THE INTERPLAY OF PHYSICAL AND MOLECULAR DETERMINANTS IN LIMB AND CARDIAC CUSHION MORPHOGENESIS

Brook Damon

Dr. Gabor Forgacs, Dissertation Supervisor

ABSTRACT

In an effort to investigate the interplay between molecular determinants and biomechanical properties during early embryonic development we have investigated two separate but similar morphogenetic processes: limb morphogenesis and cardiac cushion morphogenesis. Accurate interpretation and modeling of the physical mechanisms with which embryonic morphogenetic processes occur requires quantitative measurements of the biomechanical properties of the living embryonic tissues involved. It has previously been suggested that embryonic tissues may be described as liquids and that the biomechanical properties of individual tissue masses/populations contribute to the forces necessary for shape changes during early organogenesis. In order to quantitatively investigate the interplay between physical and molecular determinants during early vertebrate embryonic development we proposed that intact living fragments of embryonic mesenchymal tissue behave as liquids and that the biomechanical properties of living spherical explants of mesenchyme may be quantitatively measured using novel techniques and standard liquid theory.

Formation of the limbs in avian embryos occurs by changes in the properties of the somatopleural mesoderm in the limb fields whereby the limb buds are caused to bulge outwardly from the body. Signaling by FGF8 produced by the newly formed apical ectodermal ridge (AER) initiates this morphogenetic process. Employing formal similarities between embryonic tissues and immiscible liquids we have measured the apparent surface tensions of 4 day embryonic chicken wing and leg bud mesenchymal tissue, and of adjacent flank mesoderm. We found that the two types of limb tissues were significantly more cohesive than the flank tissue. The differences between the surface

tensions, a measure of relative tissue cohesivity, were consistent with the hypothesis of Heitzelmann et al. (1977) that budding of the limbs from the body wall is caused by differences between the cohesivities of limb bud and flank tissues. Besides being less cohesive than the limb buds, the flank also exhibited an active response behavior, not previously seen in tensiometry measurements of other embryonic tissues, which was dependent on an intact actin cytoskeleton. Possibly related to this active response was our finding that mRNA for α-smooth muscle actin (SMA) was expressed at significant levels in flank mesoderm, but was barely detectable in limb bud mesoderm. We performed tensiometry measurements on flank tissue before and after exposure to exogenous FGF8 to test whether the induction of limb budding by this factor is related to modification of the liquid-like properties of limb field mesoderm. We found that FGF8-treated flank tissue acquired surface tension values within the limb range, while losing its active response behavior.

During early embryonic heart development the atrio ventricular (AV) cushion mesenchyme develops to form the heart septum and valvular leaflets. Any deviation from normal AV cushion tissue development results in severe congenital heart defects. We have measured the biomechanical properties of explants of AV cushion tissue, in particular surface tension and viscosity, and found that they are developmentally regulated. Our results indicate that both the surface tension and viscosity of spherical explants of AV cushion tissue explants increase during early development. Additional tensiometry measurements of explants of AV cushion tissue incubated with exogenous TGFβ3 demonstrated an increase in tissue surface tension with decreasing efficiency with developmental stage and explants incubated with periostin over-expressing virus had an increased viscosity. Based on previous studies

which show that TGFβ3 upregulates the production of the extracellular matrix protein periostin as well as other important ECM proteins such as fibronectin, tenascin and collagen, it is possible to speculate that TGFβ3 and periostin plays a role in governing the biomechanical properties of AV cushion tissue during valvulogenesis. The biological implications of these results is that as the septum forms from the fusion of the AV cushion mesenchyme, the tissue becomes more cohesive and resistant to flow thus preserving the emerging architectural structure of the heart. Additionally we show that changes in the biomechanical properties of this tissue are in part regulated by the growth factor TGFβ3 and the ECM protein periostin.

Keywords: limb bud, surface tension, active response, cardiac, embryonic, surface tension, AV cushion, periostin, collagen, fibronectin, TGFβ3