THE UNIVERSITY OF NORTH CAROLINA
AT
CHAPEL HILL

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Dr. Brun:

I am responding to your announcement in *Science* of an Assistant Professor position in systems biology within the Department of Biology and the Biocomplexity Institute at Indiana University, Bloomington. Currently I hold a research faculty position working with Dr. Sharon Milgram, Professor in the Department of Cell and Developmental Biology at the University of North Carolina at Chapel Hill. I am actively seeking a tenure-track position in which my enthusiasm for developmental biology and my excitement for mentoring the next generation of scientists can complement the existing research and training milieu.

My research goal is to contribute to our understanding of events critical to the process of mammalian embryogenesis including early cardiovascular development and establishment of the placenta. As outlined in the enclosed research plan, my postdoctoral research has revealed an essential role for the transcriptional co-regulator/adaptor protein YAP65 in these processes. Current avenues of investigation include using the powerful *in vitro* system of ES cell differentiation as embryoid bodies coupled with a novel twist on *Cre*-lox transgenics to explore YAP's role in blood vessel development. In addition, I have generated a *Yap* conditional allele to allow tissue-specific YAP deficiency and to assist exploration of requirements for this protein in establishment of the chorioallantoic placenta. I have received a Scientist Development Grant, a four year transitional award, from the National American Heart Association to pursue aspects of these research goals. My interest in questions of embryonic development including establishment of the cardiovascular and placental systems, combined with experience in cutting-edge methods of molecular genetics and modern embryology will compliment existing research efforts in your group and fit well with your faculty search goals.

I also feel a strong commitment to training the next generation of scientists. I have mentored both students and technicians in the Milgram lab and have taken real joy in

their research accomplishments. In doing this, I have learned a great deal about issues faced by under-represented minority students in approaching a research career. This spring, one of my undergraduates, a participant in the Research Education Support Program at UNC, received an award for the poster presentation of her work at the annual NC-LSAMP Undergraduate Research Conference. In addition to mentoring at the bench, I have a sincere interest in science education as outlined in the enclosed teaching statement. I have taken advantage of a two-term opportunity to teach an Introduction to Neuroscience course to senior undergraduates at the University of North Carolina. I thoroughly enjoyed my interactions with the students and observing their growth over the term. Currently, I assist in a graduate course for students in the UNC IBMS program that is designed to facilitate a successful transition to graduate school by addressing research presentation skills and laboratory rotation goals. I approach my research with the conviction that the joy of discovery is inseparable from mentoring and training of new scientists.

Please find enclosed my *curriculum vitae* including a list of references and statements of my research and teaching interests. I have arranged for letters of reference to be sent to you at the above address. Thank you for your consideration. I look forward to meeting you soon.

Sincerely,

Elizabeth M. Morin-Kensicki, Ph. D.

enc. curriculum vitae, research plan, teaching statement, publications

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Research Plan

My research goal is to further our understanding of events critical to embryogenesis including development of the cardiovascular and placental systems. I use a combination of genetic, cellular, molecular, *in vitro* and embryological approaches in the mouse model system to address the role of the transcriptional co-regulator/scaffolding protein Yes-associated protein of 65 kD (YAP65; referred to as YAP) in development. Motivated by my characterization of the *Yap* null mouse embryo phenotype, I have defined four specific research goals to explore the role of YAP in vasculogenesis. I also am collecting preliminary data and have outlined future experiments designed to clarify requirements for YAP in a critical step in early placental development that involves attachment of the embryonic allantois to the chorion. In addition, current and future collaborations with researchers working in strong embryological systems will help dissect the role of this protein in axis elongation. The pursuit of these questions not only will help elucidate YAP functions but also will contribute to our general understanding of essential features of embryogenesis. Furthermore, this understanding may factor into understanding early pregnancy loss in mammals and also can provide significant insight into possible clinical stimulation or abrogation of blood vessel development in cases of tissue ischemia or tumorigenesis.

YAP is characterized as both a scaffolding protein and a transcriptional co-activator. YAP was first isolated based on its ability to bind the SH3 domain of the *c-Yes* proto-oncogene product. Subsequent protein interaction and cell-based assays from many laboratories indicate interactions between YAP and proteins in both cytosol and nucleus that support multiple roles for YAP in basic cellular processes. YAP may function in cytosolic modulation of signaling pathways through interactions with receptors, cytoskeleton-associated PDZ-proteins, or other cytosolic proteins and also may be involved in co-regulation of gene expression through interactions with different transcription factors. Together these assays suggest that YAP is a multi-functional protein, yet we lack an integrated view of the role of YAP in the whole organism.

YAP is essential for embryonic development. To learn about the role of YAP *in vivo*, we generated *Yap* null mice by standard targeting techniques and assessed the impact of YAP deficiency. We found that homozygosity for the *Yap* null allele (*Yap -/-*) is embryonic lethal. *Yap -/-* embryos show developmental arrest around embryonic day 8.5 (E8.5) and do not persist past E10.5 (Figure 1). The report of our characterization of the mutant phenotype is in press. Briefly, YAP appears critical to morphogenetic events both in the embryo-proper and in extra-embryonic tissues that serve nutritional roles- the yolk sac and placenta. Morphologically *Yap -/-* embryos show a shortened body axis coupled with a distinctive folding of anterior neurepithelium. In addition, these embryos fail to achieve attachment of the allantois with the chorion, an essential step in early placental development. Histochemical and marker analyses revealed that despite perturbed morphology, mutant embryos acquire remarkably proper patterning and differentiation of major cell types. In the embryo, for example, we found proper restriction of *Fibroblast growth factor 8* expression domains along the anteroposterior axis and midline expression of *Brachyury*. Significantly, however, we found that although the cellular components of the early yolk

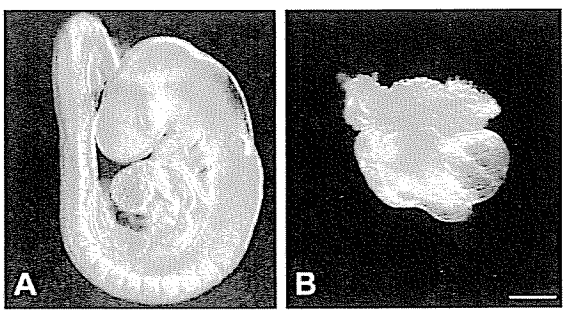

Figure 1 Mouse embryos in the 9th day of development. Wildtype (A) and Yap null (B) embryos demonstrate the mutant embryo perturbed morphology.

sac critical to formation of the first blood vessels and blood cells were present and expressed differentiation markers such as the endothelial marker CD31/PECAM1 antigen and the erythroblast marker gene *alpha-globin* , these cells failed to organize into a primitive vascular plexus (Figure 2). Thus, we conclude that in the absence of YAP, patterning and differentiation proceed relatively normally in the embryo but dramatic deficiencies exist that perturb chorioallantoic attachment and morphogenesis required for axis elongation and yolk sac vasculogenesis.

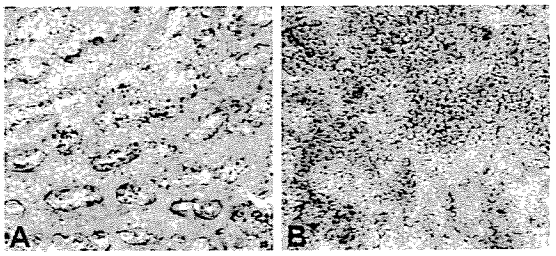

Figure 2 Mouse embryo flat-mounted PECAM1 stained yolk sacs. Wildtype (A) and Yap null (B) yolk sacs show failure of blood vessel formation in the mutant yolk sac.

ES cell differentiation as embryoid bodies models yolk sac blood vessel development and provides a powerful tool to assay YAP requirements. I have begun to pursue YAP requirements in vasculogenesis using a combination of the embryoid body (EB) model system and *Cre*-lox technology. Aspects of these experiments I have now funded under a 4 year transitional National American Heart Association Scientist Development Grant. To generate a conditional allele of *Yap* (*floxed Yap*, Figure 3C), I have used variant *lox*P sites that differ in spacer region sequence to convey specificity for recombination between homotypic but not heterotypic *lox*P sites. Doubly targeted (floxed *Yap/ Yap* null) ES cells generate *Yap* null ES cells that retain heterotypic *lox*P sites (Figure 3D). The heterotypic *lox*P sites facilitate *in vitro* insertion strategies using recombinase mediated cassette exchange (RMCE, Figure 3D-F), a technique successfully employed in many cell types including ES cells. I will use this strategy to explore the role of YAP in vasculogenesis in the EB model system. ES cells differentiating as embryoid bodies follow a developmental program very near in sequence and molecular program to that occurring during embryogenesis. In particular, the development of blood vessels in EBs appears very similar to blood vessel development in mouse embryo yolk sac. I have begun work to characterize *Yap-/-* EB blood vessel development. Next, I will (1) employ structure/function analysis of YAP requirements in vasculogenesis by exploiting RMCE in *Yap* null ES cells to introduce truncation and deletion *Yap* cDNA constructs as transgenes, (2) define pathway(s) of YAP input into vasculogenesis by introduction of pathway-specific reporter constructs in *Yap* null ES cells followed by differentiation as EBs and by comparative expression analyses with *Yap* null EBs, and (3) determine the mechanism of YAP function in blood vessel development using proliferation/apoptosis and migration/adhesion assays with *in vitro* differentiated null cells.

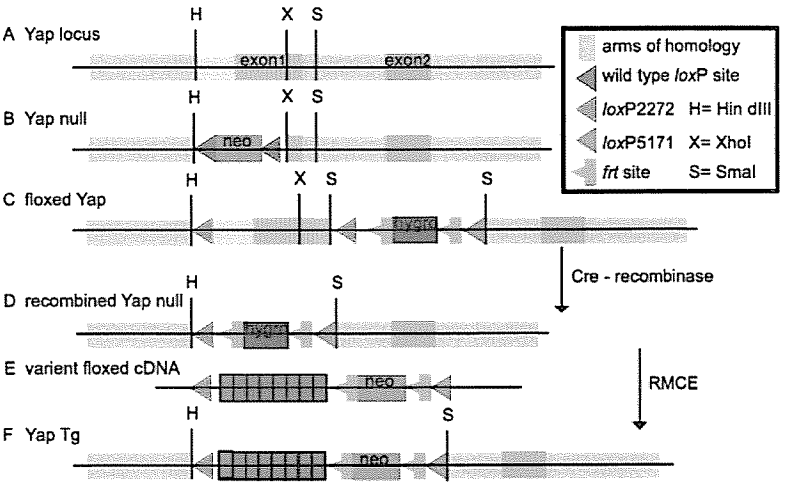

Figure 3 Yap conditional allele and targeted transgenesis

Results from *in vitro* tests can be taken back to the mouse for *in vivo* verification. While the EB model makes a powerful initial assay, a final understanding of YAP function in vasculogenesis is best supported by *in vivo* verification. Thus, I also will (4) generate mice carrying the conditional *Yap* allele and informative transgene alleles by standard homologous recombination in ES cells and RMCE to verify results from EB tests of the role of YAP in vasculogenesis. Generation of this conditional *Yap* mouse line also will serve as a powerful reagent for future tissue-specific assays for YAP *in vivo* requirements including assessment of the role of YAP in chorioallantoic fusion. Thus, the combination of *in vitro* and *in vivo* experiments characterizing YAP requirements for yolk sac blood vessel

development will provide me with the best opportunity to identify YAP domains critical to its role in vasculogenesis and reveal potential pathways influenced by YAP function. Furthermore, because these studies will explore a poorly understood yet critical aspect of blood vessel development, I anticipate that they will provide a significant advance in our understanding of vasculogenesis as an essential process of embryogenesis and adult homeostasis.

Future study of YAP requirements for vasculogenesis, embryonic axis elongation and placental development will focus on modulation of proliferation, apoptosis, cell adhesion and cell migration. The complexity of the *Yap* null mouse embryo phenotype suggests that YAP is critical for several distinct events in embryogenesis. Multiple developmental roles for YAP also is supported by a null phenotype distinct from, and more severe than, phenotypes reported for mice carrying mutations in genes that code for known interacting proteins. Thus, YAP appears to play a vital role not only in yolk sac vasculogenesis but also in an early step in placental development and in embryonic axis elongation. In these processes YAP appears to be dispensable for cell differentiation but essential for regulation of cell number and/or morphogenesis. Therefore, to learn about the molecular mechanisms of YAP function in development and to define molecular pathways employed for these functions, I have outlined future experiments that will explore the role of YAP specifically in proliferation/apoptosis, cell-cell and cell-matrix adhesion, migration and convergent extension cell movements. With these experiments, I plan to (1) fully characterize YAP requirements for regulation of cell proliferation and apoptosis using both *in vitro* and *in vivo* approaches, (2) delineate molecular pathways of YAP action in body axis elongation using genetic and embryological techniques, (3) determine cell-cell and/or cell-matrix interactions that underlie YAP requirements for chorioallantoic attachment and (4) use biochemical assays to elucidate components of YAP complexes that mediate these developmental functions. The results of these studies will provide insight into both YAP function and the underlying mechanisms of these fundamental developmental processes.

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Teaching Statement

Over the course of my teaching career, I expect to teach science courses from the introductory through the graduate level. Although course content and expectations of students will certainly vary depending on the level of the course, the foundation of my teaching goals will remain constant. My primary goal in working with students is to nurture in developing scientists the desire to learn by imparting the toolbox of skills needed to be successful "learners". By careful crafting of course content and assessment, I can help students acquire essential learning skills like problem solving and critical thinking. I have found that bringing open questions and the exploratory nature of the process of scientific inquiry to a course piques student interest and encourages active learning.

I have taken advantage of resources available at UNC in preparing for teaching aspects of my career. First, I have set up meetings with faculty who teach courses that are similar to those I might expect to teach. These courses include undergraduate and graduate level cell and developmental biology, molecular biology, genetics, structure and evolution of vertebrates and neurobiology. In my interviews with these educators I learned about their strategies for addressing different learning styles, for including the wide range of student abilities, for selecting textbooks and course content and for assessing their own success. In addition, I arranged discussions with faculty at the UNC Learning Center both to gain exposure to modern science pedagogy and assess my current skills.

Recently, I have had the extraordinary opportunity of teaching the Introduction to Neurobiology course to senior undergraduates at the University of North Carolina at Chapel Hill. I have enjoyed two wonderful terms sharing with these students the remarkable complexity and enduring mystery of nervous system structure and function. I prepared a course syllabus that allowed broad coverage of neurobiology ranging from cellular and molecular concepts of neurobiology to systems neurobiology to issues of cognitive neuroscience. In addition to coverage of textbook content, I incorporated assigned review-style scientific papers and group presentations on neuroscience topics with particularly strong social implications to serve as overt contextual links to everyday life and to emphasize the growing importance of productive group-work in career success. I used a multi-media presentation style mixing power-point lecture outlines with blackboard work, video and handout materials. Making use of a concept presented in the National Research Council's handbook "Science Teaching Reconsidered" published by the National Academy Press, I used "concept tests" to break up the lecture, encourage student interactions, promote active learning and provide an ongoing assessment tool. In addition, I made available on my course web site, the course expectations, syllabus, lecture notes, study questions, exam score distributions, and exam keys. The anonymous student feedback forms from the last week of the term indicated that students found me to be enthusiastic about the course content and sympathetic to their needs, and that they found the course stimulating and intellectually challenging. Several students indicated that they found the course to be one of the better biology courses in their experience or that their interests had changed from "biology" to "neurobiology". I have thrilled in this experience and look forward with enthusiasm to future opportunities to guide students along the path of discovery.

Currently, I assist in a graduate course offered to first year graduate students in the IBMS program at UNC. The course objectives include achieving research goals through successful laboratory rotations and acquiring professional research presentation skills. I look forward to continued teaching opportunities not only to transmit the facts and concepts of my fields of interest but also to help create the joy of discovery and learning in the next generation of scientists and educators.