THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER

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December 5, 2005

Re: Letter of Reference for Dr. Cretekos

Dear Committee,

It is my great pleasure to recommend Dr. Chris Cretekos for a faculty position in your department. I know Chris since five years when I joined the faculty at the M.D. Anderson Cancer Center. During that time I have had much contact with Chris and I have watched Chris's development closely. Before my position at M.D. Anderson, I have been a postdoc with Dr. Sean Carroll at the University of Wisconsin in Madison and I was working on evolutionary projects at that time. Chris and myself thus share a deep interest in evolutionary and developmental questions, which was the basis of many interesting and productive discussions. Chris has been an outstanding member of the Department in many respects and he has exhibited qualities indicating that he will establish a vigorous and well funded research program.

While in Richard Behringer's lab, Chris has proven that he can produce high quality data in a short amount of time. Chris has been involved in studying why bat wings grow larger than mouse limbs. He has so far focused his efforts on the Prx2 gene, although he has preliminary data on many other genes. Chris found that Prx2 has a differential expression pattern between mouse and bat limb development suggesting that the regulation of Prx2 expression contributes to the different limb morphologies. He has designed and carried out a truly beautiful experiment in switching out the mouse limb enhancer of Prx2, and he will publish a very nice paper on these results. You will notice that Chris took a very innovative and bold approach when he decided to switch out the limb enhancer in the mouse with that of the bat, rather than stopping where most researches would, namely in the description of the differential expression patterns. Thus, Chris spent the extra effort to address the function of the differential expression patterns, which is just one example of Chris's creativity, dedication, maturity and productivity.

Chris has been the driving force of the bat project in the Behringer lab, which involves several people. In this group he has been the intellectual leader and he has been the driving force behind the different projects. Chris has shown great skills in training and

supervising students and technicians. He is a master of many techniques and he likes to interact and train students. Indeed, he is an excellent teacher and mentor and he has also thought some of my students on BAC filter hybridizations (which were successful). Chris has thus been a leader in Richard's group as well as in the Department and importantly, he has the skills and patience it takes to train beginners in the field to become productive researchers.

Chris is proactive in his approach to science and he has great ability to carry out projects quickly and with high quality. Chris has shown great intellectual and practical leadership with his postdoc projects. His work in using the mouse to study growth control gave him a solid background in vertebrate developmental biology and we regularly discuss current models and problems in growth regulation. In these discussions, Chris shows a clear vision in what are the next interesting big questions, which also comes through in his very interesting research plan. My group is studying growth control in Drosophila and I think that Chris's comparative approach to study growth is not only novel and interesting but may well provide key insights necessary to understand the regulation of organ size. In vertebrates, typically k.o. alleles of genes of interest were analyzed but such alleles can only reveal a requirement for growth but do not give insights into the mechanisms that determine the size of tissues. Chris's comparative approach, on the other hand, does not generate k.o. alleles but addresses the mechanisms that are sufficient to change organ size rather than just "destroying" it. Indeed, Chris's approach has already born fruit as exemplified by his Prx2 study. In his future projects Chris wants to further harvest the power of mouse genetics to understand the mechanisms of growth control and the molecular basis of morphological evolution (allometry). I am sure he will excel in accomplishing his goals. Indeed, his training and expertise put him in an ideal position for these projects. Given the importance of understanding the molecular mechanisms of growth, I am sure that his maybe a bit unusual but very innovative project is highly fundable.

Chris is a very pleasant person to be around with and to interact, and he is very interactive and participatory in Richard's group and in the Department, qualities that I think are important for a faculty. In summary, Chris is not only a qualified researcher that has already achieved much but he is also a good mentor. Clearly, from his accomplishments, maturity and independency of his work, experience with training people and grant writing, Chris is ready to be an independent group leader.

Sincerely

Georg Halder, Ph.D.

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December 12, 2005

Dr. Yves Brun,
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142, 1001 E 3rd Str.
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Dear Dr. Brun,

I am writing to recommend **Dr. Chris Cretekos** for a tenure track assistant professor position in your department. Chris has been a postdoctoral fellow in my laboratory since 1999. Chris' thesis research, in David Grunwald's lab, focused on zebrafish early development and genetics. In my laboratory, Chris has trained to become a mouse molecular geneticist and has pioneered the molecular embryology of the bat. This makes Chris exceptionally unique, having developmental expertise in three different vertebrate systems.

In my lab, Chris has pursued a very ambitious project, using mouse genetics and bat embryology to investigate the genetic basis of organ variation between species. The basic hypothesis is that sequence differences in tissue-specific enhancers of developmental control genes lead to the morphological and physiological differences between animal species. To test this idea, Chris has focused on limb development, using the bat limb as a modified limb relative to mouse. To do this, Chris has created the field of bat molecular embryology. In collaboration with John Rasweiler (SUNY Downstate), Chris has studied the embryonic development of the short-tailed fruit bat (*Carollia perspicillata*). *Carollia* is a great model bat because there is an extensive literature on this animal, it is very abundant and easy to collect in the field, and is easily maintained and bred in the laboratory. Chris has used embryos derived from timed matings to create the first bat embryo staging system (Cretekos et al., 2005).

Chris has created many molecular reagents for his studies, including bat embryo cDNA libraries, a genomic phage library (Cretekos et al., 2001), and in collaboration with the

NIH Genome Center a BAC library that has been arrayed onto filters. Chris has also established whole mount in situ hybridization and immunohistochemistry of bat embryos and skeleton preparations of fetuses and adults.

Chris' primary focus has been to understand how genetic variation leads to morphological and physiological differences in organ formation between species. He has focused on limb development and chosen to compare the limbs of mice and bats. To this end, he has used the *Prx1* homeobox gene that has previously been shown to regulate proximal-distal outgrowth of the limbs. Chris generated transgenic mice to identify the limb-specific transcriptional enhancer for the mouse *Prx1* gene. He then cloned the bat *Prx1* locus and identified the corresponding limb-specific enhancer, using a transgenic mouse assay. But the most significant experiment Chris has performed is to create a mouse by gene targeting in ES cells in which the endogenous mouse *Prx1* limb-specific enhancer has been replaced by that of the bat. The mice he has created that express mouse *Prx1* under the control of the bat regulatory sequences have forelimbs that are about 10% longer than controls. Histological analysis also shows an expansion of the trabecular bone region at the expense of the hypertrophic chondrocytes of the growth plate. Chris' studies suggest that the bat sequences are up-regulating Prx1 activity, altering endochondral bone formation. Chris is continuing molecular and cellular studies to more precisely understand how these phenotypes develop. Importantly, Chris' studies indicate that DNA variation in cis-acting transcriptional regulatory sequences in developmental control genes contribute to the morphological differences observed in organs between species. These are novel and important findings not only for the field of evolution and development but also the biomedical field, focusing on organogenesis and birth defects.

Chris is very bright with solid and deep, training in molecular biology and developmental biology. He has always been intellectually independent yet collaborative. He has single-handedly developed his projects and is currently expanding them in anticipation of obtaining an independent position. Chris is also exceptionally meticulous. This is very evident in his data presentation in talks and papers. On a personal level, Chris is very outgoing, friendly, and participatory. Chris should also be a wonderful mentor for students. This last summer he supervised two undergraduate students and both motivated them and guided them very well. Thus, Chris will make a wonderful colleague and mentor of students and postdoctoral fellows.

In sum, I can recommend Chris to you with my **highest possible enthusiasm** and strongly encourage you to invite him to interview.

Sincerely,

Richard Behringer

Richard Behringer, Ph.D.
Ben F. Love Chair for Cancer Research