Gα8 null cell distribution in the development of *Dictyostelium discoideum*

Authors: Josh Moore and Jeff Hadwiger*
Department of Microbiology and Molecular Genetics, Oklahoma State University

Abstract

Dictyostelium discoideum is both a singular and multicellular organism and is primarily found in soil samples around the world. *Dictyostelium* is an amoeba widely used in scientific molecular biology and genetics research because of its ability to communicate between cells within the organism to move systematically as a single unit while still maintaining the ability to divide into distinct entities as well as to fuse two self-reliant entities together into a single system when necessary. Scientists use this organism to study everything from signal transduction between the different cells to the genetics of the entire population. This allows Dictyostelium Discoideum to be incredibly flexible and useful in many different types of research; specifically in determining the role of G protein-mediated signaling in the developmental process of *Dictyostelium*.

Keywords: Gα8, Dictyostelium, GFP, G proteins, Signal Transduction Pathways

Introduction

Dictyostelium discoideum is a species of amoebas generally found in soil and often referred to as a slime mold. It is a unique organism with the ability to form into an aggregate, a multicellular organism cumulated out of its single celled counterparts. Although *Dictyostelium* rarely forms into an aggregate when the environmental conditions are optimal, but when conditions change and the nutrients needed for its survival become scarce or nonexistent its cells will aggregate together to form a multicellular organism that develops spores. The spores remain dormant until resources become plentiful once again. As an aggregate *Dictyostelium* uses signal transduction mechanisms to communicate between its cells allowing them to function together where certain cells have specific roles in specific regions of the aggregate. The developmental process of *Dictyostelium* includes the aggregation of starved cells, the migration of a multicellular slug and the culmination into a fruiting body structure that contains spores.

Diseases are often the result of defects in cellular signaling and signal transduction that affect cell function, growth, differentiation, or movement. Many disease use complex chemotaxis signal transduction similar to *Dictyostelium* to communicate between their cells so they can reproduce and increase the effectiveness against their host and its immune responses; because of this similarity *Dictyostelium* is an excellent source for identifying how diseases effect the cells, the signal pathways between them, and their ability to proliferate new cells. This allows *Dictyostelium discoideum* to be incredibly flexible and

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useful in analyzing cell transduction of diseases such as cancer and others that extensively use similar communication strategies. Specifically *Dictyostelium* contains a complex network of G proteins that are vital to the signal transduction pathways during aggregation of the organism and the differentiation of the different cell types (Wu et al. 1994). These pathways allow the organism to adapt to its changing surrounding and survive during hospitable times. G proteins are instrumental in its ability to move through its own developmental process. Each G protein has a unique Gα subunit that defines the specificity of the associated signaling pathway. In total there are known to be 12 different Gα proteins; the majority of these proteins have phenotypes and functions during development that have been identified and defined through other studies except for Gα8, and one or two others. Currently, the role of Gα8 in growth and development is not well defined aside from several speculations proposed from an earlier study (Wu et al. 1994). It has been speculated that Gα8 influences the speed of proliferation and the localization of cells with the aggregate. Understanding how the Gα8 signal transduction pathway affects developmental processes such as cell localization and cell differentiation might provide insight into how other G protein signaling pathways regulate the cellular processes and development of other organisms. That information could then be applied to cell disease research to determine how the alteration of G protein signaling pathways might result in diseases.

Methodology

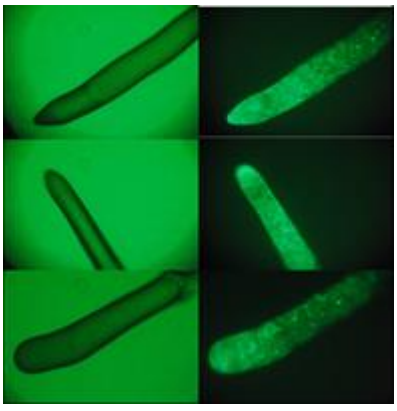
To identify the cellular localization of *gα8*$^{-}$ mutants the cells can be labeled with GFP (green fluorescent proteins). The labeled mutant cells can be mixed with wild-type cells (normal cells) and then developed. The location of GFP fluorescence will indicate the location of *gα8*$^{-}$ mutant cells. To express GFP or other reporter genes in the mutant cells the cultures should be grown in HL-5 media until there is an ample amount to use in gene transfer. When an acceptable quantity of the stock *Dictyostelium* cells has been grown then a new strain can be created through transformation of plasmid DNA into the cells; to increase the likeliness of the cells taking up the free DNA electroporation shall be used. The GFP plasmid 1058 shall be added only to the experimental test group and then both the experimental and control group shall be electroporated at 1.3Kv to create holes in the membrane of the *Dictyostelium* cells so that the free DNA has a greater chance of being taken up by the cells. The GFP plasmid 1058 gives *gα8*$^{-}$ cells green fluorescence when exposed to blue light as well as resistance to antibiotics. Following electroporation the cells must remain relatively undisturbed for a few days to allow them to heal and return to their normal vegetative state. Following several days, G-antibiotics need to be administered to both the control and experimental cultures. The addition of the G-antibiotics will kill off any of the remaining cells that failed to take up the free DNA present in the added plasmid. This should totally kill of all cells in the control group and all but the newly mutated cells in the experimental group; which allows us to guarantee that we have a pure final mutated colony with fluorescence found in the *gα8*$^{-}$ mutant cells. The two separately grown strains are essential in determining the success of the transformation of the plasmid DNA which will ultimately help us determine the function of *gα8*$^{-}$ in the developmental cycle of *Dictyosteliu.*

Once the mutated strain has been grown, individual clones can be picked from the plate to create a final pure colony of *gα8*$^{-}$ cells that can be used in the development

process of an aggregate. The *gα8*$^{-}$ cells can then be combined with other strains to identify to function of *gα8*$^{-}$ cells development. The mutant will then be added with two separate strains: normal *Dictyostelium* cells and GFP *Dictyostelium* cells and plated on non-nutrient phosphate plates forcing the cells to enter the developmental process. The slugs can then be examined under a blue light microscope to observe the location of *gα8*$^{-}$ cells in GFP cell type specific cells, the picture to the left shows what *gα8*$^{-}$ cells containing GFP look like when they are exposed to the blue light mentioned earlier. Other reporter genes can be used to identify specific cell types including pre-spore and pre-stalk cells. These will be used to assess any biasness of *gα8*$^{-}$ in cells in the chimera.

Aims

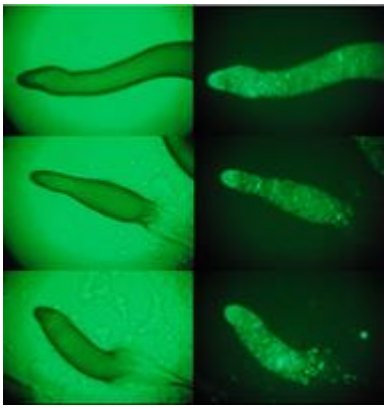
Dictyostelium slugs possess two body regions during the aggregation stage in their developmental cycle: pre-stalk and pre-spore regions. The pre-stalk region is generally located in the middle of the slug while the pre-spore region is are located at the ends of the slug. The diagram to the left shows the location of the different regions during their development cycle. It is expected that the cell type *gα8*$^{-}$ specific GFP chimaera will have the majority of the *gα8*$^{-}$ cell near one of the two regions. The region that it is localized to should leave clues to the role of *gα8*$^{-}$ in the development cycle and ultimately determine the effects on the developmental cycle and the survival of the organism if the *gα8*$^{-}$ cells are absent in the aggregation process.



GFP and Ga8-chimera

Results

Gα8 is an influential piece of the developmental cycle allowing the cells to go through the entire process in a timely matter. Gα8 causes the inhibition of proliferation, regulation of cell differentiation and increased cohesion between cells in the developmental process. To determine the role of Gα8 in the developmental cycle two different chimera strains of *Dictyostelium* were created, one with Green Fluorescence Protein (GFP) gα8- mutant cells were labeled with GFP, which are shown in the figures above. These labeled cells were developed in chimeras consisting primarily of wild-type (shown on in the right) or gα8- (shown on the left) mutant cells. The two chimeras were then plated onto a non-nutrient phosphate plate causing them to enter the developmental process. After 19 hours they were observed under a fluorescent light microscope to observe if there was any biasness of GFP-labeled gα8- cells in the chimeric aggregates. No apparent biasness existed, with GFP: Gα8- cells the labeled cells were evenly spread evenly throughout both chimeras, suggesting the mutant labeled cells were not biased in their distribution. We also observed more fruiting body formations in the gα8- chimera than in the



Wild Type and Ga8-chimera

wild type chimera; suggesting that Gα8 cells might increase the rate of development. However more experiments will need to be conducted to confirm this latter phenotype.

Conclusion

Since little is actually know about Gα8 and its role in the developmental process of GFP cells and the apparent close relation to the signal transduction, it is important that we identifying the effect on cells if they were unable to communicate between each other and go through the developmental process. Research on the Gα8 protein could result in knowledge that could be applied to the behaviors of individual cells of diseases like cancer.

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