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Tissue mechanics and adhesion during embryo development

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ABSTRACT

During development cells interact mechanically with their microenvironment through cell–cell and cell–matrix adhesions. Many proteins involved in these adhesions serve both mechanical and signaling roles. In this review we will focus on the mechanical roles of these proteins and their complexes in transmitting force or stress from cell to cell or from cell to the extracellular matrix. As forces operate against tissues they establish tissue architecture, extracellular matrix assembly, and pattern cell shapes. As tissues become more established, adhesions play a major role integrating cells with the mechanics of their local environment. Adhesions may serve as both a molecular-specific glue, holding defined populations of cells together, and as a lubricant, allowing tissues to slide past one another. We review the biophysical principles and experimental tools used to study adhesion so that we may aid efforts to understand how adhesions guide these movements and integrate their signaling functions with mechanical function. As we conclude we review efforts to develop predictive models of adhesion that can be used to interpret experiments and guide future efforts to control and direct the process of tissue self-assembly during development.

Introduction

Over several summers at the Beaufort Laboratory in North Carolina, H.V. Wilson conducted a remarkable series of experiments with cells isolated from sponges (Wilson, 1907); these cells, in isolation and when aggregated exhibited a range of phenomena including distinctive cell motility, adhesion, differentiation, development, and tissue homeostasis. Wilson dissociated cells from adult and larval sponges and by combining them he observed their ability to regenerate the structure and form of adult sponges. Wilson and others observed sorting, protrusive behaviors, tissue self-assembly, and regeneration based on the cell type origin, stage, individual, and species. These observations inspired later workers such as Holtfreter, Steinberg, Trinkaus, and others to consider the role of adhesion and cell motility in driving development and tissue-self assembly. Ultimately, this work led to the discovery of the molecules regulating cell–cell and cell–substrate adhesion, the founding of the field of cell mechanics, and a resurgent interest in the physical principles of early development, morphogenesis, organogenesis, stem cell biology, regeneration, wound healing, and disease. We focus in this review on recent efforts to understand the physical role of adhesion during development and how molecular mechanisms of adhesion generate biological form. In the following sections we introduce biophysical methods of investigating cell adhesion and its contribution to the mechanical properties and force production within developing embryos. In addition to biophysical studies on embryos we include studies with cultured cells and cells isolated from adult tissues to demonstrate how cells coordinate biochemical and mechanical signaling through cell–cell and cell–substrate adhesions.

Adhesion couples cell populations that establish mechanical support in tissues, allowing cells to be “fixed” with varying degrees of freedom to certain structures. For instance, epithelial cells can be constrained by their apical adhesive junctions to a two-dimensional plane, similarly, mesenchymal cells may form a monolayer as they bind a distinctive layer of extracellular matrix. Different cell types and diverse cell substrates can restrict cell movements along interfaces where adhesion receptors or ligands are present or direct the force they generate at these interfaces along specific directions. Context- or stage-dependent changes in adhesion may occur as cells contact new neighbors or as cells change their expression or activity of their adhesions and contacts. As tissues assemble into more complex structures adhesion can serve to couple forces produced by cytoskeletal dynamics in one cell to drive deformation and movement of a field of cells (Gardel et al., 2010; Kasza and Zallen, 2011; Parsons et al., 2010).

Adhesion is also thought to contribute to cell sorting during tissue assembly. Sorting refers to the rearrangement of scattered mixtures of two or more cell types into homogeneous clusters. Cell sorting has been observed in aggregates of different cell types

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(Steinberg, 1963), in aggregates of cells expressing different levels of adhesion molecules (Foty and Steinberg, 2005), and in aggregates of cells from different germ layers of the early embryo (Townes and Holtfreter, 1955). This later observation suggested that cell sorting might contribute to the mechanical processes that drive gastrulation. After germ layer determination in embryos, adhesions alone or adhesion-dependent cell behaviors may drive sorting to specific locations based on the type and density of adhesion proteins (Steinberg and Takeichi, 1994). For instance, assembly of extracellular matrix (ECM) at an interface between prospective notochord and paraxial mesoderm cells could attract other notochord and paraxial cells to each face of the boundary. Mixtures of notochord and paraxial cells might then sort at this boundary and then adopt specific behaviors along the interface, maintaining or strengthening that critical structure. The theories that adhesion alone is capable of driving cell rearrangement and tissue morphogenesis have been contentious (Harris, 1976) but have inspired alternative theories in which cell adhesions regulate cell behaviors, or that adhesions might be coordinated with apico-basal or planar polarity cues to create asymmetric patterns of actomyosin contractility.

Formal definitions of the mechanics of adhesion

Adhesion can be defined as the bonding of two distinct entities in a manner that resists their subsequent separation. In the context of cell biology, these entities can be held together through either homotypic or heterotypic protein–protein interactions. Cohesion is the specific adhesion formed via homotypic interactions. To understand the role of adhesion in development we must understand how cell and tissue adhesion resists or enables separation of these adhesions. Both heterotypic and homotypic adhesions resist detachment in the direction normal to the surface, e.g. tension, of the adhesive interface and can resist movement parallel to the surface, e.g. shear. In the case of shear, resistance to movement can be linear or can exhibit complex non-linear responses such as stiction when increasing force causes a tissue to “slip” along a boundary after a critical level of applied force is reached. To understand the biophysical response of cell- and tissue-level adhesion to mechanical loads found in embryos we adopt standard terminology from physics and engineering such as stress. Stress is defined in terms of the force applied over a surface (units of force/area; Newton/meter^2 or Pascal). Stress that is uniform in all three directions is pressure; a surface is under tension when the forces are applied in a direction that would cause separation and an interface is under compression when forces are applied that would bring the objects on either sides of the interface closer together. Once a force, or load is applied, a tissue can change shape or deform. Since the geometry of interfaces can take many forms, the term strain is a more useful description. Strain describes the amount of deformation per the scale of the object being deformed. The degree of strain a material exhibits when a defined stress is applied is expressed in the material's modulus. A material with a high modulus deforms less under a fixed load compared to a material with a lower modulus. The compressive modulus describes the degree a material resists compressive loads whereas the shear modulus represents how a material will change shape if a shear stress, e.g. a load applied parallel to a surface, is applied. In the practice of mechanical engineering a material may have different moduli in each of the three cardinal directions and along the six shearing surfaces. Mathematically, the modulus is a 3D tensor. When considering a material that slips at a surface we can define a yield stress which is the stress at which a material slips at the interface; such a material is referred to as a plastic and is permanently deformed once the shear stress is removed. The yield stress can be defined from the stiction force needed to overcome static friction when stationary objects are in contact.

Adhesions and biological materials can behave very differently under mechanical loads. A material is elastic if it returns to its original shape after deforming stresses are removed. The degree to which *stress* produces *strain* in a material defines the material's *elastic modulus*. By contrast, a material is viscous if it deforms over time to a new shape that is not restored after stress is removed. The degree to which *stress* produces a time-dependent *strain-rate* in a material defines the material's *viscosity*. In practice, biological materials and adhesive structures fall between the two extremes of elastic and viscous behavior and may simultaneously exhibit both viscous and elastic behavior. These intermediate behaviors can be viscoelastic or viscoplastic. The behavior of these types of materials depend critically on the rate at which a force or stress is applied. A tissue may behave as an elastic material if the force is applied rapidly but may deform more like a liquid if the force is applied over a longer time-scale. Tissues can even exhibit superplasticity – a term borrowed from descriptions of solid crystalline materials (Valiev et al., 1991); superplastic materials can deform well beyond the usual breaking point of an elastic material through rearrangements of grains at specific temperatures or strain rates. Mechanical engineers formulate theory describing such complex material behaviors from the behaviors of submicroscopic components of the materials, however, the elemental components of cells, e.g. multiple classes of interacting polarized polymers, motors, dynamic cross-linkers and their regulators may exhibit new “physics” (e.g. soft condensed matter physics) that are not well represented by the “orderly” behaviors of simple elastic or viscous materials.

Biophysical descriptions of adhesion

The dynamics of adhesions must be discussed when considering their mechanical function. Biophysical models of cell adhesion can be based on kinetic, thermodynamic and mechanical descriptions of adhesion (Zhu et al., 2000). Kinetic models represent adhesion by the rates in which adhesion receptors bind and dissociate and on their differential binding affinities. Thermodynamic models seek to explain adhesion through the differential chemical potentials of the receptors, ligands and bonds. Mechanical models approach problems of adhesion through adhesion energy density, γ, defined as the mechanical work required to separate a unit area of the adherent surface. Each of these models can be used to predict experimental properties of adhesions. For instance, mechanical engineers can measure γ using a peel-test. A peel test is a method used in materials and mechanical engineering to test adhesiveness of two materials bonded along a planar adhesive interface. In brief, one of the materials is attached to a force transducer positioned perpendicular to the planar adhesive interface and pulled away from the interface at predetermined velocities. For example, consider the removal of a piece of adhesive tape from a table top. The forces required to pull the one material from the other is measured and can be used to calculate the adhesion energy density of the interface. Peel tests have limited utility in measuring the adhesion between two tissues when adhesion strength between the tissues is greater than their cohesion strength. Analogous biophysical techniques have been used to measure cohesion using atomic force microscopy between single cells.

Another aspect of adhesion which makes it difficult to study is that the cytoskeleton and the adhesive machinery in the cell are not only coupled physically but are also coupled through intracellular signaling pathways. Changes in adhesion can alter the cytoskeleton (Kovacs et al., 2011), which in turn can alter tissue mechanics (Zhou et al., 2009). Integrins and cadherins have parallel roles in their respective adhesion complexes and involve several common scaffolding and cytoskeletal proteins such as

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