2007 Student Fellowships
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“Modulation of tear protein secretion in the lacrimal gland”

Abstract:
A classic hallmark of Sjögren’s syndrome is abnormal tear production. Insufficient total quantity and loss of protein equilibrium in the tears leads to common dry eye conditions, and in more advanced cases such as in Sjögren’s syndrome (SjS), these changes are accompanied by autoimmune symptoms in the gland. If untreated, severe dry eye associated with SjS can lead to loss of sight. Interestingly, early data suggest that Rab proteins play a significant role in the regulation of intracellular vesicle trafficking in the lacrimal gland. In particular, Rab27 is a member of the Rab family which has been implicated in human disease and has also yielded promising initial results as having functional control over the trafficking of secretory vesicles.

My career interest in research focuses on the development of treatments for dry eye symptoms and diseases. In pursuit of such a treatment, I propose an early-phase study which focuses on whether the expression of certain endogenous proteins, such as those of the Rab family, can be used to modulate tear protein secretion in lacrimal glands as a potential long-term therapy for dry eye disease. In particular, I would like to determine whether a specific Rab protein, such as Rab27, is able to modulate tear protein secretion and how it may do so. This study will be composed of a three-part approach:

First, I will investigate proteins interacting with Rab27 in the lacrimal gland acinar cell, in order to determine its placement in the secretory pathway. I propose the design of a GST-Rab27 fusion protein to be used in a series of pull-down assays in which the fusion protein will be used to identify lacrimal gland acinar cell proteins interacting with this trafficking marker. This will require reagents necessary for the design and production of the GST fusion protein as well as biochemical analysis and protein sequencing. These newly discovered Rab27 interactions will be confirmed in co-immunoprecipitation assays. In addition, I would like length of time required for the successful production and testing of the GST fusion protein, followed by conduction of the pull-down assays and confirmation of significant protein interactions, this part of the project will require approximately 5 months to complete.

The laboratory proposed for the site of this research also has available advanced microscopy imaging equipment which will enable study of Rab27 in the living lacrimal gland acinar cell. To test the mechanisms through which Rab27b partakes in vesicular trafficking, I will characterize the expression of fluorescently-tagged mutant Rab27 protein in Ad-transduced lacrimal gland
cells, before and after agonist stimulation. Data will be attained for both qualitative and quantitative analysis; the latter may focus on parameters such as the vesicles. This procedure will require 35mm glass bottom live-cell imaging plates and antibodies for the co-localization studies and will need to be conducted over a period of approximately 4 months.

Finally, I will test the *in vitro* functional effects of mutant Rab27 constructs on cargo secretion. A particular area of interest is whether supplementation of Rab27 activity in the lacrimal acinar cells can enhance regulated secretion of different products, as a first step towards improving secretory function under conditions where tear secretion has been reduced. This will require transfection of cultured rabbit lacrimal gland acinar cells with the Rab27 viral constructs along with proper controls. Following confirmed expression of the transduced Rab27 protein on the third day, lacrimal gland acinar cells can be stimulated with an agonist such as carbachol to evoke regulated apical secretion, and the concentration of protein secreted into media for each treatment will be carefully collected and measured. This procedure will require basic laboratory test equipment and chemicals such as the agonist, carbachol, and concentration assays and also likely require multiple repeats (5-8) for statistical significance, and therefore take approximately 3 months to complete. I project that the three parts to this plan will take place over the period of a year.