FINAL REPORT

1. Original aims of the project

Aim 1) Determine the fate of p57^{kip2}-expressing cells in the embryonic p57^{kip2} mutant spinal cord. Our recent results show that an excess number of post-mitotic interneurons are generated in *p57*$^{-/-}$ mutant spinal cords (Gui et al., in preparation). However, it is unclear whether these supernumary neurons are derived from cells that normally express p57 which re-enter the cell cycle inappropriately in the absence of p57, or whether the differentiation of cells that do not normally express p57 is also affected (non cell-autonomously). To distinguish between these possibilities, we will cross an existing transgenic mouse line generated from a modified *p57*-containing bacterial artificial chromosome (BAC) clone expressing *lacZ* under the control of the *p57* regulatory elements (John et al. 2001) with the targeted *p57*^{kip2}mutant line (Yan et al. 1997). Persistent expression of b-galactosidase will allow us to track the fate of p57-expressing cells in the *p57* mutant background and determine whether they alone re-enter the cell-cycle abnormally in this mutant.

Aim 2) Define the cell-cycle inhibitory activities of the p57 protein in vivo. Our preliminary data shows that transfecting a full-length p57 protein into neuronal progenitor cells in the chick spinal cord induces rapid cell-cycle arrest (Fig. 3). To further define this function of p57, we will use transfection assays to:
(a) Mis-express partial proteins lacking putative regulatory domains of p57 to identify the regions of the p57 protein that are responsible for mediating cell-cycle arrest.
(b) Test if p57 can block the activity of cell-cycle promoting cyclin proteins in co-transfection assays.
(c) Compare the activities of the p21, p27 and p57 CKI proteins in promoting cell cycle exit.

Aim 3: Define the activities of p57 that control neuronal differentiation in vivo. In addition to arresting proliferation, p57 transfected cells do not differentiate but rather remain in the proliferative zone of the spinal cord, suggesting that p57 must be down-regulated to allow normal neuronal differentiation (Fig. 3). Our preliminary transfection data implicates the C-terminal QT domain in mediating this activity, since on it's own this region is capable of blocking neuronal differentiation without affecting cell cycle arrest. As a follow-up to this, we will:
(a) Test the requirement for the QT domain in mediating differentiation-arrest phenotype by transfecting a p57 protein lacking this domain and assaying whether transfected cells differentiate.
(b) Test the ability of p57 to inhibit neuronal differentiation by restricted mis-expression in post-mitotic cells using an expression vector driven by the Xenopus b-tubulin promoter/enhancer.
(c) Identify potential co-factors that co-operate with p57 via the QT domain to regulate differentiation by performing a yeast 2-hybrid screen using the QT domain as bait. Potential factors will be selected for further examination in a follow-up study.

2. Project successes

We have successfully completed the experiments outlined in Aims 1, 2 and 3b, and have recently published these results (see below).

3. Project challenges

The experiments carried out in Aim 3a and c presented a set of challenges that we are still working on overcoming. For Aim 3a we performed all of the proposed experiments but our results were largely negative and therefore difficult to interpret in the context of our model for

the potential function of the "QT" domain of p57. For Aim 3c, we carried out 2-hybrid screens as described on 3 separate occasions, but only recovered a very small number of clones each time (<5). Unfortunately, none of these appeared to interact specifically with p57 in control experiments, nor did their annotated identities suggest any potential interactions.

4. Implications for future research and/or clinical treatment.

The long-term goal of our work is to improve the success of stem cell replacement therapies to treating spinal cord injury (SCI) by characterizing the activity of an important regulator of neurogenesis in the embryonic spinal cord. Some of the most exciting strategies for restoring function following SCI involve therapies that seek to employ immature "stem cells" to replace the function of cells that are lost as a consequence of the trauma. Neuronal stem cells possess the potential to generate the wide array of cell types that normally exist in the adult spinal cord. These cells are essentially specialized versions of normal spinal cord progenitor cells found in the developing embryo. Their utility as a therapeutic tool depends on preserving their full potential in vivo in adult injury sites, and understanding the steps that normal progenitor cells must undergo to generate neurons is critical to the success of this approach. The cell-cycle regulators investigated in this proposal play a central role in regulating the transition of multi-potent progenitors into newly-born neurons with distinct phenotypic properties in the developing spinal cord, and are likely to be critically involved in this same process in transplanted stem cells.

In the experiments carried out during the funding period, we have focused on elaborating the function of a key regulator of neurogenesis. Our work showed that $p57^{kip2}$ plays a central role in controlling the generation of neurons from multi-potent neuronal stem cells by regulating two important steps in this transition. We have also clarified the requirement of two closely-related CIP/KIP family proteins in controlling neurogenesis, $p27^{kip1}$ and $p21^{cip1}$. Our findings provide the first data examining the collective role of this important cell-cycle protein family in early CNS development, and through these studies we have discovered that CIP/KIP proteins are dispensible for neuronal differentiation. Together, this work has helped further our understanding of the mechanisms controlling neurogenesis in vertebrates, and will benefit the development of stem cell therapies to treat human patients with spinal cord injuries and other degenerative neurological disorders affecting the CNS.

5. Plans to continue research, including applications submitted to other sources for ongoing support.

We are currently planning to incorporate some of the data generated during the funding period into a grant application to a Federal funding source.

6. List and include a copy of all publications emerging from this research, including those in preparation.

Gui, H., Li, S. and **Matise, M.P.** (2007) A cell-autonomous requirement for Cip/Kip Cyclin-kinase inhibitors in regulating the timing of neuronal cell cycle exit but not differentiation in the developing spinal cord. Developmental Biology 301, 14-26.