UNIVERSITY of VIRGINIA
DEPARTMENT of BIOLOGY

December 23, 2003

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

Dear Search Committee,

Enclosed you will find my application for the Biocomplexity Institute faculty position. Since I arrived in Virginia I have been working with Ray Keller in the Department of Biology on morphogenesis in the frog *Xenopus laevis*. Since 1999 I have been working with both Doug DeSimone in Cell Biology and Ray Keller. I have recently submitted an R01 proposal to the NIH on the "biomechanics of morphogenesis" to pursue my own independent research on the mechanics of mediolateral cell intercalation and the role of physical forces and tissue mechanics during gastrulation.

As my CV and research statement show I am interested in the complex movements of cells and tissues in the early embryo. With the completion of genome-mapping projects the focus of developmental biology is now turning to the task of understanding the protein-protein interactions that drive these movements. With Doug DeSimone I have been developing techniques to identify the important molecular pathways downstream of integrin-fibronectin interactions that are responsible for these movements. My interdisciplinary background, a Biophysics Ph.D. at Berkeley with two MacArthur Fellows, combined with considerable training in advanced imaging techniques and "cut-and-paste" embryology with Ray Keller, makes me an ideal candidate to drive the field of developmental biology toward a quantitative molecular-understanding of morphogenesis.

My own interests and expertise in imaging, morphogenesis, the biomechanics of the cytoskeleton, cell motility, cell-cell and cell-matrix adhesion from the molecular to the tissue level would complement those of the Biocomplexity Institute. Thank you for considering my application and I look forward to hearing from you.

Sincerely,

Lance A. Davidson

encl: Curriculum Vitae (including references) and Research Statement.

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Research Statement
Biomechanics of Morphogenesis
Lance A. Davidson

Research Summary:

The control of morphogenesis is one of the most complex problems of modern biology. While tremendous advances have been made to understand the molecular regulation of cell motility and morphogenesis, little is understood about how these regulators control the mechanical process of morphogenesis. In order to understand the physical as well as the molecular regulators of morphogenesis we are working to "reverse-engineer" morphogenetic movements through advanced imaging, cell biological, and biophysical techniques. Advanced imaging allows us to observe cell behaviors and tissue architecture and formulate physically relevant hypotheses for driving cell and tissue movements while perturbation of cell physiology and measurement of physical forces and tissue mechanics allows us to test these hypotheses. The experimental framework established in this work will complement ongoing studies of the molecular regulators of convergence and extension by providing "nuts-and-bolts" models of morphogenesis with testable hypotheses.

As a postdoc I have carried out studies on four morphogenetic events: convergence and extension, mesendoderm migration, wound healing, and neural tube formation. While each are strong candidates for a productive research program I have chosen to focus my efforts and have a pending R01 research proposal to study the biomechanics of convergence and extension in the frog *Xenopus laevis*.

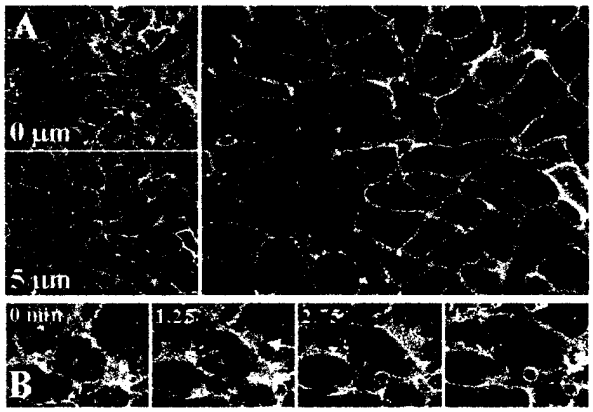

Figure 1. Two-level confocal timelapse sequence of intercalating cells. A) Frames in a timelapse collected at the level of the substrate and 5 μm above the substrate can be combined into two-color image showing show both cell shapes and lamellipodia on substrate. B) High resolution frames show initiation (arrow), extension, and retraction of a lamellipodia. With a two-level timelapse sequence, the identity of the originating cell and the orientation of the lamellipodia to its center can be determined.

Convergence and extension:
Prospective mesoderm cells drive convergence and extension of the vertebrate dorsal axis as they undergo iterations of oriented cell rearrangement known as mediolateral cell intercalation. Our studies show that productive force generation by mediolateral cell intercalation requires the presence of a complex fibrillar extracellular matrix during convergence and extension. The integrin $\alpha_5\beta_1$ appears to mediate these protrusions (figure 1). We have discovered that a major remodeling of the fibronectin-based fibrillar network (figure 2A) coincides with this cell rearrangement and is required for both spatial and temporal control of cellular protrusions that drive these coordinated movements. From this work we are evolving a more integrated understanding of the control of morphogenesis. For instance, working on the planar cell polarity genes strabismus and prickle (in collaboration with Dr. Toshi Goto at the University of Tokyo) we have found differing degrees of disorganized ECM in prickle, strabismus, and frizzled7 over-expressing embryos (figure 2B) even though over-expression produces nearly identical phenotypes. Furthermore, cell intercalation

behaviors responsible for tissue extension defects in whole embryos can be rescued by culturing explants expressing prickle but not strabismus on exogenous fibronectin substrates. A physically integrated model of convergence and extension must take into account both the capacity of cells to intercalate as well as ability of the cells to organize an extracellular matrix.

Mesendoderm morphogenesis:
Ventral and head mesoderm migrate hundreds of microns as cohesive cell sheets even though these tissues are formed from mesenchymal cells in a multi-cell-layered mass. Our work showed that this migration requires direct interactions between $\alpha_5\beta_1$ integrin and the central-cell binding domain of fibronectin. By altering the geometry of explants and their spatial organization we find that tractive forces distributed under all cells within the mass contribute to bulk explant migration rates. We further identified a "tissue-geometry" factor in the rapid closure of the mesendodermal mantle - cells *in vivo* or in "in-the-round" arrays of explants can double their migratory speed over isolated single cells or cells in single, spreading explants to over 300 μm/hour.

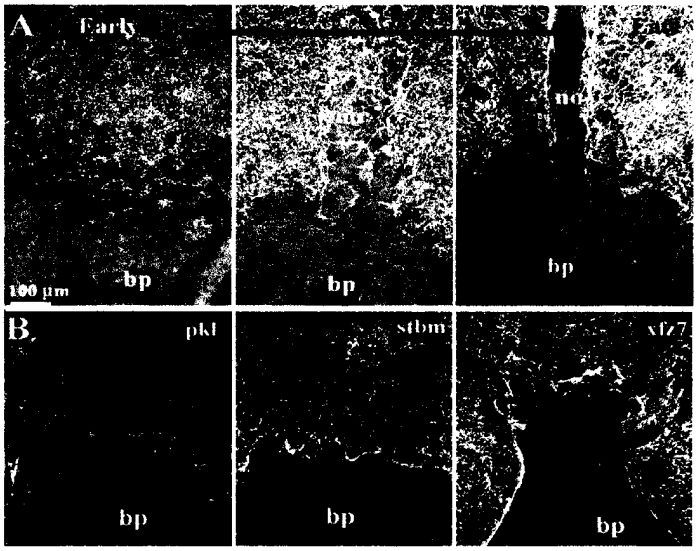

Figure 2. A) Establishment of a fibronectin *scaffold* for axial elongation. *En face* projections of confocal z-series through the dorsal axis show fibrils first assemble at the interface between the prospective neural and prospective mesoderm tissues. As gastrulation proceeds, fibrils are cleared from the surface of the notochord in a progression from posterior to anterior. (bp - blastopore, no - notochord, so - somite). **B)** Fibronectin scaffold is defective in prickle (pkl), strabismus (stbm), and frizzled7 (xfz7) overexpressing at stages equivalent to the "late" stage in (A).

Embryonic Wound Healing:
Large excisional wounds in amphibian embryos can close rapidly (less than 60 minutes) in movements that are excellent models for epithelial morphogenetic movements. Combining manipulations of the size and shape of wounds and tests of tension along the wound margin, our studies ruled out a major contribution of the contractile "purse-string" model of wound healing, instead identifying novel behaviors by deep mesenchymal cells exposed in the wound. Exposed deep cells contract their exposed apices, ingress, and draw the boundaries of the wound closed.

Neural Tube Formation:
Bending and rolling of the neural plate to form the neural tube is essential to establishing the central nervous system. In the frog embryo, Xenopus laevis, through a detailed characterization of patterns of gene expression and cell movements we identified a complex series of tissue movements during establishment of the neural tube. Like other vertebrates, apical contraction generates the neural groove, but once the epidermis fuses over the groove prospective neural cells undergo a transient mesenchymal phase as the cells facing the groove de-epithelialize, intercalate with deep neural cells, and then re-establish a neural tube lumen.

Future Plans:

The goal of my research is to integrate the biomechanics of morphogenesis across a number of size-scales from subcellular generation of forces to the macroscopic forces and bulk tissue properties that guide convergence and extension in the developing frog embryo. I want to understand, ***in mechanical terms***, how coordinated polarized cell protrusions generate force, and how these forces, through the process of mediolateral cell intercalation, are converted into tissue-scale movements. In order to understand how local force generation effects tissue movement we need to understand the mechanical context, i.e. the viscoelastic properties of tissues in the developing embryo and how they relate to tissue architecture. Lastly, we want to understand how the tissue architecture modulates conversion of protrusive activity into the bulk forces of tissue extension.

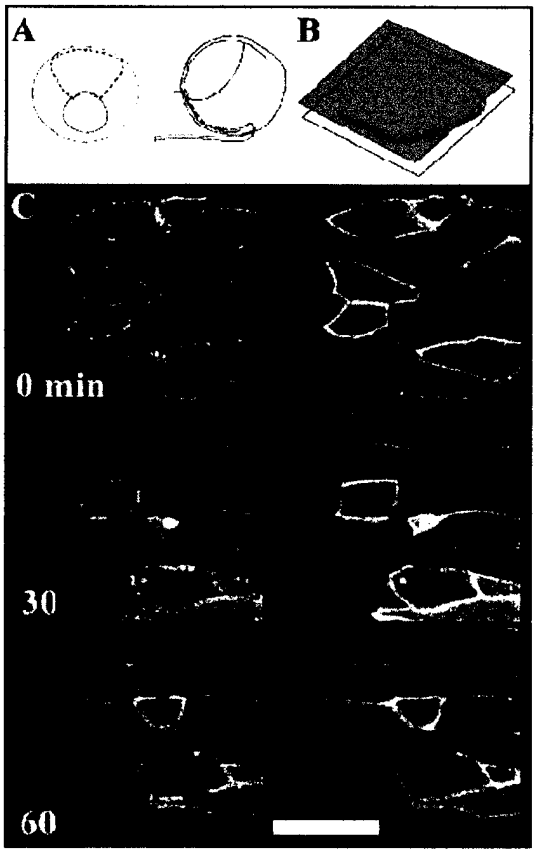

Figure 3 Fibronectin fibrils visualized in deep mesoderm explants cultured between agarose sheets. A) Schematic of deep mesoderm explant. B) Explant cultured between sheets of agarose. C) Frames from multilevel, multichannel confocal timelapse showing scattered GAP43-GFP expressing cells and fibronectin fibrils during explant elongation. Scalebar is 50 mm.

To achieve this goal and understand the mechanics of convergence and extension I am taking a multidisciplinary approach combining experimental embryological, cell biological, and biophysical techniques. Isolated tissue fragments allow us to observe cellular protrusions and cell rearrangement with high-resolution, multi-level confocal timelapse imaging techniques. We are using CY3-tagged antibodies to fibronectin to visualize fibrillogenesis and fiber handling by rearranging cells in explants (figure 3). Sensitive force transducers developed by myself in Ray Keller's lab allow us to measure the biomechanical properties of these same tissue fragments. I am currently adapting biophysical approaches, such as "force-traction" microscopy, to measure both cell-scale and tissue-level generation of forces. By complementing these biophysical approaches with cell biological techniques for disrupting "cytomechanical molecules", such as those involved in force generation, cell adhesion, ECM, and cell motility, I aim to understand, from a biomechanical perspective, the process of morphogenesis and its relevance to more clinically relevant morphogenetic events such as tumor invasion, wound healing, and the generation of birth defects.