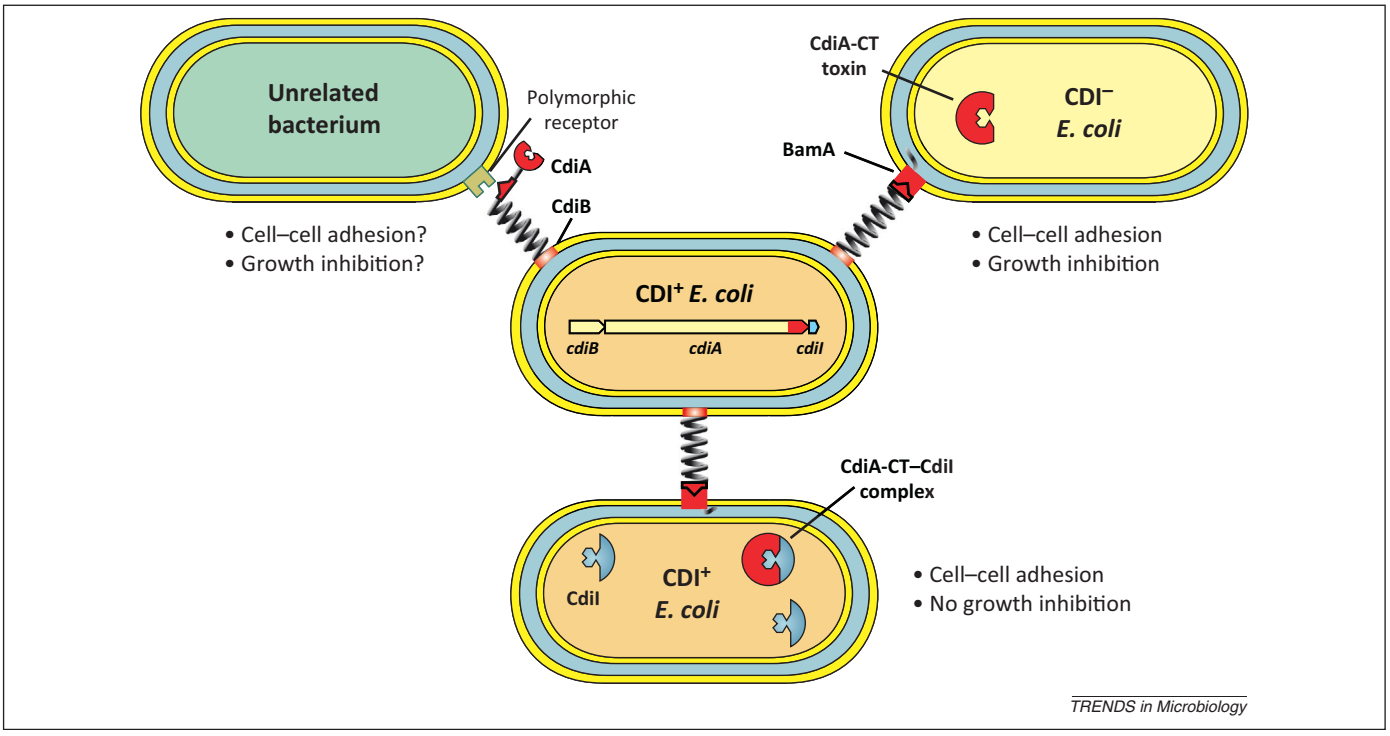

Figure 1. Contact-dependent growth inhibition (CDI) in *Escherichia coli*. CDI⁺ *E. coli* express *cdiBAI* gene clusters and present CdiB/CdiA on the cell surface. CdiA binds receptors on neighboring *E. coli* cells and delivers a toxin derived from its C terminus (CdiA-CT) into the target cell. CdiA-CT toxins inhibit the growth of CDI⁻ cells, but isogenic CDI⁺ inhibitors produce CdiI immunity proteins that protect them from toxin activity. The extracellular residues of most outer membrane proteins are highly variable between species, suggesting that the CDI only targets closely related bacteria.

systems from *Burkholderia*, *Cupravidus*, and *Variovorax* species are arranged as *cdiAIB* clusters [19–21]. CDI systems are usually encoded within genomic or pathogenicity islands. Therefore, not all strains of a given species necessarily contain *cdi* genes and some strains carry multiple loci [19,22]. For example, *cdi* loci are found in ~90 of the 576 *E. coli* genomes that have been sequenced to date. *E. coli* CdiA proteins share large regions of sequence identity, but their C-terminal regions diverge abruptly after a common VENN peptide motif [19,23], suggesting that CDI⁺ strains deploy many different toxins. There are at least 17 distinct *E. coli* CdiA-CT sequence types based on pair-wise alignments (Figure 2a); however, it is unclear whether each toxin type has a unique activity. CdiA-CT polymorphism is a hallmark of CDI in other bacteria as well [19,22]. In *Burkholderia* species, the variable CdiA-CT region is demarcated by a (Q/E)LYN motif, which appears to be analogous to the VENN sequence [20,21]. These findings imply that CDI⁺ bacteria exploit a common secretion mechanism to deploy a variety of toxins. In accord with toxin diversity, CdiI immunity proteins are also variable and specific for cognate CdiA-CT. $CdiI^{EC93}$ provides immunity to $CdiA\text{-}CT^{EC93}$ activity but not to the toxic $CdiA\text{-}CT^{UPEC536}$ tRNase from uropathogenic *E. coli* (UPEC) 536 [19]. Similarly, $CdiI^{UPEC536}$ protects cells from UPEC 536, but is ineffective against the CDI system from *E. coli* EC93 [19]. Thus, CDI constitutes a network of cognate toxin–immunity protein pairs, each with the potential to mediate interstrain competition.

Although CDI-associated toxins are highly variable, related CdiA-CT–CdiI pairs are often found in diverse bacterial clades. For example, the CdiA-CT from *E. coli* TA271 (UniProt: F4UNE5) shares identity with corresponding sequences from *Neisseria lactamica* 020-06 (UniProt: E4ZCK5), *Gallibacterium anatis* UMN179 (UniProt: F4HC25), and *Acinetobacter* species RUH2624 (UniProt: C0B226). These observations suggest that *cdiA-CT–cdiI* gene pairs are horizontally exchanged between bacteria. If so, then CdiA should be modular and capable of delivering many different CdiA-CT toxins. This hypothesis is supported by work with experimentally generated CdiA chimeras. The $CdiA\text{-}CT^{EC93}$ toxin region can be fused to $CdiA^{UPEC536}$ at the common VENN sequence to generate a functional CdiA protein [19]. Moreover, CdiA-CTs from *Yersinia pestis* and *Dickeya dadantii* can be delivered into *E. coli* target cells when grafted onto $CdiA^{UPEC536}$. Remarkably, CDI-associated toxins are found in several other protein families as well. Members of the Rhs/YD-repeat (Pfam: PF05593), WXG (Pfam: PF04740), *Neisserial* MafB, and *Mycobacterial* Ala–Pro-rich protein families all share C-terminal sequences with CdiA, suggesting that these proteins also mediate intercellular competition [22,24,25]. Analysis of the C-terminal regions of selected Rhs and WXG proteins has confirmed that these domains are indeed toxins [22,25]. Like CdiA-CT toxins, the Rhs-CT and WXG-CT activities are specifically blocked by immunity proteins encoded immediately downstream of each toxin gene. Thus, bacteria collectively carry a large repertoire of toxin–immunity protein genes that are shared between different delivery systems.

Orphan *cdiA-CT–cdiI* pairs

Often, *cdi* loci contain additional *cdiA-CT–cdiI* gene pairs in tandem arrays downstream of the main *cdiBAI* cluster

Figure 2. *Escherichia coli* CdiA-CT toxin diversity. **(a)** CdiA-CT sequences are presented beginning with the conserved VENN peptide motif. UniProt accession numbers are provided to the left of each sequence and residues are colored according to the Taylor scheme [38]. **(b)** The *cdi* loci from *E. coli* EC93, uropathogenic *E. coli* (UPEC) 536, *E. coli* 3006, and *E. coli* O157:H7 DEC9A are depicted with *cdiA-CT–cdiI* pairs color-coded to indicate family types. The downward pointing arrows indicate VENN-encoding regions. The EC93 orphan-1 (*CT/*I$_{o1}$), orphan-2 (*CT/*I$_{o2}$), and orphan-4 (*CT/*I$_{o4}$) pairs share sequence identity with the main toxin–immunity sequences from UPEC 536, *E. coli* 3006, and *E. coli* DEC9A, respectively. Open reading frames (in light blue) correspond to insertion sequence elements and transposase genes, *rtx* encodes a predicted toxin acyltransferase family member, and genes depicted in gray are unrelated to contact-dependent growth inhibition (CDI).

(Figure 2b). These toxin–immunity protein gene pairs have been termed 'orphan' modules because they appear to be DNA fragments displaced from full-length *cdiA* genes [22]. Orphan regions typically contain insertion sequence (IS) elements and transposon-related genes (Figure 2b), suggesting that the modules are horizontally transferred and integrated sequentially into the *cdi* locus. The function of orphan toxin–immunity modules and the selective pressure to retain them are unknown. Orphan *cdiA-CT* fragments often contain conserved *cdiA* coding sequences upstream of the VENN-encoding region, but do not encode secretion signal sequences or N-terminal two-partner secretion transport domains. Therefore, orphan toxins are not likely to be exported. Moreover, most orphan *cdiA-CT* sequences lack translation initiation signals, therefore it is unclear whether or not these toxins are synthesized. By contrast, orphan *cdiI* genes appear fully functional and provide immunity in at least one instance [22]. This latter observation suggests that orphan modules could persist to confer immunity to multiple CDI toxins. However, a number of orphan *cdiA-CT*

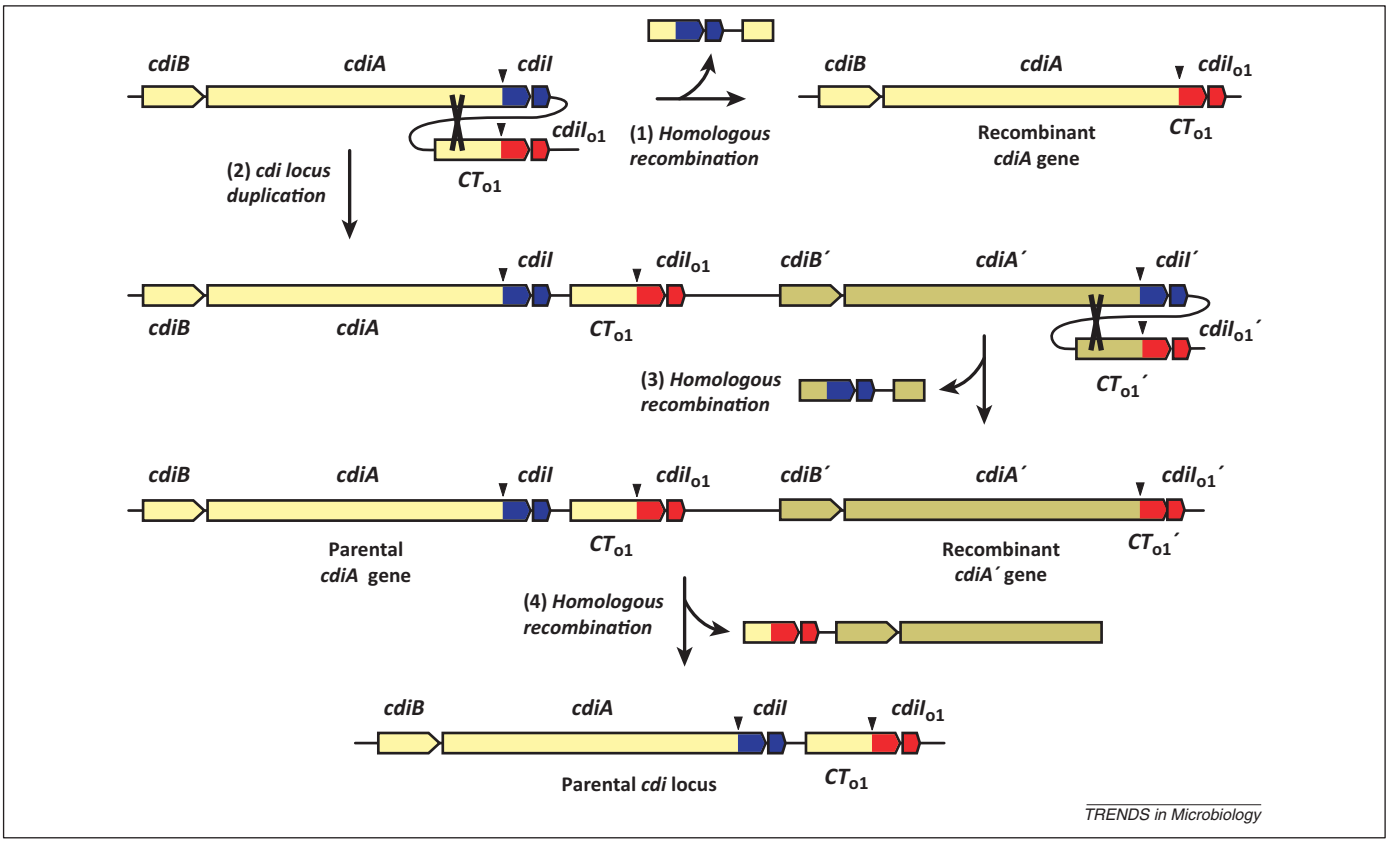

Figure 3. Models of orphan toxin–immunity protein gene rearrangement. Orphan *cdiA-CT* genes that contain conserved sequences upstream of the VENN-encoding region (downward arrow) can undergo homologous recombination with the full-length *cdiA* gene (indicated by crossover in step 1). Recombination would delete the parental toxin–immunity protein coding sequences and fuse the orphan module (red) to *cdiA*. Alternatively, the *cdi* locus could undergo spontaneous duplication (step 2) followed by homologous recombination (step 3) to generate a recombinant *cdiA* gene. Further recombination between the orphan *cdiA-CT* and the recombined *cdiA*′ could regenerate the original parental genotype (step 4).

sequences produce active toxins when expressed in *E. coli* cells [22], indicating that toxin activity is often retained. Orphan modules could also represent an arsenal of diverse toxins held in reserve. It may be advantageous for CDI$^+$ cells to deploy alternative toxins under certain circumstances – perhaps to outcompete bacteria that have acquired a *cdiI* immunity gene. Because orphan *cdiA-CT* fragments often share conserved sequences with the upstream full-length *cdiA* gene [22], homologous recombination could fuse orphan modules to *cdiA* and allow expression of new toxins (Figure 3; step 1). However, simple recombination would delete the parental *cdiI* gene and leave the recombinant susceptible to inhibition by neighboring wild-type siblings. Alternatively, recombination could occur subsequent to duplication of the *cdi* locus (Figure 3; steps 2 and 3). Large duplications are common in bacterial chromosomes [26,27] and this phenomenon could provide an opportunity for CDI$^+$ cells to deploy new toxins yet remain immune to the parental system. Importantly, the duplicated *cdi* locus could readily revert to its original structure through homologous recombination (Figure 3; step 4). Thus, iterative cycles of duplication and recombination could promote orphan toxin expression and perhaps account for the selective pressure to retain these gene pairs.

Structure and function of CDI toxin–immunity protein pairs

The first CDI toxin activity was identified before the systems were known to mediate bacterial competition. Kleanthous and colleagues discovered that the HecA (CdiA^{EC16}) adhesin from *Erwinia chrysanthemi* EC16 carries a C-terminal domain that resembles the toxic rRNase domain of colicin E3 [28]. A few other CdiA-CT toxins share obvious homology with known bacteriocins. CdiA-CT$^{3937\text{-}2}$ from *D. dadantii* 3937 is related to pyocin S3; and CdiA-CT^{K96243} from *Burkholderia pseudomallei* K96243 is related to colicin E5 [19]. In each instance, CdiA-CT activity is similar to its colicin homolog [19,21]. Aravind and colleagues have recently published a series of comprehensive analyses that predict many CDI toxins are nucleases, adenosine deaminases, ADP-ribosyl cyclases, and metallopeptidases [24,29,30]. This broad range of biochemical activities is consistent with toxin sequence diversity, and the predictions are supported by biochemical studies showing that many CdiA-CTs are RNases with unique substrate specificities. For example, the CDI toxin from *B. pseudomallei* isolate E479 cleaves tRNA between the highly conserved T54 and Ψ55 residues, and another toxin from *B. pseudomallei* 1026b cleaves within the aminoacyl acceptor stem of tRNA^{Ala} [21]. Given the diversity of CdiA-CT sequences, it seems likely that many other novel activities will be characterized, some of which may have practical applications in nucleic acid research and biotechnology.

High-resolution structural analysis has recently provided the first detailed glimpse into the CDI toxin–immunity network. Morse *et al.* solved crystal structures of the CdiA-CT$_{II}^{Bp1026b}$–CdiI$_{II}^{Bp1026b}$ complex encoded by chromosome

II of *B. pseudomallei* 1026b and the CdiA-CT_{o11}^{EC869}–$CdiI_{o11}^{EC869}$ complex encoded by the orphan-11 module of *E. coli* O157:H7 strain EC869 [31]. Both CdiA-CTs comprise at least two domains. The N-terminal domains are flexible and not completely resolved in the final models, whereas the compact C-terminal domains are responsible for toxin activity and mediate all interactions with immunity proteins. A two-domain structure is also suggested by sequence alignments, which indicate that many CdiA-CTs are assembled from independently assorting N-terminal and C-terminal regions [21,32]. The CdiA-CT_{o11}^{EC869} and CdiA-$CT_{II}^{Bp1026b}$ toxin domains share only ~15% sequence identity but both of them fold into similar structures that resemble type IIS restriction endonucleases (Figure 4a). Although the two toxins have similar structures and active sites, each exhibits a distinct nuclease activity. CdiA-CT_{o11}^{EC869} is a Zn^{2+}-dependent DNA endonuclease, whereas CdiA-$CT_{II}^{Bp1026b}$ is a specific tRNase, as described above [21,31]. By contrast, the $CdiI_{II}^{Bp1026b}$ and $CdiI_{o11}^{EC869}$ immunity proteins are not structurally related to one another and bind to independent sites on the nuclease domain (Figure 4b). $CdiI_{II}^{Bp1026b}$ binds directly over the toxin active site, forming an intricate network of direct and water-mediated hydrogen bonds with CdiA-$CT_{II}^{Bp1026b}$ [31]. $CdiI_{o11}^{EC869}$ binds its toxin through an unusual β-complementation interaction. CdiA-CT_{o11}^{EC869} extends a β-hairpin structure (β4-β5) that inserts like a lock-and-key mechanism into $CdiI_{o11}^{EC869}$ to complete a six-stranded β-sheet (Figure 4b). This interaction leaves the CdiA-CT_{o11}^{EC869} active site exposed, but DNase activity is effectively neutralized in the complex [31]. Notably, CdiA-$CT_{II}^{Bp1026b}$ lacks the extended β-hairpin (Figure 4a), and there is no detectable interaction between the non-cognate toxin and immunity proteins [31]. These initial studies show that toxins with diverse sequences can adopt similar structures, yet still exhibit unique growth inhibition activities and retain highly specific interactions with immunity proteins.

Evolution of CDI toxin–immunity protein pairs

Toxins within a given CdiA-CT family typically share significant sequence identity but also show regions of divergence. This is particularly apparent in the CdiA-CT_{o11}^{EC869}–$CdiI_{o11}^{EC869}$ toxin–immunity protein family, which is present in several *E. coli* strains and a variety of *Yersinia*, *Neisseria*, and *Photorhabdus* species (Figure 4c). Sequence alignments show that residues within the β4-β5 hairpin are the least conserved among CdiA-CT_{o11}^{EC869} family members (Figure 4c). Because the β4-β5 hairpin interacts directly with $CdiI_{o11}^{EC869}$ [31], these observations suggest that the toxin–immunity protein interface is diversifying rapidly. This hypothesis is supported by sequence

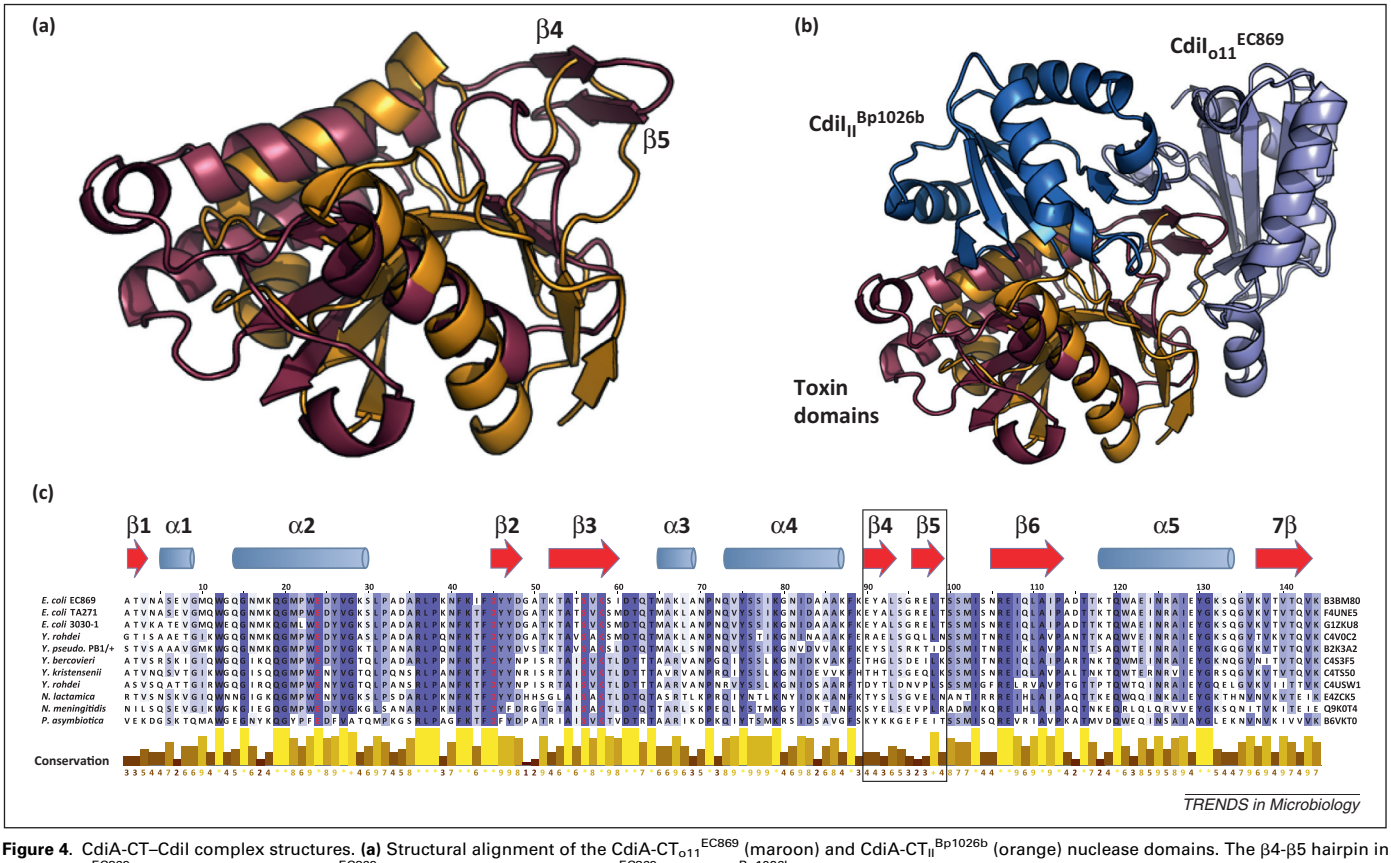

Figure 4. CdiA-CT–CdiI complex structures. **(a)** Structural alignment of the CdiA-CT_{o11}^{EC869} (maroon) and CdiA-$CT_{II}^{Bp1026b}$ (orange) nuclease domains. The β4-β5 hairpin in CdiA-CT_{o11}^{EC869} interacts with the $CdiI_{o11}^{EC869}$ immunity protein. **(b)** $CdiI_{o11}^{EC869}$ and $CdiI_{II}^{Bp1026b}$ immunity proteins bind to distinct sites on the toxin nuclease domains. Structures correspond to 4G6V and 4G6U in the Protein Data Bank and were prepared using PyMol. **(c)** Alignment of CdiA-CT_{o11}^{EC869} toxin homologs. The CdiA-CT_{o11}^{EC869} nuclease domain sequence is aligned with related toxin sequences from the indicated bacterial species. UniProt accession numbers are given to the right of each sequence. Secondary structure elements (blue α-helices and red β-strands) from CdiA-CT_{o11}^{EC869} are indicated above the alignment. The alignment was prepared with Jalview 2.8 at 30% sequence identity with progressively darker shades of purple indicating greater residue conservation. The conservation index is based on [39] and values are provided below each residue. Predicted toxin active site residues are given in red, and the β4-β5 hairpin (boxed) mediates interactions with the $CdiI_{o11}^{EC869}$ immunity protein.

alignments of $CdiI_{o11}^{EC869}$ homologs, which show that toxin-interacting residues are also poorly conserved. The same phenomenon is observed with homologs of the CdiA-$CT_{II}^{Bp1026b}$–$CdiI_{II}^{Bp1026b}$ complex, although fewer sequences are available for comparison. It appears that CdiA-CT–CdiI pairs diversify by mutation to form families of near-cognate toxin–immunity proteins. Further genetic drift probably prevents the cross-binding of diverging CdiA-CT–CdiI pairs, generating distinct immunity groups.

Within each CdiA-CT–CdiI family the immunity proteins usually exhibit much greater sequence diversity than the toxins. This suggests that CdiI evolution is rapid and may represent the mechanism for overall CdiA-CT–CdiI diversification. CdiI proteins are presumably free to diverge as long as they maintain sufficient affinity for CdiA-CT to provide immunity. Because CdiA-CT toxins are typically enzymes, their evolution is constrained by the need to retain catalytic activity. Of course, if CdiI evolution is too rapid (or radical) the cell would be exposed to unopposed CdiA-CT toxin activity. Therefore, diversification probably proceeds through iterative cycles of *cdiI* drift followed by reciprocal changes in *cdiA-CT*, such that the encoded proteins retain binding interactions through evolution. This model assumes that some missense mutations will weaken but not completely disrupt the CdiA-CT–CdiI interaction. This assumption seems reasonable for the CdiA-$CT_{II}^{Bp1026b}$–$CdiI_{II}^{Bp1026b}$ complex, which is held together by an extensive network of interacting residues. Additionally, there is evidence that a diverging CdiI protein can provide cross-immunity to a near-cognate toxin. The orphan-1 module from *E. coli* EC93 is related to the CdiA-CT–CdiI pair from UPEC 536 (Figure 2b), with 77% identity between CdiA-CT sequences and 35% identity between CdiI proteins. Both immunity proteins have significantly lower affinity for near-cognate CdiA-CT compared with their cognate toxins. Despite this lower binding affinity, $CdiI^{UPEC536}$ blocks the tRNase activity of near-cognate CdiA-CT_{o1}^{EC93} *in vitro* [22]. Conversely, the $CdiI_{o1}^{EC93}$ immunity protein is unable to neutralize the CdiA-$CT^{UPEC536}$ toxin. These results support the feasibility of the reciprocal mutation model. Moreover, it is tempting to speculate that *cdiA-CT–cdiI* modules may diversify more rapidly when present in orphan clusters. Orphan *cdiA-CT* sequences usually lack translation signals, indicating that the toxins are probably produced at lower levels than orphan immunity proteins. Excess orphan CdiI could provide a buffer against mutations that reduce affinity for toxin and thereby allow compensatory mutations to be acquired by the orphan toxin gene. The selective pressure to fix these mutations could arise through periodic expression of the orphan module following genetic rearrangement of the CDI locus (as outlined in Figure 3). Thus, competitive pressures within a CDI^+ population may drive the assimilation of new toxins as well as the gradual restructuring of existing toxin–immunity protein interactions.

Regulation and restriction of growth inhibition

E. coli EC93 is unusual among CDI^+ bacteria because its system is expressed constitutively under laboratory growth conditions. Other bacteria tightly regulate CDI expression. For example, plant pathogens appear to express their *cdi* genes only when colonizing specific hosts. Before CDI had been characterized, Collmer and colleagues found that disruption of the *virA* gene adversely affected the growth of *E. chrysanthemi* EC16 on a variety of plants [33]. Based on these results, *virA* was postulated to encode a virulence factor required for host colonization. However, *virA* lies immediately downstream of a *cdiA* homolog (*hecA*) and is predicted to encode a CdiI immunity protein. Therefore, an alternative explanation is that *E. chrysanthemi* EC16 only expresses $HecA/CdiA^{EC16}$ when invading plants; and the apparent virulence phenotype reflects autoinhibition in the absence of $VirA/CdiI^{EC16}$ immunity protein. This latter model is supported by work with *D. dadantii* 3937, another plant pathogen, which selectively activates one of its two *cdi* loci when cultured on chicory [19]. Similarly, CDI is largely repressed in *Burkholderia thailandensis* cells grown in liquid batch cultures, but strongly induced during growth in biofilms on solid media [20]. Thus, CDI expression tends to be activated at high cell densities when cell–cell contact is common. The molecular cues that induce CDI and the downstream regulatory pathways are unknown and remain outstanding problems in the field.

In at least one instance, CdiA-CT toxin delivery into target cells is not sufficient to inhibit growth. The UPEC 536 system deploys a tRNA anticodon nuclease, but this toxin is only active when bound to the metabolic enzyme CysK [32]. CysK has *O*-acetyl-L-serine sulfhydrylase activity and functions with CysE (L-serine-*O*-acetyltransferase) to synthesize L-cysteine from L-serine. CysE and CysK bind one another to form the highly conserved cysteine synthase complex [34,35]. CdiA-$CT^{UPEC536}$ and CysE share a common C-terminal peptide motif and thus the toxin appears to mimic the CysE binding interaction with CysK. CysK–CdiA-$CT^{UPEC536}$ binding is required for growth inhibition and *E. coli* Δ*cysK* mutants are completely resistant to $CDI^{UPEC536}$ [32]. These findings suggest that the UPEC 536 CDI system is only conditionally effective, allowing target cells to avoid inhibition through decreased expression or mutation of *cysK*. Alternatively, the CysK–CdiA-$CT^{UPEC536}$ interaction may serve another unknown function. CysK and the $CdiI^{UPEC536}$ immunity protein can bind CdiA-$CT^{UPEC536}$ simultaneously. Therefore, toxins exchanged between UPEC 536 cells exist as ternary complexes with CysK and $CdiI^{UPEC536}$. These complexes lack toxic tRNase activity, but have the potential to influence metabolism by modulating CysK activity. Therefore, CdiA-CT exchange between isogenic, immune bacteria could serve an intercellular signaling function.

Beyond growth inhibition

CDI has a well-established role in bacterial competition, but recent findings suggest these systems may also mediate cooperative behavior. Disruption of the *cdi* locus in *B. thailandensis* E264 abrogates biofilm formation, suggesting the system helps to establish and maintain communities in mixed microbial populations [20]. The mechanism underlying the biofilm phenotype is not known, but CDI expression in *E. coli* causes cells to autoaggregate in a BamA-dependent manner [14]. Thus, biofilm formation may be promoted through CDI-mediated cell–cell adhesion. Moreover, CDI could facilitate kin discrimination by

preventing related, but non-isogenic, strains from participating in the group behavior. CDI may have a similar function in host invasion and colonization by plant pathogens. *E. chrysanthemi* EC16 *hecA*/*cdiA* mutants are defective in adhesion to host cells [36], suggesting that HecA/CdiA^{EC16} has a direct role in pathogenesis. However, *hecA* mutants also fail to autoaggregate; and this phenotype contributes to the virulence defect [36]. Bacterial autoaggregation is associated with the killing of plant epidermal cells and may be important for host colonization. Perhaps the pathogen evades antibacterial defenses more effectively as an aggregated mass or must attain a critical cell density prior to invasion. Additionally, the coupling of *cdi* and virulence gene expression may provide a mechanism to suppress 'cheaters' that forgo production of virulence factors yet seek to exploit the niche created by their virulent siblings. This problem may be particularly acute for soft-rot pathogens, which liquefy plant tissues thereby releasing nutrients for cheaters and other competitors [37]. CDI has only been characterized in one pathogenesis model, but these systems are commonly present in a variety of bacterial pathogens [23]. We suspect that CDI coordinates multicellular activities in other pathogens as well and speculate that this may represent the primary function of these systems.

Concluding remarks

CDI represents a nexus between the competitive and cooperative forces that shape bacterial populations. CDI systems deploy a diverse array of toxin domains that mediate interstrain competition. CDI toxin–immunity evolution appears to be rapid and is probably driven by competition, horizontal gene transfer, and orphan toxin–immunity modules. CDI also appears to play roles in cooperative behavior such as biofilm formation. The coincident expression of CDI systems with bacterial group behaviors suggests that growth inhibition is used to enforce cooperation and 'punish' cheaters. Important areas for further research include determination of the regulatory networks that govern CDI expression and the mechanisms by which CDI modulates interactions in microbiomes. Additionally, metagenomic studies indicate that bacteria contain several other analogous competition systems. Most of these systems have yet to be characterized experimentally and it is not known whether they require direct cell–cell contact. However, the existence of so many systems implies that intercellular toxin delivery is a fundamental and ubiquitous process in bacterial biology.

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