New and Noteworthy-

Force bistability in adhesion switch

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The lateral junctions of epithelial cells must display mechanical stability to form barriers, but also significant plasticity to allow for reorganization during development and repair. In this issue, Biswas et al (2016) extend the use of fluid supported lipid bilayers (SLB) to reconstitute function of the key adhesion molecules that initiate lateral junctions of epithelial cells, E-cadherin, and to further study the requirements for mechanical forces in activating the cytoplasmic complex that links E-cadherin to the cytoskeleton (1). The new results suggest that conformational changes during activation of the key cytoplasmic adapter α–catenin are initially triggered in a force dependent "active nucleation" of clusters, but surprisingly, the activated clusters can persist when forces are released. This force bistability may contribute to the ability of E-cadherin to participate in broad lateral adhesions that can persist in a steady state even as forces in the epithelial sheet are borne by more specialized adhesion zones such as desmosomes (2).

Biswas et al (2016) utilized purified E-cadherin extracellular domain in SLB to test the requirements for cells expressing a full-length E-cadherin molecule to form junctions (1). Many studies have been performed with surface immobilized, purified E-cadherin to mimic various aspects of cell-cell junction formation (3), but it seemed unlikely that the substrate absorbed molecules would be able to rearrange into the interlocking lattices that are characteristic of epithelial junctions (4). Presenting E-cadherin in a laterally mobile form of SLB with disordered lipids offers a potential solution to this problem. However, when E-cadherin is presented on SLB at physiological densities with high lateral mobility, junctions are very rarely formed (5). Rarely, cells formed broad junctions with both peripheral and internal E-cadherin clusters on substrates containing the highly mobile E-cadherin. They determined that making the SLB "semi-mobile" by using more saturated phospholipids triggered robust junction formation by most cells (5). These findings led to a model of "active nucleation" in which filipodial extension and retraction allowed cell surface E-cadherin sufficient time to remodel the semi-mobile E-cadherin into clusters (5). This process resulted in a ring like junction that corresponded to the actinomyosin contractile apparatus and no visible interior clusters (5). These results suggest that active nucleation is difficult with highly mobile E-cadherin, which diffuses away faster than filipodia can gather it. Rarely, the conditions for junction formation are achieved, but it was not clear how this was accomplished. While the 2D affinity of E-cadherin is predicted to be sufficient to allow adhesion of apposed surfaces (4), the 3D nature of the filipodial structures may reinforce the need for an active process to gather low mobility E-cadherin to a critical density. While the semi-mobile SLB were effective, the new paper simply found that

increasing E-cadherin density on mobile SLB resulted in a reduced mobility of purified E-cadherin that enables efficient active nucleation of junctions by cells expressing WT E-cadherin labeled with GFP. When high density E-cadherin was presented to cells in microscale islands the pattern of cell surface E-cadherin clustering gave a readout of E-cadherin clustering. Like the spontaneous contacts formed with low efficiency on mobile E-cadherin, these junctions include both a peripheral ring the co-localized with F-actin enrichment, and interior clusters that did not concentrate F-actin to the same extent, creating an opportunity to compare adhesion associated with contractile and non-contractile domains.

The cadherin-catenin-complex (CCC) links the E-cadherin to F-actin (6). In mammals, E-cadherin cytoplasmic domain interacts directly with p120-catenin and β–catenin, which in turn interact with α-catenin, providing a link to F-actin. F-actin nucleating formins are also recruited by α-catenin. The activation of α-catenin by force results in the generation of vinculin binding sites and exposure of an antibody epitope, which are both utilized by Biswas et al (5). As expected, Biswas et al found the outer ring of E-cadherin that localizes with the F-actin and myosin rich components of the junction display strong vinculin binding. Surprisingly, they also observed vinculin binding in the central clusters that lack the contractile elements. Furthermore inhibition of contraction or F-actin didn't eliminate vinculin or antibody binding to the clustered E-cadherin. While surprising, these results immediately generated new opportunities to understand the complex requirements of E-cadherin function in epithelial sheets. These results extend the active nucleation model to include a bistable switch in which force mediated clustering facilitates initial activation of α-catenin, which can then persist in clustered E-cadherin clusters in the absence of force (Figure).

This new models system for studying junctions based on high density E-cadherin in mobile SLB raises many questions, but fortunately opens a pathway to addressing many of them. SLB have ideal optics when combined with total internal reflection fluorescence microscopy and its super-resolution derivatives (7). The density dependent behavior of E-cadherin extracellular domain is of interest and whether this is reflected at all in the behavior of cell surface cadherins. E-cadherin can undergo weak cis-interactions and these may contribute to formation of supramolecular E-cadherin complexes in the bilayer, which slow mobility, but maintain dynamics (4, 8). Biswas et al found that a mutation that eliminates the structurally defined cis interaction didn't alter performance in the semi-mobile bilayers, but this was not tested in the mobile bilayers with high E-cadherin density (5). It will also be very interesting study the dynamics of vinculin binding to the α-catenin in the two peripheral and central E-cadherin clusters. It seems most likely that this binding will be dynamic even in the low force interior clusters (9). This would suggest that the clusters offer some mode of cooperatively to keep these binding sites open in the absence of external force, and this cooperative architecture could be the key force dependent event, rather than the a force directly exposing the vinculin binding site (10). The SLB based model should be an ideal setting to dissect these questions. These explorations in hybrid junctions could then allow design

of experiments to test these ideas in epithelial sheets in vitro and developmental, homeostatic and repair models in vivo.

Figure legend- Force bi-stability. The axis arrows reflect the direction of increase in the parameters: force, E-cadherin density, and activation (vinculin binding). The head to tail orientation of the axes signifies the proposed sequence of events in active nucleation- force application, E-cadherin clustering and α-catenin activation. Once the cluster is formed the force can be relaxed with retention of activation. The model is drawn to allow for a role of force in cluster dissolution. Green arrows are pathways to active states whereas the red arrow is a pathway to eliminate the junction.

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