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Early events in the assembly of E-cadherin adhesions

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ABSTRACT

E-cadherin is a calcium dependent cell adhesion molecule that is key to the organization of cells in the epithelial tissue. It is a multidomain, *trans*-membrane protein in which the extracellular domain forms the homotypic, adhesive interaction while the intracellular domain interacts with the actin cytoskeleton through the catenin family of adaptor proteins. A number of recent studies have provided novel insights into the mechanism of adhesion formation by this class of adhesion proteins. Here, we describe an updated view of the process of E-cadherin adhesion formation with an emphasis on the role of molecular mobility, clustering, and active cellular processes.

1. Introduction

Organization of cells into tissues is at the heart of multicellular organism development. In animals, cells in different tissues are organized primarily by the interaction of cell membrane-localized receptors with either receptors on other cells or ligands in the extracellular matrix. Cadherins are a large family (> 100 members) of cell membrane-localized receptors with multiple extracellular cadherin repeats that are instrumental in the adhesion of cells in a variety of tissues. A subset of cadherins, known as classical cadherins, share a conserved cytoplasmic tail that binds catenins and links them with the actin cytoskeleton. Prominent among these are the epithelial (E)-, neuronal (N)-, and vascular endothelial (VE)-cadherins that forms adhesion between epithelial, neuronal and vascular endothelial cells, respectively. In this review we will be focusing primarily on E-cadherin.

A large number of studies have helped uncover the molecular structure of cadherins, which has been excellently reviewed elsewhere [1]. Briefly, the multidomain E-cadherin protein is expressed as a 120 kDa glycosylated protein with an extracellular domain containing five cadherin repeats (EC1-5), a trans-membrane domain, and a short unstructured intracellular domain (Fig. 1A) [1,2]. Each of the 5 EC repeats is ~110 amino acids long, and assume an immunoglobulin-like structure. Importantly, the structure of the extracellular domain is significantly altered by the binding of Ca^{2+} ions at four sites in between the five EC repeats, from a floppy to a curved, rod-like rigid conformation [3–5]. The *trans*-membrane domain of E-cadherin contains a leucine-zipper motif that may promote its oligomerization [6], and by analogy to VE-cadherin may promote interaction with other transmembrane proteins [7]. The highly conserved intracellular domain [8] is ~150 amino acids long and binds adaptor proteins such as p120- and β-catenin, which in turn interact with a multitude of structural and signaling proteins, connecting E-cadherin with a variety of cellular machineries, most notably the actin-cytoskeleton [9–11]. Thus, cadherins not only allow cells in a tissue to adhere to each other but also organize signaling in the cells [12].

Here, we describe the mechanism of E-cadherin adhesion formation from a biophysical and mechanical perspective based on recent reports in the literature. We begin by asking how the extracellular domain of E-cadherin initiates adhesion between two apposing cells. We then ask how cellular protrusions such as filopodia and lamellipodia impinge on assembly of E-cadherin-mediated adhesion sites. Finally, we ask how mechanically sensitive adaptor proteins such as α-catenin could be impacted in these processes.

2. Molecular basis of E-cadherin adhesion

At the center of E-cadherin-mediated cell-cell adhesion is the homotypic *trans*-interaction formed by its extracellular domains present on apposing cell membranes (Fig. 1B). The *trans*-interaction involves a partial swapping of the N-terminal A* β strand present in the EC1 domains [13]. These swapped strands are stabilized by the docking of a Trp (W2) residue from one monomer into a pocket in the EC1 domain of the other monomer, and other salt bridge interactions formed by positively charged N-terminal residues [4,13–20].

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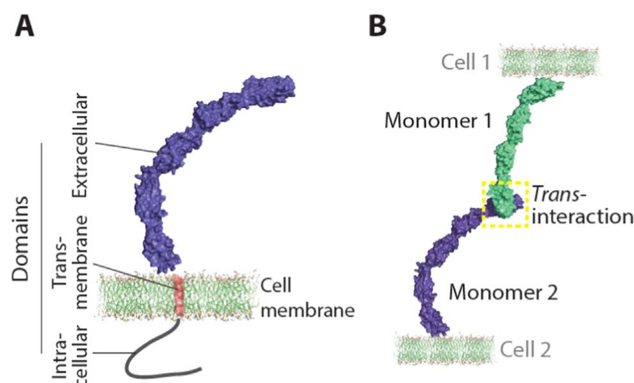

Fig. 1. Schematic representation of E-cadherin. (A) A cartoon showing the domain architecture of E-cadherin [28] on a membrane. (B) A cartoon showing a pair of E-cadherin molecules (extracellular domain) from apposing cells interacting with each. The extracellular domain cartoons were recreated using the Pymol (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC).

Importantly, the *trans*-interaction is a relatively low affinity interaction with reported K_d values ranging from 100 to 700 µM obtained from solution-based biochemical assays with the full-length versions of the extracellular domain [13,18,21,22], and involves a high activation energy barrier as determined from Nuclear Magnetic Resonance (NMR) assays [21,22]. Key to the E-cadherin homotypic *trans*-interaction is its high stability with a very slow rate of dimer-monomer interconversion [13].

The low affinity *trans*-interaction between E-cadherin extracellular domains is regulated in multiple ways at the molecular level. First, Ca^{2+} ion binding to, and subsequent structural stabilization of the extracellular domain strongly affects homodimerization, a feature which has been successfully exploited in the traditional 'calcium switch assays' wherein the adhesion of cells is controlled by reversible exchange of Ca^{2+} ions in the media [3,4,23]. Second, the final strand-swapped homodimer is formed from the conversion of an X-dimer intermediate that is formed during the initial encounter of the monomers [3,4,13,24]. The formation of the X-dimer intermediate has been implicated in the dynamics of both assembly and disassembly of cadherin adhesions based on the mutational studies of residues involved in this interaction [25]. Importantly, the X-dimer intermediate has been proposed to form a force-sensitive catch bond that could potentially be directly regulated by the mechanical tension in a cell-cell junction [26,27]. Third, the monomeric extracellular domain has been found to interact in *cis* via residues in the EC1 and EC2 domains that could potentially alter the adhesive function of cadherins by cooperatively regulating the *trans*-interaction [28,29]. Thus, it is clear that the process of cadherin adhesion formation could be regulated by different factors working at the molecular level.

3. Role of molecular mobility of E-cadherin in adhesion formation

While biochemical and biophysical studies have laid out the molecular basis of the *trans*-interaction between the extracellular domains of E-cadherin *in vitro*, the formation of cell-cell adhesions in cells is more complex. Basic molecular chemistry posits that E-cadherin molecules encounter each other by random physical translocation, and eventually form the *trans*-interacting dimers. The random translocation of E-cadherin molecules on the cell membrane will be dictated by laws of diffusion in two dimensions. However, single particle tracking experiments with E-cadherin in live cells have revealed three types of diffusion: unrestricted, free diffusion; restricted, corralled movements; and directed movements [30,31]. These different diffusion behaviors of E-cadherin could be attributed to its interaction with the actin and spectrin cytoskeleton underlying the cell membrane [32–35] or to the *cis*-interaction between non-ligated cadherin molecules [28]. Additionally, a general effect of the physical properties of the membrane on the mobility of E-cadherin, due to complex lipid compositions, cannot be ruled out. In fact, some members of the cadherin family have been found to be associated with 'lipid rafts', membrane structures that could drastically reduce their long-range molecular mobility [36–39]. Further, the mean diffusion coefficient of E-cadherin has been shown to be dependent on its oligomeric state [33,40] as well as the formation of adhesion [41].

The idea that the freely diffusing cadherin molecules encounter each other and form *trans*-interaction has been applied to *in silico* simulations, which led to the proposition of a 'diffusion trap' model, in which freely diffusing monomers from apposing membranes bind to each other and are eventually trapped at the initial point of contact between the two membranes [29]. Indeed, a reduction in the mobility of cadherin has often been utilized as a marker for stable adhesion formation [32,42]. However, this simplistic idea has been questioned by recent observations made in hybrid assays involving live cells and cadherin-functionalized supported lipid bilayers [43]. It is important to point out that the role of molecular mobility on the assembly of cadherins adhesions is difficult to assess purely from cells in culture. This is primarily due to the fact that any perturbation of molecular mobility on live cell membranes such as by changing the membrane composition or deleting the intracellular domain of E-cadherin will affect a multitude of processes in the cell. In contrast, a synthetic supported lipid bilayer allows control over the molecular mobility of proteins anchored to the bilayer by simply altering the constituent lipid molecules. The supported lipid bilayer-based assays revealed that cells rarely formed adhesion with bilayers displaying highly mobile E-cadherin molecules (diffusion coefficient, D of 1.6 ± 0.2 $\mu m^2/s$). In contrast, cells readily formed adhesion with viscous bilayers that display low mobility E-cadherin molecules. These results indicate that it is the immobile fraction of E-cadherin molecules that are capable of forming *trans*-interacting homodimers, and serve as the seed for nucleating extended cadherin adhesions [43].

4. Nanoscale E-cadherin clusters as building blocks for cell adhesion formation

A very basic observation made with cadherin adhesions is the enrichment of *trans*-interacting homodimers at the cell-cell interface [41]. This is not surprising as the local concentration of cadherin molecules at the adhesion interface is a sum of the interacting (ligated) dimers as well as non-interacting (non-ligated) monomers. The *cis*-interaction between the extracellular domains of cadherin [28] could further enhance the local enrichment of cadherins, as suggested by *in silico* molecular dynamics studies [29]. However, unlike the uniform distribution of *trans*-interacting cadherin molecules seen between two adhering vesicles *in vitro* [28], they are not uniformly distributed at the adhesive interface of living cells. Instead, they are organized into micron-scale clusters [41,43–49]. These micron-scale clusters have been further resolved in recent super-resolution (3 dimensional-Stochastic Optical Reconstitution Microscopy; 3D-STORM) studies into nanometer-scale clusters that reach molecular densities close to that seen in crystals [50,51]. Interestingly, molecular densities in these nanometer-scale clusters of E-cadherin are regulated by the *cis*-interaction between the extracellular domains, while the size of these clusters are limited by the underlying actin cytoskeleton through interaction with the intracellular domain [50]. The nanometer-scale clustering behavior of *trans*-interacting cadherins observed in the super-resolution studies is further corroborated by the Fluorescence Correlation Spectroscopy (FCS) measurements in the hybrid live cell-supported lipid bilayer experiments [43]. These experiments revealed a marked reduction in the diffusion coefficient as well as formation of large oligomers of E-cadherin at the adhesive interface [43].

One of the key observations, however, made with the super-

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