Self-identity reprogrammed by a single residue switch in a cell surface receptor of a social bacterium

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The ability to recognize close kin confers survival benefits on single-celled microbes that live in complex and changing environments. Microbial kinship detection relies on perceptible cues that reflect relatedness between individuals, although the mechanisms underlying recognition in natural populations remain poorly understood. In myxobacteria, cells identify related individuals through a polymorphic cell surface receptor, TraA. Recognition of compatible receptors leads to outer membrane exchange among clonemates and fitness consequences. Here, we investigated how a single receptor creates a diversity in recognition across myxobacterial populations. We first show that TraA requires its partner protein TraB to function in cell–cell adhesion. Recognition is shown to be *traA* allele-specific, where polymorphisms within TraA dictate binding selectivity. We reveal the malleability of TraA recognition, and seemingly minor changes to its variable region reprogram recognition outcomes. Strikingly, we identify a single residue (A/P205) as a molecular switch for TraA recognition. Substitutions at this position change the specificity of a diverse panel of environmental TraA receptors. In addition, we engineered a receptor with unique specificity by simply creating an A205P substitution, suggesting that modest changes in TraA can lead to diversification of new recognition groups in nature. We hypothesize that the malleable property of TraA has allowed it to evolve and create social barriers between myxobacterial populations and in turn avoid adverse interactions with relatives.

kin recognition | outer membrane exchange | *Myxococcus xanthus*

Kin recognition is a fundamental biological property of a diverse range of communal living organisms. Mammals (1), plants (2), invertebrates (3), and more recently, unicellular organisms (4) are known to distinguish kin from nonkin based on their genetic relatedness. Kin recognition allows close relatives to form social groups and to conduct behaviors that are beyond the abilities of the individual (5). Such behaviors include increased fitness in obtaining food and assembling societies to protect and nurture offspring. Kin recognition also allows individuals to aggregate and build multicellular organisms. Because microbes are social (6, 7) and genetically tractable, they allow for the investigation of the molecular basis of kin recognition, cooperative behaviors, and how social interactions might evolve.

Myxococcus xanthus is a soil-dwelling Gram-negative bacterium that transitions between individual and multicellular life (8–10). Individuals move by gliding motility to forage for food and to identify proximal kin. Their behaviors in motility, predation, and development are also coordinated in multicellular aggregates. Multicellularity in myxobacteria is exemplified by fruiting body development, where thousands of cells aggregate into mounds that erect into fruits wherein a subset of cells differentiate into spores. To assemble multicellular communities, cells must discriminate kin to ensure their structures contain like individuals. Previously, we described a system where *M. xanthus* recognize self upon physical contact (4, 11). This self-recognition system, termed outer membrane exchange (OME), involves bidirectional transfer of large quantities of private cellular goods between kin (12, 13). OME is thought to aid cells in the transition toward multicellularity by helping establish outer membrane (OM) homeostasis in a population. OM homeostasis in turn leads to cellular repair and building a society where all individuals contribute toward multicellular life (14). For example, OME was originally discovered based on its ability to repair a subset of gliding motility mutants through extracellular complementation (11, 15). This occurs by the transfer of missing motility proteins to a mutant from another cell that makes the corresponding wild-type proteins. Rescue is transient, as DNA is not exchanged, and following rounds of cell division the transferred proteins are diluted and turned over. In addition, OME can repair motility, development, cell permeability, and viability defects associated with OM damage caused by various lipopolysaccharide (LPS) mutants (14). Here, cellular repair occurs by a healthy donor population replenishing wild-type LPS to the damaged population. This form of wound healing provides obvious benefits to the damaged cells. Additionally, the whole population benefits when their fitness depends on a quorum or threshold population size to perform multicellular tasks such as fruiting body development (16).

To ensure that bulk sharing of cellular goods occurs among self (clonemates), myxobacteria use a polymorphic cell surface receptor, TraA, which governs self-recognition (4). Across natural isolates, we found that TraA contains a variable domain that correlates with recognition specificity (Fig. 1*A*). That is, compatible OME partners contain identical or nearly identical TraA receptors. The specificity of OME is reprogrammed simply by experimentally swapping *traA* alleles among isolates. These findings suggest self-recognition occurs through homotypic interactions between TraA receptors. TraA functions with its partner protein, TraB, where OME requires both cells to contain TraA/B (11). Overexpression of TraA/B causes tight cell–cell binding, suggesting they function as adhesins (11, 14). TraA/B

Significance

How individuals identify self (clonemates) in heterogeneous populations is a fundamental biological question. Here, we propose a mechanism by which *Myxococcus xanthus*, an aggregative multicellular bacterium, uses a cell surface adhesin to recognize clonemates. Cells selectively bind to other individuals that bear identical or nearly identical receptors. The social outcome of recognition includes bulk sharing of private cellular goods between cells. We reveal the malleable nature of this receptor that provides recognition selectivity among wild populations of myxobacteria. This is an example where single residue substitution can govern the decision for how microbes physically and socially interact.

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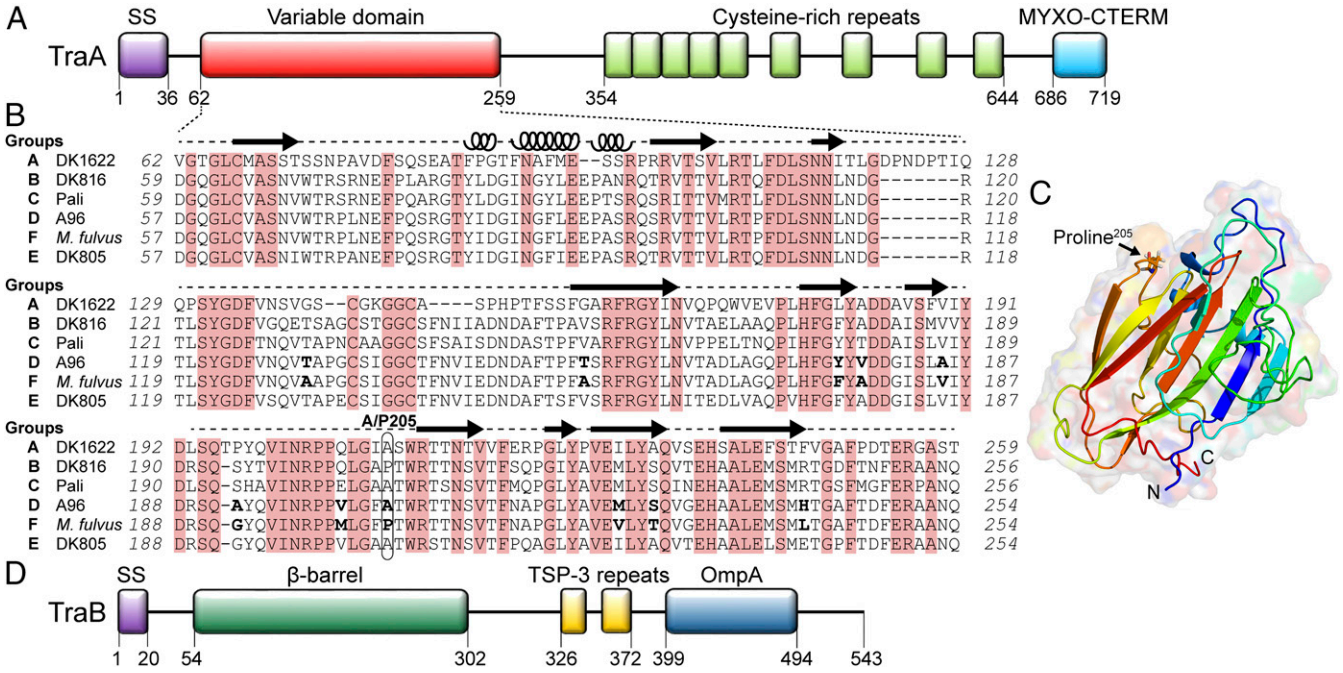

Fig. 1. TraA contains a variable domain that determines recognition specificity. (*A*) Domain architecture of TraA: Type I signal sequence (SS); Cys-rich region and MYXO-CTERM are a putative stalk and a sorting tag, respectively (11). (*B*) Sequence alignment of the TraA variable domain from six recognition groups. Conserved residues are shaded red. Amino acid differences between the TraA^{A96} and TraA$^{M.\ fulvus}$ are in bold. Predicted secondary structures by I-TASSER (22) are indicated (loops, α-helices; arrows, β-strands). (*C*) Modeled 3D structure of the TraA$^{M.\ fulvus}$ variable domain by I-TASSER where the highest C-score was selected. (*D*) Domain architecture of TraB; Thrombospondin type 3 (TSP-3, Pfam02412). β-Barrel prediction was made with BOCTOPUS2 (32).

are also hypothesized to function as fusogens, which leads to the bilateral sharing of OM components between engaged cells (9, 11, 14).

Because TraA is a specificity determinant, it functions as a greenbeard or kind recognition determinant. That is, TraA recognizes other cells that bear an identical allele and confers the preferential social behavior of exchanging private goods. Although *traA* is a greenbeard gene, OME also involves discrimination against kin because other loci impact OME. Our recent findings suggest that toxins encoded on a prophage are transferred by OME and partnering cells lacking cognate antitoxins are killed (17). Thus, TraA binding leads to self-recognition and the exchange of toxins further discriminates self from nonself. These combined functions are thought to facilitate the assembly of communities composed of highly related individuals.

Here we sought to understand how one receptor gives rise to many, potentially hundreds, of different recognition groups among natural myxobacterial isolates. To elucidate the molecular basis of TraA recognition, we first define the roles of TraA and TraB in cell–cell adhesion. Second, by chimeric allele analysis and site-directed mutagenesis, we precisely map key specificity determinants within TraA and surprisingly reveal that single residue substitutions alter recognition specificity. This finding represents a rare example where a single residue change reprograms social interactions. We suggest that the malleable nature of the TraA receptor has facilitated the evolution of social diversity within the Myxococcales order.

Results

TraA Requires TraB to Form Functional Cell Surface Adhesins. We previously showed that overexpression of TraA/B causes tight cell–cell adhesion during liquid growth (14) (Fig. S1). We suspect weaker adhesions also form when TraA/B are expressed at wild-type levels. To clearly delineate the roles of each protein in adhesion, we tested whether overexpression of either TraA or TraB was sufficient for cell adhesion. Notably, cell–cell adhesion only occurred when TraA/B were both overexpressed (Fig. 2*A*). In our model of OME, TraA serves as a receptor that recognizes clonemates by homotypic interactions, whereas the role of TraB is poorly understood. Sequence analysis predicts that TraB contains an N-terminal OM β-barrel and a C-terminal OmpA domain that presumably binds peptidoglycan (11) (Fig. 1*D*). Because of its domain features and because the *traAB* genes overlap in an operon, we speculated that TraB might facilitate the transport of TraA to the cell surface analogous to two-partner secretion systems (18). However, as shown in Fig. 2*B*, TraA was detected on the cell surface in a Δ*traB* mutant by live-cell immunofluorescence. As an experimental control, CglC, a lipoprotein that localizes to the inner leaflet of OM (19), was probed in the same manner and was not detected on the surface (Fig. S2). In a separate approach, a Δ*traB* mutant was treated with proteinase K (PK), and TraA was susceptible to proteolysis as found in the *traB*$^{+}$ strain (Fig. 2*C*). We conclude that TraA transport occurs independently of TraB. Notably, the majority of TraA receptors distribute along cell–cell adhesion junctions when TraB was co-overexpressed (Fig. 2*B*, *Inset*), suggesting TraB assists TraA to function in adhesion.

Cell–Cell Adhesion Is *traA* Allele-Specific. TraA contains a variable [a.k.a. PA14-like (11), Pfam07691] domain (Fig. 1*A*), which we suggest contains the specificity determinants (4). To test our model that TraA receptors from opposing cell membranes directly interact (9), we asked whether such interactions promoted self-adhesion. Here we overexpressed the *Myxococcus fulvus* HW-1 (20) *traAB* operon in a Δ*traAB M. xanthus* strain. As expected, this strain formed cell–cell adhesions in liquid culture, similar to cells that overexpress the *M. xanthus* (DK1622) *traAB* operon (Fig. S1). The *traAB*$^{M.\ fulvus}$ strain labeled with sfGFP was then cocultured with a *traAB*^{DK1622} strain labeled with mCherry. In support of a homotypic interaction model, we found that cells

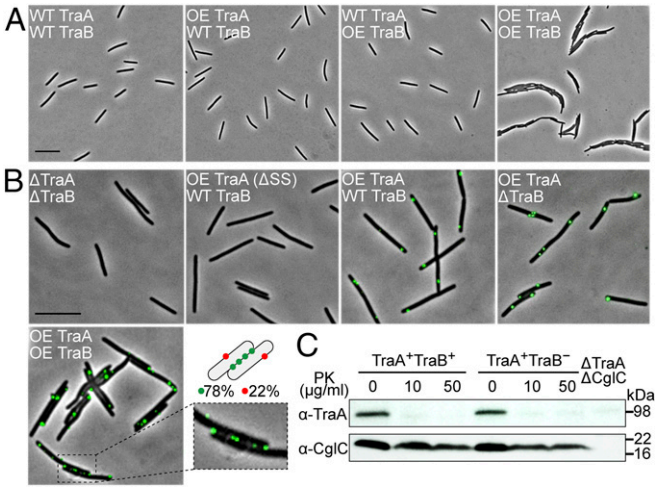

Fig. 2. TraA requires TraB to form a functional adhesin. (*A*) Wet mounts of indicated strains taken directly from shake flask cultures. Relevant properties shown: wild type (WT) and overexpression (OE). Strains from *Left* to *Right* are DK8601, DW2202, DW2203, and DW1463. (Scale bars: *A* and *B*, 2 μm.) (*B*) Cell surface localization of TraA. Immunofluorescent labeling with α-TraA serum was done on live, nonpermeabilized cells. Relevant properties are shown. Strains (*Left* to *Right*) are DW1483, DW2204 (signal sequence deletion, ΔSS), DW2202, DW2205, and DW1463. *Inset* shows TraA foci along a cell–cell junction. Among cells that clumped, 78% (379 out of 485) of the TraA foci localized along cell–cell junctions. (*C*) Protease accessibility assay of TraA in *traB*$^{+}$ (DW2202) or *traB*$^{-}$ (DW2205) backgrounds. Live cells were treated with indicated concentrations of PK and analyzed by immunoblot with α-TraA serum. The same samples were also probed with α-CglC serum as a negative control. A Δ*traA* Δ*cglC* strain (DW2220) was used as an immunoblot control.

only adhere to ones bearing identical receptors (Fig. 3*A*). To confirm that cell–cell adhesion was not the result of aberrant cell division from TraA/B overexpression, we cocultured three strains to test for selective binding. As shown in Fig. 3*B*, when *traAB*$^{M.\ fulvus}$ (sfGFP), *traAB*$^{M.\ fulvus}$ (nonlabeled), and *traAB*^{DK1622} (mCherry) strains were cocultured at a 1:1:1 ratio, interstrain binding occurred and represented ∼30% of total cell clusters. One hundred one interstrain clusters were examined from three independent experiments, and coaggregation was only found between *traAB*$^{M.\ fulvus}$ cells, whereas *traAB*^{DK1622} cells only bound to themselves. Similarly, in separate tripartite coculture experiments, *traAB*^{DK1622} (mCherry) and *traAB*^{DK1622} (sfGFP) cells were found to only cluster with one another and not *traAB*$^{M.\ fulvus}$ (nonlabeled) cells (Fig. 3*B*; 103 interstrain clusters analyzed). To test the role of TraB in binding selectivity, we constructed a strain overexpressing *traA*$^{M.\ fulvus}$*traB*^{DK1622} (nonlabeled) and cocultured it with *traAB*^{DK1622} (mCherry) and *traAB*^{DK1622} (sfGFP) cells. Binding only occurred between cells harboring the same TraA receptors (Fig. S3), suggesting TraB is not involved in specificity. In addition, we expressed *traA* alleles from two other recognition groups (4) in an isogenic Δ*traA* strain that overexpressed *traB*^{DK1622}. The resulting strains, *traA*^{DK805} (EYFP) and *traA*^{DK816} (nonlabeled), formed cell clusters in monocultures (Fig. S1). These strains were then cocultured with the *traAB*^{DK1622} (mCherry) strain, which belongs to a third recognition group. As shown in Fig. 3*C*, the expression of different TraA proteins, but identical TraB proteins, led to distinct social clusters. Consistent with this, in an extracellular complementation (stimulation) assay, which monitors the transfer of OM lipoproteins and restoration of motility to a mutant by OME, motility rescue (emergent flares) only occurred between donor and recipient strains harboring identical TraA receptors (Fig. 3*D*). We conclude that TraA serves as the sole specificity determinant, whereas TraB plays an essential role in adhesion and OME.

Chimeric Allele Analysis Defines a Specificity Region in TraA. To elucidate the molecular basis of TraA recognition, we sought to identify a subregion(s) within the variable domain that governs specificity. To this end, a series of chimeric *traA* alleles were constructed (Fig. 4*A*). Initially, we examined the *traA* alleles from environmental isolates *M. fulvus* HW-1 and *M. xanthus* A96. These two alleles are not compatible, and yet they only contain 11 amino acid differences between their variable regions (Fig. 1*B*). The first chimera fusion site between *traA*$^{M.\ fulvus}$ (*Mf*) and *traA*^{A96} (A96) was engineered after residue 205 (Fig. 4*A*), and the two resulting chimeras (*Mf*^{205}A96 and A96^{205}*Mf*) were transformed into Δ*traA* donor and recipient strains. As judged by stimulation assays, both chimeras were functional in self-recognition (Fig. 4*B*). The *Mf*^{205}A96 chimera was also found to interact exclusively with the *Mf* allele, whereas the A96^{205}*Mf* chimera interacted exclusively with the A96 allele. In other words, the chimeras recognized their parental alleles only when their *N* termini were identical. To further define the region involved in specificity, two additional chimeras, *Mf*^{192}A96 and A96^{192}*Mf*, were made in which the fusion site was moved upstream

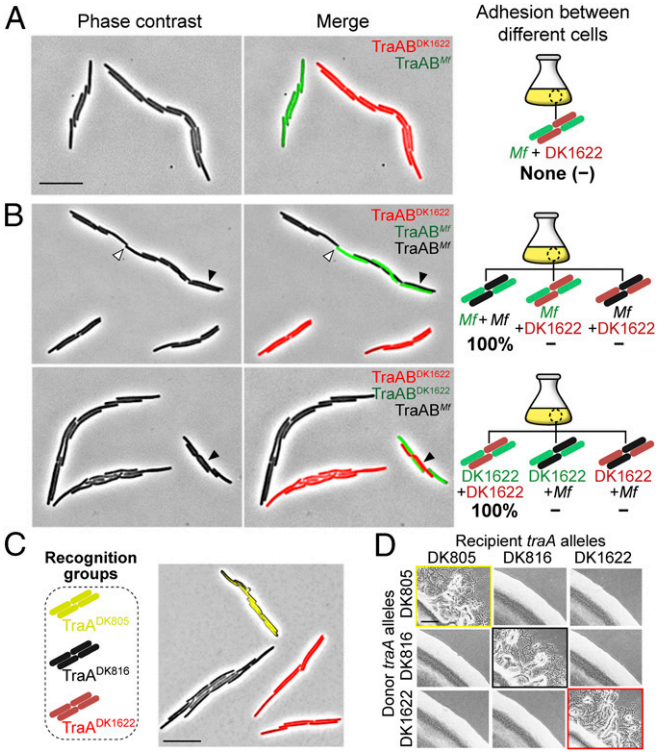

Fig. 3. Cell–cell adhesion is *traA* allelic-specific. (*A*) A *traAB*^{DK1622} strain (mCherry) was cocultured with a *traAB*$^{M.\ fulvus}$ strain (sfGFP). (*B*) Coincubation of three *traAB* strains: *traAB*$^{M.\ fulvus}$ (sfGFP), *traAB*$^{M.\ fulvus}$ (nonlabeled), and *traAB*^{DK1622} (mCherry) (*Upper*); *traAB*^{DK1622} (sfGFP), *traAB*^{DK1622} (mCherry), and *traAB*$^{M.\ fulvus}$ (nonlabeled) (*Lower*). Shown are pole-to-pole adhesion (open triangle) and side-by-side adhesion (filled triangle). Schematics of all possible interstrain adhesions are shown. Percent of each cell cluster is indicated (–, not detected). (*C*) Coincubation of strains expressing TraA receptors that belong to three different recognition groups. Strains all express *traB*^{DK1622} and are labeled (EYFP, mCherry, and nonlabeled) as indicated. (*D*) OME among cells expressing different *traA* alleles assessed by stimulation. Boxed panels highlight OME as detected by the rescue of motility defects. The recipient strains are nonmotile and stimulatable (Δ*cglC* Δ*tgl*), whereas the nonmotile donors (*aglB1* Δ*pilA*) are not stimulatable. See Table S1 for strain details. (Scale bars: *A* and *C*, 2 μm; and *D*, 200 μm.)

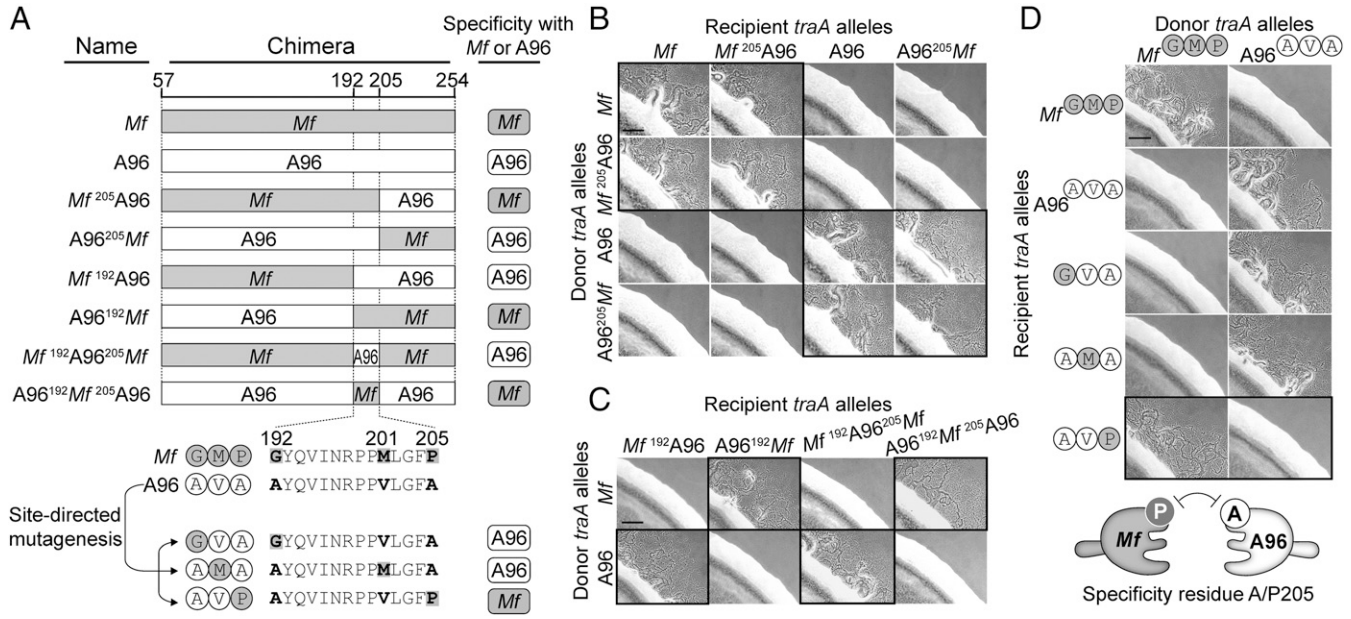

Fig. 4. A single residue (A/P205) governs the specificity of TraA^{Mf}–TraA^{A96} recognition. (*A*) Schematics of chimeric variable domains made between TraA^{Mf} (gray) and TraA^{A96} (white). The C-terminal region (not involved in specificity) is not shown. Chimeric junction sites are indicated. *Bottom* illustration shows mutagenesis strategy on a *traA* allele (pPC11) containing the TraA^{A96} subregion. Recognition compatibilities of the mutants with parental alleles are shown at the right (corresponding stimulation data shown in *B*–*D*). (*B*) Stimulation assays among chimeric and parental *traA* alleles. Mixtures that show recognition are highlighted with black frames. (*C*) Same as *B* but with different *traA* chimeras. (*D*) Stimulation assays of point mutants against parental alleles. The black-bordered micrographs show that a A205P substitution changes specificity. See Table S1 for plasmids/strain details. Cartoon summarizes the A/P205 differences that underlie incompatibility. (Scale bar, 200 μm.)

by 14 residues (Fig. 4*A*). Notably, these chimeras swapped recognition specificity; stimulation only occurred when the chimeras and parental alleles shared identical C termini (Fig. 4*C*). These combined results indicate that a small region (residues 192–205) plays a critical role in specificity. To confirm this, we created two more chimeras (Mf^{192}A96^{205}Mf and A96$^{192}Mf^{205}$A96), in which residues 192–205 from $traA^{A96}$ and $traA^{Mf}$ alleles were swapped into the parental $traA^{Mf}$ and $traA^{A96}$ backbones, respectively (Fig. 4*A*). Strikingly, Mf^{192}A96^{205}Mf now only recognized $traA^{A96}$ (Fig. 4*C*). Similarly, A96$^{192}Mf^{205}$A96 recognized $traA^{Mf}$ instead of $traA^{A96}$. We conclude that residues 192–205 play a key role in determining recognition specificity between these receptors.

Single Amino Acid Substitution (A/P205) Changes Cell–Cell Recognition. There are only three amino acid differences within the described specificity region between TraA^{Mf} and TraA^{A96} (Fig. 4*A*). To test the roles of each residue, we conducted site-directed mutagenesis. Here, the specificity region from TraA^{A96} was used as a template and the original residues were replaced individually to the corresponding residues from TraA^{Mf} (Fig. 4*A*). The 192A→G or 201V→M substitutions led to recognition with the TraA^{A96} parental receptor (Fig. 4*D*). In contrast, the 205A→P mutant recognized TraA^{Mf} rather than the parental receptor. Combined with the above results, we surprisingly found that a single residue acts as a specificity determinant between TraA^{Mf} and TraA^{A96}.

According to our previous finding (4), *M. fulvus* and A96 were assigned to the recognition "supergroup D." Supergroup D was loosely defined because in a few cases promiscuous interactions among natural isolates were detected in an assay that monitors the transfer of fluorescent lipids. Here, we sought to clarify these recognition outcomes, in terms of group members containing an A or P at the position that corresponded to residue 205 in TraA^{A96} or TraA^{Mf} (Fig. 1*B*). OME was reevaluated in stimulation assays using isogenic Δ*traA* background strains. Notably, we found that the original supergroup D members were now divided into two groups: members containing A205 (renamed D) and members containing P205 (renamed F) (Fig. S4). We suspect that the promiscuous interactions observed in prior experiments were due, in part, to the interstrain killing between natural isolates and/or toxicity associated with lipophilic dye staining. Those complications were avoided in stimulation assays with isogenic strains. In addition, poor stimulation was observed when $traA^{DK823}$ (containing A) cells were mixed with $traA^{Mf}$ (containing P) cells, suggesting crosstalk between these receptors (Fig. S4). Taken together, these results suggest that the residue A/P205 serves as a predictive feature for recognition specificity.

A/P Substitution Changes Recognition Specificity in Other *traA* Alleles. The discovery of A/P205 as a specificity determinant prompted us to study other recognition groups: A, B, C, and E. Notably, this A/P residue is ubiquitously found in all 17 *traA* alleles that

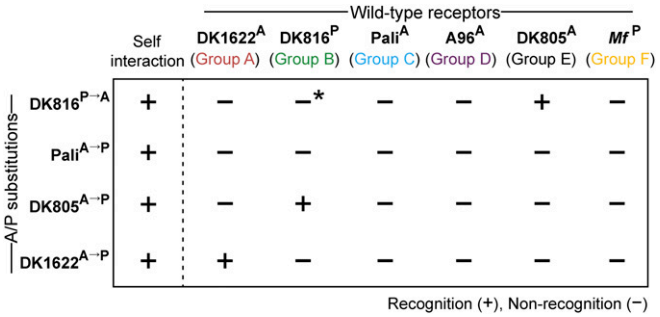

Fig. 5. Single residue substitutions (A/P) change recognition specificity in a panel of *traA* alleles. Shown are substitutions on representative *traA* alleles from four different recognition groups where the original A or P was changed as indicated (superscript). Mutants were tested for recognition against strains expressing different wild-type TraA receptors and scored for stimulation (*, poor stimulation). See Fig. S6 for experimental data.

we characterized (Fig. S5), suggesting this residue plays a broad role in recognition. To test this, site-directed mutagenesis was done on representative *traA* alleles from these groups where the original A or P residue was changed to P or A, respectively. All of the mutant alleles were functional as judged by robust self-recognition (Fig. 5 and Fig. S6). Interestingly, the P→A substitution in TraA^{DK816} (group B) resulted in poor interaction with the parental TraA^{DK816} receptor, whereas the A→P substitutions in TraA^{Pali} (group C) and TraA^{DK805} (group E) completely abolished interactions with their parental receptors (Fig. 5 and Fig. S6). In contrast, the A→P mutation in TraA^{DK1622} (group A) led to recognition with its parental receptor with little change in activity (Fig. 5 and Fig. S6), indicating the A/P residue is not critical in group A recognition. Taken together, we conclude that the A/P205 residue plays a key role in determining specificity among members of groups B, C, D, E, and F.

Unique TraA Recognition Receptor Engineered by Single Amino Acid Substitution. We next evaluated the role of A/P205 in intergroup discrimination. To this end, the interactions between the above A/P point mutants and representative *traA* alleles from all six groups were assessed. We considered three possible outcomes: A/P substitutions overcome recognition barriers between receptors from different groups, resulting in (*i*) crosstalk, (*ii*) mutant receptors with changed specificities, and (*iii*) receptors with unique specificities. As shown in Fig. 5 and Fig. S6, *traA*^{DK805} (A→P) recognized *traA*^{DK816} (containing P) but not its parental allele. In the reciprocal experiment, *traA*^{DK816} (P→A) interacted robustly with *traA*^{DK805} (containing A) and exhibited poor recognition with its parental allele. These results suggest A/P acts as a major specificity determinant between *traA*^{DK816} (group B) and *traA*^{DK805} (group E). Interestingly, the A→P substitution in *traA*^{Pali} (group C) created a receptor that only interacted with itself and not with its parental allele or any other allele (Fig. 5 and Fig. S6), thus generating a receptor with a unique specificity.

The variable domain in TraA shows distant homology to the PA14 domain found in Flo5 from yeast (11). Flo5 is a cell adhesin that mediates flocculation, and the crystal structure of its PA14 domain is known (21). Using this structure as a template, a computational model for the variable domain of TraA^{DK1622} was previously made (11). Here, a model of the variable domain from TraA$^{M.fulvus}$ was made with I-TASSER (Fig. 1*C*) (22), using multiple templates including Flo1, Epa1, Epa9, and Cea1 adhesins from various yeast strains as well as the protective antigen (PA) from anthrax toxin. This analysis suggests that A/P205 resides within a loop between two β-strands (Fig. 1 *B* and *C*). We propose that this loop acts as a recognition switch and A/P205 substitutions alter its conformation and hence specificity of recognition.

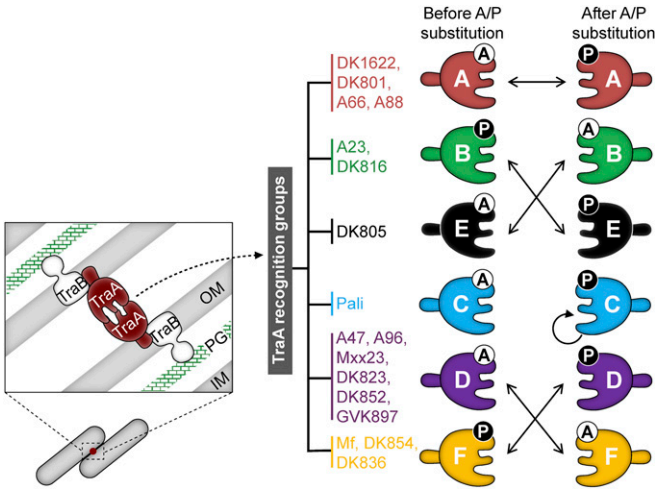

Fig. 6. Summary for how A/P205 substitutions influence TraA recognition. TraA functions with TraB to form an active adhesin wherein TraA determines the specificity of cell–cell recognition (*Left*). Inner membrane, IM; outer membrane, OM; peptidoglycan, PG. *Right* shows 17 TraA receptors are divided into six recognition groups. Solid arrows indicate how A/P205 substitutions reprogram recognition.

Discussion

OME in myxobacteria is a unique platform for cells to interact and survive as multicellular entities (9, 23). A polymorphic receptor, TraA, allows individuals to recognize clonemates and exchange their private goods. Here we show that TraA requires TraB to form an active adhesin for self-recognition. In the absence of TraB, TraA localizes to the cell surface but does not function in cell–cell binding. TraA contains a predicted sorting motif, MYXO-CTERM, which is analogous to the cell wall-sorting tag LPXTG in Gram-positive bacteria (11) and may contribute to its cell surface display. TraB, with a predicted OM β-barrel and an OmpA cell wall binding domain, may help anchor TraA to the cell envelop during adhesion and OME. We also found that heterologous expression of different TraA–TraB protein combinations were functional, and the specificity of cell–cell recognition was determined by TraA. We suspect that TraB interacts with TraA in regions that are conserved between these proteins.

Cells form distinct kin groups in liquid according to which TraA receptor they express. The ability of a single gene to identify others that bear the same allele and to confer preferential social behaviors is a hallmark of a greenbeard gene (24, 25). This is an example demonstrating that single allele variation (i.e., homotypic receptor) within a bacterium governs selective cell–cell binding. We hypothesize that selective adhesion with clonemates in heterogeneous myxobacterial populations facilitates cooperative behaviors among close relatives. In future studies, we will address the roles of TraA/B recognition and adhesion during the formation of social groups on solid surfaces, where cells glide and form structured communities.

We show that TraA is a malleable protein where chimeric alleles and amino acid substitutions are tolerated and lead to changes in self-recognition (Fig. 6). We think these qualities are important for the evolution of diversity in TraA recognition. This platform allows new recognition alleles to be created by spontaneous mutations or horizontal gene transfer that results in homologous recombination between *traA* alleles. Previously, we alluded to this latter possibility by suggesting rearrangements occurred between ancestral *traA* alleles (4). With changes in *traA* sequences and concurrent changes in recognition, populations will in turn diversify, ensuring that private good exchange only occurs with clonemates or strains that share recognition specificity. The diversification of large populations into distinct social groups also provides a selective advantage to adapt to changing environments (5, 10). For example, a homogenous population, in terms of TraA recognition, exposes itself to exploitation by selfish elements. One such element is Mx-alpha, which resembles a defective prophage and is hypervariable (17, 26, 27). Mx-alpha may harbor toxins that are transferred by OME and kill non-clonemates lacking cognate immunity. TraA polymorphisms create distinct recognition groups that prevent OME and lethal encounters from occurring between sympatric individuals. These social barriers, in turn, block exploitation by Mx-alpha.

We identified a single residue in TraA that plays a key role in specificity. For example, swapping A/P205 residues between *traA*^{DK816} and *traA*^{DK805} led to changes in recognition from the parental allele to the other allele. These findings are striking given that there are 42 amino acid differences between their variable domains (Fig. 1*B*). However, simply sharing an A or

P205 residue is not sufficient for recognition (e.g., TraA^{A96} vs. TraA^{DK805}, TraA^{Mf} vs. TraA^{DK816}), demonstrating other residues contribute toward specificity. Strikingly, an A→P substitution within *traA*^{Pali} created an allele that only recognized itself; it did not recognize its parental receptor or other receptors. This highlights the importance of A/P205 and suggests that TraA may undergo diversification and form novel recognition groups in nature. Given the high levels of heterogeneity of myxobacterial communities and *traA* alleles (4, 28, 29), we suspect the *traA*^{Pali} A→P allele is compatible with undiscovered receptors in nature. For group A receptors, which are divergent from the other five groups (Fig. 1*B*) (4), substitutions in A/P205 did not alter specificity. We suspect that the divergent sequences of group A members likely result in structural differences that will need to be elucidated elsewhere.

Given that A/P205 plays a key role in specificity, we hypothesize that this residue acts as a molecular recognition switch, where an A or P at this position changes the conformation of the loop. In support of this, a single residue was recently shown to determine the conformational position of an analogous loop (21, 30). Here, the Flo1 and Flo5 adhesins from *Saccharomyces cerevisiae* contain a distantly homologous PA14 domain (11). Crystallographic studies revealed both structures contain a loop, L3, that is flexible and adopts different conformations due to single residue variations: P in Flo1 and D in Flo5 (30). These different L3 conformations result in different mannose-binding affinities. From sequence alignments (Fig. S7), L3 from Flo1/5 corresponds with the predicted A/P205 loop in TraA. By extension, we suggest that this loop in TraA switches conformations based on the presence of an A or P residue, which consequently governs self-recognition.

The interaction of different TraA receptors does not always result in a clear OME outcomes. In a few cases, a weak response between different receptors is observed (Fig. S4). These promiscuous interactions represent crosstalk between receptors. In our system, crosstalk may arise from receptor overexpression by the strong heterologous *pilA* promoter. In nature, crosstalk might be selected against because OME between nonclonemates is lethal (17). In other molecular recognition systems, crosstalk also occurs. For example, in two-component signaling systems, histidine kinases (HKs) may interact with more than one response regulator (RR) (31). Depending on the selective conditions, this crosstalk might lead to the diversification of the HK–RR interfaces and the evolution of new specificity pairs. Similar evolutionary forces may apply to TraA–TraA interactions.

Materials and Methods

Bacterial strains, plasmids, and growth conditions are described in *SI Materials and Methods* and Tables S1 and S2.

Cell–Cell Adhesion Assay. Strains were grown to midlog phase and were mixed in indicated combinations at a density of 1×10^7 cells per mL. Co-cultures were incubated overnight with vigorous shaking, and cell suspensions were directly mounted on glass slides and observed by phase contrast/fluorescence microscopy.

Stimulation Assay. Cells were grown to midlog phase, harvested, and resuspended to the calculated density of ~2.5×10^9 cells per mL. The nonmotile donor cells (DW1467 background) could not be stimulated, whereas the recipient cells (DW2220 background) could be stimulated, were mixed at 1:1 ratio, and 5 μL of the mixtures were spotted onto casitone agar containing 2 mM $CaCl_2$. To test specificity of recognition, the endogenous *traA* gene was deleted and heterologous *traA* alleles were expressed from the Mx8 *attB* locus (Table S1). The plates were incubated at 33 °C overnight. Colony edges were imaged by phase contrast microscopy with a 10× objective lens.

Immunological Methods. Protease accessibility assay was essentially done as in ref. 19 with a few modifications. TraA immunofluorescence was performed as previously described (4). See *SI Materials and Methods* for details.

Additional details are in *SI Materials and Methods*.

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