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

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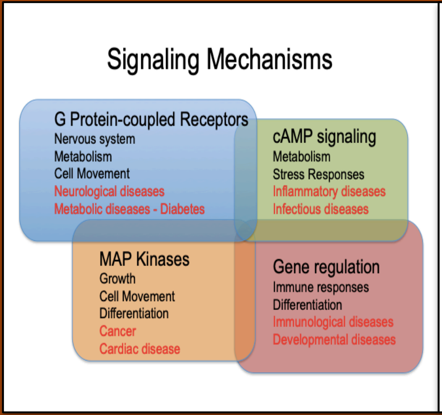
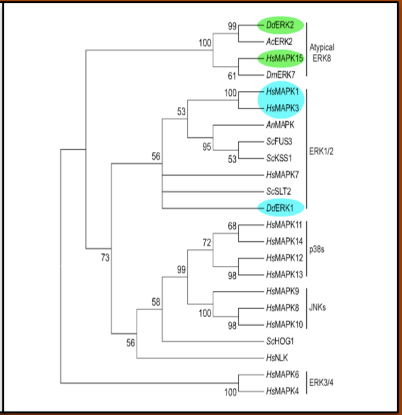
Introduction

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.



Signaling mechanisms within a cell



Classification of proteins within a cell

Methodology

The mutants were already created for the experiment, so I began by identifying the phenotypes of each variation. I studied mekA and yakA, and the differences between them, as well as the ways in which they vary from wild-type cells (or cells without mutations). The G418 drug selection allows for the blocking of a specific protein within the cells, and this was used to create the mutations. The reporter shuttling process was observed in order to test the speed and amount a cell will react to a stimulus when a certain component has been restricted. This allows us to see what role each component plays in the process of a cell's movements. 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 and observed by inserting fluorescent fluid into the cells to allow the eye to follow the movement.

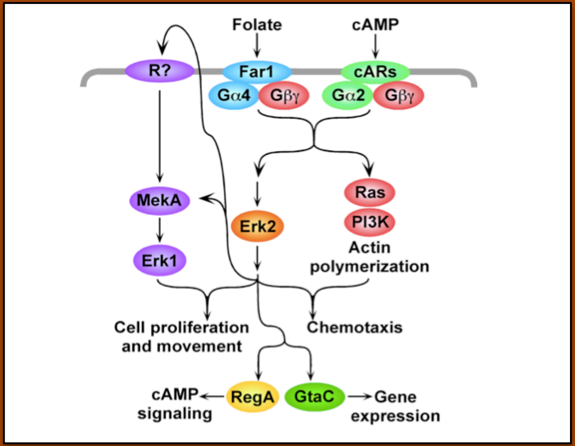


Chart of the signaling pathways within a cell

Results

- MekA may connect to Erk1, the protein necessary for adaptation when stimulated
    - It responded slowest when stimulated with cAMP
    - Whether with cAMP, or folic acid, the mutants with MekA restricted were unable to return to their unstimulated state.
- YakA does not play a clear role in the signaling process
    - It would complete the reaction quickly with either stimuli
    - The reporter protein would move quickly from the nucleus to the cytoplasm
    - The protein could then be seen returning to the nucleus as the cells adapted to the stimuli

Sources

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