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**Post-transcriptional Regulation of Neurofilament Expression  
During Axon Outgrowth**

Axon outgrowth depends critically on changes in gene expression that build the axon and establish functional connections. Although much attention has been paid to the molecular mechanisms that promote and guide axonal outgrowth, considerably less is known about how changes in the expression of proteins that are involved in building the axon are regulated intracellularly. The PI's laboratory focuses on the control of neurofilament (NF) protein expression, using developing embryos and regenerating optic axons of *Xenopus laevis* as experimental models. NF proteins are ideally suited for such studies because they are expressed in appropriate combinations to form one of the most abundant axonal structural heteropolymers, the composition of which shifts with each successive phase of axon development. Like axon outgrowth itself, this progression responds to extracellular cues encountered by the growing axons. These characteristics make studying the control of NF expression ideally suited for gaining insights into the regulation of the axonal growth program.

During the most recent funding period, the PI's laboratory established that NF protein expression during optic axon regeneration is under strong post-transcriptional control. It also established that hnRNP K, a protein that binds NF mRNAs, is essential for both efficient nucleocytoplasmic export and translation of at least one NF subunit, the middle NF (NF-M), during axon development. Consistent with this involvement in the nucleocytoplasmic export of a target RNA, hnRNP K's presence in the nucleus increased during peak times of axonal outgrowth, suggesting that it shuttles RNAs from the nucleus to the cytoplasm. hnRNP K was also found to be essential for axon outgrowth itself and for establishing neuronal microfilament and microtubule cytoarchitecture. Because these latter functions do not normally require NF-M, the effects of hnRNP K-knockdown on axon outgrowth must involve additional RNA targets. This conclusion raises the hypothesis that hnRNP K is a post-transcriptional, global regulator of multiple mRNAs whose proteins are involved in organizing the neuronal cytoskeleton into an axon.

One question related to this hypothesis is whether nucleocytoplasmic shuttling of hnRNP K is essential for its functions during axon outgrowth. To test whether this is the case, a fluorescently tagged form of hnRNP K that both localizes appropriately within and rescues neurons from the effects of hnRNP K-knockdown will be mutated at sites implicated in nucleocytoplasmic shuttling. A second question is what other RNAs are targeted by hnRNP K at the time of axon outgrowth and whether they are regulated by hnRNP K in the same way as NF-M. This question is important because up to now, the function of hnRNP K has been studied by focusing on one target at a time, primarily in cell lines. In each case, hnRNP K functioned differently in the post-

transcriptional control of the target RNA. The studies proposed here will be among the first to explore the function of hnRNP K with respect to multiple targets at once and to do so within the context of a developing organism. To this end, sixteen validated hnRNP K targets were identified from juvenile *Xenopus* brain by gene arrays. They were selected because they belong to three functional groups: neurite outgrowth-related, NF-associated, and developmentally active transcription factors. The assays developed for NF-M mRNA will be used to test whether the mRNAs in these groups depend upon hnRNP K during axon development in the same way. These studies will provide new insights into the coordinate control of functionally related genes at the time of axon outgrowth.

The work has the potential for making a broader impact along two fronts. First, the posttranscriptional control of NFs is increasingly seen as playing a role in neurodegenerative disease. The PI's laboratory is the only one working on the basic mechanisms of this control in developing and regenerating neurons. Second, it will help build the nation's scientific infrastructure by supporting the work of at least two PhD students, plus that of several undergraduates at an institution with a culturally diverse student body. The PI is active in teaching at both the graduate and undergraduate levels and has participated in scientific outreach to high school students. Spinoffs from this work will impact the development of exercises in undergraduate General Biology and Developmental Biology laboratory courses, and on the lectures, readings, and discussions of three courses in MolecularBiology, Developmental Neurobiology, and Molecular Neurobiology.