Harvard Medical School
DEPARTMENT OF GENETICS
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January 6, 2004

Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
Swain Hall West 117
Bloomington, IN 47405-7105

Re: Junior Faculty Position

Dear Professor de Ruyter van Steveninck and Faculty Search Committee,

Please accept the following letter and *curriculum vitae* as my application for the above-mentioned position. After I had completed my doctorate in Australia, I sought to broaden my experience by taking an appointment in the United States. My interests in cellular signalling led me to pursue my postdoctoral training with Professor Ernie Peralta at Harvard University. After several years broadening my experience and professional network, I am now seeking to develop my own lab so the opportunity you have advertised is of particular interest to me.

I believe that my postdoctoral training has prepared me particularly well for the next stage of my career. The research environment at Harvard is outstanding and has honed my ideas and skills, while the University's strong commitment to teaching has allowed me to advance and refine my teaching skills at both the undergraduate and postgraduate levels.

Since my arrival in Boston my main focus has been to develop and conduct an independent line of research in laboratories at Harvard University and at the Harvard Medical School. My research proposal was awarded a National Institutes of *Health National Research Service Award* in 1998 to pursue independent research under the supervision of Professor Ernest Peralta and Professor Eva Neer. Throughout this time I have been involved in a number collaborative research efforts both with postgraduate students within the lab (in a supervisory role) and other Principle Investigators.

I joined the laboratory of Professor Christine Seidman and Professor Jon Seidman in 2001 and since then I have focused on fundamental aspects of mouse perinatal heart development, with an emphasis on the regulation of gene expression, cell cycle,

differentiation and their relationship to normal heart growth and function from a molecular to whole organ level. The goal of my current and future research is to understand signal transduction pathways and the genes that regulate growth and differentiation of the heart. Defects in this process may be involved in the pathogenesis of a number of childhood and adult cardiomyopathies. A major impediment to understanding terminal heart differentiation has been the inability to study individual heart cells during their terminal withdrawal from the cell cycle. I have generated several innovative techniques to isolate and study individual perinatal cardiomyocyte through imaging at the molecular and cellular level. I am now using these new methods as well as other molecular and biochemical methods to study previously inaccessible aspects of perinatal heart development. My current research and collaboration with other investigators has led to several manuscripts being prepared for submission. See my *curriculum vitae* for more information on these articles.

In 1999 and 2000 I was employed by Professor Guido Guidotti as Head Teaching Fellow to teach and administer one of the principal biochemical undergraduate courses at Harvard; Basic Principles of Biochemistry and Cell Biology. In addition to tutoring, writing, grading problem sets and exams, and preparing and presenting eight lectures, my primary responsibility was to hire and manage a staff of 20 teaching fellows. Due to my contribution to undergraduate science education, I was then invited to become a member of the *Board of Tutors in Biochemical Sciences*. In that capacity I have been responsible for augmenting the intellectual development of four advanced placement undergraduate students each academic year. For the past three years I have served as one of their mentors by working with them to address modern biological science problems, experimental design, and encouraging the development of these students' analytical and critical thought.

With over ten years of research and teaching experience, I believe I possess the knowledge, skill, and experience to positively contribute to your department. If you have any questions or require any additional information, please do not hesitate to contact me.

Sincerely,

Patrick G Burgon Ph.D.
Research Associate

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Previous Research Accomplishments

Graduate Studies (Robertson's Lab 1992-1996)

Gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are comprised of a population of isoforms that differ in their structural and functional properties. The major structural basis for these pleomorphic characteristics of LH and FSH, was presumed to be primarily related, to variations in the oligosaccharide structure of the hormone. My graduate work involved the isolation and purification of gonadotropin isoforms from 200 human (h) pituitaries by sequential use of a variety of charge-based and size based chromatographic techniques. Twenty-four LH and 20 FSH isoforms were purified to high purity (>90%). These isoforms had different physiochemical characteristics such as apparent molecular weight, sialic acid content and mean pI. Each of these highly purified gonadotropin isoforms had their receptor and *in vitro* biopotencies, pharmacokinetic characteristics, *in vivo* bioactivities and immunological potencies determined. We found that highly purified isoform preparations of human LH and FSH exhibit a 4 to 16-fold range in specific activities. Sialic acid is a major source of the observed structural (charge and size) heterogeneity and, also a major contributor to the intrinsic activity of the isoform. The major biological points at which gonadotropin heterogeneity appears to be important in terms of regulating reproduction is at the receptor and subsequent post-receptor events. In contrast, the relationship between *in vivo* bioactivity and plasma clearance kinetics of hLH isoforms is directly related to only those isoforms with less than 3.5 moles of sialic acid/mole hLH. For isoforms with >3.5 moles of sialic acid/mole hLH *in vivo* bioactivity is independent of plasma clearance kinetics. Finally, the contribution of sialic acid content towards the intrinsic activities of hLH isoforms was shown to be important in receptor, *in vitro* and *in vivo* bioactivities, as the removal of sialic acid led to a general decrease in functional heterogeneity. The results from my graduate work, as presented in my thesis and 6 peer reviewed papers, has quantitatively established the differences in specific activities between gonadotropin isoforms, and the relationship between each of these activities to structural heterogeneity of the hormone. Since the distribution of gonadotropin isoforms vary between endocrine states, and that isoforms differ in specific activities at multiple biological endpoints, then the combination of these parameters suggests that gonadotropin heterogeneity has a physiological role in the subtle regulation of gonadal function.

Postdoctoral Studies (Peralta 1997-1999, Neer 1999-2000, Seidman 2001-to date)

Peralta & Neer labs. Shortly before joining the Peralta Lab a novel class of proteins that regulate G protein signaling (RGS) had been discovered. RGS proteins bind specifically to activated Gα proteins of heterotrimeric G proteins, potentiating the intrinsic GTPase activity of the Gα proteins and thus expediting the termination of G signaling. Although there are several points in most G protein controlled signaling pathways that are affected by reversible covalent modification, little evidence has been shown addressing whether or not the functions of RGS proteins are themselves regulated by such modifications. The aim of my post-doctoral research was to investigate the biochemical pathways that may regulate the activities of RGS proteins (RGS10 in particular) and modulate G protein-coupled signaling. We discovered the acute functional regulation of RGS10 thru the specific and inducible phosphorylation of RGS10 protein at serine 168 by protein kinase A. This phosphorylation nullifies the RGS10 activity at the plasma membrane, which controls the G protein-dependent activation of the inwardly rectifying potassium channel. Surprisingly, the phosphorylation-mediated attenuation of RGS10 activity was not manifested in an alteration of its ability to accelerate GTPase activity of Gα proteins. Rather, the phosphorylation event correlates with translocation of RGS10 from the plasma membrane and cytosol into the nucleus (Burgon *et.al.* '01).
Seidman Lab. To determine the role of cardiac myosin-binding protein C (MyBPC) in heart structure and function, the Seidman lab generated mice that expressed a truncated form of MyBPC as found in a population of humans with hypertrophic cardiomyopathy. Similar to the humans, the heterozygous mouse also develops late onset adult hypertrophic cardiomyopathy. However, homozygous mice develop dilated cardiomyopathy by 3 weeks of age due to a 10-fold reduction in MyBPC protein expression. My primary postdoctoral research in the Seidman lab investigates the molecular and signaling basis for the neonatal onset of DCM in the truncated MyBPC homozygous model ($MyBPC^{t/t}$). Data thus far generated suggests that the neonatal onset of dilation is due to alterations in cell cycle kinetics during cardiomyocyte terminal differentiation during the first 10 days of neonatal life. This aberration in $MyBPC^{t/t}$ cell cycle leads to cardiomyocyte hyperplasia resulting in an enlarged dilated heart by 10 days post-birth. The hyperplasia is transient in that there is complete withdrawal from the cell cycle like that found in a normal neonatal mouse (manuscript under preparation). The direct molecular link between the loss of MyBPC, a sarcomeric structural protein, and cell cycle is currently under investigation and form the fundamental basis of my future research plans.

Proposal of Future Research

The goal of my research is to understand signal transduction pathways and the genes that regulate growth and differentiation of the heart. Since defects in terminal heart differentiation may be involved in the pathogenesis of a number of childhood and adult cardiomyopathies, understanding the onset of these defects should provide further insight into the pathogenesis of human heart disease.

Overview

Most research on the genetic determinants of heart development and disease has been focussed on early fetal heart formation and the pathogenesis of childhood and adult heart disease. Based on these findings, it has been shown that during fetal and early perinatal development the myocardium undergoes a period of hyperplastic growth, which results in an exponential increase in the number of cardio myocytes that constitute the adult heart. Soon after birth, cardio myocytes cease to divide and all subsequent increase in myocardial mass is accomplished by cardio myocyte hypertrophy. Despite the importance of this phenomenon, little is actually known about the molecular basis for the transition from hyperplastic to hypertrophic-based myocardial growth. I am interested in understanding the mechanisms involved in such a transition. In other areas of research, the primary focus has been on the role of cell cycle genes in the development of the heart. This research has depended heavily on mouse knock-in, knockout, and transgenic models of cell cycle genes. Results from these studies have identified proteins that maintain and regulate the G1/S checkpoint as targets for the transition between hyperplastic and hypertrophic myocardial growth. While it is clear that the G1/S checkpoint genes have a role in regulating heart growth, the mechanisms by which this occurs remain largely unknown. Recent progress in this area has been made that demonstrates Cyclin E and E2F3 are important mediators for normal septal development in the heart. However to date there has been no clear link between cell cycle mediation of myocardial development and the pathogenesis of childhood and adult heart disease.

My current and proposed research bridges these broad areas of heart development. I have shown that Myosin Binding Protein C (MyBPC), a sarcomeric protein that is expressed only in cardio myocytes, is involved in the regulation of myocardial withdrawal from the cell cycle and the transition from hyperplastic to hypertrophic growth. With the long-term goal of dissecting the general mechanisms of cell cycle regulation in heart development, I have undertaken the comparative characterisation of the MyBPC deficient mouse ($MyBPC^{t/t}$) to normal mouse heart development. I also plan to further explore the interaction between MyBPC and the cell cycle proteins that influence heart development.

Specific aims

1) Determine in detail the physiological and molecular correlates, by comparative analysis of the Myosin Binding Protein C deficient mouse, of the heart's withdrawal from cell cycle.

A nine to ten fold reduction in Myosin Binding Protein C leads to perinatal heart dilation due to aberrant cell cycle regulation. I have focused on normal and abnormal perinatal heart development and the molecular basis for the neonatal onset of dilated cardiomyopathy in the truncated Myosin Binding Protein C homozygous mouse model ($MyBPC^{t/t}$). Novel techniques to isolate and study individual perinatal cardio myocytes were developed and utilised to study individual heart cells from the $MyBPC^{t/t}$ homozygous model during the hearts terminal withdrawal from the cell cycle. The data generated to date suggests that the dilation is a result of altered cell cycle kinetics in the first 10 days of perinatal life. This aberration in $MyBPC^{t/t}$ cell cycle leads to cardio myocyte hyperplasia resulting in an enlarged heart. However the cardio myocyte hyperplasia is transient in that the cardio myocytes withdraw completely from the cell cycle replicating normal mouse development. I have identified a number of candidate genes however the direct molecular link between the functional loss of a sarcomeric structural protein (MyBPC) and the cell cycle is still unknown. The data suggests that MyBPC inhibits cytokinesis within the context of a neonatal heart and forms the basis for future investigations.

Expression profile – Gene Chip expression analysis of MyBPCt/t and Normal mouse heart between E19.5 and day 10. Confirmation of chip data through northern, *in situ*, immunohistochemistry and western analysis.

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2) Determine the pathogenesis of lamin A/C derived dilated cardiomyopathy

Many of the mutations in cardiac specific genes result in cardiomyopathies some three plus decades post-birth. Clusters of mutations in the Lamin A/C gene result in dilation and conduction disease of the human heart. Lamin A/C is an integral structural protein of the nuclear membrane of most differentiated cells. In the case of the heart, Lamin A/C is only expressed in cardio myocytes that have withdrawn from the cell cycle in the week post-birth. How does the chronic expression of a mutant allele have no visible phenotype in the heart until three to four decades after the initial expression of the allele? Two approachs to identify the molecular determinants of dilated cardiomyopathy from mutations in the Lamin A/C gene will include:

i) Yeast Two Hybrid Screen - Many of the mutations identified to date result in a loss or gain of charge within Lamin A/C gene. The dominant phenotype of the disease allele suggests that the mutations result in a gain or loss of an interaction(s) between Lamin A/C and unknown proteins. To address this question a yeast two-hybrid screen has been undertaken using normal and mutated lamin (N195K) as bait.

ii) Mouse model generation - Knock-in N195K mutation & heart specific knock-out of Lamin A/C. Utilising ET-cloning a knock-in mouse that expresses lamin N195K will be generated and characterised. In addition a heart specific lamin A deleted mouse will be engineered using Cre-technology. A tissue specific mouse is necessary because the haplodeficient Lamin A mouse has no apparent recognisable phenotype and the Lamin A deleted mouse dies 6 to 8 weeks post-birth from a variety of muscular defects and organ failure. Both of these mouse models will be independently characterised and crossed to study in detail the pathogenesis of dilated cardiomyopathy as a result of mutations in the Lamin A gene.

3) Determine the role of cell cycle genes in atrial and ventricular septal formation

Malformations of the atrial and ventricular septum are one of the most common perinatal heart problems post-birth. Recent evidence points to the G1/S checkpoint in cell cycle as being an important mediator of septal development, in that mice lacking Cyclin E and E2F3 die due to atrial septal defect (ASD) and/or ventricle septal defect (VSD) *in utero* or shortly after birth. A Tbx5 haplodeficienent mouse has been shown to cause truncated limbs as well as septal defects in the heart, replicating Holt-Oram Syndrome. Recent (unpublished) evidence suggests that Tbx5 regulate BMP signalling that may indirectly modify a variety of cell cycle genes. To test if Tbx5 directly regulates cardio myocyte proliferation *in utero*, the Tbx5 deleted mouse has been crossed with a $p27^{kip1}$ knockout mouse. Mice lacking $p27^{kip1}$ are hyperplastic in all organs and tissues. By removing the inhibition CDK2 activity we may be able to rescue the Tbx5 deleted mouse by reducing the penetrance of the septal defects *in utero*. Two parallel strategies will be used to identify molecules that are involved in septal formation in the heart:

i) Genetic approach - cross Tbx5+/- x E2F3+/-; Tbx5+/- x p27+/-; E2F3+/- x p27+/-.
Analysis - Histology, Function/Structure echo (collaboration), Heart anatomy

ii) Gene expression analysis of models focused initially on cell cycle genes (Gene Chip).
Confirmation of gene chip data through northern, *in situ*, immunohistochemistry and western analysis.

Summary

My research over the next 3 to 5 years will focus on three interrelated areas: 1) Determining the physiological and molecular correlates of the hearts withdrawal from cell cycle, by comparative analysis of the Myosin Binding Protein C deficient mouse; 2) Determining the pathogenesis of lamin A/C derived dilated cardiomyopathy; 3) Determining the role of cell cycle genes in atrial and ventricular septal formation. Each research area involves detailed analysis of the expression profile of genes that impact upon the cardio myocyte transition from proliferation to hypertrophy during perinatal development, thereby furthering our knowledge of the molecular basis of heart disease. Long-term I plan to expand upon the findings of these three research areas in order to define a general mechanism for perinatal myocardial development. By doing this, I hope to answer some of the currently unanswered questions regarding the regulation and maintenance of the mammalian heart's permanent withdrawal from the cell cycle.

Teaching Report

Board of Tutors in Biochemical Sciences (2000-date)
Department Molecular and Cellular Biology, Harvard University
The tutorial program is designed to complement and expand upon the formal biochemical sciences curriculum. In response to the tutor's direction, students review current literature and present their analysis in regular meetings.
Independently directed; 4 undergraduate students

Head Teaching Fellow (1999 and 2000)
Department Molecular and Cellular Biology, Harvard University
Course: BS11 - Basic Principles of Biochemistry and Cell Biology
Instructor: Professor Guido Guidotti; 130-150 undergraduate students

Teaching Fellow (1997-2003)
Department Molecular and Cellular Biology, Harvard University
Course: MCB242 - Steroid Hormones and Receptors (1997-1998)
Instructor: Professor Matt Messelson; 20 postgraduate students

Course: BS10 - Introduction to Molecular Biology (1997-2001)
Instructor: Professor Rich Losick; 280-310 undergraduate students

Course: BS54 - Introductory Cell Biology (2001)
Instructors: Dr. Rob Lue, Professor Ray Erickson, and Professor Douglas Melton; 150 undergraduate students

Department of Continuing Education, Harvard University
Course: Biology E-1a - Introduction to Molecular and Cellular Biology (1997-2003)
Instructor: Professor William Fixsen; 100-120 undergraduate students

Course: Biology E-1b - Introduction to Organismic & Evolutionary Biology (1998-2003)
Instructor: Professor William Fixsen; 100-120 undergraduate students

Course: Biology S-1ab - Introductory Biology (1998-2002)
Instructor: Professor William Fixsen; 100-120 undergraduate students

Course: Biology S-76 - Principles of Cell Biology (2001)
Instructor: Professor Robert Van Buskirk; 40 undergraduate and postgraduate students

Course: Biology E-175 - Introduction to Cancer Biology (2001)
Instructor: Dr. Elizabeth Scott; 30 undergraduate and postgraduate students

Course: Biology E-103 - Cell Biology: Eukaryotic Experimental Models (2003)
Instructor: Dr. Cheryl Vaughn; 30 undergraduate and postgraduate students

Teaching Assistant and Laboratory Coordinator (1993-1995)
Department of Anatomy, Monash University
Course: Masters in Reproductive Biology
Instructor: Professor David M Robertson; 15 graduate students

Teaching Assistant and Laboratory Demonstrator
Department of Biochemistry, Monash University
Course: Biochemistry for 1st Year Students in Medicine (1995)