Examining the Role of MekA and YakA in *Dictyostelium* Signaling

Authors: Stormie Dreadfulwater and Dr. Jeffrey A. Hadwiger [*]

Abstract: A series of trials were conducted to determine how each protein or kinase plays a role in the signaling of a cell. Among the factors tested were response time, extent of response, and the response under varying stimuli (stimuli included cAMP and folic acid). The primary focus of my individual tests was the role of the kinases mekA and yakA, and I collaborated with my partner to study PakF. Throughout these tests it was shown that *yakA*$^-$ cells respond to either stimuli similar to wild-type cells but *mekA*$^-$ cells show a slower response or no response at all. With these results in mind, MekA was shown to play a possible role in the adaptation of the response, bringing a cell back to its unstimulated state. It is unclear what role YakA plays within a cell, but it does not seem to play a role in this signaling process.

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

Introduction

The amoeba *Dictyostelium discoideum* provides the basis for much of the knowledge of chemotaxis gathered through time. As a model organism, it maintains both a structure and behavior pattern similar to human stem cells, and the signaling pattern compares to that of blood cells reacting to their environment. Chemotaxis is the process in which cells undergo a directed migration along a chemical gradient. This involves gradient sensing, motility, and polarity (Artemenko et al. 2014). This process is important within physiology and contributes to the transportation of pathogens and sickness. *Dictyostelium* allows scientists to study motility, which is the process that cells use to move. Receptors allow cells to sense what is going on around them, what other cells are around them, and how they should react and interact (Swaney et al. 2010).

What makes the process of chemotaxis unique is that it begins with an external source. Chemicals on the outside of a cell direct a cell where to go and influence whether a cell attempts to interact or not. This process contributes to cancer, chronic inflammatory disease, immunity, and embryogenesis (Jin et al. 2008). *Dictyostelium* is a social amoeba that can form an aggregate with a slug like structure. Due to its characteristics, *Dictyostelium* is an excellent model organism. It behaves like a human stem cell, without the difficulties of isolating a specific type of cell (Loomis 2015). Within the blood, testing cells becomes difficult because there are multiple types of both red and white cells, while with *Dictyostelium,* we can study cells in an isolated state and better analyze the movement and reaction without the difficulties of outside influences. My experiment is investigating the role each kinase plays in the function and movement of a cell, while focusing on mekA and yakA, which are specific kinases within the signaling process. The theoretical framework is that each kinase or protein plays an important role within the function of a cell, and we are truly finding out how prominent that role is. The hypothesis is that the mutant cells might have altered responses to the stimuli. These particular proteins, mekA and yakA, are involved in signaling pathways and might affect the response that involves the shuttling of a reporter (GFP-GtaC) out of the nucleus or the return of the reporter to the nucleus..

Methods

The mutants were already created for the experiment, so to begin the phenotypes of each

[*] Faculty Mentor, Department of Microbiology and Molecular Genetics

variation were identified. I studied mekA and yakA, and identified the phenotypes and the differences between them, as well as the ways in which they vary from wild-type cells, or cells without mutations. Then, the reporter shuttling process was observed in order to test the speed and percentage of cells that respond to a stimulus when a certain protein has been restricted. This allows us to see what role each component plays in the process of cell movement. That details the experimental protocol, but the experiments were repeated with both kinases, and wild-type cells in order to compare the results within the data. Data was collected by testing the timing of the shuttling response within a cell, and observed by inserting fluorescent fluid into the cells to allow the eye to follow the movement. Other data included the percentage of cells that react within each test. The data also included the responses of the wild type cells.

Procedure

I tested both mutants (experimental groups) and wild-type strain (control group) of the amoeba *Dictyostelium.* A fluorescent reporter allowed me to observe the movements of the cell during chemotaxis, the shuttling process. The particular mutants I used allowed me to study how Erk1 affects the movement of cells. The mutations targeted *mekA*, *yakA* and, as an extension, Erk1. This allowed us to monitor how the cell would respond if one of these kinases was not functioning. This also shows how prominent a role the kinase plays within chemotaxis. The test was run five times on each mutant and with each stimulus. Placing the amoeba in different concentrations of folate or cAMP allowed for the observation of shuttling after stimulation, as well as after recovery. In order to measure the level of response, the percentage of shuttling cells were measured. The speed of the response was recorded as well in order to test the exact affect the mutations have on *Dictyostelium.* The results of each of these tests will be compared to the similar tests conducted on wild-type cells under the same conditions.

Results

During these tests our wild type cells would respond well to the folic acid stimuli, but the reaction often was not complete when stimulated with cAMP. Additionally, in *mekA*- cells the reporter shuttled out of the nucleus more slowly when stimulated by cAMP than it did with folic acid. Under either stimulus, the reporter could be shown shuttling into the cytoplasm but would not shuttle back into the nucleus. This offers evidence that MekA regulates Erk1, the protein in control of adaptation to a stimulus. When a cell fully adapted to the stimuli, the reporter would be seen returning to the nucleus from the cytoplasm. If Erk1 plays a part in this process, and mutants in which MekA was not functioning, did not fully adapt during the reaction, then MekA is necessary for the full adaptation of a stimulated cell.

On the contrary, yakA- mutant cells would react quickly under either stimulus and the reporter protein could be seen returning to the nucleus as the cells adapted. With this data, the purpose of yakA remains unknown. It does not play a role in the signaling or adaptation of a cell, so the need for the protein is still unknown.

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