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Development at the edge of multi-cellularity: *Dictyostelium discoideum*

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Dictyostelium is a social amoeba which grows as separate cells by consuming bacteria, but develops into a genuine multi-cellular organism. Development is triggered by starvation and, in the initial stages, thousands of cells aggregate together by chemotaxis to cyclic AMP. The resulting mound of cells elongates and falls onto its side to produce the 'slug' (Fig. 1) which migrates off in search of a suitable place to fruit. There it produces a small fruiting body, consisting of a cellular stalk supporting a mass of spores.

Dictyostelium discoideum was first isolated in 1935 by Kenneth Raper from forest leaf litter, in North Carolina, USA. Raper, John Bonner and Maurice Sussman were largely responsible for the pioneering work that attracted subsequent workers to the field. It was shown that the migrating slug had a prepattern of prestalk cells in the front and prespore cells in the rear. This pattern was regulative: if either front or rear was cut off, a smaller slug could regenerate and this would produce a properly proportioned fruiting body, with stalk cells and spores. Patterning was also allometric over a vast range of cell numbers, from about 100 to 100,000. Finally, the front of the slug, a small protuberance called the 'tip', had organising properties: when grafted onto the flank of another slug, it could organise a secondary axis, eventually leading to a splitting of the slug into two (Raper, 1940; Bonner, 1967).

Dictyostelium in Britain

The first person to study *Dictyostelium* in Britain, of whom I am aware, was Brian Shaffer in the Zoology Department of Cambridge University. Shaffer started his PhD with Victor Rothschild, who studied sperm, but became interested in *Dictyostelium*, after reading a now-forgotten review. In the free-ranging spirit then more common, Shaffer was allowed to devote his PhD to the organism. Experimental tools were rudimentary and often made by the scientist: Shaffer made his own time-lapse movies using a War-surplus gun-camera from the RAF, modified with Meccano to take one frame at a time. He made early progress in understanding aggregation. He was the first to obtain the chemoattractant (generically called acrasin and later shown by Bonner and co-workers to be cyclic-AMP) in a stable, cell-free form and was working on its identification with Todd's group in the Chemistry Department at Cambridge, the leading nucleotide chemists of the time, before unfortunate circumstances cut short the effort in about 1955. All this was well before the eventual Nobel Prize-winning identification of cyclic-AMP by Earl Sutherland. Shaffer also predicted from simple observations that acrasin would be relayed from cell-to-cell during aggregation and that it would stimulate the development of the cells it encountered. These predictions were confirmed in full detail many years later.

Somewhat later, two post-docs returned from Maurice Sussman's group and set up vigorous laboratories in Britain: Peter Newell in Oxford (where he remains) and John Ashworth in Leicester. Newell was instrumental in establishing parasexual genetics (Newell,

Abbreviations used in this paper: DIF, Differentiation inducing factor.

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Fig. 1. Migrating slugs stained with neutral red. *Neutral red is a vital dye, preferentially accumulated in the abundant lysosomes of prestalk cells. These cells form a coherent prestalk tissue at the front of the slug and also exist in the rear prespore zone, as scattered cells and as a variable 'rearguard zone'. The slugs are 1-2 mm long and contain about 100,000 cells; they crawl through a tube of extra-cellular matrix (unfairly called slime) which they secrete and leave behind themselves as a trail.*

1978) as well as studying the biochemistry of aggregation. Ashworth was a biochemist and in his laboratory Donald Watts (now at Sheffield University) developed the rich medium, which is used to this day for axenic growth of cells (Watts and Ashworth, 1970). David Garrod exploited physiological differences between cells grown in axenic medium, with- and without-glucose, to elegantly demonstrate the existence of vegetative fate biases (glucose$^-$ cells preferentially became stalk cells when developed with glucose$^+$ cells) (Leach *et al.*, 1973). Other workers from Ashworth's laboratory established themselves at Leeds University (David Hames) and at Stirling University (Michael North). Sadly, both Shaffer and Ashworth moved out of research and the next centre of activity formed independently, at the Imperial Cancer Research Funds laboratories at Mill Hill.

By this time (the early 1970's) many of the pioneers of molecular biology, considering that phage was solved, looked to development for new frontiers. True to their experience, they tended to look for simple examples to study and sought to develop genetics. John Cairns returned from Cold Spring Harbor to revitalise the ICRF Mill Hill laboratories. These laboratories were small and on a short lease from the MRC: they only lasted for about 10 years. But in this time they nurtured the careers of a group of young developmental biologists that John had recruited. They were a diverse group, including Bridget Hogan and her student Denise Barlow, who worked on mouse, David Ish-Horowicz and Phil Ingham on *Drosophila* molecular genetics and, working on amphibia, Jonathan Slack (who believed in neither genetics nor molecular biology) and Jim Smith. Julian Gross, a more senior appointment, and former bacterial-geneticist, was recruited from Edinburgh, where he had been working on *Dictyostelium* for a couple of years. He was thankfully joined by Rob Kay (who had been separately struggling to master the organism) and later by Jeff Williams, who dedicated himself to utilising cloning techniques to study developmental gene expression. A photograph of the Mill Hill *Dictyostelium* group from this time is shown (Fig. 2).

It seemed that the key to understanding *Dictyostelium* development was to understand the chemical language by which the developing cells communicate. The serendipitous discovery by Chris Town that amoebae of one particular strain could efficiently form stalk cells when plated on agar which contained cyclic-AMP, provided a new technique for investigating this communication (Town *et al.*, 1976). Though stalk cells would only form in high density populations, a factor diffusing from these cells could penetrate dialysis membrane and stimulate isolated amoebae to also form stalk cells. After almost a decade and the accumulation of factor from about 4,000 litres of axenic cells, the active ingredient, known as DIF, was identified as a chlorinated alkyl-phenone (Morris *et al.*, 1987). Jeff Williams' group isolated genes whose expression was induced by DIF and from these, the definitive markers for prestalk cells were developed (Williams *et al.*, 1987). These markers revealed a key aspect of *Dictyostelium* development: when prestalk and prespore cells first differentiate, they are largely intermingled with each other. Later the prestalk and prespore cells sort out to give coherent blocks of tissue seen in the slug. The patterning mechanism is therefore quite different from the positional mechanism, based on morphogen gradients, that many had expected to find (though there is evidence for such gradients later in development). Another key discovery at Mill Hill was of *Dictyostelium* cyclic-AMP-dependent protein kinase, by Jeff Sampson, which turned out to be a key regulator of development (Sampson, 1977).

Today

With the dissolution of the Mill Hill Laboratories, the *Dictyostelium* groups were scattered. Julian Gross, after adventures outside science, eventually joined forces with Peter Newell at Oxford (Chang *et al.*, 1996), Jeff Williams arrived at Dundee, via the ICRF laboratory at Clare Hall and University College London (Kawata *et al.*, 1997), and Rob Kay at the MRC Cambridge (Thomason *et al.*, 1998). More recently, former post-docs and students from these groups have set up their own laboratories: Catherine Pears (Huang *et al.*, 1997) in Oxford, Adrian Harwood (Williams *et al.*, 1999) and Anne Early (Early *et al.*, 1995) at University College London, Robert Insall (Tuxworth *et al.*, 1997) and Laura Machesky (Machesky and Insall, 1998) at Birmingham. Kees Weijer (Bretschneider *et al.*, 1995) and Pauline Schaap (Kim *et al.*, 1998) have also been recruited to Dundee, from continental Europe, while Mark Bretscher (Aguado and Bretscher, 1997) and John Stirling have been recruited from other fields. A list of currently active groups and their interests is provided at the end of this article. It is noteworthy that the number of such groups has approximately doubled in the last 5 years and that the total number of workers in Britain is now about 60.

In addition to these dedicated groups there have been many part-time workers who found *Dictyostelium* useful for particular purposes. Notable amongst these at the moment are the genomics groups of Bart Barrell at the Sanger Centre and Paul Dear at the MRC Laboratory of Molecular Biology, who are engaged in the *Dictyostelium* genome project.

Regular meetings of the British *Dictyostelium* groups were instigated in the 1970's. These were joyful, beery affairs and gradually expanded by attracting international visitors until they developed into the annual International Dictyostelium Meetings, that continue to this day. However, the ease and informality of the early meetings has not been lost, as they have restarted and are

Fig. 2. The ICRF Mill Hill *Dictyostelium* group in about 1976. *Back row (left to right): David Trevan, Alistair Lax, Julian Gross, Jeff Williams, Mike Peacey. Front row: Jeff Sampson, Eileen Stanford, Jennifer Trent, Rob Kay. The pleasant setting of the laboratories is quite apparent.*

currently organised by Dr. Adrian Harwood, at University College, at around Christmas each year.

The advances in molecular genetics and the genome project have made *Dictyostelium* a very attractive organism with which to work (Kay and Williams, 1999). In *Dictyostelium*, more than in most organisms, the study of development blends into cell biology, biochemistry and signal transduction, greatly adding to the interest. For this reason, in the following list I have not attempted to distinguish laboratories whose primary interest is cell biological, from those more interested in development. Since the field is not over-populated, there is scope for pioneering work and hopes that, given time and effort, definitive answers can be obtained to any question posed of the organism.

Dictyostelium laboratories in Britain and their interests

Mark S. Bretscher: MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB1 2QH. msb@mrc-lmb.cam.ac.uk. Cell motility and lipid flow.

Anne Early: MRC Laboratory for Molecular Cell Biology, University College London, Gower Street, London WC1E 6BT. dmcbaee@ucl.ac.uk. Cell-matrix interactions during development.

Julian D. Gross: Department of Biochemistry, University of Oxford, South Parks road, Oxford, OX1 3QU. gross@bioch.ox.ac.uk. Molecular genetics of development.

B. David Hames: Department of Biochemistry, University of Leeds, Mount Preston St, Leeds LS2 9JT. B.D.Hames@leeds.ac.uk. Gene expression in development.

Adrian J. Harwood: MRC Laboratory for Molecular Cell Biology, University College London, Gower Street, London WC1E 6BT. dmcbadh@ucl.ac.uk. Lithium-sensitive signal transduction pathways including those via GSK-3 regulation and via an unconventional phosphoinositide pathway.

Robert H. Insall: School of Biochemistry, The University of Birmingham, Edgbaston, Birmingham, B15 2TT. R.H.Insall@bham.ac.uk. Chemotaxis and the control of the actin cytoskeleton by signalling. Ras proteins and Ras guanine nucleotide exchange factors (RasGEFs).

Robert R. Kay: MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB1 2QH. rrk@mrc-lmb.cam.ac.uk. DIF signalling and patterning of cells in development. Two-component systems and terminal differentiation.

Laura M. Machesky: Department of Biochemistry, University of Birmingham, Birmingham, B15-2TT. l.m.machesky@bham.ac.uk. Signalling to the actin cytoskeleton via the WASp-family of proteins; how the Arp2/3 complex nucleates new actin filaments in mammalian cells and Dictyostelium.

Peter C. Newell: Department of Biochemistry, University of Oxford, South Parks road, Oxford, OX1 3QU. NEWELL@biochemistry.oxford.ac.uk. Molecular genetics of development.

Catherine J. Pears: Department of Biochemistry, University of Oxford, South Parks road, Oxford, OX1 3QU. pears@biochemistry.oxford.ac.uk. How early events in development (in particular cell cycle position) predispose cells to a particular fate in the fruiting body.

Pauline Schaap: Wellcome Trust Building, University of Dundee, Dow St, Dundee, DD1 5EH. p.schaap@dundee.ac.uk. The role of adenylyl cyclases and cAMP in development.

John Stirling: Molecular Genetics Group, Division of Life Sciences, King's College London, 150 Stamford Street, London SE1 8WA. john.stirling@kcl.ac.uk. Genetic analysis of lysosomal enzyme sorting.

Donald Watts: Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN. Mechanism of action of bis-phosphonate drugs used to treat osteoporosis

Keis J. Weijer: Wellcome Trust Building, University of Dundee, Dow St, Dundee, DD1 5EH. c.j.weijer@dundee.ac.uk. Cell movement and signalling at all stages of development; mathematical modelling of morphogenesis.

Jeffery G. Williams: Wellcome Trust Building, University of Dundee, Dow St, Dundee, DD1 5EH. j.g.williams@dundee.ac.uk. Signalling mechanisms that direct prestalk and stalk cell differentiation, especially the roles of STAT proteins that move to the nucleus in response to cAMP and DIF.

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