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Self-recognition in social amoebae is mediated by allelic pairs of *tiger* genes

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Abstract

Free-living cells of the social amoebae *Dictyostelium discoideum* can aggregate and develop into multicellular fruiting bodies in which many altruistically die as they become stalk cells that support the surviving spores. *Dictyostelium* cells exhibit kin-discrimination – a potential defense against cheaters, which sporulate without contributing to the stalk. Kin-discrimination depends on strain relatedness and the polymorphic genes *tgrB1* and *tgrC1* are potential components of that mechanism. Here we demonstrate a direct role for these genes in kin-discrimination. We show that a matching pair of *tgrB1* and *tgrC1* alleles is necessary and sufficient for attractive self-recognition, which is mediated by differential cell-cell adhesion. We propose that TgrB1 and TgrC1 proteins mediate this adhesion through direct binding. This system is a genetically-tractable ancient model of eukaryotic self-recognition.

The ability to distinguish self from non-self is observed in many organisms and is a central component of social evolution (1). During *Dictyostelium* development, individual cells aggregate to form multicellular fruiting bodies where 20–30% die while constructing a cellular stalk and 70–80% survive as spores (2). This altruistic behavior is enigmatic because fruiting bodies can contain cells with different genotypes, facilitating social exploitation (3). We have identified a self-recognition system that allows *Dictyostelium* to cooperate preferentially with relatives, providing potential defense from exploitation (4). We have also identified two polymorphic genes, *tgrB1* and *tgrC1* (previously *lagB1* and *lagC1*, respectively), that are necessary for self-recognition and for development (5).

We hypothesize that *tgrB1* and *tgrC1* encode matching transmembrane proteins (TgrB1 and TgrC1, respectively) that mediate self-recognition by heterotypic interactions between the extracellular protein domains on adjacent cells. If the hypothesis were correct, we would expect that deleting the resident alleles would render the cells unable to self-recognize. Furthermore, replacing the resident alleles with a matching pair of alleles from a different strain would restore self-recognition but abolish cooperation with the parental strain.

We deleted the *tgrB1-tgrC1* locus (Fig. S1) and mixed the mutants with the parental AX4 cells (6). The strains started to segregate within the streams of cells that form at the onset of

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aggregation and eventually formed segregated multicellular structures (Fig. 1A). In pure populations, the *tgrB1*⁻*tgrC1*⁻ cells were arrested at the aggregate stage (Fig. 1F) and exhibited compromised spore production (Fig. 1J). These results suggest that an intact pair of *tgrB1-tgrC1* alleles is necessary for self-recognition, consistent with our previous findings that *tgrB1* and *tgrC1* are individually necessary for self-recognition (5). We then reintroduced a pair of *tgrB1-tgrC1* alleles from AX4 into the deletion strain, restoring the locus to its original structure with minor changes (Fig. S1). Both alleles were fully functional because they complemented all the *tgrB1*⁻*tgrC1*⁻ phenotypes. This double-gene replacement strain (*tgrB1*^{AX4}*tgrC1*^{AX4}) mixed with the parental AX4 strain as seen in the even distribution of red and green-labeled cells (Fig. 1B). The strain also produced fruiting bodies (Fig. 1G) and spores (Fig. 1J) in pure populations.

The *tgrB1* and *tgrC1* sequences are polymorphic (5). We cloned them from four wild strains, which are different from one another and from AX4 (Fig. S2), and recombined them into the resident locus of the *tgrB1*⁻*tgrC1*⁻ cells. We then mixed the cells at equal proportions and found that the double gene replacement strains carrying alleles from QS4 (*tgrB1*^{QS4}*tgrC1*^{QS4}) or QS38 (*tgrB1*^{QS38}*tgrC1*^{QS38}) segregated from AX4 (Fig. 1C,D) and eventually formed separate fruiting bodies (Fig. S3). Segregation was observed at other proportions as well (Fig. S4). The double-gene replacement cells developed and produced spores in pure populations (Fig. G–I), as did strains carrying either *tgrB1*^{QS31}*tgrC1*^{QS31} or *tgrB1*^{QS45}*tgrC1*^{QS45} (Fig. 1J). We also tested a mixture between the *tgrB1*^{QS4}*tgrC1*^{QS4} and the *tgrB1*^{QS38}*tgrC1*^{QS38} cells and observed that the two strains segregated (Fig. 1E).

In the models (Fig. 1) we propose that cells lacking both *tgrB1* and *tgrC1* segregate from AX4 because they are not recognized as self. They also fail to develop in pure populations because they do not recognize one another as self. Reintroducing a matching pair of alleles from AX4 restores self-recognition and allows the cells to cooperate with AX4. Introducing a pair of polymorphic alleles from any wild strain restores self-recognition, as reflected in the cells' ability to develop in pure populations, but these double-gene replacement strains do not cooperate with strains with non-matching *tgrB1-tgrC1* alleles, including the parental AX4. We also conclude that self/non-self-recognition is not a result of compromised development – the double gene replacement cells develop well as pure populations, but segregate from non-self cells in mixes.

The only differences between the double gene-replacement strains are the sequences of *tgrB1-tgrC1*. Therefore, matching pairs of these alleles are both necessary and sufficient for self-recognition in the AX4 background, suggesting direct involvement in recognition.

The donor strains of *tgrB1* and *tgrC1* are genetically distinct from AX4 and from each other, and segregation between them is directly correlated with their genetic distances (4). Moreover, segregation and *tgrB1-tgrC1* polymorphism are correlated (5), suggesting that *tgrB1* and *tgrC1* are major determinants in self-recognition. To test that possibility directly, we examined recognition between the double gene-replacement strains and the donor strains (Fig. 2). In control experiments, the *tgrB1*^{AX4}*tgrC1*^{AX4} cells segregated from the wild strains (Fig. 2A and 2D). Segregation was evident at the streaming stage and later in development when the cells formed segregated fingers. Mixing QS4 with the *tgrB1*^{QS4}*tgrC1*^{QS4} strain resulted in an even distribution of cells during early aggregation and the two strains remained in the same multicellular structures although sorting was observed within the slugs (Fig. 2B). We observed similar patterns when we mixed QS31 and *tgrB1*^{QS31}*tgrC1*^{QS31} cells (with less homogeneous mixing during early aggregation, but with both strains present in the same slug; Fig. 2E). The reciprocal non-matching mixes resulted in segregation – QS4 segregated from *tgrB1*^{QS31}*tgrC1*^{QS31} (Fig. 2C) and QS31 segregated from *tgrB1*^{QS4}*tgrC1*^{QS4} (Fig. 2F). In the models (Fig. 2), two cells recognize each other via

matching *tgrB1* and *tgrC1* alleles, despite other genetic differences between them. These observations suggest that *tgrB1* and *tgrC1* are major determinants in self-recognition. The segregation observed at the finger stage may reflect physiological differences, because the wild strains are different from AX4 at various genetic loci (4).

TgrC1 is a heterotypic cell-cell adhesion protein (7) and TgrB1 is its potential binding partner (5), suggesting that self-recognition is mediated by differential binding of matching TgrB1 and TgrC1. However, *tgrC1* also affects cAMP-signaling and gene regulation (8, 9), so self-recognition could be mediated by differential signaling. To test the role of differential cell-cell adhesion directly, we developed AX4 and double gene-replacement strains separately until they aggregated, disaggregated the structures into single-cell suspensions, mixed the cells, and tested cell-cell adhesion in suspension (10). Control experiments showed even mixing between AX4 and the *tgrB1*^{AX4}*tgrC1*^{AX4} cells (Fig. 3A). However, AX4 and *tgrB1*^{QS4}*tgrC1*^{QS4} cells adhered into distinct aggregates (Fig. 3B), whereas differentially labeled *tgrB1*^{QS4}*tgrC1*^{QS4} cells formed mixed aggregates (Fig. 3C). We also tested single gene-replacement strains in pure populations and in mixes and found that *tgrB1* and *tgrC1* interact “in trans”, such that TgrB1 on one cell interacts with a matching TgrC1 on the other cell (Fig. S5). In the models (Fig. 3) we propose that TgrB1 on one cell binds the matching TgrC1 on a neighboring cell such that cells with matching *tgrB1-tgrC1* alleles adhere to one another.

Self-recognition can be mediated by attraction of self or by rejection of non-self. To test which mechanism is utilized, we tested the effect of an extra *tgrB1-tgrC1* pair. AX4 cells are haploid, so introducing extra alleles would make them merodiploid for these genes. If self-recognition were mediated by attraction of self, then introducing an extra pair of foreign alleles would allow the merodiploids to aggregate with two different strains, each sharing one pair of *tgrB1-tgrC1* alleles. In the case of non-self rejection, an extra pair of foreign *tgrB1-tgrC1* alleles would lead to self-rejection, resulting in aberrant aggregation. We constructed three merodiploid strains and found that they aggregated well and produced normal fruiting bodies and spores in pure populations. Mixing the *tgrB1*^{+/QS4}*tgrC1*^{+/QS4} merodiploid strain with either AX4 (Fig. 4A) or with the *tgrB1*^{QS}*tgrC1*^{QS4} double gene-replacement strain (Fig. 4B) resulted in even cell mixing, suggesting that self-recognition is mediated by attraction. Mixing the *tgrB1*^{+/QS4}*tgrC1*^{+/QS4} merodiploid with the *tgrB1*^{QS31}*tgrC1*^{QS31} double gene-replacement strain (Fig. 4C) resulted in segregation, suggesting that the merodiploid genotype did not result in recognition promiscuity. The *tgrB1*^{+/QS31}*tgrC1*^{+/QS31} merodiploid behaved in the same way (Fig. 4D–F). Finally, the *tgrB1*^{+/AX4}*tgrC1*^{+/AX4} merodiploid co-aggregated with AX4 but segregated from the two other double gene-replacement strains (Fig. 4G–I), suggesting that doubling the *tgrB1-tgrC1* dosage is not a confounding factor. In the models (Fig. 4) we propose that any matching pair of *tgrB1-tgrC1* alleles in one cell is sufficient for recognition of a respective pair in another cell. In addition, the presence of a non-matching pair of *tgrB1-tgrC1* alleles does not interfere with this recognition. These data suggest that *tgrB1* and *tgrC1* mediate self-recognition through an inclusive attraction mechanism.

Our data implicate *tgrB1* and *tgrC1* as central components in *Dictyostelium* self-recognition, because a matching pair of *tgrB1-tgrC1* alleles is both necessary and sufficient for self-recognition in the AX4 genetic background and because the double-gene replacement strains cooperate with the genetically different donor strains. TgrC1 is a known heterotypic cell-cell adhesion protein (7), so we propose that TgrB1 is its binding partner and that TgrB1 on the surface of one cell binds a matching TgrC1 on the surface of another cell.

D. discoideum has other cell-cell adhesion genes, mainly *cadA* and *csaA* (11), that have no direct effect on self-recognition (5), but may explain the adhesion of *tgrB1*[−]*tgrC1*[−] cells to

each other. *tgrB1* and *tgrC1* could mediate repulsion between cells with non-matching alleles by modifying other adhesion complexes (11), but the inclusive attraction of merodiploid cells to one another, to the parental strains, and to the double-gene replacement strains, suggests that *tgrB1* and *tgrC1* mediate attraction rather than repulsion.

Other self-recognition systems rely on polymorphic membrane proteins with extracellular immunoglobulin-like folds (1), including the mammalian MHC (12), *Botryllus* FuHC (13), *Drosophila* dendrite self-avoidance (14), and *Hydractinia* allorecognition (15, 16). These proteins are not related to *tgrB1-tgrC1* or to each other, suggesting convergent evolution in which similar properties arose despite the lack of extensive sequence similarity. The important properties are sequence polymorphism, which provides unique identities, and extracellular domains, which mediate cell-cell interactions. The immunoglobulin fold is common in these systems probably because it is tolerant of sequence variation (17).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and Notes

- Boehm T. Cell. 2006; 125:845. [PubMed: 16751096]
- Kessin, RH. Dictyostelium - Evolution, cell biology, and the development of multicellularity. Cambridge, UK: Cambridge Univ. Press; 2001. pp. xiv + 294.
- Shaulsky G, Kessin RH. Curr Biol. 2007; 17:R684. [PubMed: 17714662]
- Ostrowski EA, Katoh M, Shaulsky G, Queller DC, Strassmann JE. PLoS Biol. 2008; 6:e287. [PubMed: 19067487]
- Benabentos R, et al. Curr Biol. 2009; 19:567. [PubMed: 19285397]
- Materials and Methods are available as supporting material on *Science* Online.
- Wang J, et al. Dev Biol. 2000; 227:734. [PubMed: 11071787]
- Iranfar N, Fuller D, Loomis WF. Dev Biol. 2006; 290:460. [PubMed: 16386729]
- Sukumaran S, Brown JM, Firtel RA, McNally JG. Dev Biol. 1998; 200:16. [PubMed: 9698452]
- Brar SK, Siu CH. J. Biol. Chem. 1993; 268:24902. [PubMed: 8227052]
- Siu CH, Harris TJ, Wang J, Wong E. Semin Cell Dev Biol. 2004; 15:633. [PubMed: 15561583]
- Hughes AL, Nei M. Nature. 1988; 335:167. [PubMed: 3412472]
- De Tomaso AW, et al. Nature. 2005; 438:454. [PubMed: 16306984]
- Matthews BJ, et al. Cell. 2007; 129:593. [PubMed: 17482551]
- Rosengarten RD, Moreno MA, Lakkis FG, Buss LW, Dellaporta SL. Mol Biol Evol. 2010
- Rosa SF, et al. Curr Biol. 2010; 20:1122. [PubMed: 20537535]
- Bork P, Holm L, Sander C. J Mol Biol. 1994; 242:309. [PubMed: 7932691]

Fig. 1.

Cells (all in the AX4 background) labeled with GFP (green) or RFP (red) were grown separately, mixed at equal proportions and allowed to develop together (A–E). Multicellular structures were photographed with epifluorescence microscopy during streaming aggregation (8–12 hours, main pictures) and at the finger stage (14–18 hours, inserts). Bar = 0.5mm. The cartoons are models of interactions between cells. Cells are illustrated as elongated amoebae. TgrB1 and TgrC1 are illustrated as membrane proteins; the shading indicates their origins. Interactions between matching pairs are highlighted. (A) The parental strain (AX4, red) mixed with a *tgrB1-null tgrC1-null* strain (green). (B) AX4 (red) mixed with a strain in which *tgrB1-tgrC1* were replaced with matching AX4 alleles. (C,D) Strains (green) in which *tgrB1-tgrC1* were replaced with alleles from wild strains QS4 (C) or QS38 (D), mixed with AX4 (red). (E) Double gene-replacement cells carrying *tgrB1-tgrC1* from QS4 (red) mixed with double gene-replacement cells carrying *tgrB1-tgrC1* from QS38 (green). Pure populations were also developed into fruiting bodies (48h; F – bar = 1mm, G–I – bar = 0.5mm). (F) *tgrB1-null tgrC1-null* cells. (G–I) Double gene-replacement cells carrying *tgrB1-tgrC1* alleles from AX4 (G), QS4 (H), or QS38 (I). (J) Sporulation efficiencies of these and two additional double gene-replacement strains harboring *tgrB1-tgrC1* from strains QS31 or QS45 as indicated under the x-axis. The graph shows the fraction (%) of cells that became spores. The means and standard errors of 5–6 replicates are given.

Fig. 2.

We labeled the wild strains *QS4* (A–C) and *QS31* (D–F) with GFP (green text) and the double gene-replacement strains (in *AX4* background) with RFP (red text): *tgrB1-tgrC1* were from *AX4* (A,D), *QS4* (B,F) or *QS31* (C,E). We grew the cells separately, mixed them at equal proportions as indicated and allowed them to develop together. We photographed the structures as in Fig. 1. Bar = 0.5mm. Cartoons are as in Fig. 1. The shadings indicate genetic differences between cells and the respective origins of the alleles.

Fig. 3.

We developed pure populations of RFP (red) and GFP (green) labeled cells, all in the AX4 background, disaggregated and mixed them at equal proportions, allowed them to adhere in suspension and photographed with confocal fluorescence microscopy (bar = 0.1mm). **(A)** AX4 cells (red) mixed with a strain in which *tgrB1-tgrC1* were replaced with matching alleles from AX4 (green). **(B)** AX4 (red) mixed with a strain in which *tgrB1-tgrC1* were replaced with matching alleles from QS4 (green). **(C)** Strains (both red and green) in which *tgrB1-tgrC1* were replaced with matching alleles from QS4. Cartoons are as in Fig. 1.

Fig. 4.

We made merodiploid strains by introducing extra pairs of matching alleles into AX4: *tgrB1-tgrC1* from QS4 (**A–C**), QS31 (**D–F**) and AX4 (**G–I**). We labeled these cells with RFP (red) and mixed them with GFP-labeled haploid cells: AX4 (**A,D,G**), or double gene replacement strains with *tgrB1-tgrC1* from QS4 (**B,E,H**), or QS31 (**C,F,I**). Cell growth, mixing, photography and cartoons are as in Fig. 1. Bar = 0.5mm.