ANNUAL REVIEWS

Annual Review of Microbiology

Conflict, Competition, and Cooperation Regulate Social Interactions in Filamentous Fungi

A. Pedro Gonçalves,[1,2] Jens Heller,[1,3]
Adriana M. Rico-Ramírez,[1] Asen Daskalov,[1,4]
Gabriel Rosenfield,[1,5] and N. Louise Glass[1,6]

[1] Department of Plant and Microbial Biology, University of California, Berkeley, California 94720, USA

[2] Current Affiliation: Institute of Molecular Biology, Academia Sinica, Nangang District, Taipei 115, Taiwan

[3] Current Affiliation: Perfect Day, Inc., Emeryville, California 94608, USA

[4] Current Affiliation: Institut Européen de Chimie et Biologie, 33600 Pessac, France

[5] Current Affiliation: Department of Genetics, Stanford University School of Medicine, Stanford, California 94305, USA

[6] Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

Annu. Rev. Microbiol. 2020. 74:693–712

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-012420-080905

Keywords

allorecognition, nonself recognition, cell fusion, hyphal networks, kind recognition, programmed cell death

Abstract

Social cooperation impacts the development and survival of species. In higher taxa, kin recognition occurs via visual, chemical, or tactile cues that dictate cooperative versus competitive interactions. In microbes, the outcome of cooperative versus competitive interactions is conferred by identity at allorecognition loci, so called kind recognition. In syncytial filamentous fungi, the acquisition of multicellularity is associated with somatic cell fusion within and between colonies. However, such intraspecific cooperation entails risks, as fusion can transmit deleterious genotypes or infectious components that reduce fitness, or give rise to cheaters that can exploit communal goods without contributing to their production. Allorecognition mechanisms in syncytial fungi regulate somatic cell fusion by operating precontact during chemotropic interactions, during cell adherence, and postfusion by triggering programmed cell death reactions. Alleles at fungal allorecognition loci are highly polymorphic, fall into distinct haplogroups, and show evolutionary signatures of balancing selection, similar to allorecognition loci across the tree of life.

Contents

1. CONFLICT, COMPETITION, AND COOPERATION REGULATE SOCIAL BEHAVIOR

1.1 Greenbeard Genes, Altruism, and Allorecognition

Altruism is defined as an individual acting at a cost to themselves but benefiting, directly or indirectly, another individual, without the expectation of reciprocity (self-sacrifice). Self-sacrifices include complex behaviors, such as in meerkats, which watch for predators while other members of their family forage (17), or as in bacteria that absorb peptides that help the survival of the population (123). A gene-centered view of altruism provides an explanation for self-sacrifices: A gene can be favored in a population even if it is costly, if it provides benefits for other individuals carrying copies of that same gene (25). Thus, altruism is evolutionarily beneficial if the relatedness of the individual that profits from the altruistic act is higher than the cost/benefit ratio that this act imposes (Hamilton's rule) (60). This gene-centered view, in combination with kin recognition, can explain altruism in higher organisms, where genome-wide relatedness can be assessed based on a combination of visual, chemical, and tactile cues.

The concept of kin recognition is difficult to explain when considering microbes. How can microbes assess the genealogy of other individuals without seeing their surroundings? How can a microbial selfish gene (25) identify copies of itself in others? Originally envisioned to explain the genetic basis of social behavior (25), organisms containing green beards allow for easy identification by other green beard carriers. Greenbeard genes promote altruism toward individuals who share a specific phenotypic trait controlled by a given gene; an interaction defined as kind recognition or allorecognition (43). Multiple interaction modes between individuals using allorecognition are possible (e.g., cooperation versus antagonism). Allorecognition functions in phylogenetically diverse organisms (**Figure 1**): in social bacteria *Myxococcus xanthus* (132) and *Proteus mirabilis* (46); and eukaryotic colonial species including invertebrates *Hydractinia symbiolongicarpus* and *Botryllus schlosseri* (113); the slime mold *Dictyostelium discoideum* (79); and fungi *Cryphonectria parasitica* (89, 141), *Podospora anserina* (115), and *Neurospora crassa* (23, 48).

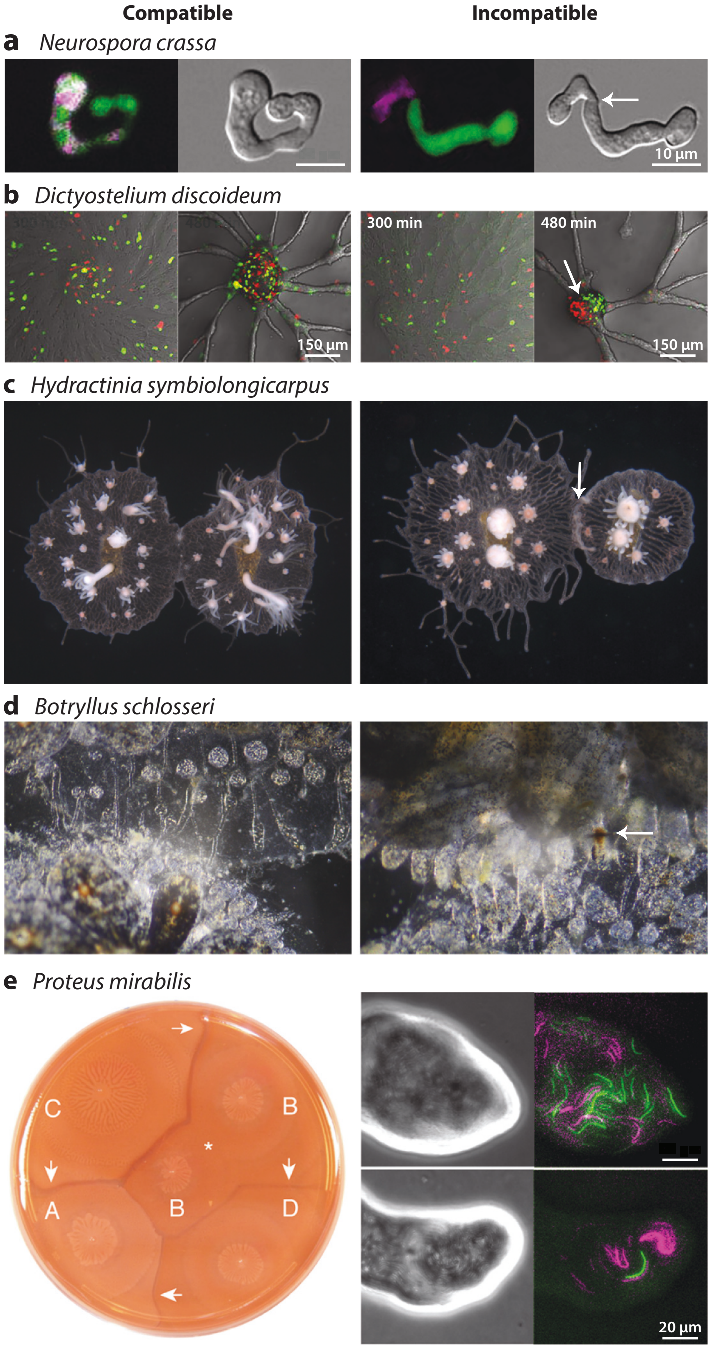
1.2. Evolutionary Features of Allorecognition Systems

Three potential drivers of allorecognition evolution in social organisms have been identified: cheaters (freeloaders), inbreeding, and disease transmission. Cheater/freeloader genotypes are named by analogy to the tragedy of the commons (107). They participate in an organism's social phase and receive social goods without contributing to their production (6), increasing the cheater's relative fitness at the expense of the social group (84). Selection for cheaters is an impediment to the progression of multicellularity and a primary driver of allorecognition evolution (6), which reduces the cheater/freeloader problem by permitting organisms to limit social behaviors to genetically similar individuals (79). The chestnut blight fungus, *C. parasitica*, provides examples for two potential drivers of fungal allorecognition. First, *C. parasitica* colonies are filamentous and syncytial, a lifestyle that selects for cheaters (6). Second, *C. parasitica* can be infected with Hypoviridae mycoviruses that reduce fitness (24). Mycoviruses lack external vectors and are transmitted via somatic cell fusion between an infected colony and an uninfected colony (45). Isolates of *C. parasitica* exhibit a form of allorecognition termed vegetative incompatibility that inhibits successful somatic cell fusion between genetically different strains (89, 141). Thus, disease transmission pressures may explain why some organisms have developed multiple allorecognition checkpoints that operate at various levels of intercellular intimacy.

Although kind recognition genes are not derived from common ancestors, they share evolutionary characteristics. Typically, genes encoding kind recognition systems exhibit evidence of balancing selection, including the long-term maintenance of multiple alleles at similar frequencies in well-mixed populations (109). Alleles at kind recognition loci are typically highly polymorphic with signatures of positive selection and fall into discrete allelic classes, termed haplogroups, which often show trans-species polymorphisms, a phenomenon observed when alleles from different species are more closely related to each other than they are to other intraspecies alleles (109). Kind recognition systems are often composed of multiple genes that are tightly linked, thus reducing the probability of recombination between a module's components and resulting in coevolution of the components and allelic diversification (9). Allorecognition systems could also represent cases of exaptation, a hypothesis developed as an explanation for allorecognition systems in fungi, where anti-pathogen defense systems are potentially harnessed for the recognition of conspecifics (57, 101).

1.3. Harming and Helping Kind Recognition

Allorecognition can be divided into harming and helping types (106). For example, bacteriocin toxins can be considered greenbeard traits of the harming type (43, 110). Some bacteria and archaea produce bacteriocins released at times of stress, with self-cells producing an antidote to the poison, which they keep private (110). Cells lacking the poison/antidote genes are killed (spite for



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Allorecognition in distinct domains of life. (*a*) Allorecognition upon cell-cell contact in *Neurospora crassa*. Germlings expressing cytoplasmic GFP (*green*) were paired with germlings stained with FM4–64 (*magenta*). Note fusion and cytoplasmic mixing on the left (compatible interaction) versus a cell fusion block upon cell-cell contact on the right (incompatible interaction) mediated by genetic differences at *cwr-1/cwr-2* (55). (*b*) Allorecognition during starvation-induced development in *Dictyostelium discoideum*. Strain pairings in which 5% of the cells are labeled with GFP (*green*) and 5% with RFP (*red*). Micrographs taken after mixing (300 min and 480 min, as indicated). Red and green cells are intermixed regardless of their allotypes (compatible and incompatible genetic backgrounds) at 300 min due to cAMP signaling for aggregation; however, at 480 min, the red and green cells segregate from each other due to expression of allorecognition determinants TgrB1 and TgrC1 (78). Panel *b* micrographs provided by Shigenori Hirose and Gad Shaulsky, Baylor College of Medicine. (*c*) Allorecognition during polyp fusion in *Hydractinia symbiolongicarpus* mediated by genetic differences at Alr1 and Alr2 (74, 94). Left and right micrographs show compatible and incompatible (rejection reaction) at early stages of fusion, respectively. Rejection causes extensive damage to adjacent colonies and may lead to the formation of hyperplastic stolons. Panel *c* micrographs adapted from Reference 94 with permission. (*d*) Allorecognition mediated by genetic differences in *fuhc(sec)* and *fuhc(tm)* (96) during colonial chimerization in *Botryllus schlosseri*. Images show extracorporeal vasculature of two colonies (*top* and *bottom*), showing interaction between the ampullae (ends of the vasculature). On the left, a compatible pairing results in fusion. In incompatible pairings, a rejection response and fusion blockage occur, as shown by the dark regions where the ampullae touch (*right*). Panel *d* micrographs provided by Anthony De Tomaso, University of California, Santa Barbara. (*e*) Allorecognition mediated by genetic differences at *ids* genes during swarming behavior of *Proteus mirabilis* (127). Petri dish shows boundaries and merging colonies of *P. mirabilis*; arrows indicate boundaries between incompatible swarm colonies, whereas an asterisk marks the merging of two compatible populations. Strains A, B, and C are independent wild-type strains, while D lacks the *ids* self-recognition genes (47). Micrographs on right show compatible (*top*) and incompatible (*bottom*) coswarmed populations of *P. mirabilis*. The two populations have been labeled with GFP (*green*) or RFP (*magenta*), with unlabeled parental strain in the mixture. Images show the leading edge of the second swarm ring; note that fewer cells of the nonself strain (*green*) are present after diverting into a swarm-incompatible state (*bottom*). Arrows indicate the zone of interaction between incompatible pairs. Panel *e* adapted from Reference 83 with permission. Abbreviations: GFP, green fluorescent protein; RFP, red fluorescent protein.

individuals lacking the greenbeard genes). Helping kind discrimination is defined by actions that provide fitness benefit to individuals that share the trait, but not to those that lack it. The tumor-inducing (*Ti*) plasmid of *Agrobacterium tumefaciens* can be considered a helping greenbeard trait. Genes are transferred from the *Ti* plasmid to plant cells, which induces tumors and production of opines (food source), which is a public good (135). However, opine production is only beneficial for *Ti* plasmid bearers, because opine catabolism is also encoded on the *Ti* plasmid (43). In *D. discoideum*, starvation leads to the aggregation of free-living ameboid cells to form multicellular structures composed of spores supported on a stalk (125). Stalk cells perform an act of self-sacrifice as they enable other cells to differentiate into spores for dispersal (87). Following the genetic logic above suggests that *D. discoideum* strains would be willing to sacrifice themselves only if they could help other individuals of the same genetic background to proliferate. When populations of *D. discoideum* contain a mixture of genetically different strains, the frequency of stalk formation is based on the likelihood of the benefit going to members of the group that share their genes (**Figure 1*b***); in this case, allorecognition is determined by the *tiger* genes (*tgrB1* and *tgrC1*) (66).

2. MOLECULAR MECHANISMS OF COOPERATIVE BEHAVIOR

2.1. Examples of Cooperation, with a Focus on Fungi

In nature, cooperation occurs at all levels and across taxa. For example, bacteria regulate their cooperative behavior in a process called quorum sensing: Autoinducers (like acylated homoserine

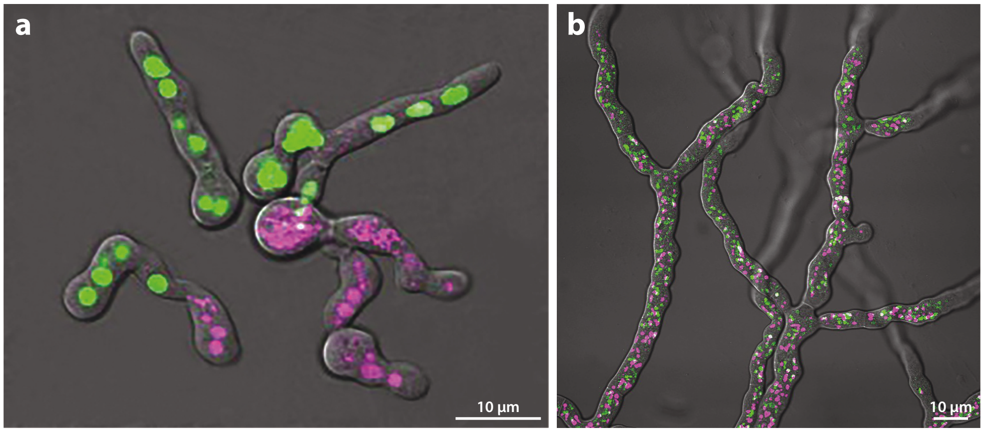


Figure 2

The syncytial lifestyle of filamentous fungi. Two genetically compatible strains of *Neurospora crassa*, whose nuclei have been labeled with either histone H1-GFP (*green*) or histone H1-DsRed (*magenta*) were paired and allowed to fuse at different developmental stages. Germlings and hyphae that have fused to form a single colony sharing a mixture of nuclei are shown in panels *a* and *b*, respectively. Abbreviation: GFP, green fluorescent protein.

lactones) increase in concentration depending on cell density, enabling bacterial communities to behave as multicellular organisms (90). The yeast pathogens *Candida albicans* and *Cryptococcus neoformans* also secrete quorum-sensing molecules such as farnesol, tyrosol, tryptophol (14, 15, 68, 81), Qsp1, and pantothenic acid (1, 67). Fungal quorum sensing is involved in many cellular processes, including morphogenesis (e.g., yeast-to-hypha transition), germination, biofilm formation, control of nutrient levels, cell death induction, antifungal activity, and pathogenicity (97).

In filamentous fungi such as *N. crassa*, mycelial growth results from tip elongation and somatic cell fusion between genetically identical germinated spores (germlings) and hyphae that are in close proximity (64) (**Figure 2**). This coordinated and cooperative behavior leads to the formation of an interconnected mycelial network. Cells deficient in somatic cell fusion show an increase in time for colony establishment (55, 72), indicating that the capacity to undergo somatic cell fusion contributes to fitness. Filamentous ascomycete colonies contain septa that are often perforated and that allow movement of cytoplasm and organelles, including nuclei, throughout the colony (111). This syncytial lifestyle makes the products of each nucleus potential social goods, which is predicted to strongly select for cheaters (6). Hyphal anastomosis and the mycelium it generates enhance fitness by increasing colony growth rates and improving the production of asexual spores (4), by distributing resources throughout the colony (122), and by increasing colony size when higher densities of spores are present (108).

2.2. Molecular Pathways Involved in Cooperative Somatic Cell Fusion in Filamentous Fungi

The molecular pathways required for somatic cell fusion between germlings/hyphae in filamentous fungi have recently been reviewed in detail (38). Here, we highlight pathways important for cooperative somatic cell fusion between genetically identical cells that are also implicated in allorecognition. In *N. crassa*, components of a mitogen-activated protein kinase (MAPK) signaling complex composed of NRC-1 (MAPKKK), MEK-2 (MAPKK), and MAK-2 (MAPK) and a scaffold protein HAM-5 assemble and disassemble at fusion tips of interacting cells during chemotropic interactions, with an ~8-min regularity and opposite dynamics in interacting cells (31, 42, 72) (**Figure 3*a***). A second protein, SOFT, also associates and disassociates at fusion tips, but with completely opposite dynamics to the MAK-2 signaling complex (42) (**Figure 3*a***). This so-called ping-pong mechanism of communication provided a hypothesis on how cells can avoid self-stimulation when undergoing chemotropic interactions with a genetically identical partner (56).

A second MAPK cascade, the cell wall integrity (CWI) MAPK pathway, is also required for somatic cell fusion in a number of filamentous fungi. The CWI MAPK pathway is composed of MIK-1 (MAPKKK), MEK-1 (MAPKK), and MAK-1 (MAPK) kinases and includes membrane-spanning sensors, such as WSC-1 and WSC-2 (38, 86). The MAK-1 complex does not show dynamic oscillation during chemotropic interactions, but once cells adhere, MAK-1 localizes to the contact zone, where it remains during fusion pore formation (134). In *Sordaria macrospora*, the ortholog of SOFT (PRO40) functions as a scaffold of the MAK-1 signaling complex (126, 134). Both the MAK-1 and MAK-2 signaling pathways regulate gene transcription through the activation of the transcription factors PP-1 and ADV-1 (40); ADV-1 is a direct activator of many of the genes required for somatic cell fusion (30, 40). Upstream of the two MAPK signaling cascades, the WHI-2, CSP-6, and AMPH-1 proteins putatively function to control endocytosis, which could be involved in the perception of chemotropic signals (53). Following cell-cell contact, cell wall dissolution at the fusion spot and plasma membrane merger is necessary to complete somatic cell fusion. A number of genes encoding proteins important for membrane merger have been identified in *N. crassa* (41, 98, 99), although a protein that directly causes membrane fusion, a so-called fusase, has not been identified.

Screening of the full genome deletion strain set available for *N. crassa* (102) revealed that ~80 genes affect or are required for somatic cell fusion, including those encoding components of signaling pathways, predicted membrane proteins, proteins that affect secretion, and a number of hypothetical proteins (38). Importantly, genes encoding the receptor or ligand involved in chemotropic interactions have not been identified or characterized. These data suggest that the genes encoding the receptor and ligand required for somatic cell fusion may have redundancy or that the receptor and ligand genes are members of the hypothetical protein gene set that have not been biochemically characterized, but that are essential for somatic cell fusion.

3. ALLORECOGNITION AT A DISTANCE

3.1. Determinants of Fungal Communication and Chemotropic Interactions

A fungal greenbeard locus that acts at a distance by regulating chemotropic interactions has been characterized in *N. crassa* (62). Germlings that share compatible alleles at the determinant of communication (*doc*) loci exhibit homing growth en route to somatic cell fusion to form a cooperative colony (**Figure 2**). Within *N. crassa* populations, five communication (CGs) haplogroups have been identified and exhibit CG-specific rearrangements, duplications, and deletions. Alleles at the linked *doc* loci, *doc-1* and *doc-2*, are ~99% identical within a CG, but only <50% identical between CG haplogroups. Strains of identical CG specificity home toward each other, while strains from different CGs ignore each other (**Figure 3*b***). Alleles at the *doc-1* and *doc-2* loci also show evidence of balancing selection and trans-species polymorphisms (62), supporting their role in mediating kind recognition in fungal populations.

Communication phenotypes of Δ*doc-1*, Δ*doc-2*, and Δ*doc-1* Δ*doc-2* mutants confirmed that the *doc* locus is necessary and sufficient to confer CG identity (62). The DOC-1 and DOC-2 proteins

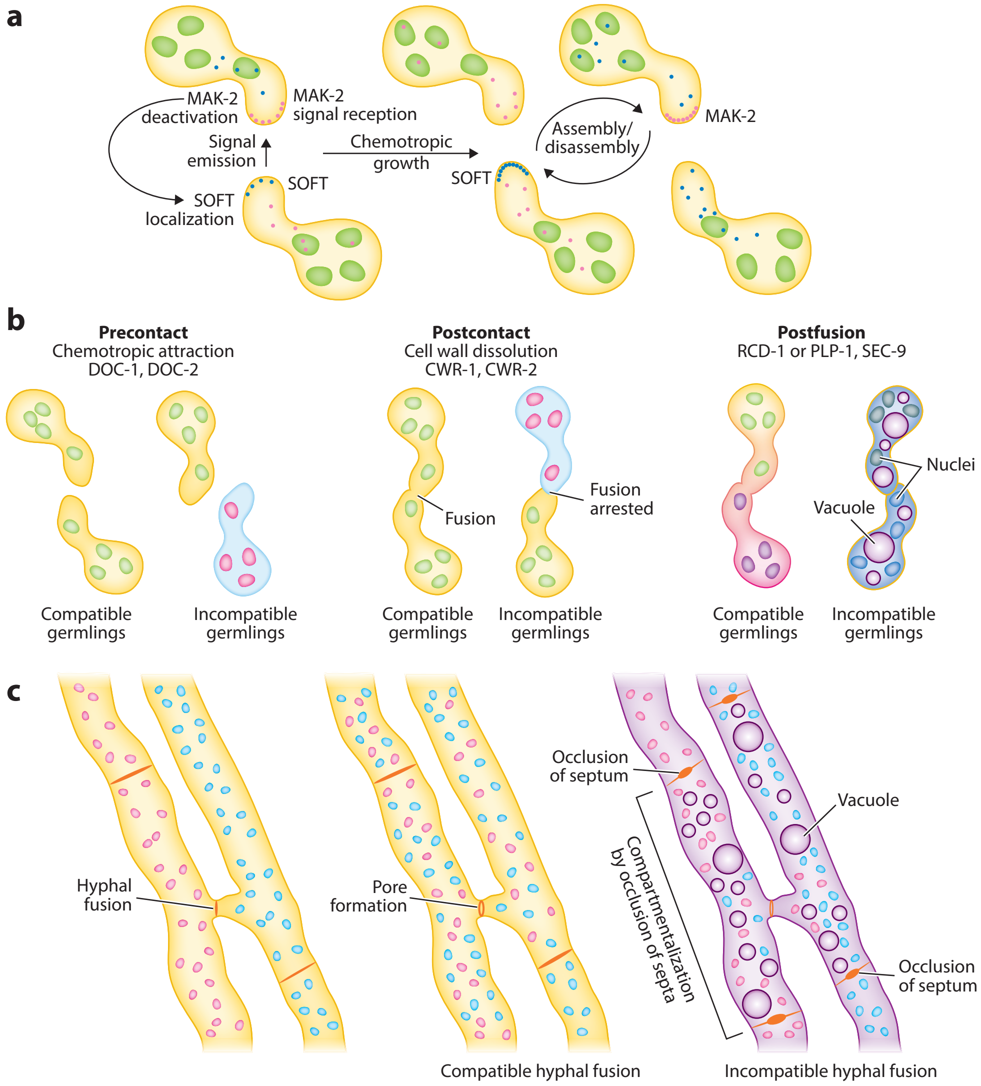


Figure 3

Somatic cell fusion and allorecognition checkpoints in *Neurospora crassa*. (*a*) During chemotropic growth that precedes cell fusion, MAK-2 and SOFT are recruited to the plasma membrane of conidial anastomosis tubes (42); both cells send and receive signals generating an appropriate cellular response that culminates in cell fusion. (*b*) Three cellular allorecognition checkpoints (precontact, postcontact, and postfusion) identified during germling fusion controlled by the *doc-1/doc-2*, *cwr-1/cwr-2*, *rcd-1*, and *plp-1/sec-9* loci, as indicated (55, 61, 62). (*c*) Allorecognition due to heterokaryon incompatibility occurs during hyphal fusion and is controlled by *het* genes (23, 115). Briefly, genetic differences at *doc-1/doc-2* result in precontact avoidance, genetic differences at *cwr-1/cwr-2* cause an arrest state upon contact, and genetic differences at *rcd-1* or *plp-1/sec-9* or *het* genes lead to postfusion cell death. In contrast, fusion between cells that belong to the same haplogroup for these loci results in stable heterokaryon formation where cytoplasmic components, nuclei, and other organelles can move through the fusion pores.

function to negatively regulate chemotropic interactions, as a Δ*doc-1* Δ*doc-2* mutant displays a high self-communication frequency, but a complete loss of communication and chemotropic interactions with its isogenic parental strain (62). The introduction of *doc-1* and *doc-2* alleles from a different CG (CG3) into the Δ*doc-1* Δ*doc-2* mutant resulted in a switch to CG3 specificity. Localization studies showed that DOC-2 localizes to the periphery of the cell while DOC-1 colocalizes and oscillates with components of the MAK-2 complex during chemotropic interactions (62). These data suggest that DOC-1 regulates reinforcement of MAK-2 complex signaling during chemotropic interactions. When cells carry different alleles at *doc-1* and *doc-2*, reinforcement of MAK-2 signaling is prevented, resulting in a decreased frequency of communication and fusion.

A link between somatic cell fusion between genetically identical cells and allorecognition by the *doc* system was recently revealed (39). The *N. crassa* Δ*ham-11* mutant fails to undergo self-fusion but will undergo chemotropic interactions and fusion with its wild-type parent. A Δ*doc-1* mutant undergoes self-fusion and fusion with its wild-type parental strain, albeit at a reduced frequency (62). However, when a Δ*ham-11* Δ*doc-1* double mutant was constructed, somatic cell fusion was completely abolished (39). These data implicate DOC-1 in regulating somatic cell fusion between genetically identical cells in a parallel pathway to HAM-11.

4. ALLORECOGNITION UPON CONTACT

4.1. Contact-Induced Allorecognition

While greenbeard genes that function at a distance offer an advantageous mechanism to recognize nonself partners, allorecognition also operates after physical contact between conspecific individuals/colonies. For example, in *P. mirabilis*, boundaries form between swarming colonies of different strains, but not between colonies of a single strain (**Figure 1*e***) (46). Strains of *P. mirabilis* that carry incompatible alleles at identification of self, or *ids*, genes induce growth arrest in interaction areas between colonies (13, 47). Growth arrest is correlated with formation of a heterotypic IdsD and IdsE complex (13). In the aggregative bacterium *M. xanthus*, contact-dependent exchange of factors that promote group motility and transition to sporulation is controlled by an allorecognition checkpoint regulated by homotypic interactions between a TraA/TraB complex (11). The *traA* gene is highly polymorphic in wild isolates of myxobacteria (12, 136).

In animal systems, the molecular basis of contact-induced allorecognition has been studied in the protochordate *B. schlosseri* and the hydroid *H. symbiolongicarpus*. In *B. schlosseri*, isogenic colonies fuse to form larger colonial chimeras, resulting in sharing of public goods (113). However, fusion between *B. schlosseri* colonies only occurs if both have allelic identity at the fusion/histocompatibility (*fuhc*) locus; if incompatibility is perceived, an inflammatory response resulting in blockage of vascular interactions followed by allograft rejection is triggered (118) (**Figure 1*d***). The *fuhc* locus contains two adjacent genes ($fuhc^{sec}$ and $fuhc^{tm}$) that show evidence of balancing selection (96); the extracellular region of the FuHC protein is highly polymorphic (26). In the cnidarian *H. symbiolongicarpus*, an analogous mechanism determines fusion of tissue projections known as stolons that arise from asexual polyps and adhere between conspecific colonies (95) (**Figure 1*c***). In this case, allorecognition is defined by *Alr1* and *Alr2*, two highly polymorphic genes that encode transmembrane proteins (74, 95, 112).

4.2. Contact-Dependent Allorecognition in Filamentous Fungi

N. crassa cells/hyphae of identical CG specificity undergo chemotropic growth and adhere upon contact. However, two phenotypes were revealed after adherence in otherwise genetically different strains: (*a*) Some strains completed cell fusion and exchanged cytoplasmic contents, and (*b*) some were unable to undergo cell wall dissolution and were thus blocked in cell fusion (55) (**Figures 1*a*, 3*b***). In cells blocked in fusion, the oscillation of MAK-2 and SOFT at fusion tips was extended, suggesting that arrested cells fail to transit from chemotropic interactions to cell wall dissolution and membrane merger. Two linked loci, cell wall remodeling 1 and 2 (*cwr-1* and *cwr-2*) are necessary and sufficient to regulate cell wall dissolution. Consistent with their role in kind recognition, alleles at *cwr-1* and *cwr-2* are highly polymorphic, fall into six discrete haplogroups within *N. crassa* populations, and show evidence of trans-species polymorphisms (55). The *cwr-1/cwr-2* loci segregate independently from the *doc-1/doc-2* loci. As with *doc-1* and *doc-2*, allelic differences at *cwr-1* and *cwr-2* negatively regulate somatic cell fusion, as Δ*cwr-1* and Δ*cwr-2* mutants are capable of undergoing both self-fusion and fusion with formerly incompatible partners.

Sequence analyses of orthologs of *cwr-1* and *cwr-2* alleles in population samples from filamentous fungal species where the two loci are linked revealed high sequence diversity in species of *Neurospora*, *Fusarium*, *Trichoderma*, and *Zymoseptoria*. Allele-specific haplogroups that show trans-species polymorphism at *cwr-1* and *cwr-2* were identified among isolates of different species of *Fusarium* (*F. tricinctum*, *F. oxysporum*, *F. fujikuroi*, *F. graminearum*, *F. proliferatum*, and *F. verticillioides*) (55). However, the *cwr-1/cwr-2* haplogroups identified in *N. crassa* were not conserved in the *Fusarium cwr-1* and *cwr-2* haplogroups, indicating convergent evolution and that polymorphisms at these loci can be repeatedly lost and gained. Genomic pairs of *cwr-1* and *cwr-2* are only present in sublineages of the Pezizomycotina, one of the only two groups where complex multicellularity has arisen in fungi (77). These observations suggest that diversification of *cwr* alleles alongside the appearance of multicellularity could be linked to formation of syncytial fungal colonies.

The *cwr-1* locus encodes a secreted polysaccharide monooxygenase (PMO) (55). PMOs catalyze the oxidative cleavage of glycosidic bonds in recalcitrant substrates, such as cellulose, hemicellulose, and chitin (124). CWR-1 is a member of the auxiliary activity (AA) 11 family homologous to a chitin-active copper-dependent PMO from *Aspergillus oryzae* (63). Genetic analyses showed that cell fusion arrest is mediated by interactions between CWR-1 in one cell and CWR-2 from a different haplotype in the partner cell. CWR-2 contains two conserved domains of unknown function and eight predicted transmembrane regions (55). These data suggest that CWR-2 may function as a membrane receptor that could interact with a haplotype-specific chitin cell wall product produced by the activity of CWR-1. A somewhat analogous situation is observed during neural self-avoidance in *Drosophila*, where alternative splicing of *Dscam* results in thousands of distinct ectodomains with self-binding specificity (137, 138).

5. ALLORECOGNITION AFTER SOMATIC CELL FUSION

5.1. Allorecognition and Germling-Regulated Death

In crosses between wild isolates of *N. crassa*, progeny that are capable of undergoing chemotropic interactions and cell wall dissolution often display rapid cell death upon fusion. At least two loci in *N. crassa* mediate germling-regulated death (GRD) (**Figure 3*b***). GRD is controlled by allelic interactions between *rcd-1-1* and *rcd-1-2* (21) or nonallelic interactions between the antagonistic and closely linked *plp-1* and *sec-9* loci (61). In germling pairs, GRD occurs rapidly (∼20–25 min) after fusion of *rcd-1*– or *sec-9/plp-1*–incompatible cells and is associated with massive vacuolization and cell lysis (61). Genetic differences at *rcd-1* or *sec-9/plp-1* also induce death upon hyphal fusion between incompatible colonies.

The allorecognition determinant *rcd-1* encodes a 257–amino acid protein of unknown biochemical function (21). Alleles of *rcd-1* fall into two haplogroups and are one of the most polymorphic genes in the genomes of wild *N. crassa* isolates; alleles of the two *rcd-1* haplogroups also show trans-species polymorphisms (21). Strains carrying a deletion of *rcd-1* form viable heterokaryons with formerly incompatible cells, while the coexpression of two antagonistic *rcd-1-1* and *rcd-1-2* alleles is sufficient to trigger cell death in fused germlings and hyphae (21). *rcd-1* belongs to a large gene family in fungi, with some species, like *N. crassa*, having only one *rcd-1* locus, while other species have multiple *rcd-1* paralogs within their genomes. These observations suggest that the function of this allelic allorecognition system might be conserved throughout the fungal kingdom.

The second allorecognition system that induces GRD upon cell fusion involves the linked loci *sec-9* and *plp-1* (61). *sec-9* encodes a t-SNARE protein, which is orthologous to a protein required for secretory vesicle/plasma membrane fusion in *Saccharomyces cerevisiae* (10); *sec-9* is an essential gene in *S. cerevisiae*, *P. anserina*, and *N. crassa*. The *plp-1* locus encodes a protein with an N-terminal patatin-like phospholipase domain, a central NB-ARC domain, and C-terminal tetratricopeptide repeats. Incompatible genetic interactions between *sec-9* and *plp-1* from different haplogroups are necessary and sufficient to induce GRD. In *N. crassa*, gene genealogies revealed four long-diverged haplogroups for *sec-9* and *plp-1*. Within a haplogroup, *sec-9* and *plp-1* show no evidence of recombination and are in the top 0.1% for the number of polymorphic sites and nucleotide diversity in population samples (61). As with other allorecognition loci, *sec-9* and *plp-1* alleles show signatures of balancing selection and trans-species polymorphism (61, 89). In *P. anserina* and *C. parasitica*, *sec-9/plp-1* also functions in hyphal incompatibility (16, 61); evolutionary analyses indicate that convergent evolution is the most strongly supported scenario for the common use of the *plp-1/sec-9* system in allorecognition in these three fungal genera (61).

In *N. crassa* and *P. anserina*, the C-terminal region of SEC-9, which includes the SNARE domains essential for protein function, is highly polymorphic between different haplogroups (61). These polymorphic SNARE domains mediate allelic specificity via interactions with incompatible PLP-1 proteins. In *N. crassa*, coimmunoprecipitation experiments showed that incompatible SEC-9 and PLP-1 from different haplogroups induces PLP-1 complex formation (61). Both the phospholipase catalytic activity of the patatin-like domain and a functional NB-ARC domain are necessary for full GRD induction. Allorecognition and cell death are dependent upon physical interaction between incompatible SNARE domains of SEC-9 and tetratricopeptide repeats of PLP-1. The tripartite architecture of PLP-1 is reminiscent of NOD (nucleotide-binding and oligomerization domain)-like receptors (NLRs) in plants and animals (33). NLRs are intracellular multidomain modular sensors in plants and animals involved in innate immunity (71) and detect pathogen-associated molecular cues or danger signals to induce downstream signaling, resulting in cell death. These observations suggest that the NLR-like protein PLP-1 monitors the essential SNARE protein SEC-9.

5.2. Allorecognition-Induced Death During Hyphal Fusion

In contrast to GRD, hyphal fusion incompatibilities have been assessed in a large number of fungal species (23, 54, 115) and are termed vegetative (or heterokaryon) incompatibility (HI). Despite limiting cooperation (i.e., resource sharing) between fungal colonies, HI prevents genome exploitation, the spread of deleterious mycoviruses, and horizontal transfer of mitochondrial plasmids (28, 29, 131). Experimental evolution studies indicate that HI evolution and maintenance is probably driven by the need to counteract selection for freeloaders (4, 20).

HI-associated programmed cell death is spatially restricted to heterokaryotic fusion cells in which cytoplasmic mixing has occurred. In many species, HI results in a barrage line that separates genetically averse strains (115). At the cellular level, HI initiates septal plugging of fusion cells, isolating them from the rest of the colony (51, 70) (**Figure 3*c***). Heterokaryotic cells undergo extensive vacuolization, reactive oxygen species production, lipid droplet formation, cell wall thickening, and hyperseptation, culminating in cell lysis and release of cellular contents to the extracellular medium (115). In fungal plant pathogens, vegetative compatibility groups (VCGs) have been used as a proxy of genetic relatedness (82), as isolates within a common VCG often share similar virulence and host-specificity functions (36, 75).

The molecular basis of HI has been investigated in three ascomycete species: *N. crassa*, *P. anserina*, and *C. parasitica* (18, 92, 115). The genes controlling HI in *P. anserina* and *N. crassa* have been named *het* (for heterokaryon), while in *C. parasitica* they are known as *vic* (for vegetative incompatibility). In *N. crassa*, the characterized *het* loci interactions are restricted to the hyphal stage and do not cause GRD. The number of identified *het* loci in the three species varies between 12 (*N. crassa*) and 6 (*C. parasitica*) (18, 51). In *C. parasitica*, disruption of *vic* genes allowed the spread of virulence-attenuating mycoviruses between formerly incompatible colonies (140).

The inability to form viable heterokaryons using auxotrophic markers correlates perfectly with induction of cell death upon fusion of incompatible hyphae (7, 44). This forced heterokaryon methodology has been used to identify *het* genes in other fungal species, including *Aspergillus oryzae* (91) and *F. proliferatum* (76). The use of strains carrying genomic rearrangements enabled the early identification and genetic mapping of *N. crassa het* loci (92, 103, 104), while more recent approaches have taken advantage of population genomics (91, 142).

As in the other allorecognition systems, genes controlling HI are highly polymorphic and multi-allelic (100). The number of alleles in wild populations varies from 2 [e.g., *het-s* in *P. anserina* (128)] to more than 10 [e.g., *het-c* in *P. anserina* (5)]. Highly polymorphic *het* loci are frequently found in hypervariable genomic regions (142). Consistent with balancing selection, alleles with different HI specificity are found in nearly equal frequency in wild populations (5, 27, 59, 89, 139). Using a comparative population genomics approach, loci with highly polymorphic alleles that displayed trans-species polymorphism and balancing selection were used to identify candidate *het* genes in *Neurospora* populations (142).

Genetic interactions triggering HI involve two or more antagonistic alleles of the same gene (allelic HI systems) or alleles belonging to different genes (nonallelic HI systems) (23, 115). At present, three incompatibility systems are strictly allelic—the *het-S/het-s* system in *P. anserina* (116) and the *het-e1/het-e2/het-e3* and *rcd-1-1/rcd-1-2* recognition systems in *N. crassa* (21, 142). Most of the characterized nonallelic HI systems involve interactions between alleles of closely linked genes; examples are the *het-c/pin-c*, *het-6/un24*, and *sec-9/plp-1* systems in *N. crassa* (59, 61, 73, 80) and *vic-6/pix-6* nonallelic incompatibility in *C. parasitica* (16). In *P. anserina het-c/het-e* or *het-c/het-d* nonallelic HI systems, the genes are located on different chromosomes (34, 114).

In a number of filamentous ascomycete species, including *N. crassa*; *Sordaria brevicollis*; *Ascobolus stercorarius*; *Aspergillus heterothallicus*; and perhaps the black truffle, *Tuber melanosporum*, the *MAT* locus functions as a *het* locus (50, 119). As with allelic differences at *het* loci, somatic cell fusion between opposite mating type hyphae results in compartmentation of the fusion cell and rapid cell death (44, 49). However, unlike most allorecognition loci, the two *mat* haplotypes in filamentous ascomycete fungi are composed of evolutionarily unrelated genes, termed idiomorphs (88). The mating type idiomorphs do not show variability within populations and are highly conserved between different filamentous ascomycete species (8). In *N. crassa*, mating-type incompatibility only occurs in the hyphal stage and is dependent on an unlinked locus, called tolerant or *tol* (121). TOL, similar to predicted proteins from other *het* loci, contains a HET domain (see below). Mutations in *tol* block mating-type incompatibility but do not affect sexual fertility (93). In *C. parasitica*, the *vic-4-1/vic-4-2* system is also composed of idiomorphic genes, although they do not play a role in mating (16).

5.3. Molecular Mechanisms of Programmed Cell Death in Allorecognition

Despite the shared evolutionary signatures of *het* genes, they show little conservation between species (100, 130). Nevertheless, molecular characterization of various HI systems shows that *het* genes encode proteins with shared domains that belong to large protein families (23, 54). A particular protein domain of unknown biochemical function, named HET, is encoded by more than half of HI genes (142). In silico analyses established a potential evolutionary relation between the HET domain and the Toll/interleukin-1 receptor/resistance (TIR) domain that plays key roles in plant and metazoan innate immune systems (33). TIR domains are involved in homotypic interactions in signaling complexes that trigger cell death by NAD^+ depletion (69, 133). NAD^+ cleavage by TIR domains has an ancient origin (35) and represents a tempting hypothesis for the function of HET domains.

The products of multiple *het* genes (*het-e*, *het-d*, *het-r*, *PaPlp1*) from *P. anserina* (34), *plp-1* from *N. crassa* (61), and *vic2* and *vic4–2* from *C. parasitica* (16) belong to the family of fungal NLR-like proteins (33). Remarkably, the HET and TIR domains are found similarly situated in the domain architectures of fungal NLR-like proteins and plant/metazoan NLRs (33). Thus, fungal NLR-like proteins may function similarly to NLR immune receptors in plants and animals, suggesting that proteins of this architecture are major contributors to innate immunity in all three kingdoms.

In *P. anserina*, the *het-S/het-s* system is unique in that it functions as a prion. The HET-S protein is a pore-forming toxin, targeting the plasma membrane when coexpressed with an alternate allelic variant termed HET-s (58, 120). HET-S and HET-s consist of two domains: an N-terminal globular α-helical HeLo domain (58) and a C-terminal prion-forming domain (PFD) (2). The inactivation of the HeLo domain allows the HET-s variant to propagate as a prion [Het-s] (19, 85). The transconformation of the PFD of the cytotoxic HET-S variant by [Het-s] aggregates activates the HeLo domain of HET-S, leading to the release of an N-terminal α helix that targets the plasma membrane to induce rapid cell death (120). Evolutionary analyses linked the HeLo domain to domains controlling cell death in plants and animals, notably the 4HB (four-helix bundle) domain of the MLKL (mixed lineage kinase domain-like) protein, which controls necroptosis (22, 65). The fungal *het-S/het-s* system and NOD-like receptors involved in nonself recognition have been reviewed in detail (117).

6. WHY DO FUNGI HAVE SO MANY ALLORECOGNITION MECHANISMS?

The relationship between protein architectures of GRD and HI determinants and proteins involved in innate immunity systems in plants and metazoans has led to the hypothesis that fungal allorecognition genes may be recruited from molecular circuits mediating broader biotic interactions in fungi, akin to a fungal immune system (129). Importantly, higher genetic relatedness appears to correlate with cooperative behaviors to avoid parasitism and to prevent the exploitation of public goods (for example, access to nutrients in a fungal colony) by cheaters (6, 32, 79). Hence, fusion between conspecific but genetically distant individuals and consequent somatic chimerization pose a dilemma. On the one hand, fusion could prove beneficial due to an enhanced ability to withstand environmental variations and eventual increase in organismal size, in turn favoring reproductive output. Moreover, heterokaryon formation in fungi can result in functional diploidy and mitotic recombination during the parasexual cycle (105). On the other hand, fusion can result in the transmission of infectious elements and in the incorporation of deleterious mitochondrial or nuclear genotypes that negatively impact fitness (3, 4, 29, 37, 141). Fungi, in particular, appear to favor the latter option, having evolved a very large number of allorecognition systems to limit genome exploitation. However, a recent study demonstrated that fusion in *N. crassa* is mutually beneficial compared to fusion blockage by allorecognition (4), suggesting a dynamic relationship between beneficial aspects of cell fusion versus the risks associated with it. Importantly, mechanisms regulating somatic allorecognition are suppressed during sexual reproduction. Indeed, wild isolates with allelic specificity differences at *doc*, *cwr*, *sec-9/plp-1*, *rcd-1*, and *het* loci are able to productively mate and produce meiotic progeny, suggesting that these allorecognition systems have evolved to specifically avoid somatic cell fusion, but allowing at the same time diversification to occur through outbreeding, potentially improving adaption to new ecological niches, as shown to occur during sexual reproduction (52).

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Meritxell Riquelme (CICESE, Ensenada, Mexico) and the National Laboratory of Advanced Microscopy at CICESE for use of their microscopy facilities for *N. crassa* images. Thanks to Shigenori Hirose and Gad Shaulsky (Baylor College of Medicine) for images of *D. discoideum*; Matthew L. Nicotra (University of Pittsburgh) for images of *H. symbiolongicarpus*; Anthony De Tomaso (University of California, Santa Barbara) for images of *B. schlosseri*; and Kristin Little, Murray Tipping, and Karine A. Gibbs (Harvard University) for images of *P. mirabilis*. Work in the Glass laboratory was supported by National Science Foundation grants MCB1412411 and MCB1818283, a Laboratory Directed Research and Development Program of Lawrence Berkeley National Laboratory under US Department of Energy contract DE-AC02-05CH11231, and a Fred E. Dickinson Chair of Wood Science and Technology award to N.L.G.

LITERATURE CITED

1. Albuquerque P, Nicola AM, Nieves E, Paes HC, Williamson PR, et al. 2013. Quorum sensing-mediated, cell density-dependent regulation of growth and virulence in *Cryptococcus neoformans*. *mBio* 5:e00986-13
2. Balguerie A, Dos Reis S, Ritter C, Chaignepain S, Coulary-Salin B, et al. 2003. Domain organization and structure-function relationship of the HET-s prion protein of *Podospora anserina*. *EMBO J.* 22:2071–81
3. Bastiaans E, Aanen DK, Debets AJ, Hoekstra RF, Lestrade B, Maas MF. 2014. Regular bottlenecks and restrictions to somatic fusion prevent the accumulation of mitochondrial defects in *Neurospora*. *Philos. Trans. R. Soc. Lond. B* 369:20130448
4. Bastiaans E, Debets AJ, Aanen DK. 2015. Experimental demonstration of the benefits of somatic fusion and the consequences for allorecognition. *Evolution* 69:1091–99
5. Bastiaans E, Debets AJ, Aanen DK, van Diepeningen AD, Saupe SJ, Paoletti M. 2014. Natural variation of heterokaryon incompatibility gene *het-c* in *Podospora anserina* reveals diversifying selection. *Mol. Biol. Evol.* 31:962–74
6. Bastiaans E, Debets AJM, Aanen DK. 2016. Experimental evolution reveals that high relatedness protects multicellular cooperation from cheaters. *Nat. Commun.* 7:11435
7. Beadle GW, Coonradt VL. 1944. Heterocaryosis in *Neurospora crassa*. *Genetics* 29:291–308
8. Bennett RJ, Turgeon BG. 2016. Fungal sex: the Ascomycota. *Microbiol. Spectr.* 4. **https://doi.org/10.1128/microbiolspec.FUNK-0005-2016**
9. Boehm T. 2006. Quality control in self/nonself discrimination. *Cell* 125:845–58
10. Brennwald P, Kearns B, Champion K, Keranen S, Bankaitis V, Novick P. 1994. Sec9 is a SNAP-25-like component of a yeast SNARE complex that may be the effector of Sec4 function in exocytosis. *Cell* 79:245–58

11. Cao P, Wall D. 2019. Direct visualization of a molecular handshake that governs kin recognition and tissue formation in myxobacteria. *Nat. Commun.* 10:3073
12. Cao P, Wei X, Awal RP, Muller R, Wall D. 2019. A highly polymorphic receptor governs many distinct self-recognition types within the Myxococcales order. *mBio* 10:e02751-18
13. Cardarelli L, Saak C, Gibbs KA. 2015. Two proteins form a heteromeric bacterial self-recognition complex in which variable subdomains determine allele-restricted binding. *mBio* 6:e00251
14. Chen H, Fink GR. 2006. Feedback control of morphogenesis in fungi by aromatic alcohols. *Genes Dev.* 20:1150–61
15. Chen H, Fujita M, Feng Q, Clardy J, Fink GR. 2004. Tyrosol is a quorum-sensing molecule in *Candida albicans*. *PNAS* 101:5048–52
16. Choi GH, Dawe AL, Churbanov A, Smith ML, Milgroom MG, Nuss DL. 2012. Molecular characterization of vegetative incompatibility genes that restrict hypovirus transmission in the chestnut blight fungus *Cryphonectria parasitica*. *Genetics* 190:113–27
17. Clutton-Brock TH, O'Riain MJ, Brotherton PN, Gaynor D, Kansky R, et al. 1999. Selfish sentinels in cooperative mammals. *Science* 284:1640–44
18. Cortesi P, Milgroom MG. 1998. Genetics of vegetative incompatibility in *Cryphonectria parasitica*. *Appl. Environ. Microbiol.* 64:2988–94
19. Coustou V, Deleu C, Saupe S, Begueret J. 1997. The protein product of the *het-s* heterokaryon incompatibility gene of the fungus *Podospora anserina* behaves as a prion analog. *PNAS* 94:9773–78
20. Czárán T, Hoekstra RF, Aanen DK. 2014. Selection against somatic parasitism can maintain allorecognition in fungi. *Fungal Genet. Biol.* 73:128–37
21. Daskalov A, Gladieux P, Heller J, Glass NL. 2019. Programmed cell death in *Neurospora crassa* is controlled by the allorecognition determinant *rcd-1*. *Genetics* 213:1387–400
22. Daskalov A, Habenstein B, Sabate R, Berbon M, Martinez D, et al. 2016. Identification of a novel cell death-inducing domain reveals that fungal amyloid-controlled programmed cell death is related to necroptosis. *PNAS* 113:2720–25
23. Daskalov A, Heller J, Herzog S, Fleissner A, Glass NL. 2017. Molecular mechanisms regulating cell fusion and heterokaryon formation in filamentous fungi. *Microbiol. Spectr.* 5. **https://doi.org/10.1128/microbiolspec.FUNK-0015-2016**
24. Dawe AL, Nuss DL. 2013. Hypovirus molecular biology: from Koch's postulates to host self-recognition genes that restrict virus transmission. *Adv. Virus Res.* 86:109–47
25. Dawkins R. 1976. *The Selfish Gene*. New York: Oxford Univ. Press
26. De Tomaso AW, Nyholm SV, Palmeri KJ, Ishizuka KJ, Ludington WB, et al. 2005. Isolation and characterization of a protochordate histocompatibility locus. *Nature* 438:454–59
27. Debets AJ, Dalstra HJ, Slakhorst M, Koopmanschap B, Hoekstra RF, Saupe SJ. 2012. High natural prevalence of a fungal prion. *PNAS* 109:10432–37
28. Debets AJM, Griffiths AJF. 1998. Polymorphism of *het*-genes prevents resource plundering in *Neurospora crassa*. *Mycol. Res.* 102:1343–49
29. Debets F, Yang X, Griffiths AJ. 1994. Vegetative incompatibility in *Neurospora*: its effect on horizontal transfer of mitochondrial plasmids and senescence in natural populations. *Curr. Genet.* 26:113–19
30. Dekhang R, Wu C, Smith KM, Lamb TM, Peterson M, et al. 2017. The *Neurospora* transcription factor ADV-1 transduces light signals and temporal information to control rhythmic expression of genes involved in cell fusion. *G3* 7:129–42
31. Dettmann A, Heilig Y, Valerius O, Ludwig S, Seiler S. 2014. Fungal communication requires the MAK-2 pathway elements STE-20 and RAS-2, the NRC-1 adapter STE-50 and the MAP kinase scaffold HAM-5. *PLOS Genet.* 10:e1004762
32. Diggle SP, Griffin AS, Campbell GS, West SA. 2007. Cooperation and conflict in quorum-sensing bacterial populations. *Nature* 450:411–14
33. Dyrka W, Lamacchia M, Durrens P, Kobe B, Daskalov A, et al. 2014. Diversity and variability of NOD-like receptors in fungi. *Genome Biol. Evol.* 6:3137–58
34. Espagne E, Balhadere P, Penin ML, Barreau C, Turcq B. 2002. HET-E and HET-D belong to a new subfamily of WD40 proteins involved in vegetative incompatibility specificity in the fungus *Podospora anserina*. *Genetics* 161:71–81

35. Essuman K, Summers DW, Sasaki Y, Mao X, Yim AKY, et al. 2018. TIR domain proteins are an ancient family of NAD$^+$-consuming enzymes. *Curr. Biol.* 28:421–30
36. Fan R, Cockerton HM, Armitage AD, Bates H, Cascant-Lopez E, et al. 2018. Vegetative compatibility groups partition variation in the virulence of V*erticillium dahliae* on strawberry. *PLOS ONE* 13:e0191824
37. Fernandez-Busquets X, Kornig A, Bucior I, Burger MM, Anselmetti D. 2009. Self-recognition and Ca^{2+}-dependent carbohydrate-carbohydrate cell adhesion provide clues to the Cambrian explosion. *Mol. Biol. Evol.* 26:2551–61
38. Fischer MS, Glass NL. 2019. Communicate and fuse: how filamentous fungi establish and maintain an interconnected mycelial network. *Front. Microbiol.* 10:619
39. Fischer MS, Jonkers W, Glass NL. 2019. Integration of self and non-self recognition modulates asexual cell-to-cell communication in *Neurospora crassa*. *Genetics* 211:1255–67
40. Fischer MS, Wu VW, Lee JE, O'Malley RC, Glass NL. 2018. Regulation of cell-to-cell communication and cell wall integrity by a network of MAP kinase pathways and transcription factors in *Neurospora crassa*. *Genetics* 209:489–506
41. Fleissner A, Diamond S, Glass NL. 2009. The *Saccharomyces cerevisiae PRM1* homolog in *Neurospora crassa* is involved in vegetative and sexual cell fusion events but also has postfertilization functions. *Genetics* 181:497–510
42. Fleissner A, Leeder AC, Roca MG, Read ND, Glass NL. 2009. Oscillatory recruitment of signaling proteins to cell tips promotes coordinated behavior during cell fusion. *PNAS* 106:19387–92
43. Gardner A, West SA. 2010. Greenbeards. *Evolution* 64:25–38
44. Garnjobst L, Wilson JF. 1956. Heterocaryosis and protoplasmic incompatibility in *Neurospora crassa*. *PNAS* 42:613–18
45. Ghabrial SA, Suzuki N. 2009. Viruses of plant pathogenic fungi. *Annu. Rev. Phytopathol.* 47:353–84
46. Gibbs KA, Greenberg EP. 2011. Territoriality in Proteus: advertisement and aggression. *Chem. Rev.* 111:188–94
47. Gibbs KA, Urbanowski ML, Greenberg EP. 2008. Genetic determinants of self identity and social recognition in bacteria. *Science* 321:256–59
48. Glass NL, Dementhon K. 2006. Non-self recognition and programmed cell death in filamentous fungi. *Curr. Opin. Microbiol.* 9:553–58
49. Glass NL, Grotelueschen J, Metzenberg RL. 1990. *Neurospora crassa A* mating-type region. *PNAS* 87:4912–16
50. Glass NL, Jacobson DJ, Shiu PK. 2000. The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. *Annu. Rev. Genet.* 34:165–86
51. Glass NL, Kaneko I. 2003. Fatal attraction: nonself recognition and heterokaryon incompatibility in filamentous fungi. *Eukaryot. Cell* 2:1–8
52. Goddard MR, Godfray HC, Burt A. 2005. Sex increases the efficacy of natural selection in experimental yeast populations. *Nature* 434:636–40
53. Gonçalves AP, Chow KM, Cea-Sánchez S, Glass NL. 2020. WHI-2 regulates intercellular communication via a MAP kinase signaling complex. *Front. Microbiol.* 10:3162
54. Gonçalves AP, Heller J, Daskalov A, Videira A, Glass NL. 2017. Regulated forms of cell death in fungi. *Front. Microbiol.* 8:1837
55. Gonçalves AP, Heller J, Span EA, Rosenfield G, Do HP, et al. 2019. Allorecognition upon fungal cell-cell contact determines social cooperation and impacts the acquisition of multicellularity. *Curr. Biol.* 29:3006–17.e3
56. Goryachev AB, Lichius A, Wright GD, Read ND. 2012. Excitable behavior can explain the "ping-pong" mode of communication between cells using the same chemoattractant. *BioEssays* 34:259–66
57. Gould SJ, Vrba ES. 1982. Exaptation—a missing term in the science of form. *Paleobiology* 8:4–15
58. Greenwald J, Buhtz C, Ritter C, Kwiatkowski W, Choe S, et al. 2010. The mechanism of prion inhibition by HET-S. *Mol. Cell* 38:889–99
59. Hall C, Welch J, Kowbel DJ, Glass NL. 2010. Evolution and diversity of a fungal self/nonself recognition locus. *PLOS ONE* 5:e14055
60. Hamilton WD. 1964. The genetical evolution of social behaviour. II. *J. Theor. Biol.* 7:17–52

61. Heller J, Clavé C, Gladieux P, Saupe SJ, Glass NL. 2018. NLR surveillance of essential SEC-9 SNARE proteins induces programmed cell death upon allorecognition in filamentous fungi. *PNAS* 115:E2292-301
62. Heller J, Zhao J, Rosenfield G, Kowbel DJ, Gladieux P, Glass NL. 2016. Characterization of greenbeard genes involved in long-distance kind discrimination in a microbial eukaryote. *PLOS Biol*. 14:e1002431
63. Hemsworth GR, Henrissat B, Davies GJ, Walton PH. 2014. Discovery and characterization of a new family of lytic polysaccharide monooxygenases. *Nat. Chem. Biol.* 10:122–26
64. Hickey PC, Jacobson D, Read ND, Louise Glass NL. 2002. Live-cell imaging of vegetative hyphal fusion in *Neurospora crassa*. *Fungal Genet. Biol*. 37:109–19
65. Hildebrand JM, Tanzer MC, Lucet IS, Young SN, Spall SK, et al. 2014. Activation of the pseudokinase MLKL unleashes the four-helix bundle domain to induce membrane localization and necroptotic cell death. *PNAS* 111:15072–77
66. Hirose S, Benabentos R, Ho HI, Kuspa A, Shaulsky G. 2011. Self-recognition in social amoebae is mediated by allelic pairs of tiger genes. *Science* 333:467–70
67. Homer CM, Summers DK, Goranov AI, Clarke SC, Wiesner DL, et al. 2016. Intracellular action of a secreted peptide required for fungal virulence. *Cell Host Microbe* 19:849–64
68. Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, et al. 2001. Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Appl. Environ. Microbiol.* 67:2982–92
69. Horsefield S, Burdett H, Zhang X, Manik MK, Shi Y, et al. 2019. NAD^+ cleavage activity by animal and plant TIR domains in cell death pathways. *Science* 365:793–99
70. Jacobson DJ, Beurkens K, Klomparens KL. 1998. Microscopic and ultrastructural examination of vegetative incompatibility in partial diploids heterozygous at *het* loci in *Neurospora crassa*. *Fungal Genet. Biol*. 23:45–56
71. Jones JD, Vance RE, Dangl JL. 2016. Intracellular innate immune surveillance devices in plants and animals. *Science* 354:aaf6395
72. Jonkers W, Leeder AC, Ansong C, Wang Y, Yang F, et al. 2014. HAM-5 functions as a MAP kinase scaffold during cell fusion in *Neurospora crassa*. *PLOS Genet*. 10:e1004783
73. Kaneko I, Dementhon K, Xiang Q, Glass NL. 2006. Nonallelic interactions between *het-c* and a polymorphic locus, *pin-c*, are essential for nonself recognition and programmed cell death in *Neurospora crassa*. *Genetics* 172:1545–55
74. Karadge UB, Gosto M, Nicotra ML. 2015. Allorecognition proteins in an invertebrate exhibit homophilic interactions. *Curr. Biol.* 25:2845–50
75. Karangwa P, Mostert D, Ndayihanzamaso P, Dubois T, Niere B, et al. 2018. Genetic diversity of *Fusarium oxysporum* f. sp. *cubense* in East and Central Africa. *Plant Dis*. 102:552–60
76. Kerényi Z, Oláh B, Jeney A, Hornok L, Leslie JF. 2006. The homologue of *het-c* of *Neurospora crassa* lacks vegetative compatibility function in *Fusarium proliferatum*. *Appl. Environ. Microbiol*. 72:6527–32
77. Knoll AH. 2011. The multiple origins of complex multicellularity. *Annu. Rev. Earth Planet Sci.* 39:217–39
78. Kundert P, Shaulsky G. 2019. Cellular allorecognition and its roles in *Dictyostelium* development and social evolution. *Int. J. Dev. Biol.* 63:383–93
79. Kuzdzal-Fick JJ, Fox SA, Strassmann JE, Queller DC. 2011. High relatedness is necessary and sufficient to maintain multicellularity in *Dictyostelium*. *Science* 334:1548–51
80. Lafontaine DL, Smith ML. 2012. Diverse interactions mediate asymmetric incompatibility by the *het-6* supergene complex in *Neurospora crassa*. *Fungal Genet. Biol*. 49:65–73
81. Leeder AC, Palma-Guerrero J, Glass NL. 2011. The social network: deciphering fungal language. *Nat. Rev. Microbiol.* 9:440–51
82. Leslie JF. 1993. Fungal vegetative compatibility. *Annu. Rev. Phytopathol.* 31:127–50
83. Little K, Gibbs KA. 2019. Analysis of *Proteus mirabilis* social behaviors on surfaces. *Methods Mol. Biol.* 2021:45–59
84. MacLean RC, Gudelj I. 2006. Resource competition and social conflict in experimental populations of yeast. *Nature* 441:498
85. Maddelein ML, Dos Reis S, Duvezin-Caubet S, Coulary-Salin B, Saupe SJ. 2002. Amyloid aggregates of the HET-s prion protein are infectious. *PNAS* 99:7402–7

86. Maddi A, Dettman A, Fu C, Seiler S, Free SJ. 2012. WSC-1 and HAM-7 are MAK-1 MAP kinase pathway sensors required for cell wall integrity and hyphal fusion in *Neurospora crassa*. *PLOS ONE* 7:e42374
87. Maree AF, Hogeweg P. 2001. How amoeboids self-organize into a fruiting body: multicellular coordination in *Dictyostelium discoideum*. *PNAS* 98:3879–83
88. Metzenberg RL, Glass NL. 1990. Mating type and mating strategies in *Neurospora*. *BioEssays* 12:53–59
89. Milgroom MG, Smith ML, Drott MT, Nuss DL. 2018. Balancing selection at nonself recognition loci in the chestnut blight fungus, *Cryphonectria parasitica*, demonstrated by trans-species polymorphisms, positive selection, and even allele frequencies. *Heredity* 121:511–23
90. Miller MB, Bassler BL. 2001. Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55:165–99
91. Mori N, Katayama T, Saito R, Iwashita K, Maruyama JI. 2019. Inter-strain expression of sequence-diverse HET domain genes severely inhibits growth of *Aspergillus oryzae*. *Biosci. Biotechnol. Biochem.* 83:1557–69
92. Mylyk OM. 1975. Heterokaryon incompatibility genes in *Neurospora crassa* detected using duplication-producing chromosome rearrangements. *Genetics* 80:107–24
93. Newmeyer D. 1970. A suppressor of the heterokaryon-incompatibility associated with mating type in *Neurospora crassa*. *Can. J. Genet. Cytol*. 12:914–26
94. Nicotra ML. 2019. Invertebrate allorecognition. *Curr. Biol.* 29:R463–67
95. Nicotra ML, Powell AE, Rosengarten RD, Moreno M, Grimwood J, et al. 2009. A hypervariable invertebrate allodeterminant. *Curr. Biol.* 19:583–89
96. Nydam ML, Stephenson EE, Waldman CE, De Tomaso AW. 2017. Balancing selection on allorecognition genes in the colonial ascidian *Botryllus schlosseri*. *Dev. Comp. Immunol*. 69:60–74
97. Padder SA, Prasad R, Shah AH. 2018. Quorum sensing: a less known mode of communication among fungi. *Microbiol. Res.* 210:51–58
98. Palma-Guerrero J, Leeder AC, Welch J, Glass NL. 2014. Identification and characterization of LFD1, a novel protein involved in membrane merger during cell fusion in *Neurospora crassa*. *Mol. Microbiol.* 92:164–82
99. Palma-Guerrero J, Zhao J, Gonçalves AP, Starr TL, Glass NL. 2015. Identification and characterization of LFD-2, a predicted fringe protein required for membrane integrity during cell fusion in *Neurospora crassa*. *Eukaryot. Cell* 14:265–77
100. Paoletti M. 2016. Vegetative incompatibility in fungi: From recognition to cell death, whatever does the trick. *Fungal Biol. Rev.* 30:152–62
101. Paoletti M, Saupe SJ. 2009. Fungal incompatibility: evolutionary origin in pathogen defense? *BioEssays* 31:1201–10
102. Park G, Colot HV, Collopy PD, Krystofova S, Crew C, et al. 2011. High-throughput production of gene replacement mutants in *Neurospora crassa*. *Methods Mol. Biol*. 722:179–89
103. Perkins DD. 1975. The use of duplication-generating rearrangements for studying heterokaryon incompatibility genes in *Neurospora*. *Genetics* 80:87–105
104. Perkins DD, Newmeyer D, Taylor CW, Bennett DC. 1969. New markers and map sequences in *Neurospora crassa*, with a description of mapping by duplication coverage, and of multiple translocation stocks for testing linkage. *Genetica* 40:247–78
105. Pontecorvo G. 1956. The parasexual cycle in fungi. *Annu. Rev. Microbiol.* 10:393–400
106. Queller DC. 2011. Expanded social fitness and Hamilton's rule for kin, kith, and kind. *PNAS* 108:10792–99
107. Rankin DJ, Bargum K, Kokko H. 2007. The tragedy of the commons in evolutionary biology. *Trends Ecol. Evol.* 22:643–51
108. Richard F, Glass NL, Pringle A. 2012. Cooperation among germinating spores facilitates the growth of the fungus, *Neurospora crassa*. *Biol. Lett.* 8:419–22
109. Richman A. 2000. Evolution of balanced genetic polymorphism. *Mol. Ecol.* 9:1953–63
110. Riley MA, Wertz JE. 2002. Bacteriocins: evolution, ecology, and application. *Annu. Rev. Microbiol.* 56:117–37
111. Roper M, Ellison C, Taylor JW, Glass NL. 2011. Nuclear and genome dynamics in multinucleate ascomycete fungi. *Curr. Biol.* 21:R786–93

112. Rosa SF, Powell AE, Rosengarten RD, Nicotra ML, Moreno MA, et al. 2010. *Hydractinia* allodeterminant *alr1* resides in an immunoglobulin superfamily-like gene complex. *Curr. Biol.* 20:1122–27
113. Rosengarten RD, Nicotra ML. 2011. Model systems of invertebrate allorecognition. *Curr. Biol.* 21:R82–92
114. Saupe S, Descamps C, Turcq B, Begueret J. 1994. Inactivation of the *Podospora anserina* vegetative incompatibility locus *het-c*, whose product resembles a glycolipid transfer protein, drastically impairs ascospore production. *PNAS* 91:5927–31
115. Saupe SJ. 2000. Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. *Microbiol. Mol. Biol. Rev.* 64:489–502
116. Saupe SJ. 2011. The [Het-s] prion of *Podospora anserina* and its role in heterokaryon incompatibility. *Semin. Cell Dev. Biol.* 22:460–68
117. Saupe S. 2020. Amyloid signaling in filamentous fungi and bacteria. *Annu. Rev. Microbiol.* 74
118. Scofield VL, Schlumpberger JM, West LA, Weissman IL. 1982. Protochordate allorecognition is controlled by a MHC-like gene system. *Nature* 295:499–502
119. Selosse MA, Taschen E, Giraud T. 2013. Do black truffles avoid sexual harassment by linking mating type and vegetative incompatibility? *New Phytol.* 199:10–13
120. Seuring C, Greenwald J, Wasmer C, Wepf R, Saupe SJ, et al. 2012. The mechanism of toxicity in HET-S/HET-s prion incompatibility. *PLOS Biol.* 10:e1001451
121. Shiu PK, Glass NL. 1999. Molecular characterization of *tol*, a mediator of mating-type-associated vegetative incompatibility in *Neurospora crassa*. *Genetics* 151:545–55
122. Simonin A, Palma-Guerrero J, Fricker M, Glass NL. 2012. Physiological significance of network organization in fungi. *Eukaryot. Cell* 11:1345–52
123. Snoussi M, Talledo JP, Del Rosario NA, Mohammadi S, Ha BY, et al. 2018. Heterogeneous absorption of antimicrobial peptide LL37 in *Escherichia coli* cells enhances population survivability. *eLife* 7:e38174
124. Span EA, Marletta MA. 2015. The framework of polysaccharide monooxygenase structure and chemistry. *Curr. Opin. Struct. Biol.* 35:93–99
125. Strassmann JE, Queller DC. 2011. Evolution of cooperation and control of cheating in a social microbe. *PNAS* 108(Suppl. 2):10855–62
126. Teichert I, Steffens EK, Schnass N, Franzel B, Krisp C, et al. 2014. PRO40 is a scaffold protein of the cell wall integrity pathway, linking the MAP kinase module to the upstream activator protein kinase C. *PLOS Genet.* 10:e1004582
127. Tipping MJ, Gibbs KA. 2019. Peer pressure from a *Proteus mirabilis* self-recognition system controls participation in cooperative swarm motility. *PLOS Pathog.* 15:e1007885
128. Turcq B, Deleu C, Denayrolles M, Begueret J. 1991. Two allelic genes responsible for vegetative incompatibility in the fungus *Podospora anserina* are not essential for cell viability. *Mol. Gen. Genet.* 228:265–69
129. Uehling J, Deveau A, Paoletti M. 2017. Do fungi have an innate immune response? An NLR-based comparison to plant and animal immune systems. *PLOS Path* 13:e1006578
130. Van der Nest MA, Olson A, Lind M, Velez H, Dalman K, et al. 2014. Distribution and evolution of *het* gene homologs in the basidiomycota. *Fungal Genet. Biol.* 64:45–57
131. van Diepeningen AD, Debets AJ, Hoekstra RF. 1997. Heterokaryon incompatibility blocks virus transfer among natural isolates of black Aspergilli. *Curr. Genet.* 32:209–17
132. Vos M, Velicer GJ. 2009. Evolution of social conflict in the bacterium *Myxococcus xanthus*: centimeter versus global scale populations. *Curr. Biol.* 19:1763–67
133. Wan L, Essuman K, Anderson RG, Sasaki Y, Monteiro F, et al. 2019. TIR domains of plant immune receptors are NAD^+-cleaving enzymes that promote cell death. *Science* 365:799–803
134. Weichert M, Lichius A, Priegnitz BE, Brandt U, Gottschalk J, et al. 2016. Accumulation of specific sterol precursors targets a MAP kinase cascade mediating cell-cell recognition and fusion. *PNAS* 113:11877–82
135. White CE, Winans SC. 2007. Cell-cell communication in the plant pathogen *Agrobacterium tumefaciens*. *Philos. Trans. R. Soc. Lond. B* 362:1135–48
136. Wielgoss S, Fiegna F, Rendueles O, Yu YN, Velicer GJ. 2018. Kin discrimination and outer membrane exchange in *Myxococcus xanthus*: a comparative analysis among natural isolates. *Mol. Ecol.* 27:3146–58

137. Wojtowicz WM, Flanagan JJ, Millard SS, Zipursky SL, Clemens JC. 2004. Alternative splicing of *Drosophila* Dscam generates axon guidance receptors that exhibit isoform-specific homophilic binding. *Cell* 118:619–33
138. Wojtowicz WM, Wu W, Andre I, Qian B, Baker D, Zipursky SL. 2007. A vast repertoire of Dscam binding specificities arises from modular interactions of variable Ig domains. *Cell* 130:1134–45
139. Wu J, Saupe SJ, Glass NL. 1998. Evidence for balancing selection operating at the *het-c* heterokaryon incompatibility locus in a group of filamentous fungi. *PNAS* 95:12398–403
140. Zhang D-X, Nuss DL. 2016. Engineering super mycovirus donor strains of chestnut blight fungus by systematic disruption of multilocus *vic* genes. *PNAS* 113:2062–67
141. Zhang D-X, Spiering MJ, Dawe AL, Nuss DL. 2014. Vegetative incompatibility loci with dedicated roles in allorecognition restrict mycovirus transmission in chestnut blight fungus. *Genetics* 197:701–14
142. Zhao J, Gladieux P, Hutchison E, Bueche J, Hall C, et al. 2015. Identification of allorecognition loci in *Neurospora crassa* by genomics and evolutionary approaches. *Mol. Biol. Evol.* 32:2417–32