Do CrlA and Gα5 Function in the Same Pathway?

Authors: Hannah L. Villines, and Dr. Jeffrey A. Hadwiger*

Abstract: G proteins have been an important factor amongst signal transduction pathways in *Dictyostelium* and most other eukaryotes. These proteins are known to connect receptors to other signaling components such as MAP kinases, cAMP, and phosphodiesterases. Even though we have identified many G proteins, we still need to determine what receptors are connected to these proteins, especially to the highly conserved Gα5 protein. As for the greater than 60 receptors in *Dictyostelium*, several have been characterized such as crlA, fAR1 and the 4 cARs. Among those receptors, there is little to no information on what external stimuli turn on the crlA receptors and whether or not this receptor has a connection with the Gα5 protein. To answer whether these two signaling proteins interact with each other, we tested to see if they both function in the same pathway. We tested this theory by determining whether phenotypes associated with the overexpression of one protein require the function of the other protein. Our results indicate that the overexpression phenotypes do not require the function of the other protein suggesting that these proteins most likely function in separate signaling pathways.

Keywords: G Proteins, Crla *Dictyostelium*, Protein Pathways, Gα5, Development

Introduction

Dictyostelium is a type of amoeba that can be found in the soil. This amoeba grows as a unicellular organism, and several signal transduction components have been shown to function in chemotactic (the movement of an organism in the response to a stimuli) and developmental processes (Raisley et al., 2004). When these cells become nutrient deprived, they aggregate together and form a mound that develops through a series of stages. Once these amoebas form a mound, they then develop into a slug and eventually into a fruiting body (a mass of spores that sit on a stalk) (Hadwiger et al., 1996). The cells that are in the central and anterior regions of the slug are designated prespore and prestalk cells, respectively, and there are also anterior-like cells (ALCs) that are scattered throughout the amoeba and they can be localized to become part of the basal end of the fruiting body. These ALCs can also act in place of the prestalk cells if they have been surgically removed (Hadwiger et al, 1996). *Dictyostelium* has also been found to have a variety of G proteins. These proteins have been found in most eukaryotic organisms and their main function is to help cells respond to extracellular signals. Because of the importance of G protein function in development, they have been extensively researched in soil amoebas because of the simple developmental life cycle.

The α subunits of these G proteins play a critical role in the regulation of active and inactive states of signal transduction components. Researchers have used genetic analysis to identify multiple pathways that are G protein-mediated that are important in this organism's development through genetic analyses (Srivinasan, Gunderson and Hadwiger, 1999). There have also been genetic analyses to determine the roles of G proteins in cyclic AMP (cAMP) specific signal transduction pathways. The regulation of cAMP is vital to the process of

* Faculty Mentor, Department of Microbiology and Molecular Genetics, Oklahoma State University

aggregation among the *Dictyostelium*, because it directs when and where they aggregate during nutrient deprivation. cAMP has also been found to stimulate different G-protein receptors and Gα subunits (Schwebs et al., 2014). Responses to cAMP are mediated by the cAR receptors and the *Gα2* G protein. It is known that the Far1 receptor and the Gα4 G protein are required to respond to stimuli such as folate and possibly other signals that are incorporated for development processes such as forming a slug or stalk and fruiting body (Schwebs et al., 2014). The Gα5 subunit is thought to be involved in the developmental processes of *Dictyostelium*. This is because the Gα5 functions in a signaling pathway that reduces folate chemotaxis, accelerates the transition from mound to slug, and accelerates gene expression during aggregation (development). It has been proposed that the Gα5 subunit could be very versatile when coupled with different signaling components (Raisley et al., 2010).

For this study, we wanted to test the specificity of G protein, α subunits, and receptor coupling. This is possible, because the roles of individual Gα subunits have been found to be quite distinct in terms of the developmental morphology and has been demonstrated by phenotypic differences in mutants that lack or overexpress the G protein (Srivinasan, Gunderson and Hadwiger, 1999; Natarajan et al., 2000). In the case of Gα5 subunit, the loss of this subunit delays development and the overexpression of the subunit impairs growth and accelerates development. Similarly, the loss of the CrlA receptor delays development and overexpression of CrlA impairs growth. These similarities in phenotypes led us to hypothesize that both the CrlA receptor and Gα5 subunit function in the same signal transduction pathway. To test this hypothesis, we will overexpress the *Gα5* gene in a *crlA* gene knockout mutant and then measure the rate of development. If our experiment shows a slow development typical of the *crlA*$^-$ mutant, then the result will suggest that the Gα5 subunit requires the receptor and that these proteins function in the same pathway. Conversely, if there is a fast development this will indicate that the Gα5 subunit does not require the CrlA receptors and will suggest that these proteins function in separate pathways. The hypothesis will be further tested by determining if the overexpression of the CrlA receptor can impair growth in cells that lack the Gα5 subunit. If the CrlA overexpression impairs growth, then this will indicate that the receptor does not require Gα5 function but if CrlA overexpression does not impair growth then this will indicate that the receptor does require Gα5 function.

Methods

We inoculated cultures of *crlA*$^-$*, gα5*$^-$, and wild-type cells (our control) in growth medium (HL5) with frozen spores of these *Dictyostelium* strains. Once the cultures grew we electroporated a Gα5 subunit expression plasmid (p485) into *crlA*$^-$ and wild-type and then select for transformants (cells containing the plasmid) by adding the drug G418 into the growth medium. The cells without the plasmid died because they didn't not have the G418 resistance gene. Once we collected our transformants we isolated several transformants from each transformation and transferred them into a multiwell tissue culture plate. We starved the clones by washing away growth medium and then transferred the cells to an agar

plate. After the transfer we began to examine the developmental morphology of the clones using a microscope as they proceeded through multicellular development. We also electroporated CrlA expression plasmids (p616 and p628) into *gα5*$^{-}$ and wild type cells. The plasmids p616 contains the *crlA* gene and p628 contains a *crlA*^{fs} mutant that does not express the CrlA protein. We also used the same techniques including the drug and electroporation, but instead of starving and transferring clones, we simply counted the clones that appeared on the bottom of the petri dish.

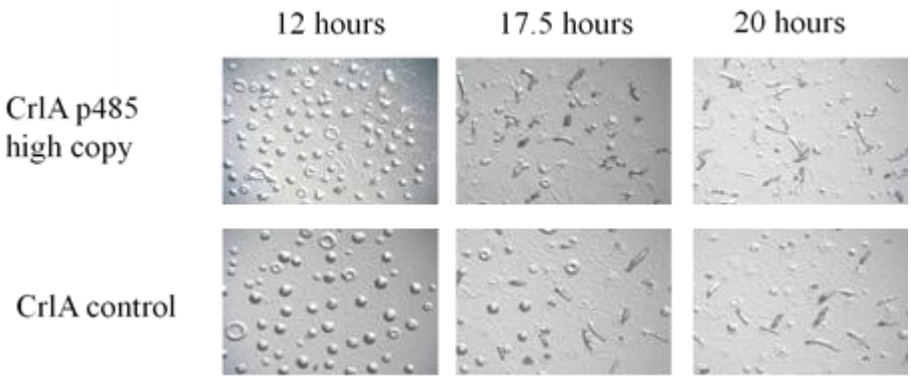

Figure 1 - A phenotypic study, showing the development of the Dictyostelium cells in a 20-hour time span. The upper panels display the developmental morphology of crlA^{-} *cells overexpressing the Gα5 subunit (p485). The lower panels display the developmental morphology of crlA*$^{-}$ *cells that do not overexpress the Gα5 subunit.*

Results

In our experiment of overexpressing the Gα5 proteins we observed a more rapid development of *crlA*$^{-}$ cells than without Gα5 overexpression (Figure 1). The cells overexpressing the Gα5 subunit formed slightly smaller aggregates that were all at the slug stage by 17.5 hours of development. In contrast, the *crlA*$^{-}$ without the Gα5 subunit expression vector produced larger aggregates and many of these were still in the mound stage at 17.5 hrs of development. These were the results of our second transformation of *crlA*$^{-}$ cells with the Gα5 plasmid because the first transformation produced no transformants due to excessive drug selection. The results from the wild type cells overexpressing the Gα5 subunit are not shown because we had difficulties in generating viable transformants with high levels of the Gα5 expression plasmid.

In our second experiment, we observed many more viable *gα5*$^{-}$ clones when we transformed the mutant *crlA*^{fs} gene (p628) than when we transformed the wild type *crlA* gene (Table 1). This difference in viable clones suggests that the wild type *crlA* gene but not the mutant *crlA*^{fs} gene impaired the growth of the *gα5*$^{-}$ cells. This result is similar to what we observed with wild type cells that contained a functional

Table 1 - Number of surviving clones of g□5^{-} *and wild type (G□5) cells transformed with a wild type crlA or mutant crlA*^{fs} *plasmid.*

	No Vector	P616 *crlA* (wt)	P628 *crlA*^{fs} *(mutant)*
ga5^{-}	25	7	139
Ga5	0	30	135

Gα5 gene. The ability of some *gα5*$^{-}$ cells to survive with the *crlA* expression plasmid is likely due to the plasmid copy number differences among the individual transformants. Transformants with a higher copy number and greater levels of *crlA* expression are likely to die but those transformants with lower *crlA* expression were capable of growth and therefore survived.

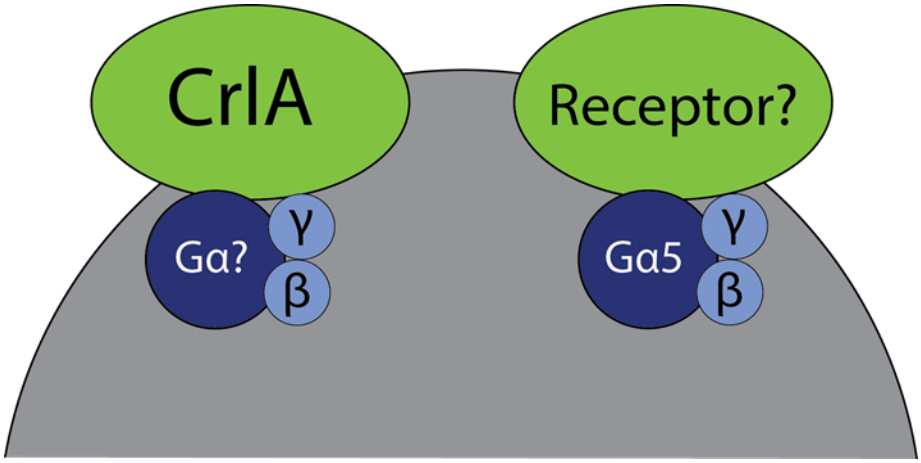

Figure 2 - New model of CrlA and Ga5 function. The experimental results suggested that these signaling proteins likely function in separate pathways to regulate growth and development.

Discussion

In collecting our data we were able to observe and conclude that the CrlA receptor and Gα5 subunit do not seem to work in the same pathway. This is because we noticed that with an overexpression of the Gα5 there was an accelerated development even when the CrlA receptor was absent, suggesting that CrlA is not required for this developmental phenotype. In the second analysis we observed that the overexpression of the CrlA receptor reduced the growth in cells while in the absence of the Gα5 subunit indicating the function of Gα5 was not required in this pathway. These results led us to reject our hypothesis that these proteins might function in the same signaling pathway. We now propose a new model for the relationship of CrlA and Gα5 function in that these proteins function in separate pathways but that both pathways are important for regulating growth and development (Figure 2). Although we did experience a few setbacks in our first experiment we learned that *crlA*$^{-}$ cells are more sensitive to G418 drug selection than initially expected. We experienced some contamination of our clonal cultures and so we had to discard some cultures and then led us to redo some of our experiments.

Literature Cited

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