Determining the Role of Gα3, Gα8, and PakF in *Dictyostelium* Signaling

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Abstract: *Dictyostelium discoideum* is a free-living amoeba found in the soil. *Dictyostelium* (commonly referred to as "slime mold") exists as a unicellular amoeba in optimal environmental conditions. In suboptimal conditions (specifically when food is scarce) *Dictyostelium* does something that many other amoebas cannot. Individual *Dictyostelium* cells release chemicals into the environment, signaling nearby cells to converge into a multicellular aggregate. This allows for the formation of spores until nutrients again become readily available. *Dictyostelium* can be useful as a model organism for studying the various pathways through which chemotaxis, the movement of cells in response to a chemical in the cell's environment, takes place. Many of the cell signaling pathways through which chemotaxis occurs in *Dictyostelium* are similar to those in mammalian cells, specifically human white blood cells (leukocytes). Not only is *Dictyostelium* simpler and less difficult to culture than leukocytes, it is haploid, which makes mutations in target genes easier to perform than in diploid human cells. For these reasons, Dictyostelium is a useful model organism to understand the cell signaling pathway when stimulated with chemoattractants, and results gained will help provide insights into the cell signaling pathway in other cells, specifically the immune cells of humans.

Keywords: *Dictyostelium*, Chemotaxis, Fluorescent Microscopy, Cell Signaling, Erk2

Introduction

Cyclic adenosine monophosphate (cAMP) and folic acid are important chemicals that often are present in a *Dictyostelium* cell's environment. When present, these chemicals signal the cell to carry out some form of chemotactic movement. cAMP is released by *Dictyostelium* cells when food is scarce, directing all nearby cells to carry out cAMP-mediated chemotaxis to form a multicellular aggregate, until optimal environmental conditions are reestablished. Folic acid is a product released by bacteria, and *Dictyostelium* chemotax to this molecule as a mechanism to find and feed on bacteria in their environment. The process of undergoing chemotaxis in response to these environmental stimuli is complex and involves many different regulatory proteins. Erk2 is an atypical MAPK required for chemotaxis and the phosphorylation of a transcription factor GtaC. *Dictyostelium* mutants with specific gene disruptions were tested for the ability to shuttle a kinase translocation reporter, GFP-GtaC, between the nucleus and cytoplasm. This phosphorylation is required for GtaC translocation from the nucleus to the cytoplasm. The reporter represents the activity Erk2, an atypical MAP kinase (MAPK) required for

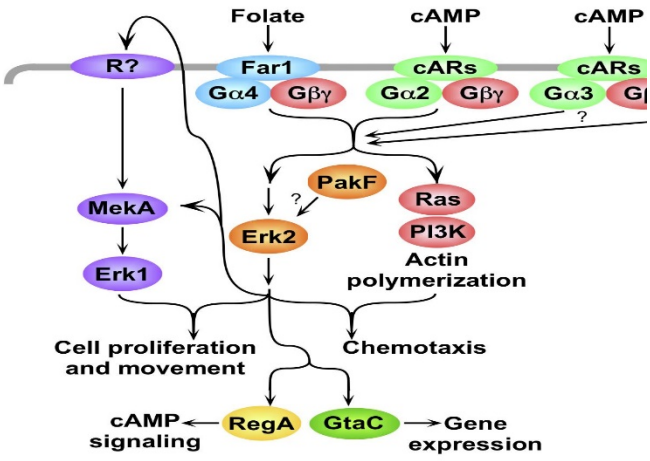

Figure 1: Dictyostelium Cell Signaling Pathway Model

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chemotaxis (Schwebs et al. 2018) and the translocation of the GtaC transcription factor from the nucleus to the cytoplasm. This study helps to define which regulatory proteins might function upstream or downstream of Erk2. When stimulated with chemoattractants, *Dictyostelium* will activate Erk2 within 30 seconds and move toward the stimulus. In response to cAMP *Dictyostelium* will adapt to the stimulation and the Erk2 will be deactivated, allowing the GtaC protein to be dephosphorylated and shuttle back into the nucleus. Gα3 and Gα8 are G protein subunits that might function upstream of ErK2 and may play a role in the cell signaling pathway. G protein coupled receptors are known to play a role in guiding chemotaxis by detecting chemokine gradients outside of the cell (Jin et al. 2008). PakF is a MAP kinase kinase kinase (MAP3K) that might also function upstream of Erk2 in the cell signaling pathway (Figure 1).

Methods

In order to elucidate the role of these proteins in chemotactic signaling pathways of *Dictyostelium*, mutants lacking Gα3, Gα8, or PakF were stimulated with 10 nM cAMP, and 1 µM of folic acid, respectively, and monitored for the shuttling of the Erk2 activity reporter, GFP-GtaC. These mutants were compared to wild-type cells with the same stimulation. Cells were monitored over an eight-minute period using confocal fluorescent microscopy for the shuttling of the reporter. Each individual group of cells was stimulated at least three times with both cAMP and folic acid, to ensure that enough data was collected to draw accurate conclusions (Figure 2).

Results

The PakF mutant showed no significant deviation from the wild type cells when stimulated with either cAMP or folic acid. The *g*α3- mutants were unable to shuttle the reporter back into the nucleus from the cytoplasm when stimulated with cAMP, suggesting that the Gα3 subunit may play a role in the adaptation response to cAMP. The *g*α8- mutants, much like the *g*a3- mutants, were also unable to shuttle the fluorescent reporter back into the nucleus from the cytoplasm, suggesting that Gα8 plays a similar role in the adaptation response. When stimulated with folic acid, the only significant deviation from wild- type cells was that *g*α8- mutants shuttled the reporter from the nucleus into the cytoplasm more quickly than the wild-type cells. This result suggests that *g*a8- mutants might be more sensitive to folic acid than the wild type *Dictyostelium* cells.

Discussion and Future Work

Although much research has gone into the signaling mechanisms of *Dictyostelium* (Artemenko et al. 2014), the cell signaling pathway in *Dictyostelium* is complex, and there are still many molecular mechanisms within it that are not yet understood or

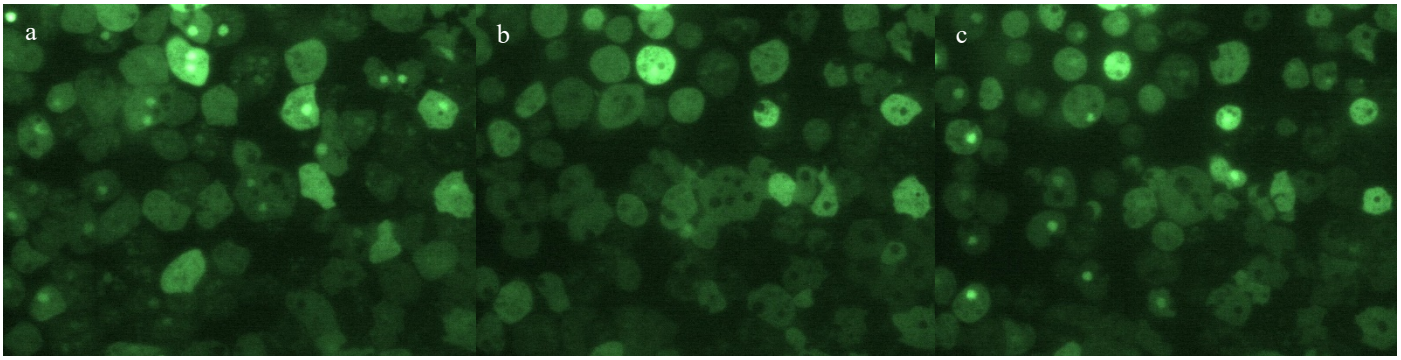

Figure 2: Wild-type Dictyostelium cells before stimulation with cAMP under a confocal fluorescent microscope, with fluorescent reporter evident in the nucleus (a). Wild-type cells 270 seconds after being stimulated with cAMP, with fluorescent reporter shuttled into the cytoplasm (b). Wild-type cells 510 seconds after being stimulated with cAMP, with fluorescent reporter shuttled back into the nucleus (c).

classified. Each individual component of the cell signaling pathway is important in some way (Loomis 2015), however not every component is important to each individual signaling pathway. Erk2 will play an important role in the understanding of atypical MAP Kinases. By determining what molecular mechanism activates Erk2, we can hopefully apply this knowledge to determine what activates other atypical MAPKs. Erk2 specifically is very similar to another atypical MAPK in human leukocytes, Erk8. Using Erk2 as a model, we might gain insights into the mechanisms of Erk8 activation, and better understand how atypical MAPKs function.

Literature Cited

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