REVIEW ARTICLE

Plasmodium development in the myxomycete
Physarum polycephalum: genetic control and cellular events

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Keywords: development, Physarum polycephalum, gene expression, cellular organization

Why study Physarum?

Physarum polycephalum belongs to the myxomycetes, or acellular slime moulds, which occur in soils throughout the vegetated land masses of the world; little is known, however, about their ecological role or abundance (Feest & Madelin, 1985, 1988). A distinctive feature of these simple eukaryotes is the presence of two vegetative phases in the life cycle, each consisting of a single cell type – uninucleate amoebae and multinucleate syncytial plasmodia. These two cell types differ in cellular organization, behaviour and gene expression. This review focuses on the development of amoebae into plasmodia in P. polycephalum.

New approaches combining molecular genetics with classical genetics and analyses of cell organization are now being used to investigate the amoebal–plasmodial transition in P. polycephalum. One long-term aim of these investigations is to understand how changes in gene expression bring about the gradual reorganization of cellular structure and behaviour that occurs as amoebae develop into plasmodia. Another aim is to understand how these changes in gene expression are regulated by the mating-type gene matA, which is known to be responsible for the initiation of development in P. polycephalum. It is hoped that insights gained by investigating such questions in this unicellular eukaryote will shed light on the processes involved in the differentiation of cells in multicellular organisms.

The two cell types of P. polycephalum

Amoebae

The amoebae of P. polycephalum are uninucleate and haploid with a diameter of 10–20 μm. They show amoeboid movement and feed by phagocytosis on bacteria, fungal spores and other micro-organisms. In the laboratory, amoebae are cultured on lawns of bacteria but strains carrying mutant alleles of the axe genes are additionally capable of growing in liquid axenic medium (Dee et al., 1989). In adverse conditions, such as starvation, amoebae reversibly transform into resistant cysts, but in favourable conditions they mate and develop into plasmodia.

Interphase amoebae possess a microtubule-organizing centre (MTOC) consisting of a pair of centrioles surrounded by amorphous material and associated with the nucleus (Havercroft & Gull, 1983). An elaborate array of microtubules radiates through the cytoplasm from this MTOC. At mitosis, the MTOC duplicates and divides, giving rise to the spindle poles; the nuclear membrane breaks down (open mitosis) and a spindle with astral microtubule arrays is formed (Havercroft & Gull, 1983). Mitosis is followed by cytokinesis. Successive mitoses with cytokinesis result in the formation of a colony of genetically identical amoebae.

In moist conditions, amoebae transform into flagellates which are unable to undergo mitosis or feed, and which revert to the amoebal form in dry conditions. When an amoeba transforms into a flagellate, the centrioles form the basal bodies of the two flagella, and the cytoplasmic microtubules are reorganized into five arrays of flagellar microtubules (Wright et al., 1988). The actin-based microfilament system is also reorganized during this transition (Pagh & Adelman, 1982; Uyeda & Furuya, 1985).

Plasmodia

The P. polycephalum plasmodium is a yellow, macroscopic syncytium with an intricate network of veins and, in the laboratory, this cell has been grown to a diameter of more than 30 cm. Locomotion in this giant multinucleate cell occurs as a result of protoplasmic streaming of the cell contents within the veins; the direction of streaming reverses every 30–60 s. Plasmodia phagocytose bacteria, myxomycete amoebae and other microbes, but also secrete enzymes to break down extracellular material, which is then ingested by pinocytosis. Plasmodia can be grown in the laboratory on bacteria, or axenically on agar or in
Table 1. Cell-type-specific gene expression in *P. polycephalum*

<table>
<thead>
<tr>
<th>Gene product*</th>
<th>Name‡</th>
<th>Expressed in</th>
<th>Evidence from</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hap-p</td>
<td><em>hap-p</em> (Lav1-1)</td>
<td>Plasmodia</td>
<td>RNA analysis</td>
<td>Martel et al. (1988)</td>
</tr>
<tr>
<td>PlasminB</td>
<td>Lav1-2</td>
<td>Plasmodia</td>
<td>RNA analysis</td>
<td>Laroche et al. (1989)</td>
</tr>
<tr>
<td>PlasminC</td>
<td>Lav1-3</td>
<td>Plasmodia</td>
<td>RNA analysis</td>
<td>Girard et al. (1990)</td>
</tr>
<tr>
<td>ProfilinP</td>
<td><em>ProP</em> (Lav1-5)</td>
<td>Plasmodia</td>
<td>RNA analysis</td>
<td>Binette et al. (1990)</td>
</tr>
<tr>
<td>ProflinA</td>
<td><em>ProA</em> (Lav3-1)</td>
<td>Amoebae</td>
<td>RNA analysis</td>
<td>St Pierre et al. (1993)</td>
</tr>
<tr>
<td>ABP-46</td>
<td>Lav3-4</td>
<td>Amoebae</td>
<td>RNA analysis</td>
<td>St Pierre et al. (1993)</td>
</tr>
<tr>
<td>P-ABP</td>
<td>Lav3-5</td>
<td>Amoebae</td>
<td>RNA analysis</td>
<td>Adam et al. (1991)</td>
</tr>
<tr>
<td>ActinD</td>
<td><em>actD</em></td>
<td>Plasmodia</td>
<td>RNA analysis</td>
<td>Reviewed in Burland et al. (1993b)</td>
</tr>
<tr>
<td>β1A-tubulin</td>
<td>betA</td>
<td>Amoebae</td>
<td>RNA and protein analysis</td>
<td>Kohama et al. (1986)</td>
</tr>
<tr>
<td>α1B-tubulin</td>
<td>altB(N)</td>
<td>Plasmodia</td>
<td>RNA and protein analysis</td>
<td>Kohama &amp; Ebashi (1986)</td>
</tr>
<tr>
<td>α2B-tubulin</td>
<td>altB(E)</td>
<td>Plasmodia</td>
<td>RNA and protein analysis</td>
<td>Uyeda &amp; Kohama (1987)</td>
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<tr>
<td>β2-tubulin</td>
<td>betC</td>
<td>Plasmodia</td>
<td>RNA and protein analysis</td>
<td>Uyeda et al. (1988)</td>
</tr>
<tr>
<td>Myosin heavy chain A</td>
<td>UN</td>
<td>Amoebae</td>
<td>Antibody staining and peptide mapping</td>
<td>KOHAMA &amp; EBASHI (1986)</td>
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<tr>
<td>Myosin heavy chain P</td>
<td>UN</td>
<td>Plasmodia</td>
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<td>KOHAMA &amp; EBASHI (1986)</td>
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<tr>
<td>Myosin 18K light chain A</td>
<td>UN</td>
<td>Amoebae</td>
<td>Antibody staining and peptide mapping</td>
<td>UYEDA &amp; KOHAMA (1987)</td>
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<td>Fragmin A</td>
<td>UN</td>
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<td>Fragmin P</td>
<td>UN</td>
<td>Plasmodia</td>
<td>Antibody staining and peptide mapping</td>
<td>UYEDA et al. (1988)</td>
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* hap-p, hydrophobic abundant protein – plasmodial; ABP-46, 46 kDa actin-binding protein; P-ABP, *Physarum* actin-bundling protein.
† Gene names are in italics and clone designations are in plain text; UN, un-named.

liquid shaken culture. Plasmodia are unable to transform into flagellated cells but, in adverse conditions, can reversibly transform into dormant sclerotia. In their natural habitat, plasmodia are most often observed when they move to the surface of the soil or leaf litter to sporulate. When plasmodia starve in the light, sporangia are formed; meiosis occurs in the spores, three of the four meiotic products break down and the remaining haploid spore is encased in a wall (Laane & Haugli, 1976). In favourable conditions, spores hatch to release amoebae or flagellates, thus completing the life-cycle.

In contrast to the situation in amoebae during interphase, plasmodial microtubules do not radiate from an organizing centre but form a sparse network in the cytoplasm (Salles-Passador et al., 1991). The mitotic spindle in plasmodia is nucleated by an intranuclear organizing centre but form a sparse network in the cytoplasm leading to a rapid increase in plasmodial size. In plasmodia, actin and myosin are organized around the veins to provide the propulsive force for the cytoplasmic streaming, and as a thin network under the cell membrane (reviewed in Stockem & Brix, 1994).

Molecular basis of the differences between amoebae and plasmodia

The differences in cellular organization and behaviour between amoebae and plasmodia are the result of differences in gene expression, the molecular bases of which are beginning to be understood. Comparisons of the proteins present in amoebae and plasmodia indicate that as many as 25% of abundant proteins are either cell-type-specific in expression, or show different levels of expression in the two cell types (Larue et al., 1982; Turnock et al., 1981). For example, there are significant differences in the proteins present in plasma membranes of the two cell types (Pallotta et al., 1984), as well as in their glyco- and phospholipid composition (Murakami-Murofushi et al., 1987; Minowa et al., 1990).

A significant proportion of genes show cell-type-specific expression; 5–10% of the genes expressed in plasmodia appear not to be expressed in amoebae, and a similar proportion of genes show amoeba-specific expression (Pallotta et al., 1986; Sweeney et al., 1987). In several cases, different members of multi-gene families are expressed in amoebae and plasmodia (Table 1). For example, amoebae and plasmodia express different myosin, fragmin and profilin genes (Kohama & Ebashi, 1986; Kohama et al., 1986; Uyeda & Kohama, 1987; Uyeda et al., 1988;
Genetic control of plasmodium development

Sexual development is the norm in natural isolates of *Physarum polycephalum* and leads to the formation of a diploid plasmodium. Plasmodium formation is under the control of three unlinked mating-type loci, *matA*, *matB* and *matC* (reviewed in Dee, 1987). Multiple alleles of all of these genes exist in natural populations (Collins & Tang, 1977; Kirouac-Brunet et al., 1981; Kawano et al., 1987b). The mating-type genes *matB* and *matC* influence the frequency with which pairs of amoebae fuse (Dee, 1978; Shinnick et al., 1978; Youngman et al., 1979, 1981; Kawano et al., 1987b). Amoebae carrying different alleles of *matB* are 100-1000-fold more likely to fuse than amoebae which are homoallelic for this locus (Youngman et al., 1981), while amoebae which differ at *matC* can fuse over a greater pH range than amoebae carrying identical *matC* alleles (Shinnick et al., 1978; Kawano et al., 1987b).

Amoebae have to acquire the ability to fuse with genetically compatible amoebae before plasmodium formation can occur. For example, when two strains of compatible amoebae are mixed and plated out, a period of amoebal multiplication precedes the onset of fusions, during which the amoebae become fusion competent (Pallotta et al., 1979; Youngman et al., 1979). Ability to undergo fusion with a compatible amoeba can also be acquired during exponential growth in clonal culture (Shipley & Holt, 1982); if both strains of amoebae are fusion competent at the time of mixing and plating out, mating can occur without a time lag. Dense cultures of amoebae can induce competence in overlying sparse cultures separated by 0.2 µm filters (Pallotta et al., 1979; Shipley & Holt, 1982), suggesting that an extracellular inducer is responsible for the acquisition of fusion competence. Preliminary analyses suggest that this inducer may be a complex molecule that acts over a short range and decays quickly (Nader et al., 1984). Addition of partially purified inducer to mixtures of compatible amoebae advances the start of plasmodium formation (Nader et al., 1984).

The third mating-type gene, *matA* (Dee, 1960, 1987), has no effect on amoebal fusion but controls subsequent development of the fusion cell (Youngman et al., 1981). When two amoebae carrying the same *matA* allele fuse, development is blocked before nuclear fusion. If, however, the fusing amoebae carry different alleles of *matA*, nuclear fusion occurs in interphase giving rise to a diploid zygote. After a period of growth, the zygote becomes binucleate by mitosis unaccompanied by cytokinesis. Larger diploid plasmodia arise rapidly as a result of further mitoses unaccompanied by cytokinesis, and fusions between genetically identical plasmodia (Bailey et al., 1990).

Mutations at *matA* give rise to apogamic strains in which haploid plasmodia arise within colonies of haploid amoebae, indicating that the diploid state is not necessary for plasmodium formation (Cooke & Dee, 1974; Shinnick et al., 1985). All the apogamic strains currently used show temperature-sensitive development; plasmodia will develop in colonies of apogamic amoebae cultured at low temperature (21-22 °C) but not at high temperature (28-30 °C). At the non-permissive temperature for development, apogamic strains are still able to mate, showing the same *matA*-specificity as the strain from which they were originally isolated (Anderson, 1979). The *gamA* (greater asexual development) mutations permitting apogamic development are dominant and genetically inseparable from *matA*. Since they do not alter mating-specificity, they are assumed to define a second function of this complex locus (Anderson, 1979, Anderson et al., 1989).

As in sexual development, apogamic amoebae undergo a period of proliferation before clonal plasmodium formation is initiated (Youngman et al., 1977). The proliferating amoebae secrete a chemical inducer and plasmodium formation is triggered when the inducer reaches a critical concentration in the local environment (Youngman et al., 1977). Addition of partially purified inducer to cultures of apogamic cells advances the initiation of plasmodium formation (Nader et al., 1984). The same preparations also affect mating (see above), suggesting that the same inducer affects the acquisition of fusion competence in sexual development and initiation of apogamic development (Nader et al., 1984).

Apogamic strains have proved valuable for identifying developmental mutants; since the cells remain haploid throughout development, even recessive mutations can easily be detected. Mutations affecting development can be identified by allowing mutagenized apogamic amoebae to develop at low temperature and screening for colonies in which plasmodium development is abnormal. The temperature-sensitivity of apogamic development permits the use of classical genetic techniques on the mutant cells; they can be crossed with compatible strains at high temperature. The *npf* (no plasmodium formation) mutations so far identified are recessive and fall into two groups. The first group, comprising the *npfB* and *npfC* mutations, block the initiation of development and are genetically inseparable from *matA* and *gamA* (Anderson, 1979; Anderson et al., 1989). Complementation analysis indicates that these mutations define two further functions at *matA* (Anderson et al., 1989), bringing to four the number of functions that have been defined at this complex locus. The second group of *npf* mutations are unlinked to *matA* and show a wide variety of phenotypes (see below).

Cellular changes during plasmodium development

The alterations in gene expression, cell organization and behaviour that accompany plasmodium formation are
initiated during a short period in development, but are not completed for several cell cycles. The majority of studies of these alterations have been carried out using apogamically developing cells, although studies on sexual development indicate that the sequence and timing of events are very similar in both types of development.

**Apogamic development**

Time-lapse cinematography of development in the apogamic strain CL (matA2-derived) shows that a single haploid amoeba can develop into a plasmodium without cell or nuclear fusion (Fig. 1a; Bailey *et al.*, 1987). Either one or both daughter cells from an apparently normal amoebal division can develop, indicating that the decision to divide or develop is not made until after the mitosis at which a cell is born. The first indication that an amoeba will develop is when it fails to divide at the end of an amoebal cell cycle. Instead, the developing uninucleate cell continues to grow for a period about 2-3-times as long as a normal amoebal cell cycle. At the end of this extended cell cycle, the uninucleate cell is twice as large as an amoeba at mitosis and becomes binucleate by mitosis without cytokinesis (Anderson *et al.*, 1976; Bailey *et al.*, 1987). About halfway through the extended cell cycle, the developing cell becomes committed to development and loses the ability to undergo the amoeba-flagellate transformation (Fig. 1a; Blindt *et al.*, 1986; Bailey *et al.*, 1987). At commitment, the developing cell becomes independent of the inducer and is able to develop into a plasmodium when re-plated at low density (Youngman *et al.*, 1977). After binucleate cell formation, larger plasmodia arise rapidly as a result of further nuclear divisions and fusions between plasmodia. Apogamic plasmodia are able to form spores of normal appearance but viability is very low since, with the exception of the occasional diploid nucleus, the nuclei in the plasmodium are haploid. Similar analyses of apogamic development in the matA3-derived strain RA376, show the same sequence of events (Bailey *et al.*, 1992).

Nuclear volume increases during the long cell cycle in apogamic development, but returns to the haploid level by the time the developing plasmodium is quadrinucleate. This alteration in nuclear volume is not due to a transient increase in DNA content (Bailey *et al.*, 1987), but may be related to an increase in the RNA content of the developing uninucleate cell (Lause *et al.*, 1982), caused by a burst of RNA synthesis from developmentally regulated genes. In vegetative cells of *P. polycephalum*, there is no G1 phase following mitosis; the 2-3 h S phase follows immediately after mitosis and the remainder of the cell cycle is occupied by G2 phase (reviewed in Burland *et al.*, 1993b). Measurement of DNA content in developing uninucleate cells gives no indication of a G1 phase, indicating that the elongation of the cell cycle is due to an extension of G2 (Bailey *et al.*, 1987).

Cell cycle regulation in *P. polycephalum* apparently operates by a size-control mechanism with mitosis being triggered when the protein:DNA ratio reaches a particular level (Laffler & Tyson, 1986). During the long cell cycle, the developing uninucleate cell grows for an extended period without any increase in DNA content, suggesting that the protein:DNA ratio is greater at mitosis in large uninucleate cells than in amoebae (Bailey *et al.*, 1987). The presence of a short cell cycle in the binucleate cell (0.7 x an amoebal cell cycle, Fig. 1a; Bailey *et al.*, 1987) during which cell volume and, presumably protein content, do not double, may serve to return the protein:DNA ratio to the usual level. Thus, the normal size-control mechanism which regulates mitosis is altered in developing uninucleate cells in a way that is unknown at present.

Many of the changes in cellular organization, behaviour and gene expression that accompany development are initiated during the extended cell cycle. For example, during the second half of the long cell cycle, the developing uninucleate cell acquires plasmodial characteristics such as the ability to ingest amoebae and to fuse with genetically identical plasmodia (Fig. 1a). It is at this stage that the plasmodial myosin heavy-chain protein is first present, as are the 18 kDa plasmodial myosin light-chain protein (Uyeda & Kohama, 1987) and plasmodial fragnments (Uyeda *et al.*, 1988). The amoeba-specific α3-tubulin isotype starts to disappear from apogamic cells during the extended cell cycle, and the plasmodium-specific β2-tubulin isotype is first detected in uninucleate developing cells (Solnica-Krezel *et al.*, 1988, 1990). In addition, a high proportion of cell-type-specific genes alter their pattern of expression during the long cell cycle (Sweeney *et al.*, 1987).

**Microtubule organization during apogamic development**

As discussed above, amoebae and plasmodia differ in the type of mitotic spindle formed. The spindle poles of the astral amoebal mitoses contain α3-tubulin, whereas plasmodial spindles do not. Conversely, the β2-tubulin isotype is present in the spine microtubules of plasmodial mitoses but is absent in amoebae (Solnica-Krezel *et al.*, 1991). In most developing uninucleate cells, the mitosis at the end of the long cell cycle is of the intranuclear plasmodial type. Other types of spindle are found in some developing cells at this mitosis, however, indicating that there may be more than one possible route for the transition from uninucleate to binucleate cell (Solnica-Krezel *et al.*, 1991). The mitosis which leads to the formation of a quadrinucleate cell from a binucleate one is always of the plasmodial type, indicating that the transition from amoebal to plasmodial mitosis is complete by this time (Solnica-Krezel *et al.*, 1991).

In many uninucleate developing cells undergoing plasmodial mitosis, an extranuclear, α3-tubulin-positive MTOC is present; this appears to be a remnant of the amoebal one, indicating that the plasmodial intranuclear MTOC is a different structure (Solnica-Krezel *et al.*, 1991). Binucleate cells contain from zero to two MTOCs, indicating that the amoebal MTOC sometimes duplicates at mitosis in developing uninucleate cells even though it
Physarum polycephalum plasmodium development

Fig. 1. Sequence of events in relation to the cell cycle in (a) apogamic and (b) sexual development.

generally does not form the spindle poles (Solnica-Krezel et al., 1990). Thus, the amoebal MTOC appears to lose the ability to form the basal bodies of the flagella and the poles of the spindle, before losing the ability to duplicate at mitosis and nucleate microtubules. In addition, there is no strict correlation between the change in expression of the a3- and β2-tubulin isotypes and reorganization of the cytoplasmic microtubules, suggesting that changes in isotype usage are not sufficient to bring about alterations in microtubule organization (Solnica-Krezel et al., 1990).
Sexual development

Analyses of the cellular events of sexual development reveal that, after zygote formation, the sequence of events is very similar to those seen in apogamic development (Fig. 1b). Fusion can occur between any two cells carrying different alleles of matB and matC, and both amoebae and flagellates are able to undergo fusion (Ross, 1957; Bailey et al., 1990). Fusion occurs at any stage of the cell cycle, except during mitosis and for a period of about 20 min thereafter (Bailey et al., 1990). In matA-heteroallelic fusion cells, commitment coincides with cell or nuclear fusion (Fig. 1b; Shipley & Holt, 1982), and ability to undergo the amoeba–flagellate transformation is lost shortly after this event (Bailey et al., 1990). Nuclear fusion, the first observable event of development, occurs in interphase about 2 h after cell fusion and appears to result from microtubule-mediated processes (Bailey et al., 1990). Since amoebal fusion does not occur at any particular stage of the cell cycle, the nuclei in the fusing cells may be at different stages of the cell cycle. The 2 h gap between cell- and nuclear fusion would be sufficient to allow a nucleus that was in S phase at the time of fusion to complete S and enter G2, ensuring that, by the time of nuclear fusion, both nuclei were in G2.

As in apogamic development, the developing uninucleate cell, in this case the diploid zygote, becomes binucleate by mitosis unaccompanied by cytokinesis at the end of an extended cell cycle (Fig. 1b; Bailey et al., 1987, 1990). There is no evidence for the presence of a G1 phase in the cell cycle of zygotes; the extension of the cell cycle appears to result from the lengthening of G2. At binucleate plasmodium formation, the zygote is about four-times the size of an amoeba at mitosis, with an enlarged nucleus but only diploid DNA content (Bailey et al., 1990): thus the protein:DNA ratio alters during sexual development in the same way as it does in apogamic development. During the long cell cycle, the developing zygote acquires plasmodial characteristics such as the ability to ingest amoebae and to undergo plasmodial fusions (Fig. 1b; Bailey et al., 1990).

Fusion cells, containing two haploid nuclei, generally have one MTOC associated with each nucleus, one from each of the fusing cells (Bailey et al., 1990). By the time nuclear fusion has occurred, there is a single MTOC associated with the diploid zygote nucleus. In some zygotes, two pairs of centrioles appear to be present at the MTOC, suggesting that the zygote MTOC results from the fusion of the two amoebal MTOCs (Bailey et al., 1990). As in apogamic development, the mitosis at the end of the extended cell cycle is usually of the intranuclear plasmodial type and the amoebal MTOC, which is often present in the cytoplasm during this mitosis, is still capable of duplicating and nucleating a microtubule network in the next interphase (Bailey et al., 1990). Thus, the switch from amoebal to plasmodial microtubule organization, as well as all the other cellular events studied, occur in a very similar way in both sexual and apogamic development. These observations indicate that the gadA mutation causing apogamic development by-passes the normal requirement for cell- and nuclear fusion and allows development to occur in a single haploid cell, without significantly altering the timing of the cellular events of plasmodium formation.

In fusion cells which are homoallelic for matA, nuclear fusion does not occur in interphase and the microtubule cytoskeletons contributed by the fusing amoebae remain separate (Bailey et al., 1990). In most cases, the fusing amoebae pull apart shortly after fusion, but in about 25% of cases, the fusing amoebae remain as a single binucleate fusion cell until amoebal mitosis occurs without any lengthening of the cell cycle (Bailey et al., 1990). Mitosis in matA-homoallelic binucleate fusion cells often results in spindle fusion and the formation of diploid amoebae (Bailey et al., 1990). Thus, the presence of two nuclei in a single cell is not sufficient to trigger interphase nuclear fusion, or the subsequent events of development, suggesting that these events are under the control of matA.

Inheritance of mitochondria

The mitochondrial DNAs (mtDNAs) from different isolates of P. polycelatum contain restriction enzyme site polymorphisms, allowing them to be identified with ease (Kawano et al., 1987a). Plasmodium formed by crossing amoebae of different mtDNA types normally possess only one of the two mtDNA types, indicating that inheritance is uniparental (Kawano et al., 1987a). Inheritance of mitochondria is independent of matB and matC, but is under the control of matA or a closely linked locus (Kawano & Kuroiwa, 1989). The alleles of matA appear to form a hierarchy such that the mtDNA present in a plasmodium is that from the amoebal strain carrying the matA allele of higher status (Kawano & Kuroiwa, 1989; Meland et al., 1991). When amoebae of different matA and mtDNA type are crossed, elimination of one parental mtDNA type is virtually complete by the time the developing plasmodia are quadrinucleate, suggesting that the lower status mtDNA is actively eliminated (Meland et al., 1991).

Some amoebal strains carry a linear 16 kb mitochondrial plasmid (mif). In crosses where one strain carries this plasmid, the mtDNA is not uniparentally inherited (Kawano et al., 1991). Instead, several mitochondria fuse into a large mitochondrion containing multiple mtDNAs; fusion is followed by several mitochondrial and mtDNA divisions. Recombination occurs during these divisions, resulting in mtDNA with a novel restriction enzyme digestion pattern; this mtDNA is passed to the normal mitochondria as they reform. The mif plasmid is transmitted unaltered to all mitochondria and subsequently to all amoebal progeny of the plasmodium (Kawano et al., 1991).

Plasmodium development in strains carrying matA-unlinked npf mutations

Strains carrying matA-unlinked npf mutations are blocked in development after initiation and show a wide variety of phenotypes (e.g. Wheals, 1973; Anderson & Dee, 1977;
Bailey et al., 1992; Solnica-Krezel et al., 1995). Amoebal growth is apparently normal in strains carrying these mutations, suggesting that the npf genes are activated during development and, thus, are directly or indirectly under the control of matA. It is possible that some of the npf genes are required for only a short time during development, while others are required throughout plasmodial growth; for most of the mutants, however, the evidence does not allow one to distinguish between these possibilities. The matA-unlinked npf mutations result in blocks at various times during development.

Mutations causing early blocks in plasmodium development

In cultures of apogamic cells carrying mutations in either npfA or npfG, the majority of cells do not initiate development, indicating that these mutations block apogamic development very early and suggesting that the wild-type product of both these genes is required at, or very close to, the time of initiation of development (Fig. 1a; Solnica-Krezel et al., 1995). In developing cultures of both strains, a few cells show positive markers of development (such as becoming binucleate, or staining for β2-tubulin) but plasmodia rarely form, indicating that cells that initiate development eventually die. In cultures of cells carrying a mutation at npfA, non-revertant plasmodia form at low frequency indicating that the mutation is ‘leaky’ (Anderson & Dee, 1977; Solnica-Krezel et al., 1995). Although the npfA and npfG mutations block apogamic development, neither affects sexual development even in a homozygote (Solnica-Krezel et al., 1995); the reason for this is unknown at present.

Mutations causing late blocks in plasmodium development

The second group of matA-unlinked npf mutations affect the later stages of plasmodium formation and block both sexual and apogamic development (npfF, npfK, npfL, npfM; Bailey et al., 1992; Solnica-Krezel et al., 1995). During development in mutant strains, abnormalities are observed at the end of the long cell cycle, about the time of binucleate cell formation, suggesting that the wild-type genes are first required at or before this time (Fig. 1a; Bailey et al., 1992; Solnica-Krezel et al., 1995). This is consistent with previous studies implicating the second half of this cell cycle as a crucial one for development, when many alterations in gene expression and cellular organization begin (e.g. Sweeney et al., 1987; Bailey et al., 1987; Solnica-Krezel et al., 1990). The terminal phenotypes in cells carrying these npf mutations are very different (Bailey et al., 1992; Solnica-Krezel et al., 1995).

In most of these npf mutants, some aspects of development continue normally while others are blocked, indicating that the events are not dependent on one another, although they occur at the same time. For example, cells carrying a mutation in the npfK gene show normal microtubule re-organization, even though the abnormal structure of the mutant cells is gradually developing (Solnica-Krezel et al., 1995). Cells carrying a mutation in npfL are still able to undergo plasmodial fusions even though the nuclei have become defective (Bailey et al., 1992). Events that are not dependent on one another may be on different developmental pathways; evidence for the existence of such pathways comes from studies of double mutants. For example, the npfK npfL double mutant has characteristics of both single mutants, indicating that these two mutations act in different developmental pathways. The npfF npfG double mutant, however, resembles npfG in phenotype, suggesting that, in this case, the wild-type genes act sequentially in the same pathway (Solnica-Krezel et al., 1995). These observations suggest that the various developmental pathways function semi-independently and some pathways may continue for some time even though other pathways are blocked.

In four of the npf mutants examined so far (npfF, npfG, npfL, npfM), the developing cells eventually die in the same characteristic manner; the nuclei condense, lose their nucleoli and stain intensely with the DNA dye 4',6-diamidino-2-phenylindole (DAPI) (Bailey et al., 1992; Solnica-Krezel et al., 1995). These features are characteristic of death by apoptosis (Kerr et al., 1972), a process of cell death observed during morphogenesis in many eukaryotic systems (e.g. Hengartner & Horvitz, 1994). In P. polycephalum, death may result from an inability to complete development, regardless of the primary lesion. Since apoptosis is often considered to have evolved in multicellular organisms (Vaux et al., 1994), these observations of apoptosis-like cell death in a unicellular organism are interesting. Investigations into the role of cell death in P. polycephalum are continuing and may help to elucidate whether this system is of value to the developing plasmodium.

The mating-type loci

Any models for the action of the mating-type loci in P. polycephalum must account for the following observations: (i) heteroallelism at matB and matC increases the frequency with which pairs of amoebae fuse; (ii) both acquisition of fusion competence in sexual development and initiation of apogamic development require the inducer; (iii) the presence of two different matA alleles in a cell triggers development; (iv) the gadA, npfB and npfC mutations all map at the matA locus and affect the initiation of development.

The mating-type loci in natural populations

In many fungi, successful development requires heteroallelism at both of two mating-type loci (e.g. Banuett, 1992) but in P. polycephalum, heteroallelism at three multiallelic loci optimizes the efficiency of development. If development occurred only between amoebae heteroallel at matA, matB and matC, an amoeba would be compatible with 12.5% (1 in 8) of the progeny from the same plasmodium and in-breeding between sibling amoebae would be infrequent. The level of compatibility
could reach 50%, however, as only matA has an absolute
effect on development and amoebae that are heteroallelic
for matA and homoallelic for matB and matC. give rise to
plasmodia at low frequency. The actual level of compat-
ibility is probably between these two estimates since all
plasmodia so far isolated have carried two alleles of matA
and matB, suggesting that, in the wild, heteroallelism at
these loci is important for plasmodium formation
(Kirouac-Brunet et al., 1981); not enough isolates have
been tested for the same conclusion to be drawn about
matC.

The presence of multiple alleles at all three mating-type
loci promotes out-breeding between unrelated amoebae.
So far, 14 matA, 13 matB and 3 matC alleles have been
identified (Collins & Tang, 1977; Kirouac-Brunet et al.,
1981; Kawano et al., 1987b), but many more alleles may
exist since every isolate so far tested has carried two
additional alleles of matA and matB (Kirouac-Brunet et al.,
1981); few isolates have been tested for matC. It is not
known, however, whether each natural population con-
tains a unique set of alleles or whether the same allele
occurs in geographically distant populations. We also
have no information about how the flow of individuals
between populations might influence the number of alleles
of each gene present in a population.

Mode of action of matB and matC

The MatC protein strongly influences the frequency of
amoebal fusion under specific pH conditions, suggesting
that the matC product is attached to the outer membrane
of the amoebae and contains ionizable groups on its
surface. The matB product influences amoebal fusion over
a larger range of conditions than MatC and is probably
also located on the amoebal surface. The MatB protein
may function as a receptor, linking events at the cell
surface to gene action in the nucleus and could be
involved in one or more of the following processes:
recognition of matB product on adjacent amoebae;
holding amoebae together prior to fusion; or subsequent
membrane fusion. The mechanisms by which hetero-
allelism at matB and matC enhance amoebal fusion are
unknown. These genes could be constitutively expressed,
but may be switched on in response to external stimuli. If
matB is constitutively expressed, the inducer could bring
about fusion competence by altering the conformation of
the MatB protein to an active form, capable of interacting
with the matB products of other amoebae. If matB and
matC are not constitutively expressed, the inducer may
activate these genes, leading to the appearance of their
products on the amoebal surface and the acquisition of
fusion competence.

Structure and mode of action of matA

The matA locus initiates plasmodium development and
controls the accompanying alterations in gene expression
and cellular organization; it is presumably a transcription
factor, or a crucial part of a transcription factor complex.
This locus could be directly responsible for all devel-
opmental alterations in gene expression but, since plas-
modium development involves a number of parallel
pathways, matA probably initiates a cascade of gene
action involving other transcription factors and structural
genes. In this case, the promoters of amoeba-specific
genes, and genes at the start of the developmental
pathways, should contain conserved motif(s) directing
binding of the MatA protein. Two candidates for genes
directly activated by matA are npfA and npfC; both block
development very close to initiation and, thus, could be at
the start of developmental pathways. It is not known if
matA is expressed constitutively, or if it is switched on
in response to an external stimulus. matA might be switched
on by the inducer when matB and matC are activated, but
could also be activated later as a result of the acquisition
of fusion competence. If the inducer activates all three
mating-type genes simultaneously, then it could also
switch on the mutant matA allele in apogamic strains,
thus permitting development in a single haploid cell.

Although several mutations at matA have been identified
by classical genetic analysis, the molecular basis of the
mode of action of matA is unknown. It has been suggested
that mating-type interactions in multiallelic systems
depend upon specific tight interactions between the
Recent studies in basidiomycete fungi have revealed how
several such systems operate at a molecular level. In the
smut fungus Ustilago maydis, for example, the a locus
mediates fusion of the haploid cells while the multiallelic
b locus governs subsequent development into the patho-
genic filamentous dikaryon (Banuett, 1992). The b locus
contains two divergently transcribed genes, bE and bW,
which are not homologous to each other although there is
homology between the bE and bW genes from different b
alleles. Both genes have a variable N-terminal domain and
a highly conserved C-terminal constant domain con-
taining a DNA-binding homeodomain motif (Banuett,
1992). The bE and bW proteins from one b locus are
presumed to form a heterodimer in which the DNA
recognition site is inaccessible, and development is not
initiated. The heterodimers formed between the bE and
bW proteins from different b alleles can bind DNA
leading to the activation of development-specific genes
and initiation of development (Banuett, 1992).

The U. maydis system serves as a useful model for the
mode of action of matA in P. polycephalum since specific
mutations in a pair of genes like bE and bW could lead to
the gadA, npfB and npfC classes of mutations. For example,
apogamic strains would result from a gadA mutation in
one of the genes, allowing the formation of an active
heterodimer in a haploid cell at the permissive tem-
perature. Mutations in either npfB or npfC would knock
out one of the paired genes, preventing heterodimers
from forming and blocking development (R. W.
Anderson, personal communication). Although this
model can explain the genetic evidence for the control of
development in P. polycephalum, since the basidiomycetes
are evolutionarily distant from the myxomycetes, the
molecular basis of the P. polycephalum system may be
totally different. Until matA has been cloned and sequ-
enced it will not be possible to determine how it operates.
DNA transformation

Reporter gene constructs based on the bacterial chloramphenicol acetyltransferase and firefly luciferase genes (Burland et al., 1992; Bailey et al., 1994) have been used to optimize transformation protocols, to study promoter function, and to ascertain the effect of DNA fragments on the stability of introduced DNA and the efficiency of stable transformation. Stable transformation vectors containing the hygromycin phosphotransferase gene under the control of a P. polypehphalum actin promoter as a selectable marker have been developed (Burland et al., 1993a). Similar vectors were recently used to carry out the first gene disruption in P. polycephalum (Burland & Pallotta, 1995); further gene disruptions are sure to follow. As transformation levels improve, it will become possible to clone genes by complementation; candidate genes for such an approach include matA and the nph mutants.

The future

The development of a DNA transformation system for P. polypehphalum was a crucial step in completing the range of molecular techniques required for the study of development in this organism. A combination of molecular genetic techniques, classical genetics and cellular analyses will allow us to answer many key questions about the control of development in P. polycephalum. This multifaceted approach should lead to the elucidation of the mechanisms by which development is initiated and how changes in gene expression and cellular organization are controlled during the amoebal-plasmodial transition.

Acknowledgements

I would like to thank Drs Jennifer Dee and Roger Anderson for sparking my interest in Physarum development, for their helpful comments and useful insights on this manuscript, and for their past and continuing support of my career. I would also like to thank the following for financial support: SERC (grant no. GR/D34530); The University of Wisconsin Graduate School; Programme Project grant CA23076 and core grant CA07175 from the National Cancer Institute; The Wellcome Trust (grants 034879 and 042524). Finally, I would like to thank my colleagues in the Physarum field who have been generous with time, discussions and sharing unpublished findings.

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