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Yves Brun
Systems Biology/Microbiology Faculty Search
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Indiana University
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Dear Dr. Brun:

I am writing to apply for the position of assistant professor in the Department of Biology. I am currently an instructor in Richard Behringer's lab in the Department of Molecular Genetics at the University of Texas M. D. Anderson Cancer Center. I am a vertebrate developmental geneticist with research interests in transcriptional regulation, organogenesis and evolution.

The ultimate goal of my research is to understand the genetic mechanisms of morphological variation between mammalian species. I use comparative embryological, molecular and genetic approaches to identify regulatory differences in organogenesis between two morphologically divergent mammals, mouse and bat, and test the function of such regulatory differences in genetically modified mice. I am currently preparing a manuscript showing that divergence of single transcriptional enhancer in bat is sufficient to elicit a change in limb morphogenesis in mouse that accounts for part of the observed difference in relative forelimb length between these species. My approach takes advantage of the grand-scale genetics experiment that is natural selection and provides insights into fundamental principles of mammalian development that are relevant to human development and disease.

I am adept in the use of three vertebrate model systems with a variety of embryological, molecular and genetic approaches to address developmental biology questions. During my graduate research, I addressed questions of cell movement and fate determination in the early zebrafish embryo. I also generated and characterized the first embryonic lethal mutant induced by DNA microinjection in zebrafish. In my postdoctoral research, I have gained expertise in all phases of generation and characterization of transgenic mice, genetic manipulation of mouse embryonic stem cells (gene targeting), generation and analysis of mouse chimeras and conditional genetic approaches. At the same time, I have pioneered the use of *Carollia persicillata* as a bat model for developmental biology research. I published a comprehensive developmental staging system for *Carollia* that serves as the basis for developmental studies in any bat species. My expertise in bat development has stimulated ongoing collaborations on a wide range of evo-devo topics with Carolyn Komar (Iowa State), Ed Laufer (Columbia), Susan Mackem (NCI, NIH), Lee Niswander (HHMI, UCHSC), John Rasweiler (SUNY-Brooklyn) and Doris Wu (NIDCD, NIH).

I am excited about the possibility of joining your group and hope you will find my background and research interests appropriate to your needs. I have enclosed my *curriculum vitae* including contact information for references, statements of my research and teaching interests, and three of my recent publications. I would very much appreciate an opportunity to interview for this position. Please contact me by telephone at 713-834-6326 (lab) or 713-794-0360 (home), fax at 713-834-6339, or email at cretekos@mdanderson.org if you would like additional information or have any questions. Thank you very much for your consideration, and I look forward to hearing from you.

Sincerely,

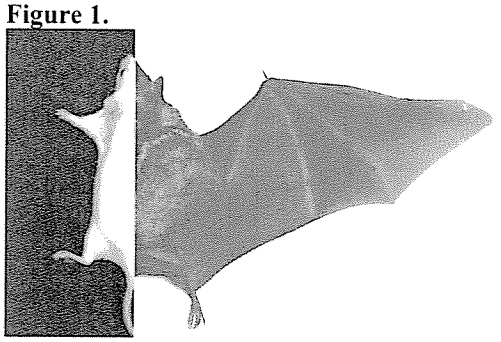
Chris J. Cretekos, Ph.D.

Evolution and Development of Organ Morphogenesis and Physiology in Mammals

Summary

How do the highly conserved genes and genetic pathways governing embryo and organ patterning and morphogenesis program divergent morphology between related species? A longstanding but largely untested hypothesis is that morphological diversity among mammalian species is the result of changes in gene regulation rather than in gene function (*1*). According to this hypothesis, the genetic differences responsible for morphological differences reside in *cis*-elements regulating the transcription of conserved developmental control genes. I am a developmental geneticist with experience using zebrafish and mouse models. My training in classical and molecular embryology, in forward and reverse genetics, and in diverse vertebrate models provides a unique combination of skills, knowledge and perspective to pursue the answers to this question.

Figure 1.



I utilize comparative embryological, molecular and genetic approaches to determine the mechanisms of morphological variation and developmental plasticity in organogenesis. I use the bat, a highly morphologically divergent model compared to the mouse (Fig. 1), to identify relative differences in developmental gene regulation that correlate with divergent morphogenesis. I also use the mouse, a highly manipulable genetic model, to determine whether such regulatory differences are functionally relevant. Studying the regulation of gene expression between species takes advantage of the grand-scale genetics experiment that is natural selection. The results and conclusions from making functional-genetic comparisons between mammalian species are likely to have significant relevance to human development and disease. What is normal in one species would in many cases be considered disease in another.

The organ I have chosen to focus on initially is the limb, a classic developmental biology paradigm. While the tetrapod body plan is conserved between all mammals, nature presents us with a fascinating array of shapes and sizes. The limbs in particular display striking morphological variety. During the course of limb induction, patterning and outgrowth, many of the same genetic pathways and tissue interactions that function in specification and morphogenesis of many other organs are redeployed and recapitulated, making the limb a compelling model for investigating a variety of critical developmental mechanisms. Moreover, limbs are dispensable under laboratory conditions, so even very severe defects can be studied in detail.

Previous Research

My graduate research focused on two different aspects of zebrafish development. First I established how the descendants of early cleavage cells contribute to the gastrula stage fate map. I found that the contribution of each early blastomere is generally unpredictable, with one exception - the region of the fate map immediately adjacent to the yolk cell, which gives rise to much of the mesoderm and endoderm (*2, 3*). I suggested that maternal mesendodermal fate determinants could be localized in this region. Several such determinants have been subsequently identified (reviewed in *4*). In my other project, I developed a transgene-based method to induce and recover insertional mutations affecting zebrafish development. I generated and characterized the first embryonic lethal mutant induced by DNA microinjection in zebrafish, *alyron*^{z12} (*5*).

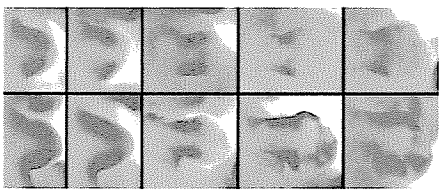
Current Research

***Carollia perspicilatta* as a model to study divergent morphogenesis and organogenesis.** Recent pioneering work using alternative model systems that have unique developmental programs initially sparked my interest in establishing a bat model for developmental biology research. Nearly 20% of extant mammal species are bats, yet surprisingly little is known about their development. To provide a

foundation for developmental studies in bats, I have generated a staging system for *Carollia perspicillata* (Seba's short-tailed fruit bat) development based on embryos collected from timed matings of laboratory-bred animals (*6*). *Carollia* has a number of advantages as a bat model. It is the most abundant mammal in the New World tropics and is easy to collect from the wild (*7*). I have made ten trips to the Caribbean island of Trinidad to collect both a large number of embryos and extensive data on seasonal breeding patterns in the wild such that I can accurately estimate when most gravid females are carrying an embryo at any stage of development. My collaborator John Rasweiler has developed efficient procedures for introducing, maintaining and breeding *Carollia* in the laboratory, and he has maintained a breeding colony of several hundred animals for over a decade (*8*). In contrast to most mammals with highly divergent limbs, *Carollia* requires only a modest amount of space and can be done at a cost comparable to rodent models. I have adapted standard molecular embryology tools such as RNA *in situ* hybridization, immunohistochemistry and skeleton histology to bat embryos and I have generated four λ-based *Carollia* libraries: genomic, adult brain cDNA, and 2 embryo cDNA libraries (*9*). Using these libraries along with a BAC library generated by Eric Green and colleagues at the National Human Genome Research Institute (NHGRI, NIH), I have isolated a number of genes that I am using as markers to characterize bat limb development and in studies of divergent developmental gene regulation.

Identifying regulatory divergence. The developmental control gene that I have chosen to initially focus on for study of regulatory divergence is *Prx1* (also called *MHox* or *Prrx1*). *Prx1* encodes a paired-class homeodomain transcription factor. Analysis of the mouse *Prx1* knockout (null) phenotype demonstrates an essential role in the morphogenesis of limb and craniofacial tissues. The limbs of mice with reduced Prx function display shortened zeugopod (forearm and lower leg) elements and numerous autopod (hand and foot) element reductions and malformations (*10, 11, 12*). Because loss of *Prx1* function in mouse leads to reduction in the length of distal limb elements, I hypothesized that divergent gain of *Prx1* function could contribute to the relative length increase in distal elements of bat limbs. *Prx1* is expressed in a complex and dynamic pattern in the limb from the early undifferentiated bud stage through outgrowth and morphogenesis, suggesting roles in multiple steps of limb development (Fig. 2; *10, 13*). I have isolated the *Carollia* orthologue of *Prx1* from my genomic library and characterized its expression pattern in bat embryos by RNA *in situ* hybridization. I found that the expression of *Prx1* in the developing bat limb shows a striking up-regulation in the distal limb compared to mouse (Fig. 2). A region in which sequences capable of regulating expression in the limb reside, just upstream of the start of transcription, had been previously identified *(14)*. I have identified the bat orthologue of the *Prx1* limb-specific enhancer by virtue of its conserved sequence and genomic position relative to mouse and human *Prx1* orthologues, and characterized the activity this enhancer by combining it with a basal promoter and lacZ reporter in transgenic mice. The *Carollia Prx1* limb enhancer shares about 60% sequence identity with that of mouse and directs the expression of the reporter in a pattern similar to that of the orthologous mouse enhancer in the limbs of transgenic mice.

Figure 2. *Prx1* expression in developing forelimbs



The regulatory switch experiment. The challenge in studying the evolution of gene regulation is to distinguish between unselected genetic drift and significant functional divergence in *cis*-regulatory sequences (*9*). To address this challenge, I have replaced the mouse *Prx1* limb enhancer with the bat limb enhancer in the endogenous locus by gene targeting in mouse embryonic stem (ES) cells and generated mice from these ES cells ($Prx1^{BatE}$). Using gene targeting I was able to replace the limb enhancer sequences without altering the coding sequence, thus any change in Prx function in $Prx1^{BatE}$ mice is directly attributable to the bat regulatory sequences. Heterozygous and homozygous mice for the bat *Prx1* limb enhancer switch are viable, fertile, segregate in the expected Mendelian ratio and show no readily apparent limb or craniofacial phenotypes. However, detailed analysis of $Prx1^{BatE/BatE}$ mice reveals that the

forelimbs of these animals are about 9% longer than their wild-type littermates by the end of gestation, and this difference is statistically significant. Histological and molecular analysis indicates that bat enhancer-driven expression of *Prx1* results in accelerated endochondrial bone formation in the mutant forelimbs. These results demonstrate that divergence in one *cis*-regulatory element is by itself sufficient to elicit a measurable change in limb morphogenesis. They further suggest that divergent expression of *Prx1* accounts in part of the observed difference in relative forelimb length between mouse and bat, and begins to address the molecular mechanism of this difference. This powerful functional-genetic approach allows me to move beyond description and correlation towards a functional demonstration of the molecular mechanisms for morphological variation and developmental plasticity in organogenesis.

Future Research

Tissue-specific modulation of endochondrial bone formation. The results of my *Prx1* regulatory switch experiment suggest that subtle alterations in *Prx1* expression lead to accelerated endochondrial bone formation in mutant mouse forelimbs. How the pathways and mechanisms that govern embryonic patterning modulate the process of bone formation to produce the specific sizes and shapes of the various skeletal elements is unknown, but such modulation has clearly been a level at which natural selection has operated. The observed limb-specific modulation of bone length; enhancement by bat enhancer-driven expression in my $Prx1^{BatE}$ mutant and reduction in the loss of function mutant; suggests that *Prx1* functions between the global mechanisms of limb patterning and the program of long bone growth in the limb skeleton. The likely mechanism is by coordinated transcriptional regulation of BMP and FGF signaling pathways (J.F. Martin, unpublished observations). To investigate the potential role for *Prx1* in this process, I plan to generate transgenic mice that over-express *Prx1* in a variety of skeletogenic tissues.

Comparative molecular analysis of bat limb development. I hypothesize that morphological diversity among mammalian species is the result of changes in the expression of conserved developmental control genes. Many of the important limb developmental control genes are known and my collaborators and I have isolated a number of these from *Carollia*. These include *Fgf8, Fgf10, Shh, Bmp2, Bmp4, Msx1, Gremlin, Twist, HoxA13, HoxD12,* and *HoxD13*. I have also established cross-species RNA *in situ* hybridization on *Carollia* embryos using mouse probes. Additional limb developmental control genes whose developmental expression will be compared in this way include *Patched1, Gli3, Hand2, Gdf5, Msx2, Wnt5a, Wnt7a, Lmx1b,* and *Engrailed1*. I will analyze the expression patterns of these genes during limb-forming stages of mouse and bat development to determine whether pattern differences exist and whether they presage or correlate with morphological differences. Comparative analysis of the expression patterns of this set of markers for limb induction, patterning, outgrowth and differentiation will provide the essential background knowledge for further studies on the relationship between regulatory divergence and the evolution of morphological variation.

Identification of other limb-specific enhancers. My choice of *Prx1* as the initial gene on which to perform the enhancer switch experiment was partly motivated by previous localization of a limb specific *cis*-regulatory element to a specific genomic region *(14)*. Besides the *Prx1* limb enhancer I found in this region, there are very few characterized enhancers that drive limb-specific expression in development. This is mainly because these were identified as alleles of limb development loci lacking mutations in the coding sequence of the affected gene. Forward genetics in vertebrate models is an inefficient way to identify enhancers. Identification of limb enhancers for a wider selection of important developmental control genes would not only be of benefit for my own interests, but would be of use to the larger community. Several recent advances create an unprecedented opportunity to identify previously unknown *cis*-regulatory elements within conserved non-coding sequences surrounding known limb developmental control genes. These advances include the sequencing of multiple vertebrate genomes, new algorithms for large-scale genomic sequence alignment, and BAC-transgenic mice.

I plan to generate modified BAC-transgenic and traditional transgenic mice to identify and characterize the limb enhancers of developmental control genes. Mice transgenic for BACs modified with

a reporter gene cassette will be used to determine if tissue specific enhancers are found on BACs containing developmental loci, and to roughly localize where such enhancers reside. Potential enhancers will be identified by comparative genomics as blocks of relatively well-conserved non-coding sequence in a region where an enhancer resides. Sequences that can function autonomously as limb-specific enhancers will be determined by fusing such blocks of sequence with a basal promoter-reporter gene construct and assaying for tissue specific reporter gene expression in transgenic mouse embryos. I will focus on developmental control genes that show expression patterns differences between mouse and bat that are suggestive of a role in divergent morphogenesis. Finally, I will test the functional relevance of divergent expression using the regulatory switch experiment as described above for *Prx1*.

Long Term Interests

A significant effort in my future lab will be to apply the comparative embryological, molecular and genetic approaches that I am currently using for limb studies to craniofacial development. As many of the genes that control limb development also function in craniofacial development, in the course of my comparative molecular analysis of limb development, I will also identify relative differences in gene expression patterns during craniofacial morphogenesis, which can then be functionally tested in transgenic and gene-targeted mice. In addition, I will carry the comparative analysis of craniofacial development to several more bat species. This will result in new insights since the greater than 1000 extant bat species show striking variation in craniofacial morphology, likely owing to dietary diversity and the advent of echolocation. NIH-funded sequencing (2X coverage) of two bat species (*Cynopterus sphinx* and *Rhinolophus ferrumequinum*) is currently in progress. Several hundred selected BACs from the *Carollia* library generated by Eric Green's lab at NHGRI (NIH) have been subjected to single pass sequencing and I am submitting a White Paper to have the genome *Carollia* sequenced. I plan to use a comparative genomics approach to take advantage of these nascent resources with the goal of identifying genomic sequence features that are either highly conserved or diverged in bats relative to other mammals, whose function can be tested in transgenic or gene-targeted mouse models. In addition finding potential *cis*-regulatory element divergence within non-coding homologies, this approach will also identify novel gene duplication and/or loss events in the evolutionary history of bats. I have preliminary evidence that at least one important developmental control gene, *Fgf10*, is duplicated in *Carollia*, but I have not yet begun to address the functional, developmental and evolutionary implications of this observation.

References

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Statement of Teaching Interests

Communicating science to nonscientists, students and colleagues has always been an important part of my scientific life. My application to Indiana University is motivated by a career choice to combine an innovative research program with scientific communication and teaching in an interdisciplinary environment.

I have enjoyed communicating my own science as well as the foundations it rests on throughout my career. Throughout my training I have organized and actively participated in teach-and-learn exchanges such as journal clubs and 'research in progress' forums. As a graduate student I took part in teaching two formal developmental biology courses for undergraduate students, a lecture and an interactive laboratory course. I enjoyed performing teaching assistant duties for the lecture course and grading exams, quizzes and papers, and I gave lectures to the class (~180 students) on zebrafish developmental genetics. I presented all of the lessons and demonstrations for the lab course. I also worked closely with the course organizer (Dr. Michael Bastiani) during the preceding semester to define the course objectives and lesson plan, prepare demonstrations and test the experiments. The material we covered included classical embryology experiments using chick and *Xenopus* embryos, molecular embryology experiments using grasshopper and zebrafish embryos, and developmental genetics experiments using fruit flies. During my postdoctoral work I gained experience in training graduate students and presenting many departmental lectures. On two occasions I have had the honor and opportunity to present interactive seminars on how basic science research helps discover new insights on cancer treatment to groups of high school students and their teachers visiting the M. D. Anderson Cancer Center. I have also been responsible for mentoring three summer students. I have been invited to prepare and present educational lectures on evo-devo paradigms as well as mouse and bat developmental biology to graduate and undergraduate students at several local universities, including the University of Houston, Rice University and Texas A&M. I particularly enjoy teaching fundamental concepts in biology, and try to engage students to think about what the data means in light of fundamental concepts.

I am looking forward to my teaching responsibilities as a faculty member of the Department of Biology. I am especially interested in contributing to the graduate and undergraduate curriculum in developmental biology. While molecular genetics of mainstream model systems must be an integral part of a basic biology education, I can add a comparative and evolutionary perspective to the coursework. In particular, I am interested in teaching a laboratory course that includes classical embryology for undergraduates. It is my experience that an understanding of classic experiments that led to fundamental concepts, such as the organizer transplant experiments of Speman and Mangold, is not only of historical interest, but greatly helps students to appreciate those concepts. There is nothing better than doing and seeing for understanding and appreciating the scientific method. At the graduate level my advanced training in vertebrate developmental anatomy and genetics including transgenic, gene targeting and conditional genetic approaches in mice will allow me to provide specialized courses. Finally I am interested in organizing seminars and discussion groups on interdisciplinary subjects. In summary, I look forward to stimulating interactions with both students and colleagues in both the lab and the classroom.