Cell fate choice and social evolution in *Dictyostelium discoideum*: Interplay of morphogens and heterogeneities

Attempts to understand the development of the social amoeba *Dictyostelium discoideum* keep throwing up surprises and drive home the point that here too, as in any biological situation, no explanation can make sense except in the light of evolution.

The life cycle of all cellular slime moulds (Dictyostelids) consists of two distinct phases, growth and development. During the period of growth, single amoebae that have germinated from different spores feed on bacteria and go through mitotic divisions until the food supply is exhausted. The developmental phase is triggered and maintained by starvation. Starved cells stop dividing, aggregate via chemotaxis and form a multicellular, motile, 'embryonic' mass, the slug (typically a tapered cylinder of about $1 \text{ mm} \times 50 \text{ }\mu\text{m}$). Within the slug of *D. discoideum* a clear cellular fate map can be discerned (Bonner 1952). Presumptive stalk (pst) cells occupy about the anterior 20% of the slug and presumptive spore (psp) cells the remaining 80% (MacWilliams and Bonner 1979); the long-held belief that the relative proportions of pst and psp cells are tightly regulated has been questioned very recently (Rafols *et al* 2001). Gene expression patterns enable a further division of the pst zone into an anterior pstA region and a posterior pstO region (Williams *et al* 1989), with the cells in the two regions corresponding to different functional classes. Thus, there is a division of labour within the slug – as it happens, in a literal sense too, because the motive force for slug movement is provided by pst cells (Inouye and Takeuchi 1980). After a facultative migratory phase, the slug transforms into a fruiting body comprising a ball of spores atop a dead cellular stalk (Bonner 1967).

How do the members of a seemingly homogenous cell population come to adopt diverse fates? An attractive hypothesis suggested itself early. It was fuelled by three considerations. Firstly, Raper (1940) showed in an elegant experiment that isolated pst or psp fragments can regulate. That is, they restore the missing cell type and go on to form normally proportioned fruiting bodies (albeit only after a period of migration in the case of pst isolates). Therefore, at least under some conditions, intercellular interactions within the slug are necessary for generating both cell types in the correct ratio. Secondly, the fate map in the slug seemed to exhibit an unambiguous anterior-posterior spatial pattern. And thirdly, on theoretical grounds, such a pattern made it attractive to think of a spontaneously arising linear gradient of morphogen concentration as being responsible for the fate map. It appeared natural to assume that an amoeba would eventually become a stalk cell or a spore depending on where it found itself within the gradient. The hope even arose that this might be the long-sought example of a Turing system in biology.

Studies that began over two decades ago made it seem plausible that one or the other member of a family of chlorinated hexaphenones made by developing amoebae could be the morphogen (Town *et al* 1976; Morris *et al* 1987). These were collectively termed DIF for Differentiation-Inducing Factor. DIF could induce stalk differentiation and inhibit spore differentiation. DIF-1, the most potent isoform of DIF, was chemically synthesized and shown to stimulate the expression of pst genes and inhibit the expression of psp genes (reviewed by Kay *et al* 1999). Based on the observation that pstA cells required a 10-fold higher concentration of DIF-1 than pstO cells (Early *et al* 1995), it was thought that varying levels of DIF-1 also induced different pst cell types. Strikingly, and in retrospect misleadingly, a mutant (HM44) that failed to make DIF did not develop beyond the tipped mound stage and formed no stalk cells, even under *in vitro* stalk cell induction conditions (Kopachik *et al* 1983).

But soon the picture started looking murky. For one thing, while there was indeed a spatial gradient of DIF-1 in the slug, it was the wrong way round. Psp cells turned out to contain more DIF-1 activity than pst cells, in fact about twice as much (Brookman *et al* 1987); unpublished results cited in Thompson and Kay (2000b) indicate that DIF-1 may be produced largely by psp cells. The shape of the gradient was later accounted for by the finding that the activity of an enzyme that degraded DIF-1, DIF-1 dechlorinase, was concentrated in the anterior of the slug (Kay *et al* 1993). (The possibility remains open that what is meaningful is the amount of DIF-1 tightly bound to an intermediate in the signal transduction pathway – as opposed to the amount of free DIF-1 – and that its level might indeed

be higher in the pre-stalk region). Another problem was that not all pst genes tested were capable of being induced by DIF-1 (Shaulsky and Loomis 1996). Recent work has gone further: it appears that the primary signal for cell type choice cannot be based on spatial gradients of DIF at all (Thompson and Kay 2000a, b). Rather, the choice of cell type appears to rest on a basis that is quite the opposite of morphogen-dependent spontaneous patterning, namely the existence of functional differences, in the form of pre-existing heterogeneities, between the members of an apparently homogeneous cell mass (Bonner 1952; Takeuchi 1969).

The evidence is growing that DIF- (or DIF-1)-related properties are one among the many indicators of early heterogeneity in *D. discoideum*. An aspect of DIF-1 related heterogeneity was demonstrated by Azhar *et al* (1997) using fluorescence-activated cell sorting (FACS). They monitored the relative distribution of "low calcium" (psp) and "high calcium" (pst) cells in pre- or post-aggregation amoebae and found that DIF-1 stimulation caused an increase in intracellular calcium only in a subset of amoebae. All of these fell in the low calcium or psp class; and, as a result of the calcium increase, entered the putative pst class (the stimulus may have also reinforced the status of pst cells but the experiment was incapable of demonstrating this). This observation raised the possibility that DIF-1 influenced cell differentiation by acting on amoebae that were *already* different in terms of how sensitive they would be to it. That possibility has now been verified by Thompson and Kay (2000a). They show that the manner in which amoebae respond to DIF-1 depends on their prior nutritional status, thereby supporting a conjecture based on an evolutionary argument (Atzmony *et al* 1997). In terms of their tendency to adopt a pst fate, cells that are raised in the absence of added glucose are more sensitive to DIF-1 than cells that are raised in a glucose-rich environment. A parallel pattern of responsiveness to DIF-1 is seen when cells that are starved early in the cell cycle are compared to those starved when late in the cell cycle (Thompson and Kay 2000a).

Astonishingly, it turns out that *D. discoideum* can develop normally – to a degree – in the absence of any detectable DIF-1. Thompson and Kay (2000b) have generated a strain (*dmtA*) lacking the des-methyl-DIF-1methyl transferase gene, the gene that encodes the enzyme which catalyses a key step in the DIF-1 biosynthetic pathway. The resulting DIF-1$^-$ amoebae aggregated with a slight delay in comparison to wild type and the fruiting bodies produced few spores. Though the slugs often broke up or laid down a stalk while migrating, the spatial and temporal expression profile of several cell type-specific genes was similar to that of the wild type. In particular, DIF-1 was sufficient but not necessary for the induction of *ecmA* (pstA cell-specific) gene expression: *ecmA* transcripts appeared not only on schedule but also in the correct location in the DIF-1$^-$ slugs. The absence of DIF-1 had an additional consequence. *dmtA* slugs lacked pstO cells, implying that DIF-1 might have been needed only for the differentiation of this particular pst sub-type. The pstO defect could be restored by supplying exogenous DIF-1 via the agar substratum, suggesting that a spatial gradient of DIF-1 is not essential for normal patterning (explicit measurements will be needed before it can be ruled out that the net intake of DIF-1 varies along the length of the slug).

These results once again raise the question of how cell fate is decided in *D. discoideum*. Inevitably, they re-direct attention towards intercellular heterogeneity as the primary factor behind cell fate choice. Pre-aggregation amoebae can differ in many ways. These include nutritional status, cell size, cell cycle phase at starvation, cellular calcium content (reviewed in Nanjundiah 1997) and, now, sensitivity to DIF-1. All act as inputs that influence the tendency of a cell to become pst or psp, but that tendency or bias can be translated into a decision only in the context of the inputs operating on other cells. Obviously, this means that a cell must be able to communicate with others and be capable of assessing another's bias relative to its own. The point is that intercellular communication-based differentiation and cell-autonomous cell type choice should not be thought of in either/or terms. At times the issue has been addressed in terms of position-dependent fate determination versus fate-dependent position determination. But the dichotomy is a false one (Bonner 1992).

In other words, the determination of cell fate involves a subtle and non-exclusive interplay between cell-autonomous factors and intercellular signalling. Nevertheless, why do most experiments on *D. discoideum* yield results indicating one or the other mechanism? The answer must be sought in the evolutionary history and ecology of Dictyostelid amoebae in general and *D. discoideum* in particular. Let us suppose that the ancestors of modern Dictyostelids evolved as clonally propagating populations – meaning that aggregates were mostly clonal. In that case, one can see how decisions

pertaining to division of labour that were advantageous to the (clonal) group as a whole would be favoured. Such decisions would have to be taken communally and would of necessity involve cell-to-cell signalling. The nature of the signals could be wholly arbitrary, dictated solely by convention. At the other extreme, suppose genetically heterogeneous aggregates were, and are, common in the wild. Then individual-level selection, rather than group selection (at the level of the clonal or kin group), would be expected to operate. The propensity on the part of a cell to adopt one or the other fate would hinge on factors intrinsic to the cell. These factors can be thought of as fitness-related qualities that depended on the cell's genotype as well as phenotype. Of course the factors would come into play only in relation to similar factors in the other cells in an aggregate. The means used by cells, both for communicating their own qualities and assessing the qualities of others, could no longer involve arbitrary signals. Rather, the signals would have to be reliable indicators of quality (Atzmony *et al* 1997). This analysis overlaps with the between-cell versus within-cell distinction drawn by Bonner (1992).

A history of clonal propagation and conventional intercellular signals, or a history of mixed aggregation and intrinsic factors, represent extreme alternatives. A less restrictive hypothesis would be to posit an evolutionary history in which Dictyostelium amoebae were sometimes members of clonal aggregates and at other times members of genetically heterogeneous aggregates. In that case one might think that natural selection would have favoured a mixed strategy wherein an amoeba could, depending on the circumstances, base its behaviour either on arbitrary signals or on signals that conveyed cellular quality. But even if Dictyostelid ancestors had always participated in clonal aggregates, phenotypic heterogeneity would be unavoidable. Individual-level selection would make it more likely that (relatively) high quality amoebae would form spores and (relatively) low quality amoebae would contribute to the stalk.

In the light of these considerations, how might one assess the developmental role of DIF-1? The production of, and sensitivity to, DIF-1 could be just one of the many measures of cellular quality (Atzmony *et al* 1997). In its absence, cells would still be capable of assessing each others' qualities. It is important to stress that what we have discussed pertains to a restricted set of facts. Integrating everything that is known regarding cellular behaviour in *D. discoideum* into a coherent evolutionary picture will be an enormously complex undertaking. Experiments directed towards understanding the natural history of cellular slime moulds should provide us with important clues behind the logic underlying cell fate choice.

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