Yale University

Albert E. Ayoub, Ph.D
Associate Research Scientist
Department of Neurobiology
School of Medicine
P.O. Box 208001
New Haven, CT 06520-8001

Campus address:
SHM C-317
333 Cedar Street
Telephone: (203) 785-5910
FAX: (203) 785-7263
email:albert.ayoub@yale.edu

March 23, 2012

RE: Faculty Position

Dear members of the search committee:

I would like to apply for the **Tenure-Track Faculty Position** at the **Assistant Professor** level as recently advertised in Neurojobs. My research is focused on transcriptional and regulatory programs that precisely orchestrate the formation of the cerebral cortex. Disruption of these developmental mechanisms is associated with neurological and psychological disorders. I apply a genome-wide systems biology approach in combination with cutting edge molecular techniques to decipher both conserved and species-specific developmental mechanisms.

I have included a summary of my research and teaching interests and current CV for your consideration. Moreover, I have requested letters of recommendations from Drs. Pasko Rakic, M.D., Ph.D., James Noonan Ph.D., and Charles Greer Ph.D. A list of my publications can be obtained online through the Web of Knowledge ResearcherID (http://www.researcherid.com/rid/G-1783-2011).

Sincerely,

Albert E. Ayoub, Ph.D.

References Requested From:

Pasko Rakic, M.D., Ph.D.
Duberg Professor of Neurobiology and Neurology
Chairman, Department of Neurobiology
Director, Kavli Institute for Neuroscience
Yale University School of Medicine
E-mail: pasko.rakic@yale.edu

James P Noonan, Ph.D.
Assistant Professor, Dept. of Neurobiology and Genetics
Kavli Institute for Neuroscience
Yale University School of Medicine
E-mail: james.noonan@yale.edu

Charles A Greer, PhD
Professor of Neurosurgery and Neurobiology
Director of Interdepartmental Neuroscience Graduate Program
Yale University School of Medicine
Email: charles.greer@yale.edu

CURRICULUM VITAE

Albert E. Ayoub, Ph.D.

Associate Research Scientist
Department of Neurobiology and Kavli Institute for Neuroscience
Yale University School of Medicine

Mailing address: Department of Neurobiology and Kavli Institute for Neuroscience
Yale University School of Medicine
333 Cedar Street SHM C317, New Haven, CT 06510
Phone: +1-203-785-5910, Fax: +1-203-785-5263
e-mail: albert.ayoub@yale.edu

Education:

2004: Ph.D. in Anatomy and Neurobiology
Department of Neurobiology and Anatomy, West Virginia University School of Medicine
1999: B.S. in Biology
Department of Biology, West Virginia University, Morgantown WV

Professional experience:

2010-pres. Associate Research Scientist
Department of Neurobiology and Kavli Institute for Neuroscience, Yale University School of Medicine
2004-2010 Postdoctoral Fellow/Associate (laboratory of Dr. Pasko Rakic)
Department of Neurobiology and Kavli Institute for Neuroscience, Yale University School of Medicine
2001-2004 Research Fellow (laboratory of Dr. Jia Luo)
Department of Neurobiology and Anatomy, West Virginia University School of Medicine
1998 Undergraduate Research Fellow (laboratory of Dr. Adrienne K Salm)
Department of Neurobiology and Anatomy, West Virginia University School of Medicine

Teaching experience:

1999-2000 Teaching Assistant, Medical Histology course, Department of Neurobiology and Anatomy, West Virginia University School of Medicine
2001-2003 Teaching Assistant, Medical Neurobiology course, Department of Neurobiology and Anatomy, West Virginia University School of Medicine
2003 Guest Lecturer, Internal Structure of CNS, Continuing Medical Education at Chestnut Hill Psychiatric Hospital, Morgantown WV
2006-pres Manager of the Advanced Optical Imaging Facility, Department of Neurobiology at Yale University

Awards and fellowships:

2008-2010 The Medical Foundation: Fellow of the Patterson Trust Fellowship Program in Brain Circuitry
1999-2003 Swiger doctoral student fellowship, WVU School of Medicine
1998 Undergraduate research fellowship, WVU School of Medicine

Research grants:

2008-2010 The Medical Foundation / Patterson Trust Postdoctoral Fellowship Award
"This project focuses on signaling mechanisms at the onset of neurogenesis in the mouse and rhesus macaque embryos".
Role: Principal Investigator

2010- pres Kavli Foundation Initiative
"Transcriptome analysis of the mouse and primate cortical layers during corticogenesis". This is a collaborative project with the Department of Genetics at Yale University on cross species comparisons of genetic mechanisms underlying corticogenesis.

Membership of academic societies:

Society for Neuroscience (SfN)
The New York Academy of Sciences
American Association of Anatomists

Reviewer for journals:

Cerebral Cortex
Proc Natl Acad Sci USA

Research Papers:

Ayoub AE, Salm AK. Increased morphological diversity of microglia in the activated hypothalamic supraoptic nucleus. J Neurosci. 2003 Aug 27;23(21):7759-66.

Salm AK, **Ayoub AE**, Lally BE (2003) Structural plasticity of nonneuronal cells in the hypothalamo-neurohypophyseal system: in the right place at the right time. Advances in Molecular and Cell Biology, Vol. 31:181-199.

Ayoub AE, Cai TQ, Kaplan RA, Luo J. Developmental expression of matrix metalloproteinases 2 and 9 and their potential role in the histogenesis of the cerebellar cortex. J Comp Neurol. 2005 Jan 24;481(4):403-15.

Gal JS, Morozov YM, **Ayoub AE**, Chatterjee M, Rakic P, Haydar TF. Molecular and morphological heterogeneity of neural precursors in the mouse neocortical proliferative zones. J Neurosci. 2006 Jan 18;26(3):1045-56.

Morozov YM, **Ayoub AE**, Rakic P. Translocation of synaptically connected interneurons across the dentate gyrus of the early postnatal rat hippocampus. J Neurosci. 2006 May 10;26(19):5017-27.

Burns KA, **Ayoub AE**, Breunig JJ, Adhami F, Weng WL, Colbert MC, Rakic P, Kuan CY. Nestin-CreER mice reveal DNA synthesis by nonapoptotic neurons following cerebral ischemia hypoxia. Cereb Cortex. 2007 Nov;17(11):2585-92.

Town T, Breunig JJ, Sarkisian MR, Spilianakis C, **Ayoub AE**, Liu X, Ferrandino AF, Gallagher AR, Li MO, Rakic P, Flavell RA. The stumpy gene is required for mammalian ciliogenesis. Proc Natl Acad Sci U S A. 2008 Feb 26;105(8):2853-8.

Breunig JJ, Sarkisian MR, Arellano JI, Morozov YM, **Ayoub AE**, Sojitra S, Wang B, Flavell RA, Rakic P, Town T. Primary cilia regulate hippocampal neurogenesis by mediating sonic hedgehog signaling. Proc Natl Acad Sci U S A. 2008 Sep 2;105(35):13127-32.

Ayoub AE, Kostovic I. New horizons for the subplate zone and its pioneering neurons. Cereb Cortex. 2009 Aug;19 (8):1705-7.

Rakic P, **Ayoub AE**, Breunig JJ, Dominguez MH. Decision by division: making cortical maps. Trends Neurosci. 2009 May;32(5):291-301.

Imamura F, **Ayoub AE**, Rakic P, Greer CA. Timing of neurogenesis is a determinant of olfactory circuitry. Nat Neurosci. 2011 Mar;14(3):331-7.

Ayoub AE, Oh S, Xie Y, Leng J, Cotney J, Dominguez MD, Noonan JP, Rakic P. Transcriptional programs in transient embryonic zones of the cerebral cortex defined by high-resolution mRNA-sequencing. Proc Natl Acad Sci U S A. 2011 Sep 6;108(36):14950-5. Epub 2011 Aug 22. **Supplemental database of transcripts expressed in the developing neocortex (http://rakiclab.med.yale.edu/transcriptome.php)**

Dominguez MH*, **Ayoub AE***, Breunig JJ, Rakic P. The onset of heterogeneity in cerebral cortical progenitors accompanies the onset of neurogenesis (2012, **contributed equally*, ***under review***).

Dominguez MH, **Ayoub AE** and Rakic P. Brn1 and Brn2 influence neurogenesis, molecular identity, and migratory destination of upper layer cells of the cerebral cortex (2012, ***under review***).

Ayoub AE, Dominguez MH, Burn K, Kuan C-Y and Rakic P. The transcriptional network of Mastermind-like controls binary cell fate in the mammalian cerebral cortex. (2012, *in preparation*).

Ayoub AE and Rakic P. Molecular Evolution of the Neocortex (2012, Review Article, ***under review***).

Ayoub AE, Xie Y, Leng J, Cotney J, Noonan JP and Rakic P. Human-specific transcriptional programs in transient embryonic zones of the cerebral cortex defined by high-resolution mRNA-sequencing (*in preparation*).

Statement of Research Interests
Albert E. Ayoub, Ph.D.

The cerebral cortex features functionally distinct classes of neurons and complex circuitry responsible for higher brain functions such as cognition, speech, learning and adaptive behavior. The regulatory mechanisms that control the formation of the cortex are poorly understood. **My primary research focus is to study the genetic and regulatory mechanisms that are essential for corticogenesis.**

The dynamic molecular processes underlying cortical development are usually studied on a microscopic level. Although numerous genes have been studied using molecular techniques, they constitute only a fraction of the transcriptional output of the mammalian genome. Conversely, studying global expression changes and transcriptional regulation is often-times achieved at the expense of spatial resolution. A new approach is therefore necessary if we are to make significant progress in understanding the development and evolution of the cerebral cortex. **I propose to combine cutting-edge genomic analysis with cellular and molecular manipulations to elucidate the function of genetic networks in cortical development.**

Technical aspects aside, one must be knowledgeable in all areas of development since genetic mechanisms underlying neurogenesis, neuronal migration, differentiation and cell death are often intertwined. **My training has led me to explore how (1) dynamic gene regulation and interactions at the molecular level instruct the proper formation of the neocortex, and (2) how transcriptional networks can be studied at high-spatial resolution using genome-wide and bioinformatic tools.**

Prior and Current Work
I first became fascinated by neuroscience when my high school biology teacher showed us a simplified version of the Broadmann map. While still an undergraduate biology student, I earned a summer fellowship in neuroscience at WVU School of Medicine. I joined the lab of Dr. Salm, who studied plasticity in the hypothalamus in response to homeostatic challenge. We found increased activation of microglial cells in the stimulated supraoptic and paraventricular nuclei, which we published in the Journal of Neuroscience (Ayoub and Salm, 2003). Soon after, I joined the laboratory of Dr. Luo to study cerebellar development. We found that matrix metalloproteases contribute to the migration of granule cells, dendritogenesis of Purkinje cells and that their function is disrupted by fetal alcohol exposure (Ayoub et al., 2005). My experience in the lab sparked my interest in cortical development but also showed me that meaningful progress requires a stronger background in genetic and molecular biology. Therefore, I seized the opportunity to join Dr. Rakic's lab at Yale University for my postdoctoral training.

Dr. Pasko Rakic is world renowned for his pioneering work on cortical development. His lab offered the perfect environment to immerse myself in the field of cortical development. I focused my intrigue on the elusive question of what genetic mechanisms allow the formation of the six-layered cortex. The dominant view asserted homogeneity of the neural stem cell population in the mammalian cortex. However, evidence from studies on the embryonic mouse, rhesus macaque and human brain supported an alternate view. I argued that the apparent morphological uniformity of the stem cell population masked widespread genetic heterogeneity. To test this idea, I began cloning and introducing different promoter-driven fluorescent proteins by *in utero* electroporation into neural stem cells lining the

ventricles. This approach allowed us to differentiate among subpopulations of neural stem cells using confocal imaging and electron-microscopy (Gal et al., 2006). Further, in collaboration with Dr. C-Y Kuan at the University of Cincinnati, we developed a tamoxifen-inducible nestin-driven GFP mouse line. Using these mice, I was able to observe neural stem cells and their progeny by 2-photon imaging in living cortical slices during different periods of cortical development (Burns et al., 2007). More recently, I provided evidence, using live multi-photon imaging, that the spatial and temporal dynamics of neurogenesis control layer specificity in the embryonic olfactory bulb as in the developing cortex. Capitalizing on previous work and to directly address genetic heterogeneity, I manipulated the fate of neural stem cells in vivo. This allowed me to put forth a model that explains the mechanism behind spatiotemporal heterogeneity of neural stem cells in the cortex (in press).

The fast tempo of corticogenesis in the mouse compared to primates does not explain the evolutionary expansion of the human and primate neocortex. I proposed that heterogeneity is coupled to species-specific genetic mechanisms, which can only be unmasked by a comparative high-resolution genomic analysis. During the last three years, I became a liaison between Dr. Rakic and Dr. Noonan's lab at Yale. Dr. James Noonan is renowned for identifying cis-regulatory regions in the genome that show accelerated evolution in the human and primate lineages through computational and mouse engineering approaches. This collaboration provided me with opportunities to train with postdoctoral fellows in genomics and bioinformatics. In my recently published study (Ayoub et al 2011), I defined, with high-spatial resolution, five transcriptional programs that govern different developmental mechanisms such as stem cell maintenance, neurogenesis, migration, and differentiation. Additionally, I was able to expose novel genes and mapped putative cis-regulatory sequences. Since publishing this work, I have made this valuable data available to the scientific community on a public website (http://rakiclab.med.yale.edu/transcriptome.php). I am currently applying this high-resolution approach to define transcriptional programs in the human and rhesus embryonic cortex. These genomic datasets and bioinformatic skills have provided me with a unique perspective and essential foundation to jump start future studies on the development of the cerebral cortex.

Future Research Plans

My research interest is to understand how layers of the neocortex are coordinately generated from neural stem cells during development. I will focus on dynamic gene regulation and interactions by bridging cellular and molecular manipulations with genomic analysis of transcriptional networks. This work has implications on both cortical evolution and neuropatholgical conditions.

1. Transcriptional networks underlying prenatal neurogenesis.

I have defined transcriptional programs that contain hundreds of network modules based on differential transcriptome analysis. Using data generated from our recently published studies, I started an independent project on the functional profiling of the transcriptional network of Mastermind-like gene (MAML), which seems to coordinate novel aspects of Notch signaling and other neurogenic pathways.

Aim 1: My preliminary data suggests that loss of MAML activates a common neuronal differentiation pathway. Indeed, overexpression of dnMAML in utero causes an abrupt exit from the cell cycle and strong neurogenesis. I propose to determine the transcriptional program initiated by the loss of MAML

in vivo. The genome-wide repercussions of this genetic manipulation will be detected at the transcript level with high spatial resolution (as in Ayoub et al 2011). A model of the transcriptional network is then built based on differential gene expression.

Aim 2. I propose to validate the network model generated in (Aim1) by testing the most significant targets differentially regulated by the loss of MAML. These targets will be validated by realtime-QPCR and in situ hybridization. My second strategy for validating target genes consists of over-expressing or down-regulating 4 genes by *in utero* electroporation, which permits efficient and precise spatiotemporal control of genetic manipulations.

Aim 3: Determine the regulatory network of MAML. Because only a small proportion of the genetic landscape contains protein-coding sequences, gene-centric studies can miss widespread regulatory events to the rest of the genome. I propose to determine a model of the regulatory network by identifying the binding sites on downstream targets using ChIP-Seq (chromatin immuno-precipitation following by sequencing). Further, I will use *in vitro* reporter assays to test the responsiveness of downstream elements harboring suspected binding sites. Highly significant targets, based on chromatin-binding signature and presence of transcription factor binding elements, will be tested experimentally by *in utero* electroporation in mice.

2. Stress-induced perturbations to transcriptional networks underlying prenatal neurogenesis.

Traumatic stress during pregnancy is known to affect the intra-uterine environment and is thought to precipitate psychological disorders in children long after birth. Disturbances to neuronal networks, synaptic signaling and plasticity have been reported but the underlying genetic causes are largely unknown. I intend to determine genome-wide modifications (including acetylation and methylation) that ensue after acute prenatal stress with specific focus on transcriptional networks underlying prenatal neurogenesis. As a model system, we induce stress and manipulate gene expression during the second half of cortical development in mice. During this period, upper layer neurons that make intra-cortical projections are forming. In humans, these layers form over a period of about one month between gestational week 9 and 13. These layers are vastly expanded in primates, especially humans, and are thought to underlie the cognitive abilities of mammals. The consequences of such experimental manipulations can be studied perinatally, with respect to developmental mechanisms, or months after birth to investigate synaptic plasticity in collaboration with other laboratories. This project will tackle a period of cortical development that is of fundamental importance to brain function with implications in many human psychiatric conditions. Although this project is still in the early stages, I am beginning to explore the functional consequences of acute stress on brain development and gather preliminary data necessary for grant applications.

Statement of Teaching Interests
Albert E. Ayoub, Ph.D.

“For the vision of one man lends not its wings to another man.” - The Prophet; Khalil Gibran

College is a critical time in student's life when leadership, excellence, and creativity are instilled through traditional and innovative teaching methods. Diversity of college students, backgrounds, aspirations, and learning styles necessitates innovative methods to drive academic and intellectual growth. Instead of taking the stage as a sage to project knowledge for the masses, I hope to inspire students through my enthusiasm, empower them through critical thinking, encourage their creativity through team-based assignments, and foster their presentation skills. These are the qualities that will set them apart as successful leaders and innovators.

In my view, a good teacher should introduce students to the way science conceptualizes our world, much of which is not directly accessible to our senses. In order to foster excellence and innovation, I will start students with reductive instructions then challenge them with inductive exercises. While important, details can be better appreciated if given after the main conceptual framework has been constructed. If teaching focuses on the art of critical, coherent and creative thinking, students are more likely to be interested in new advances long after they receive their final grades. Students in the biological sciences and/or the medical field can continue to grasp emerging concepts long after a foundation of curiosity and critical thinking is fostered.

As a doctoral student, I served as a teaching assistant in the medical neurobiology and histology courses taught to first year medical students. Most of my important contributions to the students did not come by reviewing, yet again, the specific location of the nucleus of Meynert but by placing the subject matter in a broader context. As a senior Ph.D. student, I participated in the continuing medical education series provided to psychiatry residents at Chestnut Hill Psychiatric Hospital. I prepared and presented introductory lectures on the internal structures of the CNS. I tailored my presentation to the specialized interest of the audience by emphasizing CT scans and MRI reconstructions to build upon simple efferent/afferent diagrams or cross-sectional anatomy material.

During my postdoctoral training, I supervised several graduate students at Yale. It was stimulating, challenging, but ultimately satisfying to see fledgling students emerge as scientists. Every experience was unique as one student is reluctant to deviate from lab protocols while another likes to test different experimental conditions. Despite the route chosen, eventually I was able to guide students toward becoming independent and provocative thinkers capable of designing and facilitating their own scientific inquiries. In addition to my research project, I oversaw a microscopy facility shared among several research groups including Dr. Rakic’s lab. The facility housed two confocal and multiphoton microscopes, laser microdissection and other wide-field microscopes. I had the unique experience of advising and learning from fellow researchers on how to optimize experiments, data collection and analysis from live imaging in brain slices, calcium imaging, to high-resolution 3D reconstructions.

As an assistant professor, my strengths could be best utilized by teaching Introductory and Advanced Neuroscience, Human Neuroanatomy (theory and laboratory), Brain Development and Molecular Neurobiology.