Evolution of size and pattern in the social amoebas

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Summary
A fundamental goal of biology is to understand how novel phenotypes evolved through changes in existing genes. The Dictyostelia or social amoebas represent a simple form of multicellularity, where starving cells aggregate to build fruiting structures. This review summarizes efforts to provide a framework for investigating the genetic changes that generated novel morphologies in the Dictyostelia. The foundation is a recently constructed molecular phylogeny of the Dictyostelia, which was used to examine trends in the evolution of novel forms and in the divergence of genes that shape these forms. There is a major trend towards the formation of large unbranched fruiting bodies, which is correlated with the use of cyclic AMP (cAMP) as a secreted signal to coordinate cell aggregation. The role of cAMP in aggregation arose through co-option of a pathway that originally acted to coordinate fruiting body formation. The genotypic changes that caused this innovation and the role of dynamic cAMP signaling in defining fruiting body size and pattern throughout social amoeba evolution are discussed. ***BioEssays*** **29:635–644, 2007.**

Introduction

The social amoebas or Dictyostelia represent one of nature's several independent inventions of multicellularity. The dictyostelia are members of the amoebazoans, a genetically highly diverse group that is the closest sister group to the clade containing the animals and fungi.[1] Except for the myxomycetes, all other known amoebazoans are microscopic unicellular organisms. The myxomycetes alternate a trophic amoeboid stage with a syncytial form. Here, a single cell with millions of nuclei, can grow up to several meters across.[2]

Social amoebas also have a trophic amoeboid stage, but they achieve macroscopic dimensions by aggregation.[3] This occurs in response to starvation, which triggers regulated secretion of chemoattractant by the amoebas (Fig. 1). Cellular agglomerates are formed, which can consist of up to a million amoebas. Sophisticated cell–cell signalling mechanisms between the amoebas orchestrate the differentiation of up to five different cell types and coordinate an intricate progression of cell movements. In combination with the synthesis of a flexible skin-like matrix, cell differentiation and cellular movement first generate the formation of a motile structure, called the "slug". The slug responds to chemical gradients and to light and warmth, which cause it to move to the soil's top layer. Here, the slug projects upwards and forms the fruiting body. This again involves highly ordered movement and differentiation and yields a slender column of stalk cells that bears aloft a global mass of spores. Depending on the species, the stalk can show different patterns of side branches and/or be decorated with disc, root or cup-shaped support structures (Fig. 2). Unlike the ontogeny of sessile organisms like plants and fungi, which depends largely on series of directional cell divisions, the formation of fruiting bodies in social amoebas is more similar to the ontogeny of animal form. Both depend strongly on an intertwined program of cell movement and cell differentiation.

Seminal work of Raper showed similarity of principle in the establishment of the body plan in *Dictyostelium* and vertebrate development. In vertebrate development, a small group of cells known as "the organizer" releases signals that coordinate cell movement during gastrulation and neurulation and thereby generate the animal's head-to-tail body axis.[4] Raper demonstrated that, in *Dictyostelium* aggregates, small groups of cells, recognizable as tips, secretes signals that generate anteroposterior polarity of all or a subpopulation of cells in the aggregate, yielding one or several slugs with a distinct head and body region.[5]

More recent work shows that animals and Dictyostelia share many conserved pathways for processing external signals. Particularly the elucidation of the processes that control chemotaxis in *Dictyostelium* have become a paradigm for understanding cell migration in animals.[6–8] However, the external signals that trigger movement or differentiation are rarely conserved. For instance, growth-factor-like peptides and their tyrosine kinase receptors that play such crucial roles in animal development are not present in *Dictyostelium*. The homeobox-containing transcription factors that specify segment identity in arthropods and vertebrates have only a minor function in *Dictyostelium* development.[9–11]

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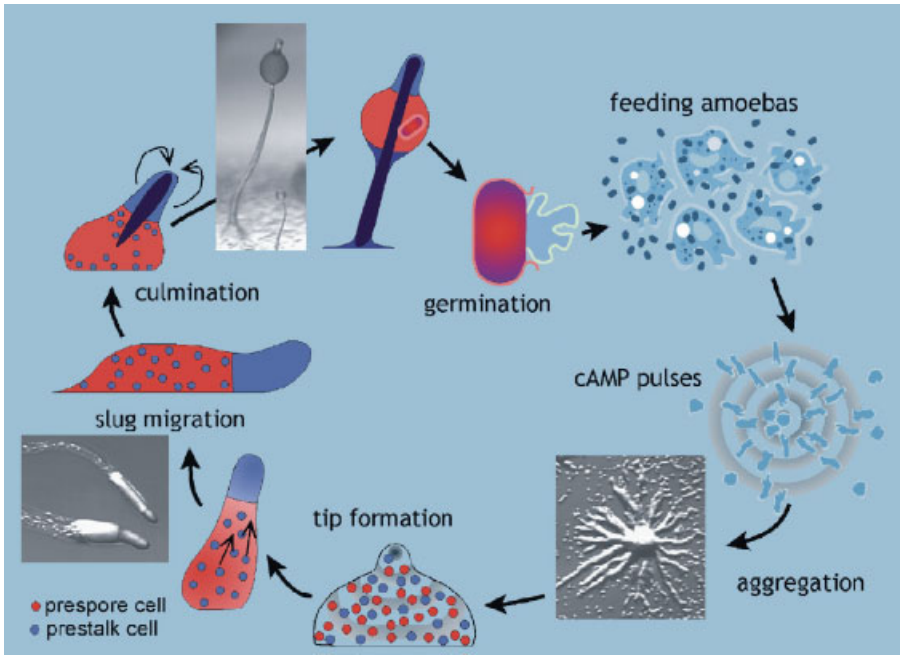

Figure 1. Life cycle of *Dictyostelium discoideum*. In the model organism *D. discoideum*, starving amoebas secrete cAMP pulses, which trigger chemotactic movement and aggregation of cells. Once aggregated, the amoebas differentiate into prestalk and prespore cells in a regulated ratio. The organizing tip continues to emit cAMP pulses, which shape the cell mass by coordinating cell movement. The cAMP pulses also cause the prestalk cells, which are chemotactically most responsive, to move towards the front. At the onset of culmination, the cells synthesize a cellulose tube, the apical prestalk cells move into the tube and mature into stalk cells, the remaining prestalk cells form support structures, such as the upper and lower cup and the basal disk. The prespore cells move up the stalk and mature into spores.

The signaling repertoire of the Dictyostelia is rather different. The model species, *D. discoideum* makes extensive use of cyclic AMP (cAMP), the ubiquitous intracellular messenger for hormone action in vertebrates. In *D. discoideum*, cAMP not only mediates the effect of a number of external signals, but is also secreted to act as a chemoattractant and inducer of cell differentiation.[12] Secreted peptides trigger maturation of spores, but are detected by sensor-coupled histidine kinases.[13] Polyketide-based metabolites and adenine-based cytokinins are secreted to regulate cell-type proportioning and spore germination respectively.[14,15]

Animals, plants and Dictyostelia evolved from different unicellular ancestors, supposedly the filter-feeding choanoflagellates for animals,[16] green algae for plants[17] and solitary amoebas for the Dictyostelia.[18] These unicellular progenitors used sensory signaling to monitor their environment and to find food and mates. The different developmental strategies that are now used by their multicellular descendants reflect how evolutionary forces differentially selected, duplicated and adapted these environmental sensing mechanisms for increasingly complex communication between cells.

Historical reconstruction as a tool to understand developmental signalling

By acting on alleles that are generated by random mutation, organic evolution is intrinsically opportunistic. It does not create optimal design, but selects combinations of traits that provide the highest probability of reproduction in a specific ecological niche. Consequently, there are no unifying schemes to explain organismal development. The underlying molecular mechanisms only truely make sense in the context of their evolutionary history. What mechanisms were used by the ancestors and how were these mechanisms modified to improve functionality in the better adapted descendants?

The evolution of novel forms in multicellular organisms requires alteration of the developmental mechanisms that shaped the earlier form. Evo-devo, short for evolutionary developmental biology, is a relatively young discipline that sets out to retrace how gene and genome modifications have altered existing developmental mechanisms to produce novel forms. Evo-devo has been predominantly applied to the development of animals[19–21] and, to a lesser degree, of higher plants.[22,23] However, an understanding of development of other multicellular organisms, such as the social amoebas or fungi will equally benefit from this approach.

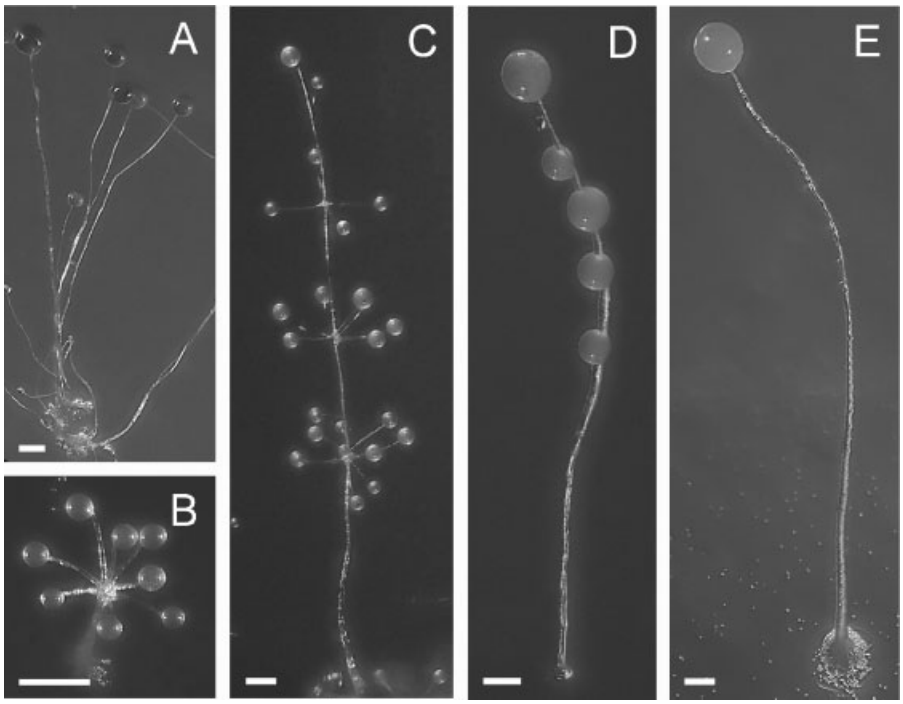

Figure 2. Fruiting body morphologies in different social amoeba species. **A:** *D. vinaceo-fuscum* fruiting bodies show a clustered habit and crampon-like support structures. **B:** *D. polycephalum* displays a coremiform (bunched) habit. **C:** *P. pallidum* fruiting bodies are decorated with regular whorls of side-branches. **D:** *D. rosarium* fruiting body with ancillary sessile sori. **E:** *D. discoideum* fruiting body with supporting basal disc. Bar lengths are 100 μ. Photographs courtesy of Andrew R. Swanson (Manatee Community College) and Frederick W. Spiegel (University of Arkansas).

Moreover, the greater genetic tractability of these organisms will greatly aid in establishing how gene modification caused novel forms to appear. The social amoebas provide other opportunities to retrace the evolution of multicellular development. All known species can be grown under laboratory conditions and complete their multicellular life cycle within a 28 hour period. They show a broad range of different morphologies, with terminal structures varying in size between 0.1 mm and several centimeters. The genome of the model species *D. discoideum* is completely sequenced[10] and sequencing of four other Dictyostelia genomes is in progress.

About 7 years ago, we initiated research into the evolutionary history of developmental signaling in the social amoebas, concentrating on cAMP signalling. A primary requisite for this project was the availability of a family tree that shows the relatedness of social amoeba species relative to the more ancestral solitary amoebazoans. We joined forces with the teams of Sandra Baldauf, an expert in protist molecular phylogeny, Thomas Winckler, who had already prepared an SSU rRNA tree of a subset of Dictyostelia species and two *Dictyostelium* field biologists, Jim Cavender and Hiromitso Hagiwara, to construct a molecular phylogeny of all known Dictyostelia.[24] We next mapped morphological traits of all species to the tree in order to determine the directionality of morphological evolution in the social amoebas. In parallel studies, the presence, regulation and function of genes that are essential for various aspects of cAMP signalling were investigated in social amoeba species that span the phylogeny.[25]

This review presents a synthesis of the outcome of these studies with current insights in developmental signaling in the model species *D. discoideum.* It highlights major trends in the evolution of multicellular complexity in the social amoebas, and correlates these trends with elaboration of function of deeply conserved cAMP signalling genes.

Molecular phylogeny and evolution of morphology in the social amoebas

In traditional systematics, the Dictyostelia were grouped with the acrisid amoebas in the division of mycetozoans in the kingdom of fungi.[3,26] However, recent molecular evidence shows that none of these groups are fungi; the acrasid amoebas are members of the discicristates, while the Dictyostelia and the mycetozoans are members of the amoebozoans.[1,18,27,28] Based on fruiting body architecture,

the social amoebas were subdivided into three genera: the dictyostelids with unbranched or laterally branched fruiting structures, the polyspondylids with regular whorls of side branches and the acytostelids with acellular stalks.

Comparison of conserved DNA or protein sequences is a more direct and reliable method to establish genetic relationships. Two family trees of the social amoebas were constructed by comparing the DNA sequences of their small subunit ribosomal RNA (SSU rRNA) gene on one hand and the amino-acid sequences of their α-tubulin protein on the other. Both trees show that the similarities in fruiting body architecture only partially reflect an underlying genetic similarity.[24] Instead, both the α-tubulin tree and the SSU rRNA tree shown here (Fig. 3) subdivide the 75 known species of social amoebas into four major groups. There are dictyostelids in all four groups. The acytostelids and all white polysphondylids are members of group 2, but the purple polysphondylid *P. violaceum* occupies a position between groups 3 and 4, and forms a small clade with the dictyostelid *D. laterosorum*. This indicates that at least two out of the three previously proposed genera are polyphyletic. Multiple origins for the polyspondylids were also predicted by a family tree that was based on 18 combined morphological traits.[29]

The DNA-based family tree was subsequently used to investigate trends in the evolution of morphology. The multicellular stages of different species of Dictyostelia show a large variety of shapes and sizes, which have been carefully quantitated and noted in the original species diagnoses, along with differences at the cellular level (see Refs 3,30 for overviews). Species use different chemoattractants and aggregate as single cells or as inflowing streams (Fig. 4). Once formed, aggregates may either produce one or several organizing tips, giving rise to solitary or clustered fruiting bodies. Secondary tips may appear in characteristic positions on rising sorogens, giving rise to secondary body axes and a range of different fruiting body architectures. Fruiting body stalks may develop a variety of support structures, such as discs, crampons or triangular supporters, while their tips can vary from thinly pointed to bulbous. Many species form motile slugs, which may optionally form a stalk while migrating. At the cellular level, spores can be round or oblong and, in the latter case, display conspicuous granules at their poles, which are either loosely grouped or consolidated. Some species have retained the ancestral survival strategy of encystation, or display the capacity to mate and form sexual macrocysts.

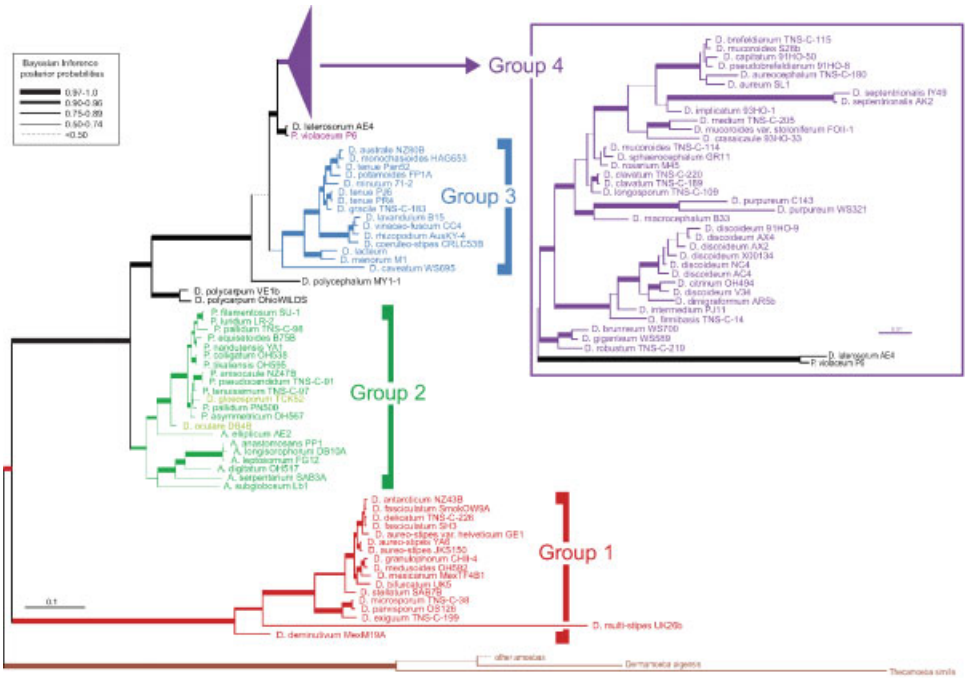

Figure 3. The family tree of the social amoebas. The phylogenetic tree of the social amoebas, based on conserved SSU rDNA sequences from 1655 aligned positions, was constructed by Baldauf and coworkers,[24] using Bayesian inference. The tree shows subdivision of nearly all known species of *Dictyostelium* (D.), *Polysphondylium* (P.), and *Acytostelium* (A.) into four major groups, which are indicated by separate colors. Separate analyses were conducted on group 4 sequences with an additional 300 nucleotide positions that were more divergent. The tree is rooted on SSU rDNA sequences of closely related solitary amoebas. (Previously published in Schaap P, Winckler T, Nelson M, Alvarez-Curto E, Elgie B et al. 2006 Science 314:661–663 with permission of *Science*).

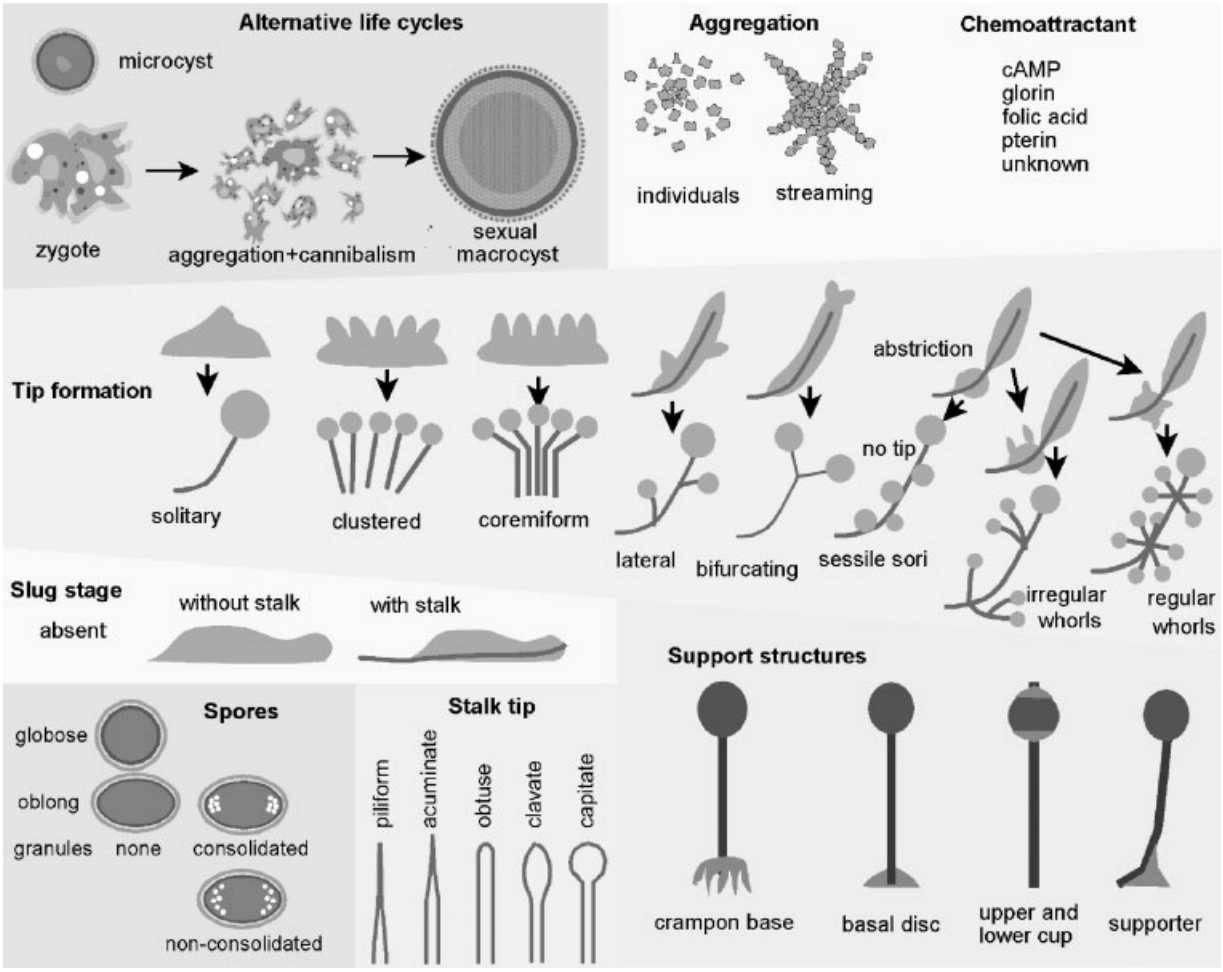

Figure 4. Phenotypic variation in the social amoebas. Cartoon representation of morphological and behavioural variation at the cellular and organismal level in social amoeba species.

A mapping of all these characters to the molecular tree indicates which characters are shared between close relatives, and yields information about the order in which characters evolved (Fig. 5). Amoeba size shows no strong group-specific trend, but spores are consistently smaller in the most basal group 1. Of all traits, spore morphology follows the phylogeny most strongly. Spores of the evolutionary youngest group 4 species have no polar granules, in group 2, polar granules are loosely grouped, and in groups 1 and 3, they are consolidated. The acellular stalk evolved only once. The shape of the stalk tip also marks relatedness; species in group 2 usually have pointy or blunt tips, while in groups 1 and 4 stalk tips tend to be club- or head-shaped. Species with similarly coloured stalks and spore heads are often related, but no color is specific for any of the four major groups.

Encystation of individual cells is lost from group 4 species, but retained in the evolutionary older groups, while sexual macrocysts are made by species scattered over all four groups. The chemoattractant that is used for aggregation is known for only a few species. It is cAMP for all investigated group 4 species while, in the other groups, at least three other compounds are used. Most species aggregate as inflowing streams of amoeba, but groups 1–3 contain some species that aggregate as individuals. Slug migration also occurs in all groups, but is most common in group 4. Stalkless migration is shown by a small cluster of group 4 species and a single non-group 4 species, *D. polycephalum*. Fruiting structures of most but not all species throughout the phylogeny veer towards light (phototropism).

Fruiting bodies (sorocarps) tend to be clustered or grouped in groups 1 to 3 and solitary in group 4, while branched structures are also more common in the basal groups. Specific branching patterns do not show strong group-specific trends and laterally branched, rosary-type and whorled morphologies

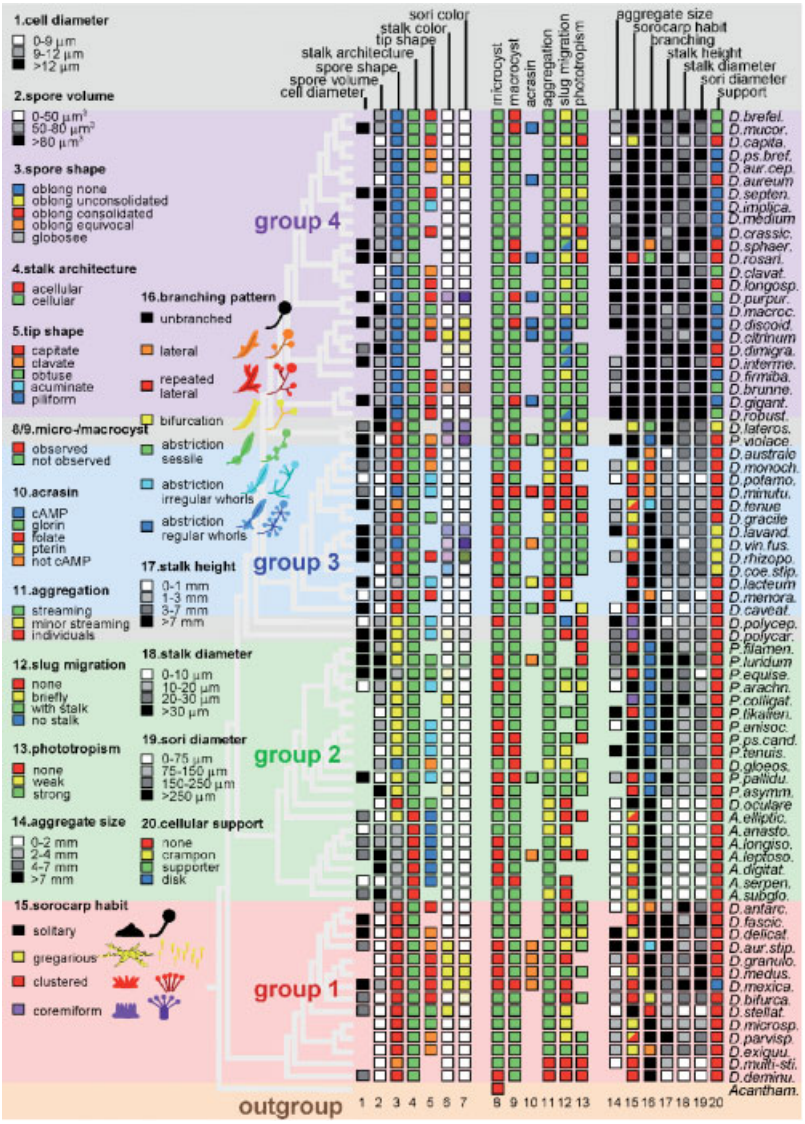

Figure 5. Trends in character evolution in the social amoebas. 20 characters that were most-consistently noted in the original species diagnoses and species monographs[3,30] were mapped onto the SSU rDNA phylogeny, here represented as a cladogram. The code key for the character states is shown on the left side of the figure. (Previously published as online material in Schaap P, Winckler T, Nelson M, Alvarez-Curto E, Elgie B et al. 2006 Science 314:661–663 with permission of *Science*).

appear, respectively, six, two and five times across the tree. There is a modest trend towards taller sorocarps in the more-derived members of groups 1–3, and a very strong trend towards sorocarps with long thick stalks and large spore heads (sori) in group 4. These large sorocarps are usually buttressed by cellular support structures, such as basal disks and supporters. The crampon-base is almost uniquely associated with a tight cluster of group 3 species.

In summary, the most-obvious trend in the evolution of social amoebas appears to be related to size. Evolutionary younger species both have larger sized spores and larger sized fruiting bodies. The latter is particularly evident in group 4 where large stalk and sori size is correlated with a tendency to form solitary and unbranched fruiting bodies. In addition to large size, group 4 displays other distinguishing features, such as formation of cellular support structures, loss of individual encystation, loss of spore granules, and the use of cAMP as attractant. The correlation of the latter two traits was also noted earlier by Traub and Hohl.[31] These workers also associated the presence of polar granules with a tendency to form clustered and/or branched sorocarps and a tendency for those sorocarps to be smaller than in species without polar granules, both of which are borne out by the recent analysis.

The adaptive advantage of larger spores and fruiting bodies can be surmised. Larger spores may store more nutrients to survive dormancy, while larger fruiting bodies may aid spore dispersal, both contributing to species propagation. Individual encystation may have become redundant after the sporulation mode of survival became more robust. It is less easy to envisage how loss of spore granules and use of cAMP as

attractant improved fitness. In the following paragraphs, we explore how the latter character may have been a means to achieve an end.

Evolution of developmental signalling

The appearance of new morphologies in multicellular organisms requires alteration of existing developmental pathways. In the social amoebas, developmental pathways have only been studied in detail in the model organism *D. discoideum,* where cell-to-cell communication is largely mediated by secreted signaling molecules.

cAMP plays a primary role; it is secreted in periodic waves by aggregation centres to mediate the aggregation of starving cells.[32] Later, organizing tips become the sources of cAMP waves (Fig. 1), which direct the movement of cells in multicellular structures.[33] Secreted cAMP also triggers the differentiation of the prespore cells.[34,35] In turn, the prespore cells secrete a chlorinated polyketide, DIF, that induces regulated redifferentiation of prespore cells into prestalk cells.[14] Ammonia, which is produced by protein degradation in the starving cells, represses terminal spore and stalk cell maturation during slug migration,[36,37] and is implicated in cell sorting, slug phototaxis and fruiting body phototropism.[38,39] Two secreted peptides, conditioned medium factor (CMF) and prestarvation factor (PSF) induce the growth to development transition.[40] Other peptides, the spore differentiation factors (SDFs), trigger the maturation of spores.[13]

There is no information on the conservation of the peptide signals in other social amoeba species. Ammonia is always produced by starving amoebas, and at least some of its roles are therefore likely to be conserved. DIF was identified in another group 4 species, *D. mucoroides*, which also has the DIF-degrading enzyme, DIF dechlorinase. *D. minutum* and *P. violaceum,* which reside in group 3 and between groups 3 and 4, respectively, synthesize chlorinated factors that induce stalk cell differentiation in *D. discoideum*. However, they do not have DIF dechlorinase, indicating that the DIF signaling pathway is at best partially conserved.[41,42]

More information is available on the conservation of cAMP signalling. All tested group 4 species use cAMP to aggregate, but no species outside group 4. However, early biochemical work showed that species that use other attractants to aggregate, nevertheless display cAMP binding sites and cAMP phosphodiesterase on their cell surface after aggregation.[43,44] The group 3 species, *D. minutum*, aggregates by continuous release of folic acid, but shows cAMP waves emerging from the tip region after aggregates have formed.[43,45] This suggested that the role of the tip as a pacemaker of cAMP waves is deeply conserved in the social amoebas.

Cell surface cAMP receptors (cARs) mark the use of cAMP as an extracellular signal. *D. discoideum* has four homologous cARs. cAR1 is expressed shortly after starvation, while cAR3, cAR2 and cAR4 are expressed at progressively later stages.[45,46] Recent studies show that the cAR1 gene is deeply conserved in social amoeba evolution and is present in all four taxon groups.[25] The gene duplications that gave rise to cAR2, cAR3 and cAR4 only occurred in group 4 (Y. Kawabe and P. Schaap, unpublished results). The basal cAR1s are functionally identical to *D. discoideum* cAR1, but there is a marked difference in their developmental regulation. The cAR1s from the basal groups are expressed as a single mRNA after aggregation while, in group 4 species, a second cAR1 mRNA is expressed before and during aggregation.[25] Transcription of this early mRNA is driven by a second promoter that is more distal from the cAR1 coding sequence than the promoter that drives expression after aggregation.[47] Also in the gene encoding the extracellular cAMP phosphodiesterase, the promoter that drives late expression is proximal to the coding sequence and the promoters that drive expression before and during aggregation are more distal.[48] This arrangement suggests that the use of cAMP as chemoattractant by the evolutionary younger group 4 species was achieved by addition of distal promoters to existing cAMP signalling genes.

Loss of cAR1 function in group 4 species blocks aggregation and further development. In the basal groups 1–3, aggregation is unaffected, but the subsequent formation of slugs and fruiting bodies is disrupted.[25] This indicates that, in the more basal species, extracellular cAMP signalling is required for slug and fruiting body morphogenesis.

cAMP signaling and size regulation

In addition to using cAMP for aggregation, group 4 species also stand out by having large solitary unbranched fruiting structures, as opposed to the clustered and branched smaller structures that are common to the other groups. Are cAMP signalling and size related?

The segmentation of aggregates into clusters of fruiting bodies and the formation of side branches all represent the formation of multiple body axes that are initiated by newly emerging tips (Fig. 4). Analogous to the phenomenon of apical dominance in plants, where lateral shoots are suppressed by the primary shoot,[49] *D. discoideum* tips suppress the formation of ancillary tips. *D. discoideum* tips are self-organizing pacemakers for cAMP waves. The waves are propagated through the cell mass by cAMP-induced cAMP production, also known as cAMP relay.

Tip dominance can be established in different manners: (1) higher frequency oscillators entrain cells that oscillate at lower frequency,[50] and (2) tips produce a diffusible inhibitor that reduces the excitability of surrounding cells.[51] The cAMP hydrolysis product adenosine was proposed to fulfill this role.[52,53] Dominance will break down if there are physical or biochemical barriers that prevent propagation of cAMP waves or diffusion of the inhibitor.

Irrespective of the exact mechanism, dominance is intrinsic to oscillatory cAMP signalling. Group 4 species have larger fruiting bodies because their cAMP oscillators are better at suppressing competitors. Two aspects of cAMP signalling are specific to group 4: (1) oscillatory cAMP signalling occurs much earlier in development than in groups 1–3, and (2) the cAMP receptor gene was duplicated three times, and both expression and affinity of the daughter cARs were altered. It is conceivable that either of these novelties may have "improved" cAMP signalling to allow it to control larger numbers of cells and make it generally more robust.

The plasticity of fruiting body architecture

The DNA-based phylogeny of the social amoeba did not reproduce the earlier classification into three genera that was based on fruiting body architecture. In fact, it appeared that many similar fruiting body branching patterns evolved several times independently (Fig. 5). This implies that specific architectures cannot be under extensive genetic control.

As discussed above, fruiting body branching patterns reflect how and when competing pacemakers for cAMP waves appear on multicellular structures. The production of cAMP waves by *D. discoideum* cells consists of a positive feedback loop where extracellular cAMP acting on cAR1 stimulates further cAMP production by adenylyl cyclase A (ACA), and a negative feedback loop where cAMP inhibits ACA and stimulates its own hydrolysis.[54–56] Variation in a range of parameters, such as the relative expression levels of the component proteins, diffusion or cell movement barriers generated by structural components, and the relative motility or cohesiveness of responding cells can potentially affect the dynamics of the signalling process in a such a way as to allow competing pacemakers to arise in a variety of configurations.

For instance, *D. gloeosporum*, which owes its name to its extremely sticky spore matrix,[57] is the single dictyostelid member of the clade of white polysphondylids (Fig. 3). The branched whorls of the polysphondylids are formed when a group of cells detaches from the rear of a rising cell mass and then forms new tips (Fig. 4). *D. gloeosporum* may owe its consolidated single spore head to the fact that, due to the highly adhesive matrix, detachment of cell masses does not occur. Similarly, other fruiting body architectures are likely to result from interaction of the cAMP signaling network with different biophysical environments, rather than being controlled by architecture-specific genes.

Does branching have any adaptive value at all? I believe it does. Within groups 1–3 there is a trend towards taller fruiting bodies in the more-derived species. Being carried in the air on tall stalks may not only aid spore dispersal but also contribute to spore preservation, away from the decomposing agents in wet humus. However, the construction of a robust stalk comes at a cost of reducing the spore-to-stalk ratio. Group 4 species resolve this problem by additional cell-type specialization to form support structures. For the basal groups, branching and particularly whorl formation may provide a solution for the problem of building tall well-balanced fruiting bodies without sacrificing too many spores.

Conclusions

This review summarizes the recent construction of a systematic framework to study causal relationships between genotypic and phenotypic change during evolution of the social amoebas. The foundation of this framework is the first DNA-based phylogeny for all known species of social amoebas. The phylogeny, which is based on SSU rDNA sequences and confirmed by α-tubulin protein sequences shows subdivision into four major groups and a molecular depth that is equal to that of all animals.[24]

A plotting of the most consistently noted species characters onto the phylogeny shows unexpected trends in character evolution with the greatest changes occurring at the transition between the youngest group 4 and the evolutionary older groups 1,2 and 3. Group 4 species are characterized by large solitary and unbranched fruiting structures as opposed to smaller, clustered and branched structures in the other groups. Group 4 species have also lost the ancestral survival strategy of encystation and gained the use of cAMP as chemotactic signal for aggregation.

A study into the evolutionary origins of extracellular cAMP signalling revealed that this strategy is used by all social amoebas to coordinate the process of fruiting body formation. Group 4 species have recruited this mechanism to additionally control the aggregation process. This occurred by adding aggregation-specific promoters to existing cAMP signalling genes.

Many intriguing questions remain unresolved. Social amoebas are the only known organisms that use cAMP as extracellular signal. How did this role of cAMP originate in the first place? cAMP signals are produced by oscillating pacemakers. Are these dynamics unique for cAMP or are other chemoattractants, such as glorin, also released in an oscillatory manner? Are other *D. discoideum* signal molecules, such as DIF, ammonia, SDF, PSF and CMF conserved throughout the phylogeny?

Thus far, only those features were plotted to the phylogeny that were observed by standard light microscopy. One cell-associated character, the presence of granules in spores proved to be the strongest group-defining determinant. This suggests that there are other characters at the cellular level that define species within groups. More detailed (ultra)microscopic analysis would be required to identify such features.

In *D. discoideum*, the proportion of prespore and prestalk cells in slugs are regulated to the approximate proportions of stalk and spores in the fruiting body. However, in other species such as *P. violaceum, P. pallidum* and *D. lacteum,* cells first differentiate into prespore cells only to dedifferentiate into

stalk cells at the tip.[58,59] Apart from stalk cells and spores, *D. discoideum* has three more cell types, the basal disc, upper cup cells and lower cup cells, that each display specific patterns of gene expression. When and how did cell-type proportioning and greater cell-type specialization evolve?

In addition to these development-related aspects, the molecular phylogeny provides a framework to investigate conservation and divergence of any protein with an important function in the cell biology of *D. discoideum.* This is useful for identification of conserved domains and/or amino-acids in proteins that are thus far not well characterized, but also to outline how protein modification gave rise to novel protein functions. Projects are now in progress to sequence the genomes of at least four group-representative social amoeba species. Combined with detailed information of phenotypic evolution, this information on the evolution of genotype will provide tremendous opportunities to retrace how this particular form of multicellular life evolved.

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