UNIVERSITY of VIRGINIA
DEPARTMENT of BIOLOGY

15 October, 2005

Whitehead Search Committee
D.C. Page
Whitehead Institute for Biomedical Research
Nine Cambridge Center
Cambridge, MA 02142-1479

Dear committee,

Dave Shook has asked me to write in support of his application for a position in your institution. I am happy to do so. I recommend him very highly and without reservation.

Dave is independent, self-driven, and has his own ideas, concepts, and goals in research and teaching. He came to my lab with his own project, a project funded by an NIH NRSA postdoctoral fellowship, which he got without any help from me, other than offering my lab as a host. His idea was to learn something about morphogenic mechanisms in general by looking at variations in morphogenesis, specifically, variations in the gastrulation and early development of amphibians. He has a very well thought out rationale as to why this will contribute to understanding developmental mechanisms, and especially morphogenic mechanisms, and also to understanding evolution of developmental mechanisms.

He has succeeded beyond my expectations. His work is turning out to be more important to developing the perspective and outlook of my lab than any work since the early work of John Shih, Paul Wilson and others showing the cell intercalation underlying convergent extension. His main accomplishment was to define and characterized the expression and morphogenic function of epithelial mesenchymal transitions (EMTs) in early development of urodele amphibians, as well as in anurans, where this process is much less important, particularly for gastrulation. His work identified the process of EMT, which occurs in a bilateral primitive streak around both sides of the blastoporal lip, as a major force generating process in gastulation of urodele amphibians. It has been known for a long time that the urodele and anuran blastopores share a common superficial appearance, and Dave's recent biomechanical experiments show that similar patterns of convergence forces close and internalize mesoderm. But he has also discovered that the underlying cellular processes producing these convergence forces are vastly different. Like the anurans that have been studied in detail, the urodeles Dave has studied use intercalation of deep, mesenchymal cells to drive the convergence of the notochord. But in the somitic region, the urodele uses EMT and ingression of a massive field of superficial somitic mesoderm to generate these convergence forces, whereas the anurans, having small fields of superficial somitic mesoderm, use cell intercalation of deep cells instead, and reserve EMT and ingression of what surface mesoderm they have to neurula stages. This conservation

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of patterns of forces but diversification of force-generating mechanisms during gastrulation encouraged us to re-think our concepts of the mechanics of blastopore closure, the cellular and molecular processes involved, and the diversification of these processes across the amphibians. In this re-thinking was motivated by Dave's results described above, his unpublished work on *Epipedobates*, and work he an others have done on *Xenopus* and *Gastrotheca*. We know believe that the process of "convergent thickening", which occurs in a restricted area in Xenopus, is more prominent in Epipidobates, and appears to span the entire marginal zone of *Gastrotheca*, is a common underlying process of gastrulation that is used to a variable degree in gastrulation of most, if not all amphibians. Convergent thickening is perhaps an underlying essential process in convergent extension and perhaps its predecessor. In short, Dave's discovery of the role of EMT in convergence and blastopore closure in urodeles, and his role in identification of convergent thickening as a widely distributed mechanism in amphibians has been the stimulus for a major revision of our concept of gastrulation, a revision that promises to be experimentally productive and to yield a deeper understanding of both gastrulation and morphogenesis in general. We are one stop closer to a general synthesis, a predictive theory of gastrulation, that accounts for the underlying cell biological bases for the various tissue movements, the biomechanical integration of these movements necessary to bring about gastrulation in eggs of vastly different architectures, and for a seamless evolutionary transition between these mechanisms.

In a larger context, it is now clear from his work that morphogenic processes evolve in the context of a biomechanical role that is defined by the geometric and material properties of the tissues and the movements that are to be accomplished, and their diversity stems from the fact that, as in a soccer game, or a football game, for example, there are a lot of ways to put the same basic players on the field, and there are a lot of different plays that can be run. Many of these mechanisms are biomechanically integrated and compatible, such that rapid evolution from one to the other could occur using compatible, intermediate combinations, brought about by coupling different downstream cell behaviors to conserved, underlying patterning processes.

Although many high-minded ideas have been proposed and kicked around in this area of comparative gastrulation, they have been mostly just that- plausible ideas. In contrast, Dave has been unusually successful in getting down to the cell biological and biomechanical reality of gastrulation in diverse species with eggs of different architectures, largely because he can get things to work in two regards where others have either been unsuccessful are haven't seen the importance: imaging and mechanics. First, Dave was able to label cells and image cell behavior at high resolution in the urodele gastrula, something that had not been done very well. His direct imaging of EMT and ingression in the urodele, which is the highest resolution, most informative picture we have of this process in any organism, is a key element understanding how the urodele differed greatly in cell behavior but not in patterning or biomechanics from the anuran, and how it differed greatly in biomechanics but shared cell behavior with the amniote gastrula. Second, Dave has been able to test the function of regional cell behaviors by directly measuring the mechanical forces they develop. In ongoing, unpublished work, he and Lance Davidson have recently shown directly with biomechanical tensile force measurements that the EMT in the urodeles and cell intercalation in *Xenopus* generate similar convergence forces. In work in progress, he has been similarly successful in working with a number of other species, moreso than anyone else. Being able to relate observable cell behaviors to patterns of forces is what defines morphogenic machines, and these machines are the core of morphogenic mechanism and the clay that the evolutionary process must work with. Dave's

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package of skills in these areas, along with a development of molecular biological resources, such as a urodele (axolotl) EST project by Elly Tanaka at Dresden, and others, opens up a grand new opportunity for studying variations in the coordinate regulation of morphogenic processes versus regulation of tissue type, variation in cell behaviors, and variation in biomechanics of gastrulation in eggs of different architectures.

Beyond gastrulation, Dave's comparative work is also leading to fundamental insights about basic mechanisms. His work relates cell behaviors, force generation, geometries of forces, in the overall development of an understanding of biomechanical, morphogenic strategy. This general framework is proving very useful in thinking about the cellular and molecular mechanisms of morphogenesis, the nature of developmental constraints on morphogenesis, and explaining why the common, cellular force-generating mechanisms are conserved but their application, function, and consequence in morphogenesis are very diverse. It advances our understanding of the logic of morpho-mechanical strategies in development and the evolution of morphogenesis.

This work will also be important in biomedical research on "morphogenic diseases", or birth defects, such as neural tube defects, in which the underlying genetic control mechanisms appear to be similar but the mechanical and geometric circumstances of the most relevant model systems, particularly the mouse, differ substantially from humans. By working effectively on "non-model" systems, I think Dave's work is important is advancing our knowledge of the morphogenic landscape- what is possible with a given machine, its performance parameters, and its necessary contexts of utilization. This constitutes a strong rationale for biomedical funding of comparative morphogenesis, something that is not commonly done, even in good times, and Dave has been successful in this regard, being funded by an NIH postdoctoral NRSA for his work. I did not think they would fund this type of work, but he provided a great rationale then, and was successful, and he can provide a stronger one today. He has an independent RO1 in review, which I am sure he will get funded, even in this climate. He great strength is that he is a broad and original thinker, but he is also technically talented and can do experiments most others can't, thus bringing a solid experimental basis to the field

The key limitation in comparative morphogenesis is the difficulty in working with non-model systems, which are just that usually because they were thought too difficult to use, but Dave is a very talented experimentalist and has shown that success simply involves working with each type of embryo on its own terms rather than expecting common procedures to work on all species. He has been creative in his approach, his choice of species, and his techniques, and is making great progress on a variety of species and egg designs. He is not afraid to use any method he needs to answer a question. He was trained as a biochemist as an undergraduate, as a developmental biologist and a population biologist as a graduate student, and has applied a bit of everything to his postdoctoral work. He is persistent, tough-minded, and shows a strong instinct for pursuit; he keeps his eye on the goal, but he knows when an approach is a waste of time, and it is time to take a new tack. He also has the big-picture perspective to ask important questions

Dave is an excellent mentor and has been very successful in working with, and teaching, and leading others in research. He has published two papers with an undergraduate research student, whom he mentored. He has been the organizing figure in bringing together the biomechanical expertise of Lance Davidson, and work on myosin heavy chain IIA and IIB function by Paul Skoglund, a postdoc, and Ana Rolo, a graduate student, in a biomechanical analysis of the function of this molecule in developing convergence forces. He

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can bring together cell biology, development, and evolution in the arena of morphogenesis in a way that few can, a skill that I believe will make him a major leading figure in the field. He has teaching experience and works well with undergraduates and graduates. He taught the advanced developmental biology course here with two other postdocs several years ago and did quite well. He is very perceptive about how others are thinking about a problem and gives good guidance and suggestions.

He is an excellent colleague and citizen in the scientific community. He is very much an activator and initiator. He has organized seminars and interest groups since his days as a graduate student in Colorado. Here at Virginia, he organized a grounds-wide (Arts and Sciences and the School of Medicine) series of talks, once a month in the evening on morphogenesis, the "morphogenesis supergroup". He will be an excellent colleague and public citizen. He has broad interests, interacts with people in diverse areas, asks excellent questions, often far out of his field, and makes contributions to others' work beyond their expectations. He does projects for the local schools, and is quite good at holding the attention of youngsters and teaching them about complex subjects. He is an excellent ambassador to the larger community. He is generous in his help and appreciates thanks, but does not hold a grudge or cause problems if mistreated. As a population biologist, he made a major contribution to setting up the in-breeding strategy in the local *Xenopus tropicalis* genetics project. He tends to ignore people he doesn't like, rather than antagonize them, but he is assertive and can certainly stand up for himself in any situation if it is necessary. Personally, he is lively and engaging, and has a good sense of humor. He is more direct than many people and does not mince words, but at the same time he is considerate, well liked, and a popular participant in many enterprises. He will be a good colleague.

In summary, Dave is bringing a new combination of cell biological and biomechanical approaches to problems of morphogenesis and evolution of morphogenesis with results of general significance to the development and evolution communities, as well as to the biomedical field as well. He is the best I have had in my lab in this area, and one of the very best overall. I recommend him very highly and without reservation.

Sincerely,

Ray Keller
Professor
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