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Induction of Stalk Cell Differentiation by Cyclic AMP in the Cellular Slime Mold *Dictyostelium discoideum**

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Abstract. Cyclic AMP, which is a cell attractant (acrasin) for *Dictyostelium discoideum*, will cause isolated, unaggregated cells to turn directly into stalk cells containing thick celluloselike walls and large vacuoles. From previous work we know that in the cell mass, acrasin is produced solely in the region of stalk formation during fruiting, that stalk formation involves a high level of catabolism, and that cyclic AMP stimulates catabolic enzymes in other systems. These facts obviously suggest that in the development of *D. discoideum*, cyclic AMP might be a key factor in stalk cell differentiation.

The morphogenetic stages of the cellular slime mold begin with the aggregation of separate, independent amoebae to cell masses which ultimately differentiate into fruiting bodies with two basic cell types: stalk cells and spores. In the species *Dictyostelium discoideum*, the stalk cells are large, vacuolate, dead[1] cells with thick cellulose[2] walls, and are found both within the stalk proper and in the basal disk which surrounds the base of the stalk (Fig. 1).

Previously we have shown that cyclic AMP is a chemotactic agent (or acrasin) normally produced by *D. discoideum* and responsible for the aggregation of the cells.[3] Already, in 1949, I had demonstrated that acrasin was present in cell masses at later stages of development and asked the question of whether or not its presence was related to the process of differentiation.[4] Now, 20 years later, we can definitely answer the question: cyclic AMP does induce the differentiation of amoebae into stalk cells.

Materials and Methods. A haploid strain of *Dictyostelium discoideum* NC-4 (kindly supplied by Dr. K. B. Raper) was grown on *Escherichia coli* on buffered nutrient agar containing 1% peptone and 1% dextrose at 21°C for 38 hr. If the cells were still vegetative at this time, they were washed from the plate and centrifuged at 50 × *g* for two 5-min periods in a 1% physiological salt solution.[5] The tests were run in small plastic Petri dishes (50 × 12 mm—Falcon no. 1006) containing the cyclic AMP and 1% salt solution in 2 ml of 2% Difco agar. The amoebae were placed on the surface of the agar, either in drops or dispersed evenly over the entire surface.

Results. If the agar in the small Petri dishes contains a high concentration of cyclic AMP (10^{-3} *M*), and if the amoebae are placed in different size drops on the surface (3–8 mm in diameter), the cells will begin to move outward[6] and a strong ring will appear somewhere near the middle of this advancing edge.[7]

Twenty-four hours after inoculation, some of the cells in and near the advancing ring turn into separate, and often isolated, stalk cells. By 48 hours, this is

true of a large number of cells (Fig. 1). In controls with no cyclic AMP in the agar, no such isolated stalk cells are formed, but, instead, the vast majority of the cells aggregate and form normal fruiting bodies. The few remaining cells that fail to aggregate never differentiate into stalk cells. The timing of the formation of stalk cells is about the same in the experimentals and the controls.

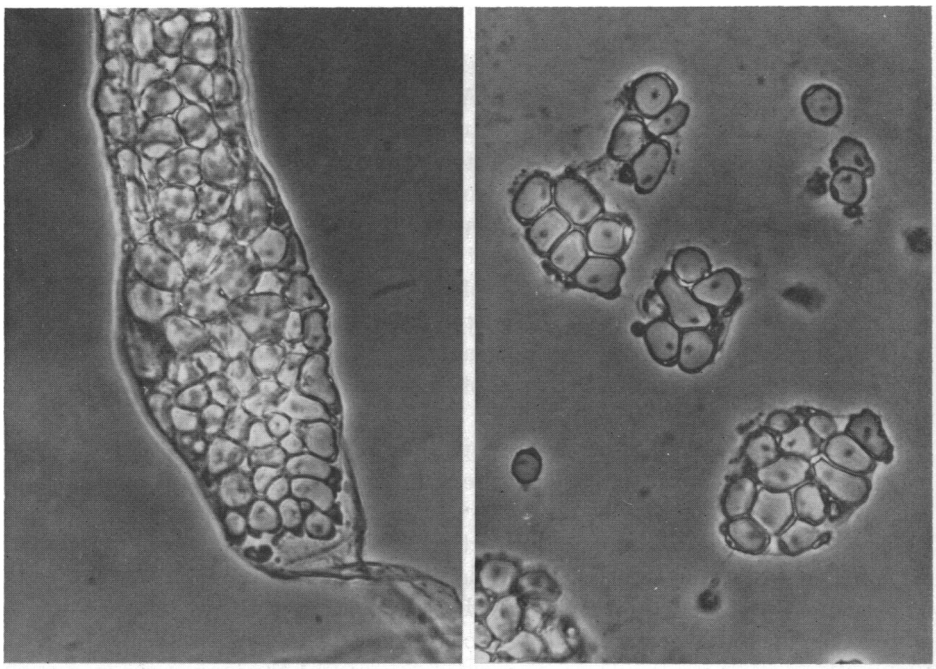

FIG. 1.—*Left:* A phase-contrast microphotograph of the base of a normal stalk on 2% agar. Reading upwards one first sees the slime sheath, then a flat basal disk, and at the top, one can see the stalk itself partially surrounded by the disk.

Right: A group of artificially induced stalk cells on 2% agar containing 10^{-3} *M* cyclic AMP.

Both photographs are the same magnification and both were taken 48 hr after the amoebae were placed on the agar. Magnification: rounded single cell in the upper righthand corner is *ca.* 10 μ in diameter.

The same result can be obtained by spreading the amoebae in a sparse, even layer over the surface of the agar containing 10^{-3} *M* cyclic AMP. If the amoebae density is sufficiently low, the amoebae will not aggregate, and between 1 and 50 per cent of the isolated cells will differentiate into stalk cells, while none will appear in the controls lacking cyclic AMP.

It is also possible to induce stalk cells with cyclic AMP at higher densities of amoebae by adding inhibitors which interfere with normal development. If 10^{-3} *M* puromycin,[8] 10^{-2} *M* ethionine,[9] or 10^{-5} *M* actinomycin D[10] are added to the agar, stalk cell induction by cyclic AMP is striking, while none of these inhibitors alone in the agar will produce isolated stalk cell differentiation.[11]

Because 10^{-3} *M* puromycin in the agar prevents normal development, it is possible to induce stalk differentiation of isolated cells with a lower concentration

of cyclic AMP (10^{-4} M). Since the cells are producing not only their own cyclic AMP, but also an extracellular phosphodiesterase,[12] the cyclic AMP concentration in any one microregion of a Petri dish over the long period of time of the experiment would be exceedingly difficult to determine.

To test the possibility that a product of cyclic AMP might be active, a series of concentrations of 5′ AMP were tried and found to have no effect in inducing stalk differentiation.

Some simple histochemical tests for celluloselike substances were performed to compare the composition of the cyclic AMP-induced cells with those of normal stalk and basal disk cells. Using Calcofluor white ST[13] (kindly supplied by American Cyanamid Co.), a fluorescent brightener, both the normal stalk cells and the experimentally induced cells showed comparable fluorescence. Furthermore they both stained positively with iodine-potassium iodide and sulfuric acid (65%) by swelling and turning distinctly blue.[14] Finally, the walls of the cells exhibited birefringence, a property characteristic of normal stalk and basal disk cells.

Discussion. It is clear from these experiments that externally applied cyclic AMP induces stalk cell differentiation in cells that have not aggregated. These cells are indistinguishable from normal stalk (or basal disk) cells; they have large vacuoles and thick celluloselike walls.

The cell mass normally produces acrasin after aggregation, and in early fruiting it is only produced at the anterior of the cell mass, in the region where the stalk cells are in the process of formation.[3]

Some years ago, Gregg *et al.*[15] showed that there was a 50 per cent reduction in certain protein fractions between the migration stage and the final fruiting body in *D. discoideum*. Furthermore they showed that some 82 per cent reduction occurred in the anterior stalk cells. In other words, these organisms are burning their own reserves for their morphogenetic phases and the largest share of such catabolism occurs primarily in the prestalk region. If we couple this information with the fact that cyclic AMP is known to promote catabolism in other systems,[16] we can propose that cyclic AMP induces stalk cell formation by a general stimulation of catabolism.

These different lines of evidence strongly suggest that in the normal development of *Dictyostelium* cyclic AMP induces the differentiation of stalk cells.

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[11] Some aggregation occurs in the presence of ethionine and the cell masses show some stalk cell differentiation. This is consistent with the fact that these masses are undoubtedly secreting their own cyclic AMP. Mitchell, J. L. A. (senior thesis, Oberlin College (1966)) showed that ethionine, in lower concentrations, had the effect of producing fruiting bodies with disproportionately large stalks, a matter which deserves further investigation.

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[16] For example, the recent work on cyclic AMP overcoming catabolite repression in *Escherichia coli;* see Perleman, R., and I. Pastan, *Biochim. Biophys. Res. Commun.*, **30**, 656 (1968); *J. Biol. Chem.*, **243**, 5420 (1968); Ullman, A., and J. Monod, *FEBS Letters*, **2**, 57 (1968); Monard, D., J. Janeček, and H. V. Rickenberg, *Biochem. Biophys. Res. Commun.*, **35**, 584 (1969). Also there is a large literature for mammalian systems.