Two Proteins Form a Heteromeric Bacterial Self-Recognition Complex in Which Variable Subdomains Determine Allele-Restricted Binding

Citation

Published Version

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:17295588

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Self- versus nonself-recognition in bacteria has been described recently through genetic analyses in multiple systems; however, understanding of the biochemical properties and mechanisms of recognition-determinant proteins remains limited. Here we extend the molecular and biochemical understanding of two recognition-determinant proteins in bacteria. We have found that a heterotypic complex is formed between two bacterial self-recognition proteins, IdsD and IdsE, the genes of which have been shown to genetically encode the determinants for strain-specific identity in the opportunistic bacterial pathogen *Proteus mirabilis*. This IdsD-IdsE complex forms independently of other *P. mirabilis*-encoded self-recognition proteins. We have also shown that the binding between IdsD and IdsE is strain- and allele-specific. The specificity for interactions is encoded within a predicted membrane-spanning subdomain within each protein that contains stretches of unique amino acids in each *P. mirabilis* variant. Finally, we have demonstrated that this *in vitro* IdsD-IdsE binding interaction correlates to *in vivo* population identity, suggesting that the binding interactions between IdsD and IdsE are part of a cellular pathway that underpins self-recognition behavior in *P. mirabilis* and drives bacterial population sociality.

**IMPORTANCE** Here we demonstrate that two proteins, the genes of which were genetically shown to encode determinants of self-identity in bacteria, bind *in vitro* in an allele-restricted interaction, suggesting that molecular recognition between these two proteins is a mechanism underpinning self-recognition behaviors in *P. mirabilis*. Binding specificity in each protein is encapsulated in a variable region subdomain that is predicted to span the membrane, suggesting that the interaction occurs in the cell envelope. Furthermore, conversion of binding affinities *in vitro* correlates with conversion of self-identity *in vivo*, suggesting that this molecular recognition might help to drive population behaviors.

---

**ABSTRACT** Self- versus nonself-recognition in bacteria has been described recently through genetic analyses in multiple systems; however, understanding of the biochemical properties and mechanisms of recognition-determinant proteins remains limited. Here we extend the molecular and biochemical understanding of two recognition-determinant proteins in bacteria. We have found that a heterotypic complex is formed between two bacterial self-recognition proteins, IdsD and IdsE, the genes of which have been shown to genetically encode the determinants for strain-specific identity in the opportunistic bacterial pathogen *Proteus mirabilis*. This IdsD-IdsE complex forms independently of other *P. mirabilis*-encoded self-recognition proteins. We have also shown that the binding between IdsD and IdsE is strain- and allele-specific. The specificity for interactions is encoded within a predicted membrane-spanning subdomain within each protein that contains stretches of unique amino acids in each *P. mirabilis* variant. Finally, we have demonstrated that this *in vitro* IdsD-IdsE binding interaction correlates to *in vivo* population identity, suggesting that the binding interactions between IdsD and IdsE are part of a cellular pathway that underpins self-recognition behavior in *P. mirabilis* and drives bacterial population sociality.

**IMPORTANCE** Here we demonstrate that two proteins, the genes of which were genetically shown to encode determinants of self-identity in bacteria, bind *in vitro* in an allele-restricted interaction, suggesting that molecular recognition between these two proteins is a mechanism underpinning self-recognition behaviors in *P. mirabilis*. Binding specificity in each protein is encapsulated in a variable region subdomain that is predicted to span the membrane, suggesting that the interaction occurs in the cell envelope. Furthermore, conversion of binding affinities *in vitro* correlates with conversion of self-identity *in vivo*, suggesting that this molecular recognition might help to drive population behaviors.
recognition gene cluster, ids, does not contribute to competitions against other P. mirabilis strains and instead encodes proteins necessary for nonlethal interactions within a clonal population (11, 13). Studying the ids locus, therefore, addresses mechanisms for recognition of self (kin) cells as opposed to the inhibition of nonself cells.

Insights into the functions of the Ids proteins have arisen primarily from genetic and bioinformatic analyses. Briefly, deletion of the six ids genes, idsABCDEF, is sufficient to prevent P. mirabilis strain BB2000 from merging with an otherwise genetically identical parent population, indicating that the Ids system is necessary for establishment of self-identity in this strain (11). The introduction of ids genes (or of just idsDEF) from strain BB2000 and driven by the native ids promoter (also from BB2000) into strain HI4320 is sufficient to cause the recipient HI4320 strain to form a boundary with both parental strains, indicating the formation of a new identity (11). Likewise, in trans expression of the ids genes from strain HI4320 in strain BB2000 is sufficient to alter self-identity and induce boundary formation (11). Therefore, expression of foreign ids genes in either of these P. mirabilis strains can confer a new identity.

Both the idsD and idsE genes, which are carried adjacently, must originate from the same strain for two P. mirabilis swarming populations to merge and for a conversion of strain identity to occur (11). Compared across several P. mirabilis strains, the idsD and idsE genes each contain a region of reduced sequence conservation that is flanked by highly conserved sequences (11). Together, these data suggest that the idsD and idsE genes encode the information determining self-identity (11). Neither IdsD nor IdsE has a known function outside self-recognition-dependent boundary formation. Prior to this current research, there was no structural or localization prediction for either IdsD or IdsE, and no interaction partners have been described for either protein. Therefore, we hypothesized that IdsD and IdsE may comprise a complex whose function is to convey and/or determine self-identity within a bacterial population. Here we examine the in vitro protein-protein interactions between IdsD and IdsE, as well as determine the allele and strain specificity of the IdsD-IdsE binding interaction, including critical residues that contribute to binding specificity. We also provide evidence that in vitro binding affinities positively correlate with in vivo self-identity.

**RESULTS**

**IdsD and IdsE interact with each other.** Given the limited information available about either IdsD (1,034 amino acids) or IdsE (312 amino acids), we explored the predicted domains of these proteins. The N-terminal domain of IdsD, from amino acids 1 to 750, is predicted to consist of several components: a disordered region, a putative T6S-associated motif (19), a coiled-coil region, and a series of sequential alpha-helices (Fig. 1A). The IdsD C-terminal domain is predicted to contain two transmembrane domains from approximately amino acids 695 to 708 and 786 to 814 and an unstructured C-terminal tail from amino acids 815 to 1034 (Fig. 1A). Likewise, IdsE appears to consist of two discernible domains: an N-terminal domain that is predicted to contain two transmembrane domains from approximately amino acids 61 to 78 and 154 to 171 and a C-terminal domain (amino acids 172 to 312) that is predicted to contain six beta-strands and two alpha-helices (Fig. 1A). From this analysis, we proceeded with the hypothesis that IdsD and IdsE are membrane-associated proteins and therefore may be localized to similar cellular environments.

We next queried whether IdsD and IdsE encoded by strain BB2000 would interact with one another. We constructed plasmids containing the entire ids operon under the control of the native promoter with a FLAG-tagged epitope attached in-frame to either idsD or idsE and then introduced the plasmids individually into a strain containing a chromosomal deletion of the ids genes (Δids). The resultant strains exhibited a wild-type boundary phenotype, indicating that the gene fusions with the FLAG epitope were functional (see Fig. S3 in the supplemental material [20]). We then performed pulldown assays with cellular extracts derived from actively migrating cells expressing either FLAG-tagged IdsD or FLAG-tagged IdsE using anti-FLAG antibody resin and analyzed the precipitates by Western blot analysis using IdsD- and IdsE-specific antibodies. IdsE was pulled down at higher levels by FLAG-IdsD than by the control protein, FLAG-BAP (Fig. 1B). Similarly, IdsD was present in the FLAG-IdsE sample, with only
FLAG immunoprecipitation in the presence of IdsD-His6. Neither genes separately expressed in Each IdsE variant (IdsE2 and IdsE3) was individually fused to ant IdsDBB-His6 (Fig. 2A). We observed similar results with IdsDCW-FLAG-IdsEHI only bound trace levels of the BB2000-originated vari-

Two Proteins Form a Strain-Specific Complex

FIG 2 IdsD and IdsE exhibit strain-specific binding. Variants of FLAG-IdsE and IdsD-His6 were subjected to pulldown assays and analyzed as described in the legend to Fig. 1. Interactions were tested between FLAG-IdsE and IdsD-His6 originating from strain BB2000 or HI4320 (A) and FLAG-IdsE originating from BB2000, HI4320, or CW977 and IdsD-His6, originating from strain CW977 (B). Representations of IdsD and IdsE are based on protein secondary structure predictions (Fig. 1): for IdsE, two transmembrane alpha-helices (rods) and a C-terminal domain (oval) and for IdsD, an N-terminal domain (long rectangle) and two transmembrane helices (smaller rectangles). Colors correspond to the source gene; the lighter shades indicate variable regions.

sequence from strain HI4320, resulting in FLAG-IdsEHI to H. We expressed this protein in E. coli and assayed for binding interactions by immunoprecipitation with anti-FLAG antibodies. In FLAG-IdsEHI to H, we did not detect IdsEIHI but did detect IdsDHI (Fig. 3A), indicating that the variable region of IdsE is sufficient to define allele-specific binding with IdsD.

Similarly, we probed for which residues in IdsD mediate binding specificity. The 33 amino acids of IdsD unique to either the BB2000 or HI4320 variants are located across the primary sequence (see Fig. S1 in the supplemental material [22–25]). Therefore, we constructed a hybrid epitope-tagged IdsD protein, IdsDlarge BB to H, consisting of amino acids 1 to 442 and 866 to 1034 of IdsDBB, where 4 amino acid polymorphisms lay, and amino acids 443 to 865 of IdsDH, which contained the remaining 29 polymorphisms and is part of the C-terminal domain (Fig. 4). We expressed this protein in E. coli and assayed for binding interactions by anti-FLAG immunoprecipitation and found that hybrid IdsDlarge BB to H-His6 was pulled down robustly by both FLAG-IdsEHI and FLAG-IdsEHI, but not by FLAG-IdsEBB (see Fig. S6 in the supplemental material). This result suggested that the residues sufficient to convert IdsD to a new binding specificity were found in the C-terminal domain.

To more narrowly define the variable region of IdsD, we reasoned that the variable region we identified for IdsD mapped to a region predicted to be surrounding a membrane-spanning portion; therefore, we predicted that the complementary surface on IdsD would map to a similar position (Fig. 4). As such, we replaced

trace amounts in the control (Fig. 1B). Since IdsD and IdsE co-

IdsD and IdsE bind to each other independently of other Ids proteins. IdsD and IdsE binding might be direct or, alternatively, may require the other Ids proteins. To distinguish between these possibilities, we expressed both proteins individually in Escherichia coli strain BL21(DE3), which does not contain any ids-like genes. We engineered a His6 epitope tag on the C terminus of IdsD, expressed IdsD-His6, and FLAG-IdsE in separate E. coli strains, and performed anti-FLAG pulldown assays on mixed cell extracts followed by Western blot analysis. IdsD-His6 was efficiently detected in the immunoprecipitate of FLAG-IdsE but not in that of the control protein, FLAG-BAP (Fig. 1C). Therefore, the interaction between IdsD and IdsE does not require other Ids proteins and is most likely direct.

IdsD binds specifically to the adjacenty encoded IdsE vari-

ant. In several sequenced P. mirabilis genomes, multiple alleles of idsE can be identified; however, we have observed at most only one allele of idsD (see Table S1 in the supplemental material). For example, strain BB2000 contains two additional idsE alleles immediately downstream of the idsABCDDEF gene cluster (see Fig. S5 in the supplemental material), as well as two other alleles at a distant chromosomal position, all of which have unique sequences (21). Two hypotheses for the binding interactions between IdsD and IdsE are that (i) IdsD can bind any IdsE variant found within a single genome or (ii) IdsD can bind only the IdsE protein encoded immediately adjacent on the genome. To distinguish between these hypotheses, we queried whether either of the IdsE variants encoded directly downstream of ids would bind IdsD-His6 in vitro. Each IdsE variant (IdsE2 and IdsE3) was individually fused to FLAG, separately expressed in E. coli, and subjected to an anti-FLAG immunoprecipitation in the presence of IdsD-His6. Neither downstream IdsE variant was able to effectively bind IdsD-His6 (Fig. 1C). Taken together, we conclude there is a restricted specificity to the IdsD and IdsE binding.

IdsD-IdsE binding is strain-specific. To further examine the hypothesis that IdsD-IdsE binding specificity is restricted, we replaced idsD and idsE in the E. coli expression plasmids with adjacently carried alleles (see Table S1 in the supplemental material) from the independent P. mirabilis strains HI4320 and CW977, resulting in the production of IdsDHI-His6, FLAG-IdsEHI, IdsDCW-His6, and FLAG-IdsEHI. Strains BB2000, HI4320, and CW977 form boundaries against one another and as nonself (11, 12). Anti-FLAG pulldown assays were performed on all samples. Trace levels of IdsDHI-His6 were detectable in pulldown assays with the BB2000-originated variant FLAG-IdsEHI whereas IdsDHI-His6 was readily detected in the FLAG-IdsEHI sample (Fig. 2A). Conversely, FLAG-IdsEHI only bound trace levels of the BB2000-originated variant IdsDHI-His6 (Fig. 2A). We observed similar results with IdsDCW-His6, which was only co-immunoprecipitated by FLAG-IdsEHI and not by FLAG-IdsEBB or FLAG-IdsEHI (Fig. 2B). Therefore, we conclude that the IdsD-IdsE binding interaction is primarily strain-specific.

The variable regions of IdsD and IdsE mediate binding specificity. Alignments between IdsD and IdsE variants highlight a region of high sequence variability in each protein; therefore, the strain-specific binding might be determined by these distinctive amino acid sequences. As such, we replaced the variable region in IdsE from strain BB2000 (amino acids 147 to 169) with the analogous
the membrane-spanning region containing distinctive amino acids in IdsD from strain BB2000 (amino acids 777 to 865, where 16 of the 33 polymorphisms reside) with the analogous sequence from strain HI4320, resulting in IdsDsmall BB to HI-His$_6$ expressed this protein in E. coli, and assayed for binding interactions by anti-FLAG immunoprecipitation. Surprisingly, we did not detect IdsDsmall BB to HI-His$_6$ in pulldowns with FLAG-IdsE$_{BB}$, FLAG-IdsE$_{HI}$, or the VR-swap variant, FLAG-IdsE$_{BB}$ to HI (Fig. 3B). These data indicate that the exchanged residues in IdsD were sufficient to disrupt binding interactions but not sufficient to confer a different binding specificity.

Several additional amino acid polymorphisms flank the predicted IdsD variable region (Fig. 4). Using this as a basis, we introduced 2 amino acid exchanges (A761V and A765T) into the IdsDsmall BB to HI-His$_6$ variant; this new construct, IdsD$_{BB}$ to HI-His$_6$, was expressed in E. coli and assayed for binding interactions by anti-FLAG immunoprecipitation. IdsD$_{BB}$ to HI-His$_6$ was detected in FLAG-IdsE$_{BB}$ but not in FLAG-IdsE$_{BB}$ pulldowns (Fig. 3C), indicating that conversion of these A761 and A765 residues in addition to the membrane-spanning variable region was sufficient to convert the binding specificity of IdsD from the IdsE variant of BB2000 to the IdsE variant of HI4320.

Strikingly, the IdsD$_{BB}$ to HI-His$_6$ protein was also detected in FLAG-IdsE$_{BB}$ to HI protein pulldowns, indicating that exchanging both variable regions in tandem can rescue binding between the two hybrid proteins (Fig. 3C). Therefore, exchanging the variable region in either IdsD or IdsE from strain BB2000 to that of strain HI4320 is sufficient to abrogate interactions with a cognate binding partner and, importantly, to switch binding specificity to that of a foreign variant.

**In vitro binding correlates with self-identity in vivo.** To determine whether the in vitro binding interactions correlated with in vivo behaviors, we used an in vivo ids expression system in which all ids genes are expressed from a plasmid under the native control of the ids promoter (pIds$_{BB}$) in a BB2000 mutant strain lacking the ids genes (Δids) (11). We chose this simplified system in which all other genes are identical except for the expressed ids genes so as to remove contributions to self-recognition-dependent boundary formation due to differences at other loci. To test the hypothesis that in vitro binding interactions correlate to in vivo self-identity, we replaced the now-defined variable regions (residues 761 to 865 of IdsD and 147 to 169 of IdsE) in the ids and IdsE genes, individually or together, in plasmid pIds$_{BB}$ with those from strain HI4320 and introduced each construct into the Δids strain.

These strains were subjected to boundary formation assays, which are currently the standard assay for studying self-identity in *P. mirabilis* (7–9, 11, 12). When two migrating populations merge to form a single swarm upon meeting, they are described as “self,” and when a boundary forms between the two populations they are described as “nonself” (7, 11, 12). Expression of the ids genes from BB2000 (pIds$_{BB}$) in a Δids background results in a strain that merges with BB2000, indicating that BB2000 is recognized as self (11). In contrast, expression of the ids genes from strain HI4320 (pIds$_{HI}$) in a Δids background led to a boundary with the BB2000, HI4320, and Δids strains carrying pIds$_{BB}$ (Fig. 5; see Fig. S7 in the supplemental material). The boundary formation with strain HI4320 likely results from multiple factors independent from the ids genes, such as the putative cytotoxic idr (13) and/or pef (8) genes.
Surprisingly, strains expressing an individual exchange, whether in idsD or in idsE, did not clearly merge or form a boundary with the strains expressing ids genes from either BB2000 or HI4320 and exhibited reduced swarm expansion (Fig. 5; see Fig. S7 in the supplemental material). Strikingly, the strain carrying ids alleles in which both the idsD and idsE variable regions were exchanged with those from strain HI4320, pIdsBB-idsD-BB to HI-idsEHI-BB to HI, merged with the strain expressing the ids genes from HI4320 (pIdsHI) and formed a boundary against the strain carrying the ids genes from BB2000 (pIdsBB) while also exhibiting a wild-type swarm expansion (Fig. 5; see Fig. S7 in the supplemental material). This observation is consistent with the observed in vitro binding interactions between the hybrid IdsD and IdsE proteins and the Ids_{EHI} and Ids_{DHI} Proteins, respectively. From this, we conclude that the presence of cognate variable regions in both IdsD and IdsE, in otherwise isogenic strains, led to the conversion of strain-specific identity from that of one isolate to another in vivo, indicating that these binding interactions are one factor that contributes to the definition of strain identity.

**DISCUSSION**

Here we have shown that IdsD and IdsE bind to one another in vitro without the necessity of additional Ids or *P. mirabilis*-derived proteins (Fig. 1). Furthermore, we demonstrated that the in vitro IdsD-IdsE binding interaction is restricted by both allele (between proteins encoded within strain BB2000) and strain (between proteins encoded by strains BB2000, HI4320, and CW977), indicating the presence of allele-specific (i.e., cognate) IdsD and IdsE pairs in nature (Fig. 1 and 2). The information for the binding specificity between the IdsD and IdsE proteins is encoded in a short stretch of distinctive amino acids within each protein that comprises the variable region (Fig. 3 and 4). Strikingly, a positive binding interaction between IdsD and IdsE in vitro directly correlates with self-identity in vivo (Fig. 5).

The molecular recognition site between IdsD and IdsE overlaps with at least one predicted transmembrane domain for each protein (Fig. 4), suggesting that IdsD and IdsE may interact via an interface within the membrane. Exchange of residues within these

---

**FIG 4** The IdsD and IdsE residues that mediate binding specificity overlap with predicted transmembrane domains. Presented are 2D projections (39) of IdsD_{HI} from amino acids 441 to 1034 (A) and IdsE_{HI} from amino acids 1 to 180 (B). Residues colored blue indicate BB2000 residues that were substituted for those from HI4320 in the IdsD_{small BB to HI-His6} and FLAG-IdsE_{BB to HI} variants, while the residues colored in gray indicate amino acid polymorphisms between IdsD_{HI} and IdsE_{HI}, as well as between IdsE_{BB} and IdsE_{BB} that were not exchanged. Positions A761 and A765 in IdsD (IdsD_{BB to HI-His6}) are colored green. The membrane is colored light gray bordered by black lines.
In fact, the *in vitro* binding specificity between IdsD and IdsE appears to be preferential to not only an endogenous *idsE* variant but also the *idsE* allele immediately adjacent to *idsD* (see Table S1 in the supplemental material). The role of the IdsE-like proteins encoded by nonadjacent alleles remains unknown. These orphan IdsE variants may interact with foreign IdsDs from the environment or may serve as reservoirs for alternate identity by replacing the canonical *idsE* gene via allelic exchange. Interestingly, not all *P. mirabilis* genomes contain *ids* genes, and it is unclear whether this is due to a recent acquisition of *ids* genes within the *P. mirabilis* species or whether the *ids* genes have been lost in some strains. Alteri et al. have demonstrated that a HI4320-derived strain with a disruption in the *idsD* gene is able to merge with its parental strain; however, the effect of a full *ids* deletion or a deletion of *idsE* in strain HI4320 remains to be examined (8). The diversity of IdsD and IdsE likely extends beyond *P. mirabilis*, as genes with similarity to *idsD* and *idsE* are adjacent to each other in other bacterial species (see Fig. S8 in the supplemental material [22–25]), raising the possibilities that IdsD and IdsE may encode strain-specific information in other species and that IdsD and IdsE may coevolve. The specific binding between adjacently encoded IdsD and IdsE pairs observed here supports a hypothesis for selective pressure on *idsD* and *idsE* to maintain a complementary protein interaction interface. It remains to be determined how each distinctive amino acid contributes to binding specificity and how the remaining portions of the IdsD and IdsE proteins contribute to biological function.

Here we have reported new biochemical information on two self-identity proteins of unknown function and structure. This information is a necessary contribution for developing a mechanistic model of self-recognition, as well as for a fuller understanding of protein-protein interactions in *P. mirabilis* and other bacteria. However, many questions remain unresolved. For example, multiple modes for the IdsD and IdsE interaction *in vivo* are possible. The IdsD and IdsE interactions could occur (i) between neighboring cells, (ii) within a single cell, or (iii) through a combination of both.

We prefer a model in which IdsD from one cell is communicated to a neighboring self cell; a positive binding interaction between the transferred IdsD and the recipient’s encoded cognate IdsE would then cause a signaling cascade in the recipient cell, ultimately resulting in behaviors that are beneficial for kin, such as swarming, which is a cooperative method of motility. In support of this hypothesis, we have observed reduced motility in strains in which only the IdsD and IdsE proteins are noncognate and thus do not bind *in vitro* (see Fig. S7 in the supplemental material). Given that the T6S system is necessary for boundary formation (8, 13) and for the export of IdsD from liquid-grown cells into the extracellular medium (13), IdsD may be transported directly into (or onto) a neighboring cell via the T6S system to elicit a response. Indeed, T6S systems in *Pseudomonas aeruginosa* can transfer macromolecules known as effectors directly from one cell into its neighbor, resulting in the recipient’s cell death (16). However, we have not observed transfer of IdsD directly into neighboring cells in liquid or on surfaces and as such cannot definitively conclude whether IdsD is transported into neighboring cells. Further, the presence of IdsD in the extracellular supernatant despite the predicted transmembrane domains (13) raises the question of whether multiple isoforms of IdsD are present within a cell; a cell envelope-localized isoform and an exported isoform. Alternatively, since IdsD and IdsE

---

**FIG 5** *In vitro* binding interactions between IdsD and IdsE correlate with self-identity *in vivo*. Boundary formation between strains expressing different IdsD-IdsE pairs was tested. Close-ups of contact regions between approaching swarms are shown. The arrowheads below indicate where two swarms meet, and the green outlines indicate swarms that have merged. The dashed box defines a region of contact between indicated swarms when more than two swarms are visible within the frame. Full images of swarm plates are shown in Fig. S7 in the supplemental material. Notations as follows: ovals represent the tested swarm with the chromosomal *ids* locus at the top and the plasmid-contained *ids* locus at the bottom, and rectangles show six *ids* genes in sequence with alleles from strain BB2000 (orange), strain HI4320 (blue), or missing (white). Blue boxes within an orange box indicate a variable region exchange. The absence of bottom rectangles signifies that the strain carries the empty parent vector, pKG101.

transmembrane domains as well as in the predicted periplasmic loop was sufficient to disrupt native binding interactions for both IdsD and IdsE and was also sufficient to confer a new binding specificity for IdsE. Interestingly, for IdsD two additional predicted cytoplasmic residues (A761 and A765) were needed to convert binding specificity (Fig. 3 and 4), suggesting that the variable region for IdsD might be extended to the regions flanking the transmembrane domain. These two residues may also contribute to the stability of IdsD or to the fold of the variable region.

Binding specificity between two proteins is crucial for many intracellular processes, e.g., bacterial histidine kinase (HK) and response regulator (RR) proteins (reviewed in reference 26). Multiple variants of homologous HK and RR proteins are simultaneously present in a single *Caulobacter crescentus* cell, yet signaling via phosphorylation is restricted to cognate HK-RR protein pairs that are often encoded by adjacent genes (27). Specific residues in the HK and RR proteins define the specificity of these interactions, restricting the ability of a given protein to interact with a cognate variant and permitting the predictive redesign of a protein’s signaling specificity (28–33). As the specificity of signaling interactions is sufficient to alter intracellular processes, it stands to reason that variant-specific binding interactions between proteins may also drive population and social behaviors. Since no additional proteins are required for the IdsD-IdsE interaction, we posit that the binding between these two proteins is the central protein-protein interaction determining *P. mirabilis* Ids-mediated self-recognition and most likely occurs in the cell envelope.
each have two predicted transmembrane domains, IdsD and IdsE could potentially form an envelope-spanning complex within a single cell that in turn interacts with a similar complex in a neighboring cell. Nonetheless, it remains likely that adjacent cells share identity information through the actions of IdsD and IdsE. Future work will be aimed at determining the topology, subcellular localization, and three-dimensional structure of the native IdsD-IdsE complex.

Without knowledge of the topology for IdsD-IdsE binding interactions in vivo, we cannot predict how these interactions contribute to boundary formation. However, these data are consistent with our current model for self-recognition in P. mirabilis strain BB2000, in which the absence of cognate Ids proteins denotes that the interacting cell is missing self-identifiers (13). Defining self-identity is at the foundation of many group behaviors mediated by self- versus nonself-recognition. Features of P. mirabilis self-recognition are shared with recognition systems like those of other social microbes, e.g., kin-specific binding interactions between proteins to define identity. In this study, we definitively link recognition are shared with recognition systems like those of other social microbes, e.g., kin-specific binding interactions between proteins to define identity. In this study, we definitively link recognition with our current model for self-recognition in E. coli and P. mirabilis strains were maintained on LB and LSW agar, respectively (34). CM55 blood agar base agar (Oxoid, Basingstoke, England) was used for swarm colony growth. All strains were grown in LB broth under aerobic conditions at 16, 30, or 37°C. Antibiotics were used at the following concentrations: carbenicillin, 100 μg/ml; tetracycline, 15 μg/ml; kanamycin, 35 μg/ml; and chloramphenicol, 50 μg/ml.

Boundary assays. All boundary assays were performed as previously described (13) on swarm-permissive nutrient plates with kanamycin. FLAG immunoprecipitations from P. mirabilis cell extracts. P. mirabilis strains carrying pIds plasmids were inoculated from overnight cultures onto three swarm agar plates and incubated for ~20 h until the population almost reached the edge of the petri dish. Cells were resuspended in LB, harvested by centrifugation, and stored at ~80°C. Pellets were resuspended in 1 ml cell lysis buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% Triton X-100, 1 mM EDTA) supplemented with Complete protease inhibitor cocktail (Roche, Basel, Switzerland) and lysed by vortexing with cell disruptor beads (0.1-mm diameter; Electron Microscopy Sciences, Hatfield, PA). Lysates were cleared by centrifugation and applied to 20 μl preequilibrated anti-FLAG M2 antibody resin (Sigma-Aldrich, St. Louis, MO). Control lysate (not containing a FLAG-tagged protein) was supplemented with 2 μg of FLAG-BAP protein (Sigma-Aldrich, St. Louis, MO). Lysates were incubated with resin for 2 h at 4°C. Unbound cell extract was removed. Resin was washed five times in wash buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% Triton X-100), and bound proteins were eluted with 50 μl of elution buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 200 ng/ml 3× FLAG peptide) for 45 min at 4°C. The elution was centrifuged, and the top 40 μl of the original 50 μl was retained. Samples of load (L), flowthrough (F) (i.e., proteins in supernatant after incubation with resin), and elution (E) were separated by SDS-PAGE and analyzed by Western blotting.

Bioinformatics analysis and construction of 2D projection graphs. Bioinformatics analysis of the IdsD and IdsE amino acid sequences from strains BB2000 and H4320 were performed using the web interfaces of PredictProtein (35), TMFinder (36), Hammer (37), and Phyre2 (38). Two-dimensional (2D) projections of IdsD and IdsE were prepared using the web-accessible Proter software (http://wlab.ethz.ch/protter/start/) (39), which employs Phobius (40, 41) to predict transmembrane domains and orientation. Colors and red lines were added using Adobe Illustrator (Adobe Systems, San Jose, CA). The sequence alignment methods are described in the supplemental material.

SUPPLEMENTAL MATERIAL

ACKNOWLEDGMENTS
We thank members of the Gibbs laboratory for experimental tools and advice, as well as Richard Losick, Bodo Stern, and Alexander Schier for comments on the manuscript.

The Canadian Institutes for Health Research (L.C.), Harvard University, the George W. Merck Fund, and the David and Lucile Packard Foundation, funded our research.

REFERENCES