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Sex and macrocyst formation in *Dictyostelium*

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ABSTRACT Sex in Dictyostelia involves a remarkable form of cannibalism in which zygotes attract large numbers of surrounding amoebae and then ingest them. Before they are consumed, the attracted amoebae help the zygote by synthesising an outer wall around the aggregate that traps them inside and helps to protect the mature developed zygotic structure, the macrocyst. Competition between cells vying to contribute genetically to zygotes and through to the next generation seems likely to have promoted the evolution of several unusual features of dictyostelid sex: individual species often have more than two mating types, increasing haploid cells' chances of matching with a compatible partner, and fusion of many gametes to form transient syncytia allows cytoplasmic mixing and lateral transmission of mitochondrial genomes. This review will summarise recent advances in our understanding of mating-type determination, gamete fusion, and inheritance in *Dictyostelium*, and highlight the key gaps in our understanding of this fascinating set of phenomena.

KEY WORDS: *social amoeba, meiosis, syngamy, karyogamy, zygosis*

Introduction

The sexual cycle of dictyostelids shares a number of features with their better-known asexual developmental cycle. Dictyostelid sexual development is triggered, in part, by depletion of bacterial prey; it involves the aggregation of amoebae responding to secreted chemoattractants; mesoscopic structures containing cellulose and other biopolymers are constructed collectively; and individual cells have dramatically different fates, with some being sacrificed for the ultimate benefit of others (Bloomfield, 2013; Fukuzawa, 2018; Raper, 1984). However, the differences between these two developmental programmes are profound: in sexual aggregates, zygote cells consume the cells that surround them as the latter lay down walls around the outer surface of each aggregate. Each zygote grows in this way, without dividing, to produce a single large cell called a macrocyst, which can contain thousands of cannibalised amoebae within its food vacuoles (Fig. 1). Furthermore, sexual development is favoured when starving cells are submerged, and, unlike asexual development, only occurs efficiently in the dark (Bloomfield, 2013; Fukuzawa, 2018; Raper, 1984).

Importantly, the dictyostelid sexual cycle displays features that are unique or rare among eukaryotic sexual biology, which presumably reflects in large part from the biological imperatives imposed by these organisms' unusual life history. First, each species (at least in the genus *Dictyostelium* as recently redefined (Sheikh *et al.*, 2018)) often has more than two mating types, perhaps resulting from selection to increase the probability of sexual compatibility between conspecific cells that encounter each other at the initiation of sex (Bloomfield, 2011). Second, there appears to be no block to multiple fusion of gametes during zygosis: tens or hundreds of gametes can fuse together over a period of several hours before measurable nuclear fusion occurs, allowing mixture of cytoplasms and exchange of mitochondria (Bloomfield *et al.*, 2019; Ishida *et al.*, 2005; Saga *et al.*, 1983). Third, meiosis occurs in the absence of the key Spo11 enzyme, which has a very widely conserved essential function in initiating meiotic recombination in other sexual eukaryotes (Bloomfield, 2016; Bloomfield, 2018a; Bloomfield *et al.*, 2019; Malik *et al.*, 2007). This review will discuss recent progress in understanding these critical aspects of dictyostelid biology, and raise the questions that remain outstanding.

Sex and multiple mating types

Most sexual eukaryotic species have two sexes or mating types. In anisogamous species, in which one gamete class is normally larger than the other, this is typically the only possible stable scenario (Lessells *et al.*, 2009). However, even among isogamous eukaryotes, the occurrence of two mating types is thought to be most common (Whitfield, 2004). Dictyostelids and their close rela-

Abbreviations used in this paper: Hgr, HAP2-GCS1 related.

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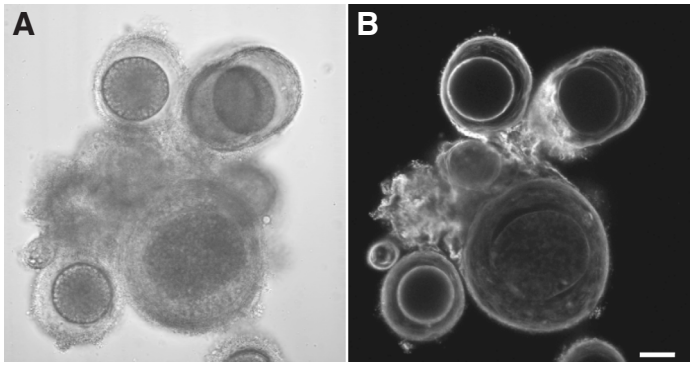

Fig. 1. Macrocysts of *Dictyostelium discoideum*. *Mature macrocysts formed in a cross between strains AX2 (mating type I) and HM1560 (type II). Structures were visualised using* **(A)** *digital interference contrast microscopy to show overall morphology under visible light illumination and* **(B)** *fluorescence microscopy to show cellulose bound by calcofluor white. Scale bar, 25 μm.*

tives the myxogastrids are among the exceptions to this rule, along with certain ciliates and basidiomycete fungi (Hurst and Hamilton, 1992). Relatedly, certain dictyostelid and myxogastrid isolates (and many fungi) are homothallic, so that genetically identical gametes are sexually compatible, unlike heterothallic isolates in which only gametes of different mating types can mate (Bloomfield, 2011). The main evolutionary driver for increased number of mating types (and homothallism), as mentioned above, is thought to be selection to maximise the probability of compatibility with potential sexual partners. This tendency for selection to increase the number of mating types is countered in part by the competition between mating type alleles or idiomorphs: if one mating type mates less efficiently than others it will be eliminated from the population (Hadjivasiliou and Pomiankowski, 2016; Power, 1976). The overall frequency of mating among theoretical sexual populations has also been found to affect the number of mating types (Constable and Kokko, 2018).

In *Dictyostelium discoideum*, mating type is determined by the *mat* locus, which is a small (<6000 basepair) region on chromosome 5 (Bloomfield *et al.*, 2010). This species has three mating types (I, II, and III), each of which possesses a different *mat* idiomorph. The NC4 strain is type I, as are its many widely used axenic derivatives such as AX2, AX3, and AX4. V12 is an example of type II, and WS2162 is type III (Bloomfield, 2011; Erdos, Raper, *et al.*, 1973). All three mating types of this species are commonly found in nature, often together in the same soil sample, and all produce equally sized gametes, on average (Douglas *et al.*, 2016; Francis and Eisenberg, 1993). In a number of regions of North America the frequencies of the different mating types isolated from nature deviates from equality, suggestive of drift, but at the same time balancing selection appears to prevent extreme disparities (Douglas *et al.*, 2016); along with earlier population genetic evidence of recombination among wild *D. discoideum* isolates (Flowers *et al.*, 2010), this finding provides evidence of the frequent occurrence of sex in wild dictyostelids.

The type I idiomorph of *mat* contains a single gene, *matA*. The type II idiomorph contains a gametologue of *matA,* called *matB*, along with two unrelated genes, *matC* and *matD.* Finally, the type III idiomorph contains *matS* and *matT*, which are gametologues of *matC* and *matD* respectively. The three idiomorphs are shown schematically in Fig. 2. Deletion of *matA* abrogates macrocyst formation in crosses involving type I strains, and the introduction of *matB* together with *matC* into such *mat* null cells converts them into functional type II strains (Bloomfield *et al.*, 2010). Equally, introduction of *matS* alone into *mat* null cells switches them to type III. Neither *matD* nor *matT* are necessary for macrocyst formation (Bloomfield *et al.*, 2010).

The MatA and MatB proteins are small (107 amino acids) and possess a homeodomain-like fold (Hedgethorne *et al.*, 2017). They do not have strong amino acid sequence similarity to canonical homeoproteins, but the presence of several residues widely conserved in homeoproteins, the ability to bind DNA with an α-helix corresponding to the DNA-binding helix of the homeodomain, and the occurrence of homeoproteins within sex-determining regions in other eukaryotes all support the argument that MatA and MatB are divergent forms of an ancestral homeoprotein gene (Hedgethorne *et al.*, 2017). MatC and MatS have no recognisable sequence homology to known proteins, except for each other, and their functions remain unknown. However, when tagged with certain fluorescent proteins, these proteins can be seen to be enriched within the nucleus, and it is likely that these small, charged proteins, along with MatA and MatB, will prove to be transcription factors (Hedgethorne *et al.*, 2017). MatD and MatT are larger proteins with predicted GPI anchors, and contain sequences distantly related to the gamete fusogen HAP2-GCS1 (see below). Given that these proteins are dispensable for gamete fusion, this raises the possibility that these proteins might be involved in cell surface interactions during mating that are important for optimal gamete recognition (Bloomfield *et al.*, 2010).

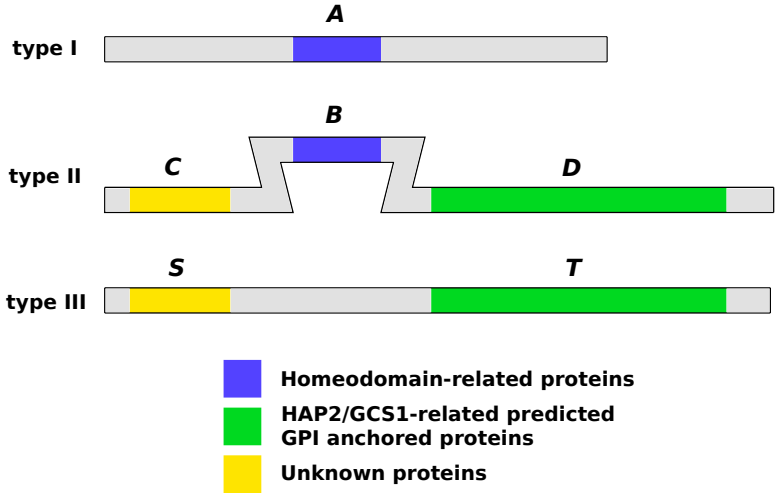

Fig. 2. The *D. discoideum* mating type locus. *The six genes found at the* D. discoideum mat *locus are shown schematically, not drawn to scale. 'A' is* matA, *' 'B' is* matB, *and so on. Gametologues (homologous genes found in the different idiomorphs) are paired according to colour. The characteristics of the polypeptides encoded by these genes are also indicated.*

The machinery of gamete fusion

The highly efficient pairwise fusion of gamete of three different mating types implies difference in cell surface composition that can be recognised and acted upon, as well as membrane fusogens or proteins that

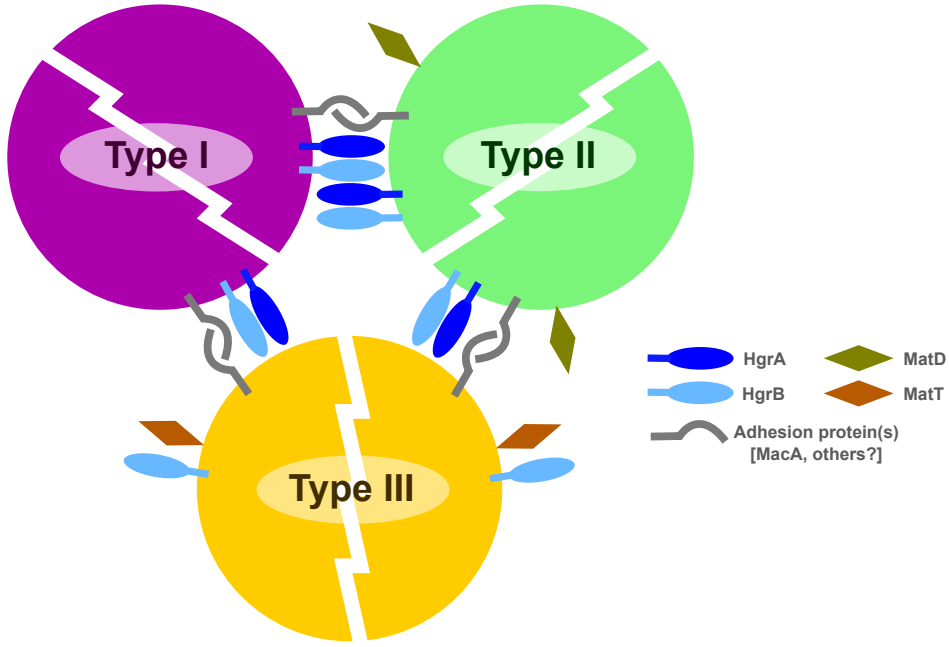

Fig. 3. Cell-cell interactions in *Dictyostelium* gametes. *Proteins implicated in gamete recognition and fusion in* D. discoideum *are shown. MatD and MatT are encoded by genes within the mating-type locus and so can only be expressed in type II and type III cells respectively; HgrA is only translated at measurable levels in type I and type II gametes. The protein-protein interactions that allow gametes of different mating types to recognise each other are not known; proximity of elements in this cartoon should not be understood as indicating physical proximities.*

function both in recognition and membrane fusion (Urushihara, 1996; Urushihara and Muramoto, 2006). As well as MatD and MatT, dictyostelids possess two other proteins related to HAP2-GCS1, which are encoded by genes outside of the mating type locus (Bloomfield *et al.*, 2010; Okamoto *et al.*, 2016). One of these, HgrA, is orthologous to canonical HAP2-GCS1 proteins in other eukaryotes and contains a single predicted transmembrane helix. The other, named HgrB, is a larger protein conserved only within dictyostelids with a single predicted transmembrane helix; like MatD and MatT, HgrB displays weak homology with the extracellular region of HAP2-GCS1. Transcripts of *hgrA* and *hgrB* can be detected in fusion-competent cells (gametes) of all three *D. discoideum* mating types; however, while HgrB protein is detectable in all three mating types, HgrA has only so far been found in type I and type II gametes. HgrA is expressed at lower levels than HgrB, and it is possible that very low levels of the former protein are present in type III cells (Okamoto *et al.*, 2016).

Null mutants in which either *hgrA* or *hgrB* are disrupted in a type I background are unable to fuse at high frequencies with cells of the other mating types, and do not form macrocysts, indicating that the ancestral gamete fusogen function of HAP2-GCS1 is conserved in this species. Strikingly, both *hgrA* and *hgrB* are also necessary for macrocyst formation in type II strains, but dispensable in type III (Okamoto *et al.*, 2016). This suggests that both proteins have essential roles during gamete fusion on both fusing membranes in crosses between type I and type II cells, but are only required on one membrane in crosses involving type III cells. In plants, green algae, apicomplexans, and very likely some animals, HAP2-GCS1 is required only in male gametes (Besser *et al.*, 2006; Ebchuqin *et al.*, 2014; Liu *et al.*, 2008; Mori *et al.*, 2006), but in the ciliate *Tetrahymena* it functions on both membranes (Cole *et al.*, 2014). It is important to recall that type III *D. discoideum* gametes express the HAP2-GCS1-related protein MatT (mentioned above) that presumably functions on the plasma membrane facing HgrA and HgrB; although MatT is not required for macrocyst formation, it remains possible that it interacts physically with either HgrA or HgrB or both (Fig. 3).

Another membrane protein named MacA that is unrelated to HAP2-GCS1, also functions during gamete fusion in *D. discoideum* and may be important for cell-cell interactions prior to fusion (Araki *et al.*, 2012). In the homothallic DM7 isolate, which is related to *D. clavatum* and *D. longosporum* (Mohri *et al.*, 2018) ethylene promotes zygote formation, likely helping to trigger gamete fusion (Amagai, 1989). The genes responsible for ethylene production are present in other dictyostelids (Amagai, 1992; Eichinger *et al.*, 2005), suggesting that this function is likely to be conserved; the identity of the ethylene receptor and other downstream targets remain unclear. Extracellular calcium ions are also necessary for cell fusion (Chagla *et al.*, 1980); this likely explains earlier observations of inhibitory effects of phosphate (Raper, 1984), which precipitates calcium ions.

Syncytial prozygotes and nuclear fusion

Heterothallic *Dictyostelium* gametes, as defined by their ability to fuse at high frequency with cells of complementary mating type, can be prepared in clonal cultures, that is in the absence of signals from cells of other mating types (Saga *et al.*, 1983). When gametes prepared in this way are mixed with gametes of another mating type, fusion begins within minutes (Saga *et al.*, 1983). Remarkably, fusion is so extensive that large syncytia containing tens or hundreds of haploid nuclei are often formed (Bloomfield *et al.*, 2019; Ishida *et al.*, 2005; Saga *et al.*, 1983). These large cells can persist for several hours; gradually they break apart (Fig. 4), and after six to nine hours, nuclear fusion occurs (Ishida *et al.*, 2005; Okada *et al.*, 1986). In these syncytial prozygotes, nuclei and mitochondria appear to be thoroughly mixed: when three strains, marked with fluorescent proteins localised to their nuclei or mitochondria, are mated, distinct organelles from all three ‘parents’ can be observed within syncytia for several hours (Bloomfield *et*

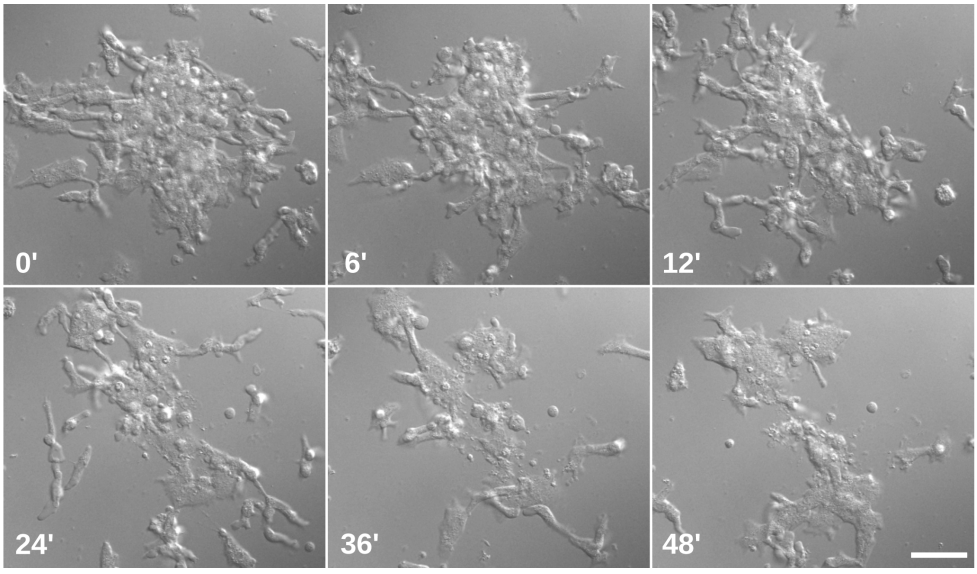

Fig. 4. Division of a syncytial prozygote. *A syncytium formed by the homothallic* D. aff. discoideum *isolate AC4, imaged by digital interference contrast microscopy, gradually break into several smaller cells. The time is shown in minutes; scale bar, 25 μm.*

al., 2019). Mitochondria have not been observed to fuse within syncytia (but might do so at low frequency).

These behaviours differ strikingly from the typical pattern of gamete fusion in eukaryotes, in which pairwise mating is strongly favoured. Often mechanisms exist that prevent fusion of more than two gametes; if more than two gametes fuse, and in some organisms supernumerary nuclei are destroyed to ensure that only two go on to fuse (Bianchi and Wright, 2016; Rothschild, 1954; Tekleyohans *et al.*, 2017). These restrictive mechanisms most likely exist because polyploidy is strongly disadvantageous. One can speculate that the lag of several hours between gamete fusion and nuclear fusion in *Dictyostelium* has evolved at least in part because polyploidy in costly in this genus. The molecular basis of this lag period is not known. It is not clear what advantages syncytium formation might give to mating cells, given that it would seem to increase the risk of polyploidy and of the lateral transmission of deleterious cytoplasmic elements (however see the section on mitochondrial inheritance below).

Growth and development of macrocysts

After zygotes are formed by nuclear fusion, a developmental program is initiated: zygotes (at least in *D. discoideum*) secrete the chemoattractant cyclic AMP (Abe *et al.*, 1984) and quickly start ingesting nearby cells (Filosa and Dengler, 1972; Fukui, 1976; Lewis and O'Day, 1986). If zygotes are not already inside cell aggregates, they can be observed actively preying on other cells, crawling and extending pseudopodia that attempt to attach to other cells. This mode of growth is unlike that of haploid cells not only because haploid cells prey on bacteria, not on other amoebae (if haploid amoebae were to feed frequently on conspecific amoebae, their population growth would be limited), but also because it involves no mitotic divisions: zygotes increase in volume as they ingest thousands or tens of thousands of other cells without undergoing division. Mature macrocysts can be one hundred micrometres or more in diameter (Raper, 1984). Zygotes, also called 'giant cells' as they grow in size cannibalistically, also ingest other zygotes (Fukui, 1976).

This program of zygotic development depends on the *mat* genes (Hedgethorne *et al.*, 2017). When haploid cells of different mating types fuse, the resulting sexual diploids do not undergo mitosis, as far as is known, being instead committed to meiotic divisions once macrocyst development has completed. In contrast, diploids formed by the fusion of cells of the same mating type and therefore homozygous at the *mat* locus, known as parasexual diploids, continue to grow and divide mitotically in the same way as haploid cells for as long as prey bacteria are present. The inability of sexual diploids to proliferate using bacterial food is known as 'vegetative incompatibility'. This phenomenon was first discovered during attempts to select for parasexual diploids in experimental crosses between strains of different mating types; the only proliferative diploids that could be obtained were rare clones that appeared to have undergone loss of heterozygosity at *mat* (Robson and Williams, 1979). Crosses involving engineered *mat* null strains: when parasexual diploids hemizygous at the mating type locus (that is, with one functional *mat* idiomorph and one null version) are selected, they do not differentiate as zygotes and instead grow and proliferate in the haploid manner, as diploids homozygous at *mat* do (Hedgethorne *et al.*, 2017). The *mat* genes induce zygote development in pairs: *matA* can function in combination with either *matC* (from type II) or *matS* (from type III); *matB* functions only in combination with *matS*, not with *matA* nor *matC* (Hedgethorne *et al.*, 2017). In certain fungi and in green algae, homeodomain-containing protein expressed specifically in different gametes induce zygotic gene expression after they heterodimerise after gamete fusion (Bowman *et al.*, 2016). The interactions that *Dictyostelium* Mat proteins undergo with other polypeptides are not known, but genetic data are intriguingly consistent with activation of zygotic functions by heterodimeric proteins formed by the binding of the homeodomain-related MatA and MatB proteins with as-yet unidentified partners.

Before they are ingested, the amoebae that surround zygotes cooperate in laying down the outer primary wall on the outside of the precyst aggregate. After the zygote eats its way to this wall, it secretes a thicker secondary wall and then, at least in some cases a tertiary wall that displays similarity to the dictyostelid spore coat (Erdos, Nickerson, *et al.*, 1973; Nickerson and Raper, 1973). Macrocyst walls vary considerably in thickness even within a single species; variations in structure between species have not yet been examined carefully. The walls are fibrillar and contain cellulose; the primary wall appears to be similar in construction to the slime sheath of asexual aggregates and slugs (Larson *et al.*, 1994). Macrocysts remain semi-dormant within their walls for up to several weeks; ingested amoebae are gradually assimilated over this period (Raper, 1984).

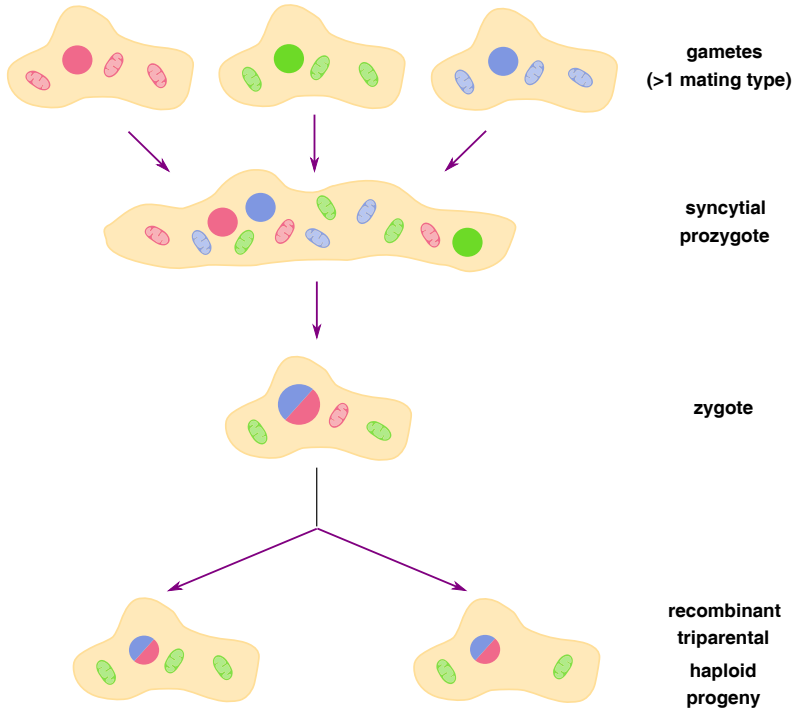

Fig. 5. A model of nuclear and mitochondrial inheritance in *D. discoideum*. *Gametes of three strains and of at least two mating types fuse to form a syncytial prozygote. A zygote forms from fusion of two (pro-)nuclei after division of the syncytium and contains mitochondrial from some or all of the gametes that fused. After meiosis, progeny have recombinant nuclear chromosomes derived from the two gametes whose nuclei fused, and often have mitochondrial genomes from a third gamete.*

Inheritance and emergence of progeny

Genetic studies of recombination in haploid progeny obtained from macrocysts found patterns of segregation consistent with meiosis, albeit with the complication that products of more than one meiosis were apparently sometimes present in progeny from a single macrocyst (Erdos *et al.*, 1975; Francis, 1998; Macinnes and Francis, 1974; Wallace and Raper, 1979). This anomaly could be explained by the presence of more than one zygote within the same macrocyst primary wall, or by aggregation of progeny from more than one macrocyst prior to harvesting of progeny. The detection of structures resembling synaptonemal complexes within zygote nuclei further supports the contention that standard meiotic divisions occur as macrocysts mature (Erdos *et al.*, 1972; Okada *et al.*, 1986). However because macrocysts are large and surrounded by refractile walls it has proven impossible so far to monitor the nuclear divisions directly. Further, after the genomes of multiple dictyostelids were sequenced, their lack of any sequences corresponding to orthologues of the otherwise highly conserved transesterase Spo11 cast some doubt on whether a conventional meiosis could in fact occur in dictyostelids (Bloomfield, 2016; Malik *et al.*, 2007). Spo11, a protein related to topoisomerase VI enzymes of archaea and eukaryotes, is normally required to initiate meiotic recombination: it introduces the DNA double-strand breaks that are used to form chiasmata between homologous chromosomes (Bergerat *et al.*, 1997; Keeney *et al.*, 1997). In several model organisms, Spo11 null mutants are sterile, although in some cases the meiotic block that occurs in the absence of Spo11 can be bypassed in DNA breaks are formed by some other means (Bloomfield, 2016).

A recent study that characterised *D. discoideum* macrocyst progeny genome-wide for the first time confirmed and extended earlier findings that recombination occurs at high frequencies (Bloomfield *et al.*, 2019). Despite the absence of any recognisable Spo11 enzyme, recombination rates were found to be very high compared to other eukaryotes; at least one crossover occurred on every chromosome, and occurred on average approximately once per megabase. Sites of recombination were scattered near-randomly across the genome, with some apparent bias towards regions enriched in A and T nucleotides, and some decrease in frequency in regions distal to the centromeres (*D. discoideum* chromosomes are telocentric). No obvious indications were found that recombination might be sequence specific, and the mechanism of recombination remains mysterious. As found in some earlier studies, all clones recovered from a single macrocyst were identical as assessed by genotyping three loci (whole genomes of such 'sibling' clones have not yet been sequenced), consistent with the survival of only one meiotic product; the fate of the other three potential meiotic products is not known.

In this recent study, as well as conventional crosses between two strains of different mating types, 'three-way' crosses between two wild type strains and the *mat* null strain that is unable to complete macrocyst formation were also performed. These crosses were designed to test for any lateral transmission of genetic material from cannibalised cells into progeny. Remarkably, progeny in these 'three-way' crosses frequently have three parents, with nuclear chromosomes recombined from the two wildtype strains and mitochondrial genomes from the *mat* null strain. In several cases progeny were heteroplasmic, having more than one mitotype. It is suggested that this unusual mode of mitochondrial inheritance results from exchange of mitochondria within syncytial prozygotes (Fig. 5); it remains possible, though less likely, that mitochondria might also pass from cannibalised amoebae into zygotes out of ruptured food vacuoles.

This lateral spread of mitochondrial genomes raises the possibility that mitochondrial genes might be able to promote their transmission into progeny; any variant that can achieve this will be expected to increase in abundance, all other things being equal. Interestingly, dictyostelid mitochondrial genomes contain certain highly divergent, enlarged, and split mitoribosomal genes (Iwamoto *et al.*, 1998; Ogawa *et al.*, 2000), and one gene whose transcript is specifically enriched in *D. discoideum* gametes and encodes a predicted transmembrane helix that has no recognisable homology to known proteins (Muramoto *et al.*, 2003). The possible self-interested behaviours of mitochondria during dictyostelid sex invite comparison with reproductive manipulation by cytoplasmic endosymbionts in other organisms, notably cytoplasmic male sterility in plants that is caused by mitochondrial genes (Chen *et al.*, 2017). Cytoplasmic male sterility genes persist in plants because nuclear suppressor genes permit sufficient individuals with male reproductive organs to be maintained (Schnable and Wise, 1998); if selfish cytoplasmic elements, mitochondrial or of any other kind,

take advantage of syncytium formation to spread in dictyostelid populations, defence mechanisms will be expected to evolve in nuclear genomes to counteract any deleterious effects.

It is also worth noting that prolonged prozygote stage in dictyostelids could provide an opportunity for mitochondrial quality control: if defective mitochondria could be distinguished at this stage and removed, zygotes and progeny would benefit. In some metazoa, such quality control involves a genetic bottleneck in the female germline (Floros *et al.*, 2018; Lieber *et al.*, 2019; Wei *et al.*, 2019); it is not known whether similar changes in mtDNA turnover occur in dictyostelids. The null hypothesis, that sampling of mitochondria by pro-nuclei within *Dictyostelium* prozygotes (and their endoplasmic reticulums) within syncytia is random, cannot currently be rejected. Equally, although in the few cases when the mitotypes of progeny from single macrocysts have been tested all have been identical, it is possible that in zygotes possessing more than one mitotype progeny randomly sample mitochondria as the coenocytic cytoplasm is divided among the haploid nuclei.

A final noteworthy aspect of inheritance revealed by this recent study concerns extrachromosomal nuclear DNA. Dictyostelid ribosomal DNA in growing cells exists largely as extrachromosomal palindromic molecules (Cockburn *et al.*, 1978; Eichinger *et al.*, 2005; Sucgang *et al.*, 2003). Meiotic progeny appear to inherit ribosomal DNA almost entirely uniparentally (Bloomfield *et al.*, 2019). This is consistent with the existence of a chromosomal 'master copy' that is inherited in a Mendelian manner, and either the physical exclusion or destruction of extracellular copies during the sexual cycle. A circular extrachromosomal element, a nuclear plasmid that stably infects WS2162, one of the wildtype strains used as a parent (Rieben *et al.*, 1998), was also found to be absent in progeny from crosses involving this strain. The molecular basis (or bases) of these phenomena are unknown.

Germination of macrocysts, which follows meiosis and multiple rounds of mitotic divisions of progeny, is not well understood. At least in some isolates, the mitotic divisions preceding germination are coenocytic (Filosa and Dengler, 1972; Nickerson and Raper, 1973). Germination frequencies vary between strains, but with very few exceptions tend to be very low in the laboratory (Nickerson and Raper, 1973). The triggers of germination in wild macrocysts is not known.

Conclusions and prospects

Although good progress has been made in recent years, many aspects of dictyostelid sex remain mysterious. To list some of the most pertinent questions: What are the downstream targets of the *mat* genes? What are the signals that induce and repress gamete differentiation and maturation? What is the nature of the post-transcriptional control on HgrA expression? How is karyogamy controlled? Do genes in the mitochondrial genome have any influence on gamete fusion or on mitochondrial survival into progeny after gamete fusion? What are the functions of the unusual mitochondrial genes? What signals govern macrocyst development and entry into the first meiotic division? How does meiotic recombination occur? What is the fate of each meiotic product? What controls on mitochondrial inheritance are there post-fusion (is there a quality control element; is there a bottleneck in mtDNA copy number)? What happens to heteroplasmic cells? How are plasmids eliminated? What are the triggers for germination?

It ought to be possible to answer most of the more mechanistic questions using the standard toolsets of molecular and cell biology to study the well-established model dictyostelid *D. discoideum.* In order to address some of the deeper questions, broader considerations of life-history and evolutionary contexts of the sexual cycle are likely to be particularly valuable. Macrocysts seem likely to promote the survival of dictyostelids when they starve in submerged conditions that make fruiting body construction impossible (Bonner, 1959), and their ability to remain dormant for several weeks could be beneficial during periods of wet or cold. The transfer of resources to the zygote from cannibalised cells could be connected to these imperatives, providing nutrients for a long starvation period, and similar predatory zygotic behaviours in myxogastrids, which are close relative of the dictyostelids, suggests that this could be an ancient feature of mycetozoan biology (Bloomfield, 2018b). Aggregative behaviours shared between the sexual and asexual cycles of dictyostelids have undoubtedly co-evolved closely since the first dictyostelid common ancestor emerged (Shibasaki *et al.*, 2017); and game-theoretic considerations help to explain how the various strategies of cooperation and non-cooperation arise and are maintained (Shibasaki and Shimada, 2018). Ultimately, it seems certain that several of the unusual features of dictyostelid sex have evolved as more or less stable strategies among cells competing for sexual success in this remarkably social (or antisocial) form of sex. Further investigations of diverse dictyostelids and related mycetozoans should help to shed further experimental light on these intriguing questions.

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