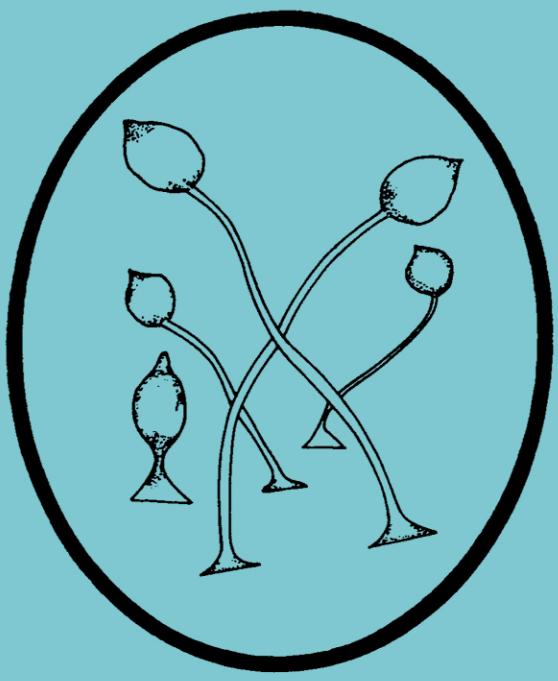
P. Cappuccinelli and J.M. Ashworth
(editors)



Development and Differentiation in the Cellular Slime Moulds

Developments in Cell Biology, Volume 1

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Development and Differentiation in the Cellular Slime Moulds

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EDITORS' PREFACE

Most international conferences are either organised around an academic discipline (Biochemistry, Genetics, Ecology etc.) or a research problem (Control of Gene Action, Management of Tidal Estuaries etc.). The Conference we organised in Sardinia from 12 April - 16 April was unusual in that it was restricted to a single group of organisms, the cellular slime moulds; indeed for most of the time to one particular representative of this group, Dictyostelium discoideum.

We thought that it would be of interest, both to those who work directly on these organisms and to those whose interests are in disciplines such as Developmental Biology or Mycology, to see how the whole variety of techniques of modern biological research can be brought to bear on a single small group of organisms and to see to what extent the whole of this effort was more than the sum of its parts. Reading these contributions and listening to the participants at the conference, there is no doubt in our minds that this kind of venture is both rewarding and very worthwhile for those who take part. The participants at Sardinia were, however, experts in the sense that they had all either worked with the cellular slime moulds or knew the basic biological facts about these organisms. We see it as our task here to describe sufficient of these basic facts to enable others, less expert than those for whom the Conference papers were initially intended, to profit, as we have done, from this published record. Others have attempted this task before us and for a fuller account Bonner's classic monograph [1] or Loomis' more recent account of Dictyostelium discoideum [2] should be consulted.

Dictyostelium discoideum is by far the most studied and the best known of the cellular slime moulds and its life cycle is represented in Figure 1.

The eliptical spore (~6μm long) germinates when placed in a medium containing amino acids or following a short heat shock, producing a uninucleate, haploid (n = 7) amoeba, which is sometimes called a myxamoeba to distinguish it from other amoebae. The slime moulds are soil organisms and, in the soil, the amoebae feed on bacteria. In the laboratory it has proved possible to obtain, by mutation, so-called axemic strains which will also grow in simpler media containing yeast extract, peptone and salts. When feeding on bacteria one amoeba eats about 1000 bacteria at 22°C (the optimum temperature for growth) before dividing by binary fission.

This growth and division cycle continues for as long as nutrients are provided but, when removed from nutrients, the second, aggregation stage occurs. On a

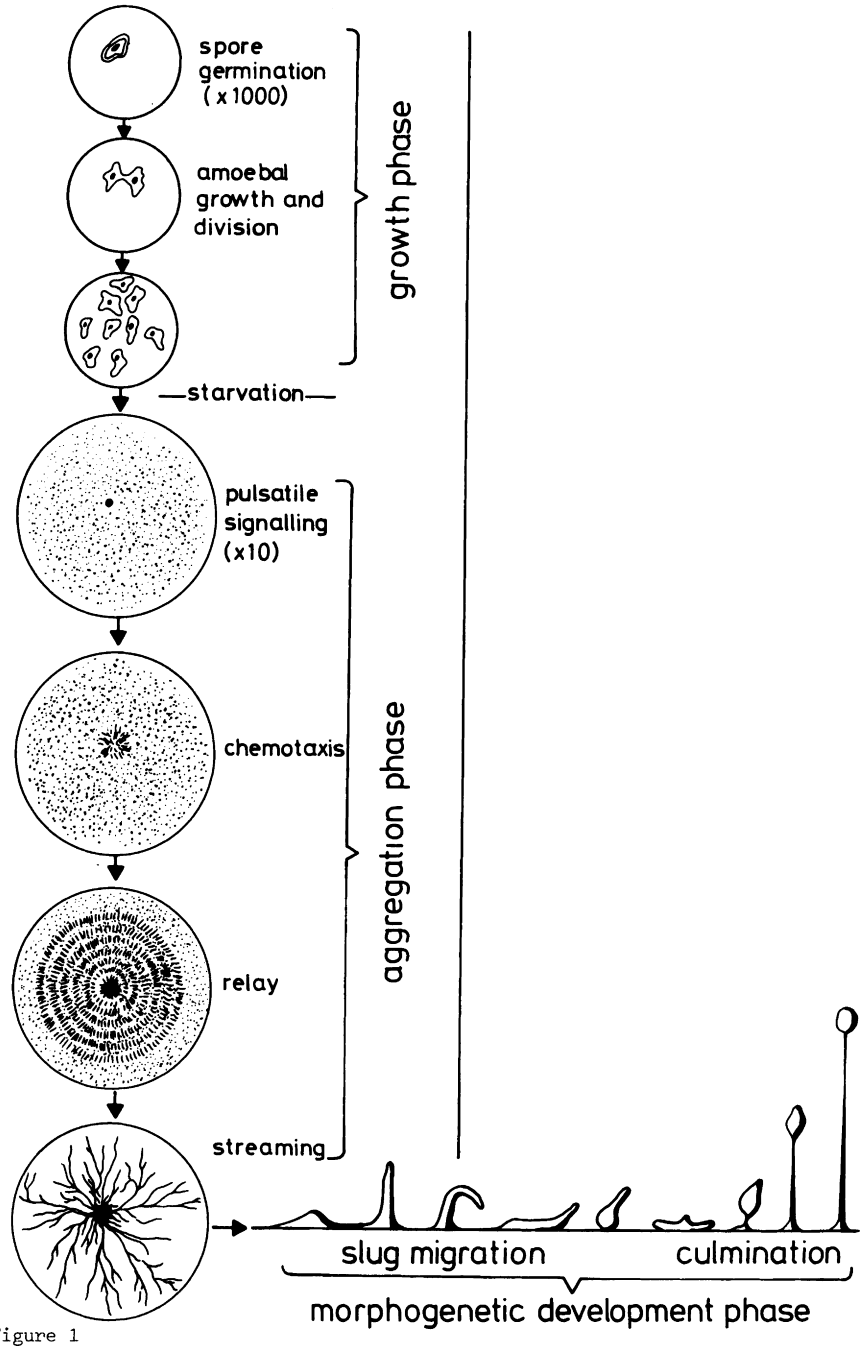

Figure 1

Life cycle of Dictyostelium discoideum (Reproduced, with permission, from Newell[3])

solid surface (which is usually agar but can be plastic or glass as long as the cells are not allowed to dehydrate) the amoebae are seen to move, initially at random. Within a few hours, however, autonomous aggregation centres develop which consist of one or more cells from which chemotactic signals originate. The chemotactic substance was called "acrasin" (since the botanists have given the taxonomic name Acrasiales to the cellular slime moulds) and in the case of Dictyostelium discoideum, but not all other species, acrasin is known to be 3', 5' - cyclic AMP. The cyclic AMP is known to be produced discontinuously in a pulsatile fashion by these centres and the responding cells are seen to move in a correspondingly discontinuous manner towards regions of highest cyclic-AMP concentration.

Amoebae which have just finished feeding also respond chemotactically to folic acid and some of its derivatives. Their responses to these compounds, although not so well studied, resemble in many respects their responses to cyclic - AMP. However, since the threshold for a chemotaxic response to folic acid increases as the cells are kept without nutrients whilst the threshold for the cyclic - AMP mediated response declines it seems likely that the response to folic acid represents a mechanism whereby amoebae "hunt" bacteria and the response to cyclic - AMP represents a mechanism whereby amoebae attract one another.

After they have sensed the cyclic-AMP pulse, responding cells move towards the aggregation centres, act as secondary sources of the cyclic-AMP signal itself (relay) and are unable to respond to another pulse for a time known as the refractory period. In the early stages of aggregation what is seen (at say X 10 magnification) is thus a rhythmical movement of cells towards a common centre which, due to the combined action of the relay and refractory properties of the cells, often takes the appearance of concentric circles of moving and stationary cells. Occasionally single or double spirals are produced and petri dishes in which some areas contain concentric circles and others spiral structures have great, if transient, beauty. This inherent beauty is certainly part of the reason why this early aggregation phase has been so intensively studied but there is also the obvious analogy with other morphogenetic processes, such as gastrulation, which involve concerted and co-ordinated cellular movements. At least in the early stages, aggregation in the slime moulds is a two dimensional process and the path of every cell can be plotted exactly and recorded on film. Analysis of such films both of wild type strains and of mutants deficient in various aspects of the aggregation process has enabled the times of the various periods (movement, refraction etc.) to be determined and provided a wealth of experimental data against which various biochemical and cell biological descriptions of the process can be judged. The beautiful early aggregation patterns soon break up as cells adhere one to another and form three-dimensional "streams" of cells. The typical

dendritic aggregation stream (as seen in Figure 1) can be up to a centimetre or more in diameter and contain half-a-million cells.

As cells arrive at the aggregation centre they force those there initially up above the sruface and become encased in the slime sheath which covers the developing aggregate. A critical stage in the next, morphogenetic development, phase is the appearance, on the top of this structure of a definite "tip".

If amoebae are placed not on a moist solid surface, but gently shaken in a dilute salt suspension then they undergo many of the changes seen in the aggregation phase. Cyclic-AMP is produced by such cells and they also respond to externally applied pulses of cyclic-AMP by changes in shape which can be detected as changes in light absorption or scattering. Depending on the rate of shaking cell clumps of varying size can also be formed and in some circumstances differentiation of the component cells of these aggregates into spore and stalk-like cells has also been observed. However, it seems that an air/water interface is needed for tip formation and it is this tip which defines the polarity of the cell aggregate and thus plays, or appears to play, a key role in the true morphogenesis which is the next development stage after aggregation.

When all the cells in the aggregate have reached the centre a finger-like structure is produced called the slug or grex. The subsequent fate of this slug depends critically on the environmental conditions. Under "normal", standard laboratory conditions of humidity, pH and ionic strength the slug bends over and begins to migrate, tip cells leading, towards light sources, down humidity and up temperature gradients. (These properties are such that, if one imagines aggregation to occur in the interstices of the soil particles, then the slug will move to the soil surface). If, however, the ionic strength and the pH are both rather high then no migration occurs and, conversely, if the ionic strength and the pH are both rather low then the subsequent, "culmination", phase does not occur.

After a period of time (which is experimentally variable) slug migration stops, the tip and front cells of the slug round up and force their way through those cells which were at the back of the slug. As they do so these cells become encased in a cellulose sheath which thus forms a stalk up which those cells which were at the back of the slug move and, to a degree, are forced by the vacuolation of the stalk cells. These cells are characterised by the possession of a pre-spore vacuole (PSV) which contains carbohydrate material representing the precursors (probably) of the spore wall. These PSV structures develop quite early in the slug stage and their presence is characteristic of a pre-spore cell.

When the developing fruiting body is about half its final height these pre-spore cells also begin to synthesise a characteristic, yellow pigment (some other species have different colours e.g. D. purpureum has purple spore masses). The final fruiting body thus consists of a lemon coloured, and lemon shaped, spore mass borne aloft on a stalk which is typically some 2-5 mm high. The whole structure is easily visible to the naked eye. The spore mass contains, in addition to the spores and spore pigments, at least two compounds which specifically inhibit spore germination. These germination inhibitors are readily water soluble and, on the surface of the soil, one can imagine the fruiting body being hit by rain drops and the spores being carried, in the soil water, to areas where more bacterial food may be plentiful. The spores themselves are much more resistant to a number of environmental stresses such as desiccation and heat than are the amoebae and the whole of this life cycle can be rationalised in terms of a dispersal mechanism whereby the amoebae move from an area where they have just eaten all the available food, across environmentally inhospitable areas, to other places where bacteria may be plentiful.

There is no necessary connection between this life cycle and any process of genetic recombination but, in fact, parasexual recombination of genetic markers can be demonstrated to occur during the process of spore formation if two genetically distinct populations of amoebae are mixed initially. This parasexual cycle is the one most used for genetic investigations so far but there also exists a sexual process whose occurrence is controlled by a mating type locus such that recombination only occurs between strains of different mating type (+, -).

If two strains of different mating type are mixed, under water in the dark, then large assemblages containing several hundred cells, are produced. Fusion of two haploid cells occurs and the resulting diploid cell engulfs all others and, at the same time, the developing macrocyst (as this structure is called) secretes about itself a thick cellulose cell wall. The diploid cell then undergoes meiosis and the daughter haploid cells are released following germination of the macrocyst. Unfortunately this germination process is extremely inefficient in the case of D. discoideum and this has prevented the development of genetic analysis using meiotic recombination.

The life cycle of D. discoideum thus offers a number of experimental advantages to those interested in studying, at the most fundamental level, the basic processes involved in cell differentiation in simple eukaryotes. The spores and stalk cells are markedly different biochemically and structurally, the fruiting body has a form and shape (apparently) every bit as closely determined as the shape and form of a more complex organism and yet the amoebae are truly microbial organisms with all

that that implies in terms of biochemical and genetical convenience. We hope these points will become even clearer in the papers that follow so we will not belabour them here.

We would like to close by thanking our colleagues for the tremendous care and help they have taken in preparing their manuscripts, which has made our task as editors much less arduous than it might have been, to Mr. Paul Taylor and his colleagues at Elsevier for being so prompt in producing this book and to the European Molecular Biology Organisation, the University of Sassari, the Italian Research Council, Sardinian Regional Government and the Sassari Provincial Government who, together, provided us with the money which made it all possible.

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