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Developmental regulation and evolution of cAMP signalling in Dictyostelium

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Summary for the non-biologist

Developmental biology

Developmental biologists study the processes that lead to the formation of a complex multicellular organism starting from a much simpler form such as an embryo or an egg. A variety of organisms like frogs (*Xenopus*), fruit flies (*Drosophila*) and social amoebas like *Dictyostelium discoideum* are used as model systems to study these processes. Although humans and amoebas, or flies and frogs, do not physically resemble each other, the mechanisms controlling important developmental processes are very similar between them from a molecular point of view. Therefore, the study of these processes in simpler systems (like the social amoebas) can help us understand how they work in more complex ones.

Developmental biologists also investigate how cells communicate the right message to each other. All living organisms are exposed to different types of stimuli, to which they react or give a response to. For example, the increase of sugar in the blood stream causes the release of a particular type of hormone. The correct response comes after the signal has been processed by the right cell type or organ. *Dictyostelium discoideum* responds to certain changes on the environmental conditions such as lack of nutrients congregating to form a multicellular structure. Therefore, efficient cell communication is paramount for the system to work.

The components of the machinery involved in cell communication and development act through what we know as *signalling pathways*. The main parts of any signalling pathway are: proteins or *enzymes*, which will carry out the reactions, and *signalling molecules* that will pass on the message or the instructions of what needs to be done and where.

Dictyostelium discoideum life cycle and developmental program

Organisms must find a way of adapting to a constant changing environment to ensure surviving. The social amoeba *Dictyostelium discoideum* is found normally in the soil and although this is a rich environment where to live it is also a place that is subject to great changes in temperature, pH, humidity, and where food might not always be available. Through evolution the social amoebas have developed the mechanisms to adapt to those changes and to ensure survival.

Dictyostelium cells normally feed on bacteria and other soil-dwelling micro-organisms but when their food source is depleted they are able to aggregate and form a fruiting body. More than 100.000 cells participate to form a single fruiting body that consists of a mass of spores on top of a cellulose stalk made of dead cells. *Dictyostelium* amoebas that are initially all the same *differentiate* (change their properties) in response to the signalling molecules that they produce and get exposed to during development. During *Dictyostelium* life cycle about 80% of the cells become spores, whereas the other 20% will differentiate into dead stalk cells. The dead stalk is used to lift the spores off the ground facilitating dispersal of the spores by other soil organisms. The spores containing dormant amoebas inside are resistant structures that can withstand harsh environmental conditions. They germinate to a new generation when favourable conditions are met, such as right humidity or replenishment of food. Under normal conditions this life cycle takes about 24 hours to complete. An important intermediate stage of the life cycle is the formation of a so-called slug. The slug is originated from cells in the *aggregate* and is a cylindrical shaped, motile structure organized to the equivalent of a "head to tail" plan. The slug is able to move and it migrates through the soil until it finds the right place to culminate (erect to form a fruiting body). The slug is attracted to light (it is phototactic) and guided by temperature too (it is thermotactic). It is during slug migration that cells determine their fate (*differentiate*) to become either a spore or a stalk cell.

Upon starvation, during the process of aggregation and later on during slug migration *Dictyostelium* amoebas produce and secrete a molecule called cyclic AMP or cAMP. This is the main signalling molecule that controls most of the developmental decisions throughout *Dictyostelium* life cycle. The cAMP secreted initially by a small group of cells accumulates in the extracellular medium and is detected by neighbouring cells attracting them to the

aggregate. This process of moving towards a chemical compound is called chemotaxis (chemo=chemical, taxis=movement) and cAMP acts in this instance as *chemoattractant*. Once a few amoebas start secreting it, the rest will move towards them to form an aggregate at the same time that they relay the signal (*or pass on the message*). This is how multicellular development is triggered and its dependence on extracellular cAMP is a unique feature of *Dictyostelium* development.

The proteins or *enzymes* that produce cAMP are called adenylyl cyclases and we find three in *Dictyostelium discoideum*: ACA, ACB and ACG. The cAMP produced by these enzymes plays different roles depending on whether it is secreted by the cells and found extracellularly (outside the cells), or kept inside the cells then remaining intracellular. The regulation of cAMP production in time and space is essential for correct cell differentiation and survival.

The aims and conclusions of this thesis

cAMP seems to be the most prevalent molecule in *Dictyostelium* development therefore I have focus my attention to the regulation of cAMP production during particular developmental stages. A number of complex processes take place during the slug stage of which a big majority are controlled by cAMP. There are morphogenetic movements, which means that groups of cells move to build up a particular form or structure such as the slug and later on the fruiting body. These movements are mainly controlled from the tip of the slug through the emission of pulses of cAMP that will orient the cells. Cell differentiation also occurs during this stage. This means that cells acquire different characteristics becoming either prespore (later spores) or prestalk (later stalk) cells. From the tip of the slug to the rear a “head to tail” axis is established. Along this “head to tail” axis the prestalk cells are closest to the tip occupying one third of the structure and the prespore cells are in the posterior most part, occupying the other two thirds of the slug. *Dictyostelium* has developed mechanisms to achieve and maintain this pattern through the differential expression of the different components of the cAMP signalling machinery.

The production of cAMP by the adenylyl cyclases (ACA, ACB and ACG) at different times and in different places of the slug is involved in controlling cell differentiation (Chapter One). ACA controls early development and aggregation and it is only found at the tip of the slug. From here it emits pulses of cAMP regulating slug movement. The pattern of expression of adenylyl cyclase B was not known but I have shown in Chapter One that ACB is specifically expressed in the prestalk region of the slug, (anterior part closest to the tip). ACB mainly controls events during late development such as the entry into culmination, which is the transition from slug to fruiting body. In combination with other components ACB also contributes to the proper encapsulation of prespore cells into spores and the final formation of the stalk. ACG is expressed in the spores where it is activated by the high osmolarity (high concentration of solutes) found in the spore head fluid. The main function of ACG here is to stop spores from germinating under these adverse conditions. I have discovered that ACG is also present during the slug stage (Chapter One). During this stage ACG is present at the rear most part of the slug. From here it produces cAMP that is responsible for triggering prespore differentiation, a novel role for this enzyme. In summary the cyclases are expressed at the tip (ACA), the prestalk region (ACB) and the prespore region (ACG). This pattern of expression will directly reflect on the regulation of prespore and prestalk differentiation. In Chapter One I also show that the functions of the adenylyl cyclases are partially redundant, so one cyclase can overcome the absence of any of the other three. For example when ACG is not present the expression of ACB changes taking over the role of ACG in the prespore region. This makes it difficult to differentiate between adenylyl cyclase activities at the stages in which more than one is expressed.

The creation of specific gene knockouts by disrupting a particular gene using molecular biology techniques can be useful to analyse the function of the protein encoded by that gene. For example if a gene is involved in maintaining spores dormant under high osmotic conditions, like ACG, and we disrupt this gene, the resulting predicted phenotype of the mutant

(the physical appearance or resulting defect) would be spores that cannot remain dormant and that will germinate under high osmolarity. However, knocking out a gene is not always possible or practical as the gene of interest might be lethal or block development completely. A pharmacological approach can then be used instead using synthetic compounds that inhibit specifically the activity of a particular enzyme as an alternative to gene disruption. The advantage of this approach is that the drug can be used at the time or dose that we are interested in. Chapter Two shows such a pharmacological study performed to find potential inhibitors of the adenylyl cyclases. These studies have yielded compounds that inhibit ACA (SQ22536) and ACG (Tyrphostin A25) specifically.

cAMP degradation originates by-products that can also act as signalling molecules. Adenosine, produced as a result of cAMP degradation, is such a molecule. Because adenosine is quite similar to cAMP and it accumulates significantly during development, it can interfere with cAMP functions such as aggregation or prespore differentiation. In Chapter Three I show a study on an enzyme called adenosine kinase that phosphorylates adenosine (which is a type of chemical modification) potentially reducing the overall adenosine concentration. In this Chapter I show that the lack of adenosine kinase activity can have an effect determining slug size but not in prespore differentiation.

Dictyostelium discoideum is the species that we use in the laboratory as a model organism but it is only one of many described species. *D. discoideum* belongs to a bigger group called Amoebozoa, found as a sister branch to Animals and Fungi on the tree of life. Therefore *Dictyostelium* is in a very interesting evolutionary position to study the origins of multicellularity. All *Dictyostelia* species have in common that they form multicellular fruiting bodies originated from unicellular amoebas. However the shape and sizes of these fruiting bodies can vary greatly and whereas some species have only one spore head (*D. discoideum*), others have many along regular whorls (*Polysphondyliids*). How these different phenotypes have evolved and what are the molecular mechanisms that control fruiting body formation on the other species has been one of the interesting questions I have asked in the last two chapters of this thesis. To analyse this we need to know about phylogeny, about the relationships between all the *Dictyostelium* species. Phylogenies are normally represented as trees, similar to a genealogical tree, and they show the sequence of events in the evolution of the species. Looking at the phylogeny one can determine relations between different species. We have built first molecular-based phylogenetic tree using DNA sequence information from more than 80 *Dictyostelium* species. Furthermore, mapping and following species characteristics through the tree highlights interesting evolutionary traits such as that during the Dictyostelids evolution the size of the fruiting body tended to increase in the most evolved species (“younger”) and this is directly linked with their use of cAMP during aggregation.

I have used this phylogeny to track down when certain traits appeared for the first time during evolution, such as the use of cAMP as chemoattractant (Chapter Five). In this Chapter I show that whereas the younger species like *D. discoideum* use cAMP as chemoattractant during aggregation at the onset of development, most “ancient” species such as *D. fasciculatum* or *D. minutum* use cAMP to control later events such as fruiting body morphogenesis, which represents the ancient function of the molecule.