powders consisting mainly of nano-scale icosahedral phase embedded in an FCC phase. This area promises to open up new application such as a new type of Al-base composite alloys which may replace many commercial composite alloys. Ti-based QC alloy was shown by Kelton's group to possess the potential for hydrogen storage. The use of Al-base QCs was being used as efficient coating material with increased wear-resistance and non-sticking property and it was patented by J. M. Dubbois's group (France) earlier. Surface properties of these QCs were discussed with interest.

Concluding remarks

The activities on quasicrystals are expected to continue and throw more light on unresolved issues. Apart from finding new systems of quasicrystals, a new type or class of quasicrystals along with more insight towards structure, stability, properties and new directions for potential technological applications seems to be happening. Therefore one can hope to get a better understanding and exciting results in this field by the 7th International Conference on Quasicrystals which is scheduled to be held in Stuttgart, Germany in 1999. It is pertinent to point out that the Indian scientists have made pioneering contributions in the area of quasicrystals[6]. As a recognition to Indian science, it has been recommended by the International Advisory Board to hold the 8th International Conference on Quasicrystals in India in the year 2001.

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RESEARCH NEWS

Is cAMP necessary for *Dictyostelium* development?

M. Azhar and Vidyanand Nanjundiah

'Redundancy' has become a major puzzle in this age of genetic engineering: if doing away with the activity of a gene does not cause any difference to the phenotype, why is that gene there in the first place?[1] The situation is especially embarrassing when an organism appears capable of carrying on, to all appearances normally, without a supposedly essential gene product. A recent paper by Wang and Kuspa[2] makes this point forcefully by showing that haploid amoebae of *Dictyostelium* can complete a normal life cycle in the absence of *acaA*, the gene that encodes aggregation-specific adenylyl cyclase. To understand why this is so startling, one needs to go back to the past.

Ever since 1967, when it was first discovered[3], a series of elegant experiments have added evidence upon evidence in favour of the thesis that adenosine 3′–5′ cyclic monophosphate (cAMP) is *the* agent of communication between *Dictyostelium* amoebae. Indeed, cAMP-based signaling in *Dictyostelium discoideum* came to be regarded as a paradigm for intercellular communication in all of developmental biology. (Here we are talking of an unusual 'first messenger' role for cAMP over and above that of the ubiquitous 'second messenger'.) The life cycle of *D. discoideum* involves feeding, aggregation (following starvation) and differentiation into two cell types. Differentiation is initially apparent as a spatially segregated pattern of presumptive stalk (prestalk) and presumptive spore (prespore) cells within the multicellular aggregate (the slug), and latter as a mass of spores supported by a stalk of dead cells[4]. Each spore can germinate and give rise to an amoeba that can feed, grow and divide by mitosis and the cycle begins anew. Aggregation is caused by the secretion of a diffusible chemical attractant[5] and by the cell-to-cell transmission of an oscillatory signal[6,7]. cAMP synthesized and released periodically by starved amoebae, is capable of attracting competent cells from a distance and can be relayed from one cell to another[8]. The beautiful concentric and spiral waves of cell density that are seen during aggregation are overlaid by waves of cAMP concentration[9]. This last finding appeared to clinch the case for cAMP as a combined chemoattractant and transmitter that both mediated long-range intercellular signaling and was responsible for aggregation.

More was to follow. Genes that encoded products required for aggregation were shown to be specifically inducible by extracellular cAMP, and by pulsatile stimuli at that[10]. Harking back to classic experiments that demonstrated a positive spatial correlation within the slug between the ability of cells to release chemoattractant and their eventual fate[11], it appeared that cAMP, by evoking a differential chemotactic response in the two presumptive cell types, could also be responsible for the spatial patterning of cell types in the slug[12]. Finally, in combination with another small molecule, DIF, cAMP was shown to act as an inducer of cell type-specific gene expression[13] – though, surprisingly, the cell type that was induced corresponded to regions in the slug where cAMP levels appeared to be, relatively speaking, on the lower side.

Adenylyl cyclase is the enzyme that catalyses the formation of cAMP from ATP. *D. discoideum* has two adenylyl cyclase genes. One is expressed during development (*acaA*) and other during spore germination (*acaG*). *acaA*$^-$ mutants are unable to aggregate, but the deficiency can be overcome by subjecting cells to a regime of extracellular cAMP pulses followed by a steady concentration[14]. Extracellular cAMP cannot enter the cell[15]

and works via binding to surface receptors[16]. cAMP binding induces a rapid transient increase of intracellular cAMP that is released in turn as a pulse, thereby extending the effective range of attraction[17].

A cAMP-dependent protein kinase (PKA) appears to play a central role in the signal transduction pathway within the cell. *Dictyostelium* PKA has single regulatory (R) and catalytic (C) subunits. The C unit becomes dissociated from R, and thereby activated, upon the binding of cAMP to R. Details of the 'downstream' pathway remain to be understood but a host of mutant phenotypes testify to the central role of PKA. When the gene that encodes R is mutated (Rm) to a condition in which it can no longer bind cAMP or is constitutively overexpressed, a dominant negative phenotype results: cells do not aggregate[18]. Similarly, a *pka-C*$^-$ strain also does not aggregate[19]. Expression of Rm under prestalk- or prespore-specific promoters yields a phenotype in which differentiation of mature stalk or spore cells is blocked[20,21]. The absence of a functional R subunit, or overexpression of *pkaC*, leads to rapid development and the formation of spores at low density[22,23]. Overexpression of *pkaC* under a stalk-specific promoter blocks development[24]. Further testimony to the importance of PKA is provided by the observation that a steady level of a membrane-permeable cAMP analogue and PKA agonist, 8-Br-cAMP, can induce terminal differentiation of both cell types. What all this tells us is that the main effect of intracellular cAMP, formed in response to the activation of acaA by extracellular cAMP, is channelled via the activation of PKA. As regards extracellular cAMP, the picture that has been built up so far is that upon binding to surface receptors it induces (a) cell aggregation, (b) expression of aggregation-specific functions, (c) cell-type specific gene expression, and (d) the spatial patterning of aggregated cells.

The paper by Wang and Kuspa[2] casts serious doubts on this picture. They started with the *acaA*$^-$ mutant and transformed it such that the C subunit of PKA was expressed constitutively [*acaA*$^-$(*pka-C*)]. Astonishingly, *acaA*$^-$(*pka-C*) cells produced no measurable cAMP but showed normal development, generated streams during aggregation, and went on to differentiate and gave rise to morphologically normal fruiting bodies. In particular, they formed migrating slugs that displayed a normal prestalk–prespore pattern and, later on, a normal proportion of differentiated cell types. Except that they appeared slightly later, a number of cell-type specific genes (*cotA*, *ecmA*, and *spiA*) displayed expression patterns that were very similar to those in the wild type. Apart from the glaring discrepancy between these findings and the belief that extracellular cAMP is essential for aggregation, the hypothesized role of extracellular cAMP for proper prestalk–prespore patterning[25] is also called into question.

These findings bring to mind puzzles thrown up by earlier studies on *Dictyostelium*. Based on the observation that cell movement and cell orientation seemed to be regulated independently, Gerisch *et al.*[26] had hypothesized that amoebae of *D. discoideum* might release two factors capable of evoking one or the other of the two responses. With hindsight, we can see that one of the factors may be something other than cAMP. The non-aggregating mutants Agip53 and HSB1 can express developmentally-regulated gene products and (in the case of Agip53) aggregate and differentiate when stimulated by cAMP pulses. But they do so without any increase in intracellular cAMP, and so presumably without activating PKA (however, cAMP pulses cause a transient increase in the cGMP level in these mutants[27,28]). Pitt *et al.*[14] found that in *acaA*$^-$ cells, treatment with 2′-deoxy cAMP (a cAMP analogue) can activate cell surface cAMP receptors, though not cAMP-dependent PKA, and lead to the expression of prespore- and prestalk-specific genes as well as fruiting body formation. Therefore cells can differentiate in the absence of intracellular cAMP, meaning either in the absence of PKA activation or with the help of an as yet unknown activator of PKA. Thus, stimulation of PKA by cAMP is not necessary for development. Because there is no doubt that the effects of cAMP are mediated by cell surface receptors and signal-transducing G-proteins, this implies that there is a signal transduction pathway that does not require intracellular cAMP[29]. It has been suggested that cAMP-independent PKA activity could also be due to mismatching in the levels of the R and C subunits or to stimulation by compounds other than cAMP, such as cGMP or calcium. (Given the enormous range of effects that it has on intercellular communication, gene expression and cell differentiation in *D. discoideum*, Ca^{2+} is a good candidate[30,31].)

The chief lesson of the Wang–Kuspa paper is that signaling molecules other than extracellular cAMP can mediate cell sorting and patterning in *D. discoideum*. There may be precedents in other Dictyostelids: the dipeptide glorin[32,33] and pterin derivatives[34] are chemoattractants that drive cell aggregation in *Polysphodylium* and *D. lacteum*, respectively. It will be interesting to see whether they are used as backups in *D. discoideum* when extracellular cAMP is missing. On the other hand, there is a positive aspect to the findings: all the effects of intracellular cAMP would appear to be channeled via PKA. PKA alone can derive aggregation of cells, cell-type proportioning, post-aggregative gene expression and differentiation. There is a discrepancy as well, in the sense that earlier works had shown that overexpression of *pkaC* under stalk-specific promoters[24] could prevent aggregation; the explanation might lie in the precise levels of expression in the two sets of experiments. PKA is a key player in Drosophila cell fate determination[35] and the present findings may have a bearing, not only on *Dictyostelium* research, but also on issues pertaining to the development of higher organisms. Needless to say, all speculations carry a caveat. Namely, they stand or fall depending on the reliability of the observation that *acaG* remains inactive in the *acaA*$^-$ line – something that is known not to be true under certain conditions[36].

Coming back to the point that we started with, what do the present results tell us about redundancy? In order to answer this question we need to draw attention to the two phenotypic effects that Wang and Kuspa[2] did observe in *acaA*$^-$(*pka-C*) cells. The first was a slowing down in development. From starvation to completion of fruiting body formation, it took 36 h as against 24 h in the wild type (this delay was also reflected in a delay in developmental gene expression). The second effect was more interesting. Wild-type cells can aggregate at densities of about 5×10^4 amoebae/cm^2 and above; in the case of the mutant, on the other hand, the critical density was 5.5×10^5 amoebae/cm^2. Thus, aggregation in the mutant may be driven more by cell–cell

contacts than by attraction at a distance. Extracellular cAMP may be crucial for the formation of aggregates at the low cell densities that may occur under natural conditions. Also, because aggregation is the first step of a defensive response to a stressful environment, it stands to reason that more rapidly the subsequent development ensues, the better. In short, there is no doubt that the wild type combination of *acaA* and *pkaC* confers a higher fitness under certain conditions than the *acaA*⁻ (*pkaC*) construct does. The implication is that cAMP is not 'redundant' in the usual sense of the term (the *acaA*⁻ mutant is unable to develop). But, because normal development can be restored by over-expressing *pkaC* in an *acaA*⁻ background, the system must have means available whereby it can make do without cAMP: not a backup pathway, perhaps, but certainly other gene products whose functions overlap with those of cAMP. The upshot is an organism that exhibits a degree of resilience far beyond anyone's expectation.

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Micro-organisms as fish feed in fish industries

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Aquaculture has emerged as an important industry during last decade and is practised in more than 150 countries in the world. Global aquaculture industry is worth about 30 billion US $ (ref. 1) and is growing at the rate of about 10%. Asia is considered to be cradle for aquaculture. Asian countries contribute 85% of the total production and Japan shares a major part of it. Considerable part of Japanese economy relies on the fish market. Various new efforts are always in action to improve the quality of the fish as well as to flourish the market. Feed and feeding are crucial elements of aquaculture. Feed cost ranges from 30 to 60% of the total culturing cost depending upon the type of fish and culture system. Various rotifers, plant extracts, stout's viscera, soyabean meal, etc. are normally employed as the feed. Although there are reports of micro-organisms causing mortality of fish fauna[2], from last few years thrust has been on the use of micro-organisms as the feed.

Various algae, yeasts and bacteria have been employed as a primary and/or secondary feed in the recent years. Algae such as *Chlorella*, *Haematococcus*, *Spirulina* were found to be useful for growing young fish. In the recent times, purple sulphur bacteria (PSB), *Rhodobacter capsulatus*, has been employed in the artificial feed of the fish larvae. Microbial cells as food supply additional nucleic acids, proteins, vitamins and various minerals along with the carotenoids. Micro-organisms are nutritious source of energy. *Phaffia rhodozyma*[3,4], *Rhodotorula* and other pigmented yeasts impart red fleshy colour to the meat of salmon, trout and Red seas bream and thus are used as the product quality feed. Astaxanthin is a major pigment present in *Phaffia*