The VGN Proteomics Facility Highlights Successful Scientists

Ion Channel Study

Ion channels are determinants of the electrical activity that lies at the core of nervous and cardiovascular system function. The dynamic nature of that excitability derives, in large part, from the regulation of ion channels by post-translational mechanisms. The voltage-gated potassium channel Kv1.2 is widely expressed in the nervous and cardiovascular systems where it has key roles in regulating excitability. The molecular mechanisms behind Kv1.2 regulation are complex and remain largely unknown. In our recent publication, Connors et. al., we report that the cAMP pathway has dual roles in the regulation of Kv1.2 trafficking. Our results show that channel trafficking involves both PKA-dependent and PKA-independent pathways. In addition, we report a shift in the electrophoretic mobility of the channel protein derived from cells treated with the adenylate cyclase activator, forskolin. This suggested that elevated cAMP levels lead to a post-translational modification of the channel that elicits a change in channel structure correlated with altered channel trafficking. Biochemical and mutational analysis identified phosphorylation of the C-terminal serine, S449 as a likely cause of the change in the channel’s electrophoretic mobility. However, interpretation of mutation studies is limited by the potential mutational effects on channel structure or function, independent of effects on channel phosphorylation. Mass spectrometry provided an invaluable solution to this limitation by allowing the positive identification of the sites within Kv1.2 and confirming the forskolin-induced phosphorylation of S449. A key advantage of mass spectrometry is its ability to discover modifications at sites not targeted by mutagenesis. In this study, mass spectrometry analysis revealed that, in addition to S449, another C-terminal serine, S440, was also phosphorylated in response to forskolin treatment. Both serines identified by mass spectrometry were shown to be critical for regulation of channel expression at the plasma membrane and interestingly, for determining channel glycosylation patterns. Future studies will examine the role of these serines in specific protein-protein interactions important for channel trafficking and will study in detail their effects on neuron function.


Drosophila Aging Study

Aging is a natural biological process that results in a progressive loss of cellular, tissue and whole body function with the passage of time. One of the most common manifestations of aging is reduced mobility due to loss of muscle strength. Our research is focused on understanding the changes that occur in muscle at the molecular level, and how these changes are manifested at the level of muscle function and organismal locomotion. We used fruit flies, or Drosophila melanogaster, since comparative studies between humans and insects have been particularly adept at uncovering fundamental mechanisms of aging. Fruit flies provide an excellent model system for aging research due to their short life span, ease of maintenance, powerful genetics and the available in-depth knowledge of numerous aging processes due to the multitude of studies performed in this species. We investigated the effects of aging on the indirect flight muscles, the large thorax muscles which allow the insect to beat its wings and fly, through a combination of biochemical, biomechanical, and biophysical approaches. We found middle-aged flies had decreased flight performance and wing beat frequency compared to young flies, most likely driven by damage to their mitochondria, which provides the energy for the muscles to perform. Middle-aged muscles compensated for this lack of energy by increasing their stiffness so that their force generation and power output was actually improved over young flies. To explain this unexpected improvement, we used mass-spectrometry to identify the individual proteins that were changing during aging, and found that most of the large changes due to aging were in proteins responsible for muscle fiber stiffness. Knowledge of the specific proteins involved in the aging process will allow us to identify potential strategies for treating the age-related decrements in human muscle mass and performance.

Cellular Signaling Pathways Study

A variety of intracellular signaling pathways can be stimulated when cells respond to extracellular stimuli. This cellular response is complicated set of pathways. One pathway, the p38 mitogen-activated protein kinase (MAPK) pathway, is known to be activated by stress responses. This pathway functions in the control of cell differentiation, proliferation, and in the induction of cell death, or apoptosis. The p38 MAPK pathway has also been shown to promote survival by unknown mechanisms. In our recent study\(^3\), we have identified glycogen synthase kinase 3-beta (GSK3\(\beta\)) as a novel target of p38 MAPK signaling. Inhibition of GSK3 activity is required to activate the \(\beta\)-catenin-mediated survival pathway. It is well established that GSK3\(\beta\) is negatively regulated by phosphorylation at serine-9 within its N-terminus by Akt. However, the role of this mechanism of inhibiting GSK3 has been called into question because knock-in analysis of the serine-9 to alanine-9 mutants resulted in only a minor defect in insulin metabolism in skeletal muscle. In this study\(^3\) we showed that p38 MAPK phosphorylates GSK3\(\beta\) at a different site than serine-9, providing evidence for an independent mechanism for GSK3\(\beta\) inhibition. In collaboration with the VGN proteomics facility, we found that p38 MAPK phosphorylates a novel site within the C-terminus of GSK3\(\beta\) at threonine 390. Drs. Matthews & Deng were able to identify specifically which of several potential sites in this region of the protein were phosphorylated and provide specific evidence via mass spectrometry for site-specific phosphorylation. Phosphorylation of GSK3\(\beta\) at threonine-390 resulted in the inactivation of GSK3\(\beta\) and activation of the \(\beta\)-catenin-mediated survival pathway. p38 MAPK phosphorylated GSK3\(\beta\) was more abundant in the brain and thymus of mice. Thus, phosphorylation of threonine-390 by p38 MAPK represents an alternative mechanism to regulate GSK3\(\beta\) activity and a novel mechanism by which p38 MAPK can promote survival. Hyperactivation of GSK3\(\beta\) has been linked with several human neurological diseases such as Alzheimer’s and bipolar disorders. GSK3\(\beta\) inhibitors are being used to treat some of these neurological disorders. The identification of this novel mechanism to inhibit GSK3\(\beta\) activity through phosphorylation of its C-terminal domain by p38 MAPK will provide avenues for the development of alternative therapeutic drugs.

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VGN Graduate Student Chosen to Attend NIH Festival

VGN funded graduate student, Sunandan Banerjee, was chosen to attend the 2nd Annual Graduate Students Research Festival at the National Institutes of Health.

The National Institutes of Health (NIH) organized its 2nd Annual Graduate Students Research Festival (NGSRF 2007) in Bethesda, Maryland on the October 11-12, 2007. The event provides a platform for advanced graduate students in the U.S.A to explore post doctoral training as part of the intramural research program at NIH. The festival is highly competitive with 250 graduate students selected out of an applicant pool of 1000 applicants. As a 5th year PhD candidate in the Department of Chemistry at UVM, I am exploring various laboratories for my post doctoral training and this festival provided me the perfect chance to visit NIH and interview with investigators whose research matches my interests. The festival was extremely well organized with scientific talks from NIH investigators and poster sessions from participating graduate students. The students were given time to schedule meetings with potential advisors and discuss their research interests. The students also saw the impressive research facilities at the Bethesda campus and came to know the positives of being a post doctoral fellow at NIH in terms of career opportunities. I came back from the event with the prospect of post doctoral training in two labs and I intend to make a final decision as I approach my graduation.