Cellular Communication during Aggregation of *Dictyostelium*

The Second Fleming Lecture

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The fundamental difference between a population of individuals and a community is that within a community there is communication between the members. This interaction enables organization to be achieved such as we see in human or animal societies. It also occurs, however, at a more basic level between specialized tissues to form a co-ordinated living organism and between the individual cells that make up these various tissues. Failure of such communication at any level leads to anarchy.

While we know a great deal about the forms of communication used between whole organisms and an increasing amount about the hormonal and neuronal communication that is used between tissues, we know extremely little about the way cells within such tissues can communicate. The principal difficulty of studying local cell-cell communication results from the short intercellular distances that the weak and evanescent signals have to cross when these communicating cells lie extremely close to each other.

The cellular slime moulds* have attracted attention in the field of cell communication because of the ability of their cells to emit and to relay chemical signals while spatially separate from one another and to exhibit rapid and easily observable responses to such signals. During the aggregation phase of their life cycle (Fig. 1) the previously independent population of amoebae aggregates towards central collecting points and assembles itself into an organism. The study of communication between cells in the newly formed organism is possibly as difficult as a similar study in tissues of higher organisms, but during the process of aggregation the signals are synchronized in waves and the cells conveniently respond by fluctuations in their absorbance profiles. It is the mechanism and the biochemical and genetic components of this aggregational system that I now wish to discuss.

Communication during the amoebal aggregation phase

When slime mould amoebae begin to starve, some of them start to emit rhythmic pulses of a chemical – given the general name of acrasin – that in the case of *Dictyostelium* is the nucleotide cyclic AMP (cAMP) (Konijn, 1972). [For another slime mould, *Polysphondylium violaceum*, it is a small peptide (Wurster *et al.*, 1976; Bonner, 1977).] These pulses are slowly relayed outwards from the centres and bring about a progressive and usually intermittent movement of the amoebae toward the centres. The process may be subdivided, for convenience, into seven ‘acts’ (Fig. 2) and the evidence for these acts will now be considered.

1. *Pulsatile signal generation.* Evidence that the signalling is pulsatile rather than a continuous gradient of cAMP in *D. discoideum* came originally from a number of observations of spiral or concentric light and dark bands around the aggregation centre when dense populations of amoebae were allowed to aggregate on agar surfaces. These bands were

* Subclass Dictyostelia of the Mycetozoa (Olive, 1975), formerly the Acrasieae (De Bary, 1887).

Fig. 1. The life cycle of the cellular slime mould *Dictyostelium discoideum*.

The haploid spores germinate to produce amoebae (about $10\ \mu\text{m}$ diam.) that grow in their natural forest environment on soil bacteria (Raper, 1935) or under laboratory conditions on lawns of *Klebsiella aerogenes* grown simultaneously on agar-solidified media (Sussman, 1966). Some mutant strains also grow in liquid axenic medium (Watts & Ashworth, 1970) or in chemically defined medium (Franke & Kessin, 1977; Watts, 1977).

Upon starvation, the amoebae enter the aggregation phase. This is initiated by some of the amoebae producing pulses of cyclic AMP that cause periodic movement of the responding amoebae towards the pulsating centre and relay of the signal outwards from the centre (see text). This motion is unstable and eventually breaks down to produce streams of cells that finally move into the aggregation centre. A slime sheath is produced that covers the aggregate as the aggregation phase comes to an end.

During the morphogenetic development phase the aggregate is transformed into a migrating 'slug' by upward movement of the amoebae (within their slime sheath) off the substratum and a bending movement of the resultant 'finger' back down on to the substratum again. The slug, which is responsive to heat and light gradients, can migrate for 0 to 20 days depending upon environmental conditions (Slifkin & Bonner, 1952; Newell, Telser & Sussman, 1969). It eventually settles back on to its rear end and transforms itself first into a sombrero-like body and then, by means of cell movements and elongation, into a round mass of spores elevated on a slender, cellulose-ensheathed stalk. (For more details of the life cycle, see reviews by Bonner, 1967; Newell, 1971; Loomis, 1975).

seen to move slowly outwards at the same time as the amoebae aggregated towards the centre (Arndt, 1937; Bonner, 1944; Shaffer, 1957; Gerisch, 1968; Alcântara & Monk, 1974). The light bands seen with dark-field optics are considered to be the moving amoebae and the dark bands the stationary amoebae awaiting the arrival of the next signal from the centre (Fig. 3). The same phenomenon may also be observed using a spectrophotometer cuvette containing oxygenated suspensions of starving amoebae (Gerisch & Hess, 1974).

- 1 Pulsatile signal generation
- 2 Signal reception
- 3 Signal destruction
- 4 Signal relay
- 5 Chemotactic response
- 6 Developmental initiation response
- 7 Cell docking

Fig. 2. The seven acts of aggregation. The circle on the left represents a signalling amoeba in the aggregation centre (the three curved lines representing a single pulse being emitted) and the circle on the right represents a responding amoeba. Note that acts 1 to 5 may be repeated several times before acts 6 and 7 occur. (Redrawn from Newell, 1977*a*.)

Fig. 3. Diagrammatic interpretation of the light and dark bands (moving and stationary amoebae respectively) seen by dark-field optics of dense populations of amoebae on agar surfaces. The circular field shown on the left of the figure is approximately 5 mm in diameter. (Photography by courtesy of M. Peacey and J. Gross. Reprinted from Newell, 1977*b*.)

Such amoebae show autonomous oscillation in their absorbance with a periodicity matching that of the waves seen to emanate from the aggregation centres on agar plates. This technique, which effectively allows a synchronous entrainment of the chemotactic cell shape change throughout the contents of the cuvette, has enabled Gerisch and co-workers to assay the concentration of cAMP and the activity of the cAMP-forming enzyme, adenylate cyclase, at precisely controlled times during the oscillation cycle (Gerisch & Wick, 1975; Roos, Sheidegger & Gerisch, 1977). Their results indicate that the activity of the adenylate cyclase enzyme and the internal and external concentrations of cAMP oscillate in phase

Fig. 4

Fig. 5

Fig. 4. Binding of cAMP to species of *Dictyostelium*, plotted by the method of Scatchard, showing different receptor affinities (calculated as equilibrium dissociation constants, K_d , from the gradient of the curves) at different cAMP concentrations. *Dictyostelium discoideum*, $K_d = 10$ to 100 nM; *D. purpureum*, $K_d = 10$ to 580 nM; *D. mucoroides*, $K_d = < 10$ to 95 nM. (Upward curvature was observed for *D. mucoroides* at very low, but reproducible, cAMP concentrations.) (Data from Mullens & Newell, 1978.)

Fig. 5. Dissociation of $[^3\text{H}]$ cAMP from cAMP receptors in the presence or absence of excess unlabelled cAMP. The dissociation of 1 nM- $[^3\text{H}]$ cAMP from aggregating amoebae was induced by rapidly diluting amoebae suspensions 80-fold into buffer (●) or buffer plus excess ($100\ \mu\text{M}$) unlabelled cAMP (○). The extent of dissociation was determined at the times indicated by rapid filtration of the diluted suspension on to membrane filters followed by scintillation counting of the filters and trapped amoebae. (Redrawn from Mullens & Newell, 1978.)

with the absorbance changes seen with the same cells. Such data convincingly support the notion that the oscillations of absorbance seen in washed suspensions of cells are indeed a reflection of the pulse relay system seen on plates, and also that such oscillations involve regulation of the activity of adenylate cyclase – an enzyme thought, from histochemical studies, to be located on the inside face of the plasma membrane (Farnham, 1975; Cutler & Rossomando, 1975). How such an oscillating enzyme is produced is unknown, although interesting models have been proposed by Rossomando & Sussman (1973), Goldbeter (1975) and Goldbeter & Segal (1977). The primary source of the oscillations may, however, depend on oscillations of the cytochrome chain such as were observed by Gerisch & Hess (1974) and found to be in phase with the autonomous absorbance oscillations. The system is clearly not rigidly connected to such an internal oscillator, however, since externally administered pulses of cAMP (see below) can alter the phase of autonomous oscillations.

2. *Signal reception.* The cAMP signal is recognized and temporarily bound by receptor molecules on the surface of the amoebae (Malchow & Gerisch, 1974; Green & Newell,

Fig. 6. Models of cAMP receptors.

1. Two distinct types of protein with different and fixed affinities. Such fixed affinities are not favoured by the kinetic data.

2. Negative co-operative subunit interactions (arbitrarily shown as four subunits). A shift from the high affinity form (squares) to the low affinity form (circles) is induced by binding of cAMP (probably through a series of intermediate complexes).

3. Binding of cAMP to the high affinity form causes dissociation of the subunits to the low affinity form.

4. Binding of the low affinity form of the receptor to its effector (shown in black) activates the effector and simultaneously converts the receptor to the high affinity form. (The effector is the hypothetical membrane molecule that causes effects inside the cell in response to cAMP binding.)

1975; Henderson, 1975; Mato & Konijn, 1975). The binding characteristics of the receptors are complex and in many ways resemble those of certain hormone receptors in mammalian tissues (Mullens & Newell, 1978). For example, a Scatchard plot of the degree of binding of cAMP at a range of concentrations to the amoebal receptors is strongly curvilinear and this has been observed for several species that employ cAMP as an acrasin (Fig. 4) (Green & Newell, 1975; Mullens & Newell, 1978). Such curvilinearity is seen with insulin binding (De Meyts, Bianco & Roth, 1976) and with a number of other hormone binding systems (Jacobs & Cuatrecasas, 1976; Bradshaw & Frazier, 1977) and is generally thought to indicate either the presence of various molecular species of receptor with fixed affinity or the possession by the receptor of variable affinity controlled by subunit or effector interactions.

In a recent study, Mullens & Newell (1978) investigated the effect of excess unlabelled cAMP on the rate of dissociation of a small amount of labelled cAMP. Again, in a similar way to hormone binding, the excess ligand increased the rate of dissociation of the bound ligand (Fig. 5). This effect of excess cAMP on the receptor favours the notion of variable affinity receptors. Models to account for such variability include negative co-operative subunit interactions, dissociation of subunits or receptor-effector interactions (Fig. 6).

The finding that cAMP binding to its receptors in a primitive eukaryote such as *Dictyostelium* resembles the binding of certain hormones to cell surface receptors in several respects may be of some significance in understanding the evolution of cell communication systems. It may also point to a requirement, common to both systems, for graded sensitivity to a large range of ligand concentrations. Such wide sensitivity might be most conveniently generated by variable affinity receptors.

3. *Signal destruction.* For a pulsatile system to operate, there must exist a mechanism for rapidly destroying the signal after it is produced. Only in this way can the signal be prevented from accumulating and becoming a continuous gradient. The cAMP destruction

mechanism comprises two known phosphodiesterases: one is membrane-bound (mPD) (Pannbacker & Bravard, 1972; Malchow *et al.*, 1972) and the other is soluble and extra-cellular (ePD) (Chassy, 1972; Riedel *et al.*, 1972; Malkinson & Ashworth, 1973). The enzymes differ in their properties and their capabilities. For example the ePD is markedly inhibited by an extracellular proteinaceous inhibitor that is produced during aggregation, whereas the mPD is not so inhibited. (This casts some doubt on the usefulness of the ePD as a signal destroying agent as its activity in some strains is inhibited at precisely the time one might expect it to be most needed.) The mPD, which remains active in the presence of excess inhibitor, also differs from the ePD in that, unlike the ePD which shows Michaelian kinetics, the mPD shows a pronounced downward curving double reciprocal (Lineweaver-Burk) plot suggestive of negative co-operative subunit interactions (Malchow, Fuchila & Nanjundiah, 1975; Green & Newell, 1975). Such negative co-operativity is considered by Malchow and co-workers to reduce the cAMP signals more rapidly to concentrations below a critical threshold than would a simple phosphodiesterase with Michaelian kinetics.

4. *Signal relay.* Although the pulse of cAMP is very short-lived and is able to diffuse only a short distance from its source (probably less than 100 μm) before being destroyed, enough cAMP transiently binds to the receptors of nearby amoebae to induce the production of a new pulse of cAMP.

A study of time-lapse films of aggregation on agar plates (Alcântara & Monk, 1974) has shown that there is a delay of about 12 s between receipt of the incoming pulse and generation of a new one. This delay in part determines the centrifugal velocity of the signal (Cohen & Robertson, 1971), which Alcântara & Monk observed to be of the order of 6 $\mu\text{m s}^{-1}$ at a cell density of 10^5 amoebae cm^{-2} .

Signal relay has also been studied using artificial pulses of cAMP liberated at regular intervals on to agar surfaces from a hollow microelectrode by the passage of a minute electrical current. Using this technique Robertson, Drage & Cohen (1972) and Gingle & Robertson (1976) measured the time at which starving amoebae became able to relay a pulsed cAMP signal emitted by the electrode. The first response of the amoebae, after only a few hours of starvation, was a movement toward the electrode tip of those amoebae in its immediate neighbourhood. After 7 to 8 h starvation, however, the signal was suddenly generated throughout the field of amoebae, and amoebae from much larger distances began to converge on the pulse-generating electrode.

The relay phenomenon has also been studied very successfully by Gerisch and co-workers using oxygenated suspensions of starving amoebae in cuvettes. If a small pulse of cAMP is added to such amoebae (under conditions where spontaneous oscillations are not occurring) then a rapid negative spike in the absorbance trace can be observed followed by a slower wave (Gerisch & Hess, 1974): an example of this is shown in Fig. 7. Further work of Roos *et al.* (1975) and Roos & Gerisch (1976) has shown that the wave corresponds to a period of cAMP synthesis by the enzyme adenylate cyclase which is briefly stimulated at least five- to eight-fold, as measured in extracts made a few seconds after the exogenous cAMP pulse. It is interesting that such cAMP pulses could produce this activation only if they were applied to whole cells; the extracts themselves were not sensitive to stimulation. Two recent studies of this system by Wurster *et al.* (1977) and by Mato *et al.* (1977) have implicated guanylate cyclase as an intermediary enzyme between the cAMP binding to the plasma-membrane receptors and the activation of the adenylate cyclase. The important observation was made that a pulse of cAMP triggered an intracellular rise in the cGMP concentration within 3 to 9 s of addition of the cAMP and that this clearly occurred before any significant rise in the internal cAMP concentration. It is therefore an exciting possibility that this induced spike of cGMP synthesis (which is roughly one-tenth of the later cAMP peak concentration) induces the subsequent increase in activity of the adenylate cyclase. If this is correct then one might also suggest that the guanylate cyclase could be the

Fig. 7. Oscillation of absorbance measured in oxygenated suspensions of amoebae of *D. discoideum* strain x22 after 5.5 h starvation using the technique of Gerisch & Hess (1974). Pulses of cAMP (indicated by the arrows) elicit a rapid spike followed by a slower wave. Control pulses of salt solution (SS) produced only small changes in absorbance due to dilution of the cell suspension. (Data of Wright & Newell; reprinted from Newell, 1977b).

membrane effector molecule with which the cAMP receptor binds in the fourth model of receptor action depicted in Fig. 6.

5. *Chemotactic response.* Once set in motion by receiving a pulse of cAMP the amoebae move toward the signal source for approximately 100 s travelling during this time about 20 μm , i.e. about two cell diameters (Alcântara & Monk, 1974) (Fig. 3). The mechanism of this movement is little understood but almost certainly involves the actomyosin system that Clarke & Spudich (1974) and Spudich (1974) have shown exists in these cells.

Somehow the cAMP receptors must be coupled to activation of the actomyosin. A possible coupling mechanism could involve Ca^{2+} ion release from vesicles, triggered by the cGMP rise alluded to earlier, although there is at present no evidence for such release in *Dictyostelium*. It is significant, however, that the actomyosin ATPase has been observed by Mockrin & Spudich (1976) to be activated by Ca^{2+} ions.

6. *Developmental initiation response.* It may be of some relevance to other developmental systems that several workers have found that receipt of cAMP pulses by *Dictyostelium* amoebae not only causes chemotaxis but also brings about the onset of other cellular events associated with differentiation. For example, the time of formation of the cell surface receptors, called contact sites A, that are involved in cell adhesion during the multicellular stage of development, is governed by the number of pulses of cAMP that the aggregating amoebae have experienced. If starving amoebae in suspension culture are given premature pulses of cAMP then the contact sites are formed prematurely (Gerisch *et al.*, 1975); this finding is also true for other developmental events (Darmon *et al.*, 1975; Klein & Darmon, 1976). The chain of events linking receipt of the pulses and the switching on of genes for proteins required for development is at present unknown although the recent finding by Sampson (1977) of an increase of cyclic nucleotide-activated protein kinases early in aggregation suggests the possibility of regulation by such kinases of specific nuclear protein phosphorylation.

7. *Cell docking.* The final act of aggregation may be considered as the linking together of cells to form a multicellular aggregate. This happens to some extent during the later phases of aggregation as chains and streams of cells form from the originally radially moving amoebae. The proteins or glycoproteins involved in the specific cell adhesion or recognition are: the contact sites A, mentioned earlier, found by Beug, Katz & Gerisch (1973), and the discoidins (for *D. discoideum*) and pallidins (for *Polysphondylium pallidum*) found by Barondes and co-workers (Rosen *et al.*, 1973; Frazier, 1976). The properties and developmental roles of these molecules have recently been reviewed elsewhere (Newell, 1977a).

Genetic complexity of aggregation

Mutants that are defective in various aspects of the aggregational process but which retain normal growth abilities may be readily found after treating haploid cultures of the cellular slime moulds with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. These mutants, when studied as clones growing on bacterial lawns, show several clearly recognizable phenotypes (Fig. 8).

Such mutants may be fused in pairs to form stable heterozygous diploids and selected using temperature sensitivity for growth (Loomis, 1969; Williams, Kessin & Newell, 1974*a*; Kessin, Williams & Newell, 1974; Newell, 1975) or *Bacillus subtilis* sensitivity (Newell *et al.*, 1977). They may then be tested for the ability of the aggregational mutations to complement each other genetically. After such a study in which 419 diploids were isolated by fusing pairs of aggregation-deficient mutants, Williams & Newell (1976) found five complementation groups (*aggA*, *B*, *C*, *D* and *E*). Using a statistical treatment of the data, it was estimated that there are about 50 genes that are specific and *essential* for aggregation in *D. discoideum* such that their mutation stops the process. Such results agree with a similar analysis by Warren, Warren & Cox (1975, 1976) using *Polysphondylium violaceum* and with another study on *D. discoideum* by Coukell (1975, 1977). So far 10 complementation groups have collectively been assigned for aggregational mutations in *D. discoideum* and, taken together, the complementation data suggest a figure of about 50 to 150 genes that are specifically involved in *some way* in the aggregational process. This figure is supported by an independent mutational frequency analysis by Loomis *et al.* (1977).

Progress has also been made recently in positioning some of these mutations with respect to known markers on the seven linkage groups in *D. discoideum* using drug resistance haploid selection techniques (Katz & Sussman, 1972; Williams, Kessin & Newell, 1974*b*) and analysis of mitotic recombination products (Williams *et al.*, 1974*b*; Gingold & Ashworth, 1974; Katz & Kao, 1974; Mosses, Williams & Newell, 1975). In this way, the relative order of several of the aggregation complementation groups has been defined (Williams & Newell, 1976) (Fig. 9).

A task that is at present largely unmastered is the correlation of mapped genetic mutations with proteins known to be involved in aggregation. For progress in this direction, however, may be cited the experiments of Brachet *et al.* (1977) indicating the location of a gene for synthesis (or control of synthesis) of the extracellular phosphodiesterase enzyme (ePD) on linkage group IV.

Cellular communication during the morphogenetic stage

An interesting question related to communication during aggregation is whether the same system is used during the subsequent phase of morphogenetic development. For the reasons outlined in the introduction, very little is known about cell interaction when cells are in contact with each other. It is, however, known that continuous cell contact is necessary and that mechanical disruption of the cell contacts at any stage stops further development until contacts are restored. Keeping the cells dispersed at low population density halts the programmed increase of, for example, the developmentally required enzyme UDP-glucose pyrophosphorylase, while allowing the cells to reaggregate restarts a new round of accumulation of this enzyme (Newell, Longlands & Sussman, 1971). The pattern of formation of several other enzymes that can be used as markers of development has confirmed this notion that intercellular signals are continuously required for the progress of development (Newell, Franke & Sussman, 1972; Sussman & Newell, 1972).

Whether in *D. discoideum* a metabolite such as cAMP plays a role as intercellular mediator is still an open question. However, certain lines of evidence support this possibility. For example, several groups of investigators have reported that cAMP causes amoebae to

Fig. 8. Phenotypes of the parental strain x22 and mutant strains of *D. discoideum* growing as clones on nutrient agar plates inoculated with *K. aerogenes*. The amoebal growth ring can be seen expanding towards the lower left-hand side of each picture and various types of aggregation phenotypes are visible behind this zone: (A) parental (aggregation competent) strain x22; (B) NP134, showing formation of distinct aggregates containing amoebae that fail to link up or 'dock' together; (C) NP143, showing loose irregular mounds of amoebae; (D) NP140, showing a plateau of swirling amoebae, usually with a matt surface appearance; (E) NP139, showing faint ripples but no defined aggregates; (F) NP129, showing a completely flat lawn of amoebae without any sign of aggregation. The clones are approximately 15 mm in diameter. (From Newell, 1978; reproduced with permission of The National Foundation, March of Dimes.)

differentiate directly into stalk cells (Bonner, 1970; Chia, 1975; Hamilton & Chia, 1975; Town, Gross & Kay, 1976). The concentrations necessary for this phenomenon are high ($100\text{ }\mu\text{M}$ to 1 mM) but this may be due to inefficient transport into the amoebae from outside (compared with that between touching amoebae) and to the presence of the phosphodiesterase enzymes. Whether the observed phenomenon actually occurs with totally separate amoeba is debatable, as the conditions used invariably allow some degree

Fig. 9. Map of linkage groups I and II of *D. discoideum*. The figure shows the relative order of the markers and, for *whiA* and *acrA*, the approximate relative positions as derived from mitotic recombination studies. Symbols: *aggA*, *aggB* and *ago-41*, mutations for aggregation deficiency; *cycA*, ability to grow in the presence of cycloheximide ($500 \mu\text{g ml}^{-1}$); *tsgE* and *tsgD*, temperature-sensitive growth mutations; *sprA* and *sprB*, spore shape mutations; *whiA*, formation of white spore masses lacking the wild-type yellow carotenoid pigment; *acrB*, ability to grow in the presence of acriflavin ($100 \mu\text{g ml}^{-1}$); *acrA*, ability to grow in the presence of acriflavin ($100 \mu\text{g ml}^{-1}$) or methanol (2%, v/v). (From Newell, 1978; reproduced with permission of The National Foundation, March of Dimes.)

of cell clumping. Moreover, Town *et al.* (1976) found that the efficiency of stalk formation was greatly increased as the amoebal population density increased. It is clearly possible that cAMP is only one of two or more metabolites that are used by such cells.

Stemming from observations that high concentrations of cAMP disrupted the migrating 'slug' stage of *D. discoideum* causing loss of normal polarity and the formation of bizarre structures (Nestle & Sussman, 1972), Sussman has recently proposed a model in which polarity and pathways of differentiation are regulated by the two molecules ammonia and cAMP (Sussman, Schindler & Kim, 1977). Although such work is in its early stages it seems not unreasonable that cAMP and other small ubiquitous metabolites such as ammonia play roles in cellular communication beyond the stage of aggregation.

Conclusion

The aggregation phase of the cellular slime moulds presents a tractable system with which to study cellular communication. While it is certainly true that such aggregation is a very unusual process and could involve a unique form of cellular interaction, it seems intuitively more reasonable that the interaction involved bears some resemblance to cellular communication in other situations.

At the risk of generalizing from slime moulds to man it is perhaps worth contemplating the possibility that a pulse relay system using nucleotides or small peptides may be a cellular communication system that is not just restricted to the cellular slime moulds. Had it not been for the stepwise movement of the *Dictyostelium* amoebae and the visualization of dark and light bands expanding from the aggregation centres, the pulsatile nature of the interaction would not easily have been observed, even in this system, and in the absence of such dramatic gestures it may easily go unnoticed in higher embryonic tissues.

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