Determining the Role of Ga3, Ga8, and PaKF in Dictyosteium signaling.

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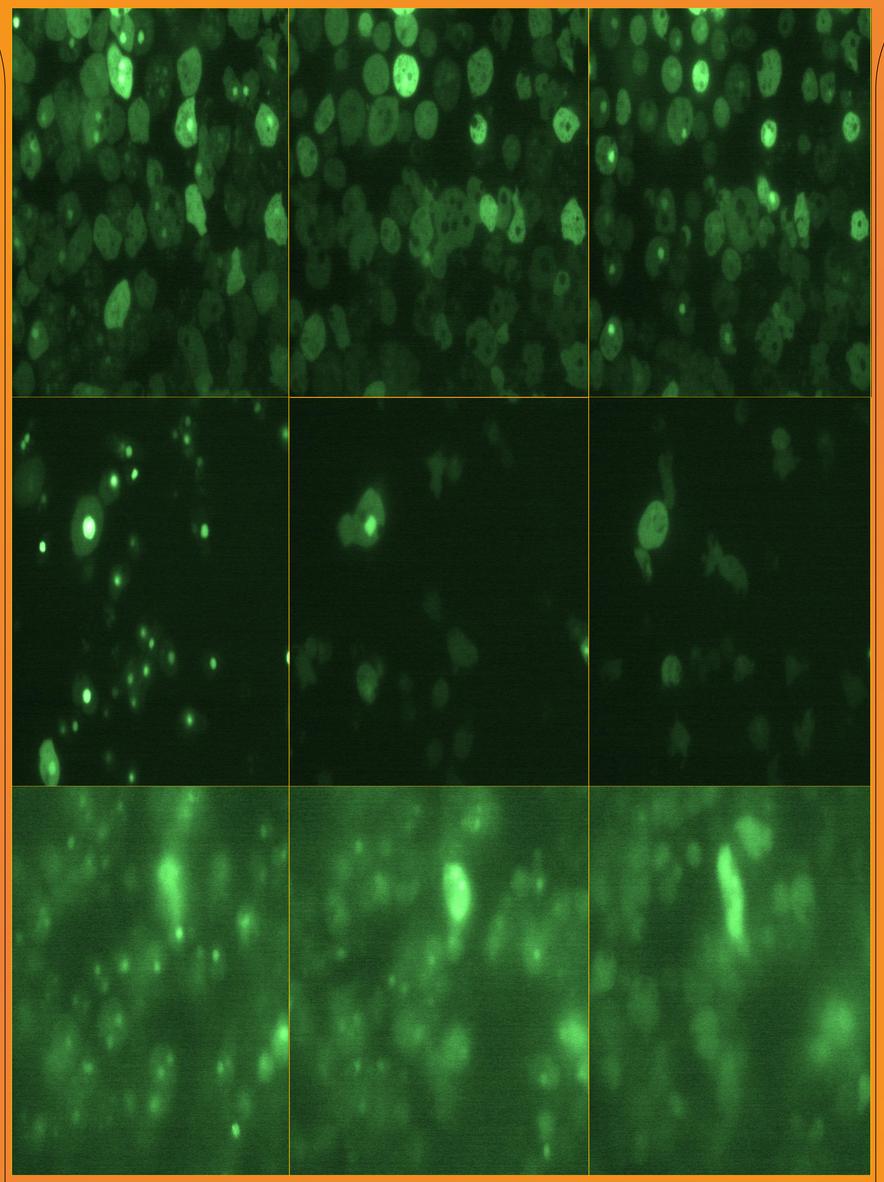
Introduction:

- *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.
- When food is scarce, *Dictyostelium* does something that 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). *Dictyostelium* is simpler and less difficult to culture than leukocytes, and it is also haploid, which makes mutations in target genes easier to perform than in diploid human cells.
- *Dictyostelium* mutants with specific gene disruptions were tested for the ability to shuttle a kinase translocation reporter, GFP-GtaC, between the nucleus and cytoplasm. The reporter represents the activity Erk2, an atypical MAP kinase (MAPK) required for chemotaxis 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.
- For these reasons, Dictyostelium is a useful model organism to understand atypical MAPK regulation in response to chemoattractant stimulation and results gained will help provide insights into the role of atypical MAPKs in other systems, such as the immune cells of humans.
- The PakF mutant showed no significant deviation from the wild type cells when stimulated with either cAMP or folic acid.
- The gα3- 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. When stimulated with Folic Acid, the gα3- mutants did not show any significant deviation from wild type cells.
- The gα8- mutants, much like the ga3- 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 gα8- mutants shuttled the reporter from the nucleus into the cytoplasm more quickly than the wild-type cells. This suggests that ga8- mutants might be more sensitive to folic acid.



Fluorescent *Dictyostelium* Cells:

- WT cells stimulated with cAMP after 0 seconds, 270 seconds, 510 seconds
- ga3- mutant cells stimulated with cAMP after 0 seconds, 210 seconds, 510 seconds
- ga8- mutant cells stimulated with cAMP after 90 seconds, 210 seconds, 390 seconds

*Any deviations in the timing of images shown is due to a loss of focus in the microscope, or a bump of the cell plate, causing cells to become out of focus. The differences in times are not significant.

Abstract:

- Cyclic adenosine monophosphate (cAMP) and folic acid are two 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 in order to form a multicellular aggregate, until optimal environmental conditions are reestablished.
- Folic acid is a product released by bacteria, and *Dictyostelium* move 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. This phosphorylation is required for GtaC translocation from the nucleus to the cytoplasm.
- 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 shuttled 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; PaKF is a MAP kinase kinase kinase (MAP3K) that might also function upstream of ErK2 in the cell signaling pathway.
- In order to elucidate the role of these these proteins in chemotactic signaling pathways of *Dictyostelium*, mutants lacking either the Gα3, Gα8, and PaKF Subunits 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.

Conclusion :

- The PakF mutant showed no significant deviation from the wild type cells when stimulated with either cAMP or folic acid.
- The gα3- 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. When stimulated with Folic Acid, the gα3- mutants did not show any significant deviation from wild type cells.
- The gα8- mutants, much like the ga3- 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 gα8- mutants shuttled the reporter from the nucleus into the cytoplasm more quickly than the wild-type cells. This suggests that ga8- mutants might be more sensitive to folic acid.