

Survival by cAMP in social amoebae: an intersection between eukaryote and prokaryote signalling systems

Pauline Schaap

Dictyostelium has a fascinating strategy for disseminating its spores to ensure survival. Pauline Schaap describes the sophisticated system of cell communication that enables this to happen.

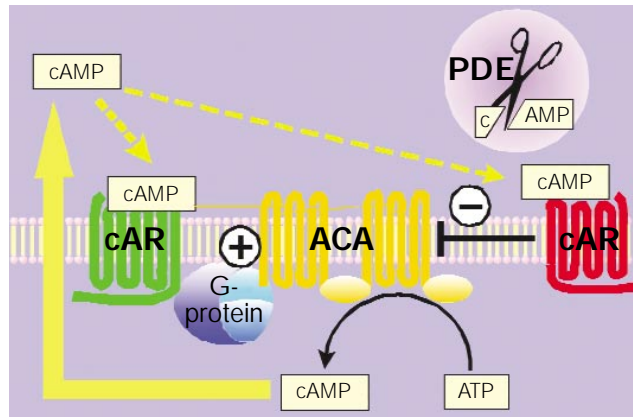
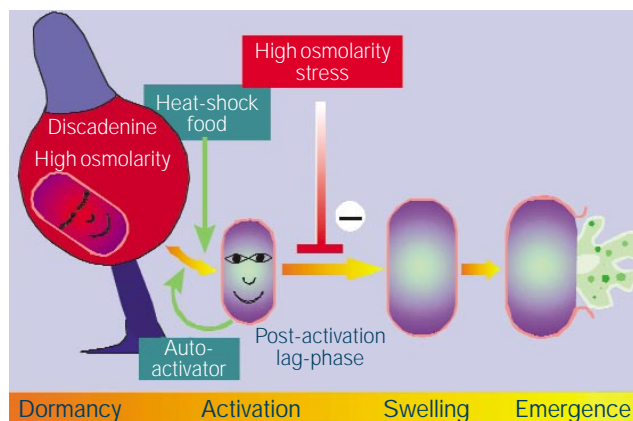
Sporulation is a common strategy for most prokaryotic and eukaryotic microbes to survive food shortage and other environmental challenges. This essential response ensures the long-term survival of microbes in a quiescent state until conditions that are suitable for growth resume. Typically, sporulation involves the active elimination of water from the cell, cessation of metabolism and finally encapsulation in a protective cell wall. The process is remarkably effective as shown by the recent discovery and subsequent germination of spores that had been preserved in amber for millions of years.

Most spores do not need to wait millions of years for their environments to improve, and growth resumes predictably in a short amount of time. Some other microbes, rather than passively waiting around, tip the scales in their favour by placing their spores in locations that will allow their transport to better pastures. One of the most elaborate and magnificent examples of this latter strategy is seen in the social amoeba, *Dictyostelium discoideum*. These single-celled soil inhabitants first get together when food runs out. Up to 100,000 independent cells then collaborate to build a fruiting body that keeps the spores aloft and facilitates their dispersal by insects and other soil creatures. A sophisticated system for cell-cell communication has evolved to co-ordinate the functions, such as metabolism, movement and differentiation, that allow fruiting body formation to occur. cAMP, a well known mediator of hormone action in vertebrate cells, plays a crucial role at almost every stage of the sporulation process.

● Extracellular cAMP

In the first stage, the starving amoebae use cAMP as a chemoattractant. Some cells start to secrete a pulse of cAMP every 6 minutes. The cAMP pulse induces surrounding cells to move towards the source of the signal and to secrete a cAMP pulse themselves. As a result, waves of cAMP propagate through the field of starving cells, which move collectively towards each other to form a multicellular aggregate (Fig. 1).

The aggregate then undergoes intriguing shape changes. A small extrusion, the tip, appears, which extends into a finger-shaped structure, the slug. The slug topples over and migrates towards the light until it reaches a location that is suitable



for fruiting body formation. Meanwhile, the cells start to differentiate. About 75 % of the cells express genes that will ultimately cause them to transform into spores fated to survive. The remaining 25 % express genes that will cause their transformation into the stalk cells that are doomed to die. At first the two cell types are intermixed, but when slugs start to form, they sort out. The prestalk cells (blue in Fig. 1) move forwards to the front of the slug, while the prespore cells (red) remain at the back.

During fruiting body formation, the prestalk cells at the front synthesize a central cellulose tube and then move into it. While doing so they differentiate into stalk cells. This is quite a dramatic process; the cells swell by taking up water and deposit a shared layer of cellulose fibres that allows them to assemble into a rigid stalk. The prespore cells move up the stalk. They mature into spores by losing water and by constructing a thick wall from prepackaged spore coat materials.

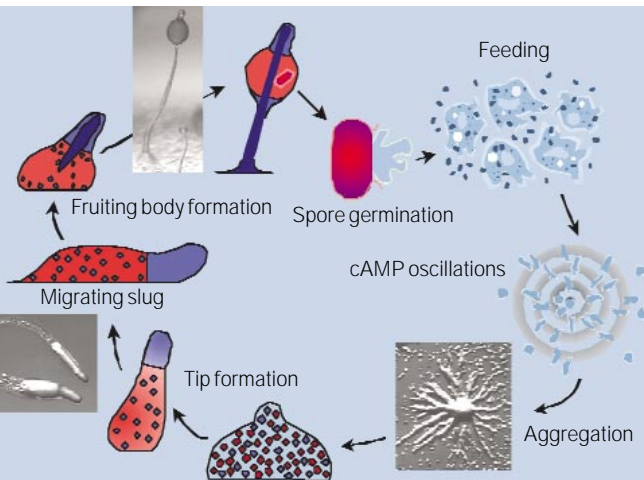
The cell movements that allow slugs and fruiting bodies to form are controlled by the tip cells. They continue to secrete cAMP pulses, which travel through the slug in waves and cause cells to move towards the tip. The cAMP waves also cause the prestalk and prespore cells to sort, because the prespore cells gradually lose chemotactic responsiveness to cAMP and are left behind to occupy the rear of the slug.

BELOW:
Fig. 1. The *Dictyostelium* life cycle.

TOP RIGHT:
Fig. 2. Regulation of spore germination.

BOTTOM RIGHT:
Fig. 3. Adenyl cyclase A is regulated by positive and negative feedback loops.

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Extracellular cAMP has a second important function: it induces the differentiation of the prespore cells. The prespore cells in turn secrete a small lipophilic factor, DIF, that induces the differentiation of the stalk cells.

● **Intracellular cAMP**

Social amoebae are the only known organisms that use cAMP as an extracellular signal. Most prokaryotes and eukaryotes use cAMP as an intracellular messenger for a wide variety of physical and chemical external stimuli. In prokaryotes cAMP is usually detected by transcription factors, such as the catabolite repressor protein. In eukaryotes the most common target is the cAMP-dependent protein kinase (PKA). In

(Fig. 2). In the fruiting body, spores are kept dormant by the presence of a plant cytokine type molecule, discadenine, and by high osmolarity, which is also a common inhibitor of the germination of plant seeds and fungal spores. Young spores require a stimulus such as food to germinate. Old spores secrete an autoactivator of germination and germinate in the absence of any stimuli. After being activated for germination, spores enter a lag phase before swelling and emergence are initiated. During the lag phase, spores sense whether the environment is suitable for germination, and if not, they return to dormancy. High osmolarity is also a constraint factor that induces return to dormancy and it does so by increasing intracellular cAMP.

● **Adenylyl cyclases**

Dictyostelium cells express three adenylyl cyclases that synthesize cAMP from ATP. Adenylyl cyclase A (ACA) is regulated by a positive and negative feedback loop to produce the cAMP pulses that control cell movement. This works as follows: starving cells secrete a small amount of cAMP, which acts on surface cAMP receptors (cARs) to stimulate ACA activity and initiate a cAMP pulse (Fig. 3). With a small delay, extracellular cAMP also triggers an inhibitory pathway that blocks further activation of ACA. An extracellular phosphodiesterase then degrades cAMP. cAMP receptors are now freed from cAMP and the cells become resensitized to respond to cAMP once more.

Adenylyl cyclase A (ACA) is homologous to the vertebrate adenylyl cyclases. These enzymes harbour two sets of six transmembrane domains interspersed with two catalytic domains. They are regulated by serpentine receptors that are coupled to heterotrimeric G-proteins. In vertebrates the β -adrenergic receptor, coupled to the G-protein G_s is the archetypal example of a signalling pathway that via activation of an adenylyl cyclase and PKA leads to glucose production. Glucose is required for the so-called fight or flight response that is triggered by an increase in blood adrenalin levels. In *Dictyostelium*, the serpentine cAMP receptors activate the G-protein (Fig. 3).

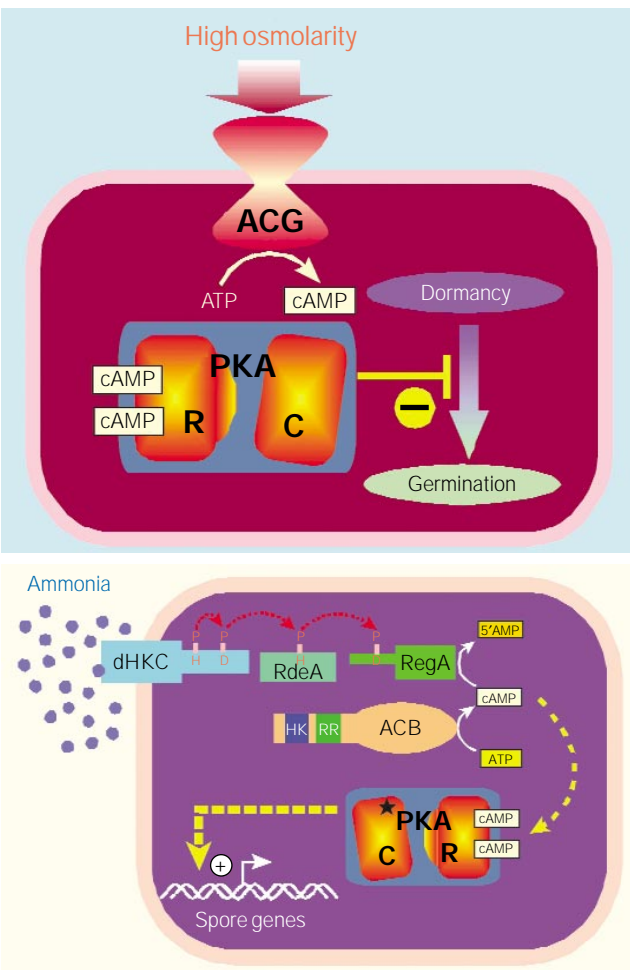
Adenylyl cyclase B (ACB) produces the intracellular cAMP for spore maturation. The ACB gene is homologous to that of adenylyl cyclases in cyanobacteria. In addition to the cyclase catalytic domain, it harbours a response regulator and a histidine kinase domain. These are very common components of the phosphorelay signal transduction systems in prokaryotes. The significance of these regions for regulation of ACB enzyme activity is not yet clear.

Adenylyl cyclase G (ACG) controls spore germination (Fig. 4). It is structurally homologous to adenylyl cyclases in *Trypanosoma* parasites with a single catalytic domain and a single transmembrane domain. ACG is strongly stimulated by high osmolarity. It produces

TOP LEFT: Fig. 4. Adenylyl cyclase G is an osmosensor that controls spore germination.

BOTTOM LEFT: Fig. 5. RegA and ACB control the maturation of spores.

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Dictyostelium, intracellular cAMP acting on PKA induces the maturation of spore cells. Intracellular cAMP also regulates the very first step in the life cycle, the germination of the spores. Germination is a critical step in the life of any organism with a dormant phase, such as a cyst, spore or seed, because it must only occur under conditions that allow proliferation. Several constraints regulate spore germination in *Dictyostelium*

Book Review

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cAMP which activates PKA. PKA in turn blocks the transition from dormancy to germination.

● Phosphodiesterases

The phosphodiesterases (PDEs) that degrade cAMP are of crucial importance for every step in the life cycle. PDE is an extracellular enzyme that degrades cAMP between pulses. This is necessary to generate steep gradients of the chemoattractant and to resensitize the cells for the next cAMP pulse.

RegA is an intracellular cAMP phosphodiesterase; its vertebrate-like phosphodiesterase domain is activated by its prokaryote-like response regulator domain. RegA acts antagonistically with ACB to control the process of spore maturation (Fig. 5). In migrating slugs, spore maturation is inhibited by ammonia which is produced in large amounts as an end product of protein degradation. Ammonia is detected by the histidine kinase dHKC. dHKC initiates a chain of events in which phosphate is first carried over to an intermediate, RdeA, and subsequently to the response regulator of RegA. RegA is then activated to degrade cAMP, which is continuously being produced by ACB.

When the spores are carried aloft during fruiting body formation, ammonia will be removed by diffusion into the atmosphere. dHKC, and therefore RegA, become inactive. At this point cAMP can accumulate and activate PKA. PKA triggers the expression of spore genes and mature spores are formed shortly afterwards.

In essence the function of cAMP during *Dictyostelium* development is to bring starving cells to the spore stage and to maintain dormancy until conditions improve. Signalling strategies from both the prokaryote and eukaryote kingdoms have been united to perform this unique feat of self-organization.

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This is the first attempt to publish an encyclopaedia, which exclusively deals with environmental microbiology. The Senior Editor, Professor Gabriel Bitton, has assembled an impressive editorial board, who are well respected in their fields of microbiology and include Robert Burlage, Douglas Capone, Charles Gerba, Mark Le Chevalier, Kate Scow and David White.

This six-volume set, with over 3,500 pages, contains 320 contributions from more than 420 scientists in 25 countries. As you would expect, the material in the volumes is presented alphabetically, with good use of cross-referencing of subject matter. At the front of each volume, the subject content is indexed and, in the final volume, there is a comprehensive index for the entire encyclopaedia. The information has been divided into 14 areas, which the editorial board considers to be the key areas on environmental microbiology: groundwater, freshwater, marine and estuarine waters, biofilms, soils, environmental biotechnology, air, wastewater, drinking water, pathogens, parasites and viruses, biodegradation, extreme environments and methodologies. Professor Bitton sees these volumes as a quick reference guide for environmental microbiologists at all levels, from undergraduate students to academics and other professionals working in this vast field.

The information is presented as small, peer-reviewed chapters, which are well structured. The authors make good use of sub-headings within each chapter, making it easier to isolate specific information. Furthermore, the chapters are well supported by the inclusion of figures and tables to augment the information contained within. Each chapter is well referenced, acting as a secondary source of information with which to delve further into the subject of interest.

My only criticisms of the encyclopedia are (i) a little difficulty in finding specific information; however, cataloguing and indexing are clearly difficult things to achieve for such a publication. (ii) The cost of the encyclopaedia is a significant constraint, precluding purchase by most interested parties. However, I think that for most academic libraries, this is a must for their reference sections. Perhaps something that the author and the publishers should consider is a CD-ROM version, containing a good search engine, which would be a useful asset to this extensive publication, allowing a lowering of the cost price, thereby providing greater accessibility to end-users as well as increased user-friendliness.

To summarize, this is a significant contribution to the environmental microbiological literature, which is well constructed and extremely useful to all interested in the subject area.

■ *Kirk Semple, University of Lancaster*

