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Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142
1001 E 3rd Street
Bloomington, IN 47405-7005

Dear Dr. Brun,

I am an Associate Research Scientist (non-tenured Assistant Professor) in the laboratory of Dr. Lloyd Greene in the Department of Pathology and Center for Neurology and Behavior, Columbia University College of Physicians and Surgeons. I write to apply for the position of Assistant Professor in your department.

My research centers on understanding the molecular and cellular mechanisms underlying the differentiation and survival of neurons in mammals. Specifically, my work has been focused on cell cycle regulation in neural stem/progenitor cells and in neurons themselves that impinges on both the formation and demise of functional neurons. Indeed, the cell cycle machinery must be progressively restricted to permit neural differentiation and it must be actively suppressed to keep neuron survival. If neural progenitor cells fail to exit the cell cycle and to differentiate at the appropriate moment, specific classes of neurons and/or glia may be misrepresented in the adult tissue, which could lead to debilitating neurological defects. On the other hand, failure of cell cycle suppression in mature neurons will lead to neuron death, which is an underlying cause for various neurodegenerative diseases. My work also involves the understanding of the formation and survival of brain tumors.

I have attached my CV and Research Statement for your review. Recently, I had started several experiments that were planned for my future research (please see my Research Statement) and the results are exciting. These data indicate that my research plan will likely succeed as planned. I hope that I will have an opportunity to fully develop my ideas and make a significant contribution to your Department and to science as a whole.

I appreciate your consideration and look forward to hearing from you.

Sincerely,

David X. Liu, Ph.D.
Associate Research Scientist
Department of Pathology

David X. Liu, Ph.D.

STATEMENT OF RESEARCH INTERESTS

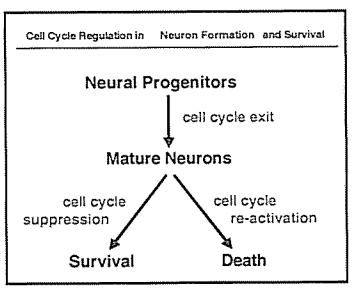
The cell cycle machinery plays an important role in neuron development and survival. On the one hand, the cell cycle must be progressively restricted in neuroprogenitor cells for them to mature and the identity of mature cells depends on the timing of cell cycle exit; on the other hand, inappropriate activation of the cell cycle machinery in post-mitotic neurons is causally involved in mediating neuron apoptotic death under both normal developmental and pathological conditions. In spite of this fundamental importance, relatively little is known about how the cell cycle machinery engages with other key regulators in cell fate determination and in neuron development and survival. And indeed, the present paucity of knowledge about cell cycle-dependent cell fate acquisition represents a major opportunity for advancing our understanding in stem cell regulation, neural differentiation, tumor formation and cell death.

Recent Research Accomplishments

My work in Dr. Lloyd Greene's laboratory centered on the molecular mechanisms of neuron differentiation and survival with a particular focus on the involvement of cell cycle regulators in these processes. I demonstrated that E2F acts as a gene silencer in neurons and that repression of E2F-responsive genes is required for neuron survival. E2F-dependent gene derepression rather than E2F activation causes neuron death (*Liu and Greene, Neuron 2001*). I further discovered that B- and C-myb are downstream mediators of E2F derepression pathway (*Liu et al., J. Neurosci. 2004*) and with my colleague showed that B- and C-myb evoke apoptotic neuron death by up-regulation of Bim, a pro-apoptotic Bcl-2 family member (*Biswas et al., 2005*). I also found that p130, but not Rb, is solely responsible for control of E2F repression/derepression and of neuron survival/death. The E2F4/p130 complexes bind and silence pro-apoptotic genes such as B- and C-myb by tethering chromatin modifying enzymes HDAC1 and Suv39H1 to the vicinity of affected promoters. Apoptotic stimulation causes cdk-dependent p130 phosphorylation that leads to loss of nuclear hypophosphorylated p130 and to disassociation of HDAC1 and Suv39H1 from the complexes. The consequent acetylation and phosphorylation at the N-terminal of histone H3 makes it possible for pro-apoptotic genes to be expressed (*Liu et al., Genes&Dev. 2005*). Together, these works showed how neurons utilize the cell cycle machinery to propagate apoptotic signals and to activate the core apoptotic machinery (also see reviews *Liu and Greene, Cell Tissue Res. 2001; Greene et al., Cell Death Diff. 2004*).

Future Research Plan

The work that I have described above, although important, is one small piece of the much larger question of neuron development and survival. While a variety of approaches are likely to yield insights into these questions, there are several reasons to extend my previous work studying the cell cycle machinery. First, the cell cycle machinery is linked to many other cellular functions and plays a commanding role in cell fate determination that includes cell differentiation and death. And indeed, accumulating evidence in the last several years shows that neuronal differentiation and death processes depend on active participation of the cell cycle machinery. Second, our experience in the last several years has convinced us of the direction we take and of several related generalizations which will add my ability to identify the right questions in the field. These include the realization that the invovlement of cell cycle machinery in neuron formation and survival is much more complex than initially anticipated and that despite of the complexity and multiplicity of the mechanisms invovled, there are certain cell cycle pathways that are encountered again and again when one dissects how neurons are formed and die. Third, we have identified a few molecules/pathways that play critical roles in neuron differentiation and survival, holding promise to address major questions such as how extracellular and intracellular signals interplay in regulation of stem cell maintenance and differentiation? Or how brain tumors are formed and what are the cells of origin and cell lineage associations for distinct tumor types? I will focus on two critical transcription factors/pathways (E2F and ATF5) which are master regulators for cell proliferation, apoptosis, maintenance of stem cell, and differentiation. My choice on these two molecules/pathways respects some of our generalizations stated above and also considers reasons given below.



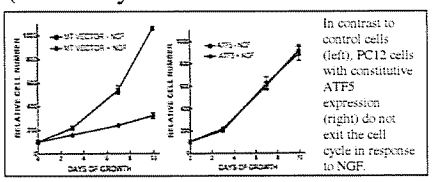
1. E2F pathway

a. Down-stream targets of the neuronal E2F derepression pathway While we had identified myb and Bim as sequential downstream targets of the E2F derepression pathway, blockade of Bim expression (and even in Bim null mice) only delays neuron death, indicating existence of other apoptotic genes. One challenge to uncover regulated death-associated genes is that most apoptotic stimuli also set off responses un-related to death. For

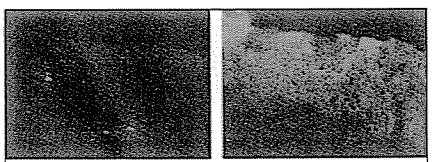
example, NGF has many actions in addition to promotion of neuron survival, and its withdrawal leads to changes in gene expression associated not only with death, but also with de-differentiation and metabolic rearrangement. Because E2F1(1-368) kills neurons effectively by E2F de-repression without even invoking E2F transactivation, an action proved irrelevant to neuron death (*Liu and Greene, Neuron* 2001), a major advantage of using E2F1(1-368) to uncover death-associated genes is that many fewer genes are likely to be regulated by such a focused stimulus. I had made an adenovirus containing E2F1(1-368)-RFP under control of a Tc-inducible promoter, and plan to use it to infect SCG neurons and PC12 cells, and identify critical down-stream target genes that mediate neuron death.

b. Prevention of neuron death and treatment of neurodegenerative disorders in animal models Having shown that E2F1-Rb and E2F4-p130ΔCDK can protect several types of neurons from various apoptotic stimuli in culture, I want to test if they can protect neurons and improving diseased conditions in neurodegenerative diseases. Dr. R. Burke at Columbia University had compared adeno associated viruses (AdAV) with adenoviruses (AdV) in expressing target genes in dopamine neurons (DN) in rat substantia nigra and found that AdAV is a superior gene delivery system. We plan to make an AdAV containing E2F1-Rb and test its efficacy in DNs protection in an acute PD model. I also want to test the feasibility of using the E2F1-Rb virus to treat spinal cord injury (SCI). Neurite regeneration after spinal cord injury is hindered by neuronal death and the formation of scars resulted from gliogenesis after SCI. Current approaches have not been able to deal with the two issues simultaneously. I hypothesize that expression of E2F1-Rb by viral infection, however, will both protect neurons from death and at the same time inhibit proliferation of glial cells, hence promoting neurite regeneration.

2. ATF5: How ATF5 coordinates with key cell cycle regulators in prevention of cell cycle exit and neural differentiation One of the key processes that govern neural development involves the maintenance of neural stem/ progenitor cells. ATF5, a bZIP transcription factor of the CREB family, is among a few molecules that seem to play major roles in maintenance of neural stem cells. ATF5 is markedly down-regulated in PC12 cells treated with NGF (a model system for neuronal differentiation) and is turned off in fully differentiated neurons, astrocytes, and oligodendrocytes in the developing brain. Moreover, unabated ATF5 expression blocks cell cycle exit and neuronal differentiation of PC12 cells induced by NGF (right upper, clone 15). It also promotes hyperplasia of VZ and inhibits differentiation of neural stem cell *in vivo* (right lower; also *Angelastro et al., 2003; 2005a*). These findings establish ATF5 as a key player in the growth and maintenance of neuroprogenitor/stem cells. We also found that ATF5 knock-down can specifically induce death of various (such as rat C6) glioma cell lines and brain tumors *in vivo* but spares ATF5-expressing glial cells, indicating a pro-survival role of ATF5 in glioma cells (*Angelastro et al., 2005b*). The challenge now is to understand the mechanisms. I will use a combination of molecular, cellular, and mouse genetic approaches to gain the insights.



In contrast to control cells (left), PC12 cells with constitutive ATF5 expression (right) do not exit the cell cycle in response to NGF.



Constitutive expression of ATF5 in SVZ cells *in vivo* promotes hyperplasia and movement of VZ. SVZ of P2 rats were injected with retrovirus encoding eGFP alone (left) of eGFP+ATF5 (right). Animals were maintained for 3.5 months before examination for GFP expression. Lateral ventricle is at the top of image.

To understand how ATF5 works in neuroprogenitors, stem cells and gliomas, I want to take a sytematic approach to identify the ATF5 cognate binding sequences, down-stream target genes, and the proteins that interact with ATF5 in neuroprogenitors, PC12 cells and gliomas (C6) cells. An initial ChIP strategy has yielded promising results on ATF5 binding sequences. This and other strategies will also help indentify ATF5-regulated target genes in those cells. To gain insights on what proteins work together with ATF5, I will use a double-tag strategy perfected by Dr. W. Gu at Columbia University (please see *Chen et al. Cell* 121:1071), and yeast two hybrid strategy as a backup. Potential players that had been reported to be related to ATF5 include LKB1, PRL-1, cyclinD3, DISC1, HIV protein Tax and the β-catinin/Tcf pathway. I also plan to create ATF5 KO mice and use an *in utero* gene transfer strategy developed by Dr. A. Kriegstein at UCSF (please see *Noctor et al., Nat Neurosci.* 7:136) to study ATF5 role in neuronal differentiation in the brain. Undoubtedly, my work on ATF5 will impact on fields of stem cell regulation, tumor metastasis, neural differentiation and apoptosis.

My research has yielded a number of unexpected findings about the regulation of the cell cycle machinery and the complex roles that can be played by cell cycle components during neuron differentiation and death. Considering that similar cellular processes are likely to be common in the CNS *in vivo*, these results should have broad implications for our understanding of how the CNS works and why it sometime malfunctions. The next several years should mark an exciting time to build on the existing principles and expand our basic understanding of stem cell regulation, developmental processes, and their contribution to human CNS disorders.

David X. Liu, Ph.D.
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Teaching Philosophy and Interests

My teaching experience began at Qingdao University, China, where I conducted lectures for undergraduate students in Biology/Biochem majors. Later, during my tenure from 1991-1996 as a Graduate Teaching Assistant at Hunter College, City University of New York, I again taught undergraduates several courses. At New York University and at Columbia University, I continued my teaching role as mentor to first and second year graduate students. These experiences gave me opportunities to improve my teaching skills and, in retrospect, allowed me to understand the core elements of my teaching philosophy. They also led me to realize that teaching is the opportunity to make a real difference in students' lives, and that it carries awesome responsibility.

I believe that an effective teacher must have a great understanding of the taught subject and of the underlying theory. This helps in a good selection of the teaching materials and a logical and an organized way to present them. A good teacher must not only effectively communicate facts and information to the students but also motivate them to think critically and independently about the processes and methods that underlie the fundamental principles. I believe that teaching must be an interactive process. Encouraging participation and questions from students helps me gauge their level of understanding and tailor my teaching closely for their need.

As a bench biologist myself for so many years, I believe that it is essential to expose students with real world questions and challenge them to discover their own solutions. It is important that students understand that biologists do not have all the answers to biological questions and that theirs could be the best. I found that paper studies and critiques, peer-review process, and research that include my own provide perfect learning opportunities for students in this process. These activities serve a great deal to promote students' curiosity and inner drive and to foster their confidence.

My varied research and teach experience has provided me with a deep understanding of molecular biology, cell biology, biochemistry, microbiology and neurobiology. I believe this diverse background will add to my ability to teach a variety of subjects. Topics that I would like to teach are as follows: 1) signal transduction pathways from the cell surface to the nucleus that lead to cell proliferation, cell-fate determination, differentiation, or apoptosis; 2) lectures that are related to Cell Biology, Molecular Biology, Biochemistry, Molecular Genetics, Molecular Neurobiology, or Cancer Biology; and 3) special topic courses that would be open to hosting seminar speakers within the university scientific community, and from other institutions.