Guilt by association: What p120-catenin has to hide

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Members of the p120-catenin family associate with cadherins and regulate their stability at the plasma membrane. How p120-catenin limits cadherin endocytosis has long remained a mystery. In this issue, Nanes et al. (2012. *J. Cell Biol.* doi:10.1083/jcb.201205029) identify a conserved acidic motif within cadherins that acts as a physical platform for p120-catenin binding. However, in the absence of p120-catenin, the motif acts as an endocytic signal. These results provide new insight into p120-catenin's role as guardian of intercellular junction dynamics.

Adhesion receptors of the classical cadherin family have a major role in establishing tissue organization and maintaining tissue homeostasis (Gumbiner, 1996). Classical cadherins are transmembrane glycoproteins that use their extracellular domains to establish calcium-dependent trans homophilic interactions with cadherins in neighboring cells. To enhance adhesive strength, cadherin ectodomains oligomerize through lateral (cis) interactions, whereas their cytoplasmic domains anchor to the actomyosin cytoskeleton. The cytoplasmic domain of cadherins is highly conserved and binds to proteins called catenins. p120-catenin (p120) associates with the transmembrane adjacent domain (juxtamembrane; JMD) of the cadherin cytoplasmic tail, whereas β-catenin interacts with the more distal portion of cadherin's cytoplasmic domain. β-Catenin in turn, binds α-catenin, which, through multiple interactions, both indirect and direct, can associate with the actin cytoskeleton (Perez-Moreno and Fuchs, 2006).

Cellular rearrangements are orchestrated by dynamic assembly/disassembly of cadherin complexes. The process is fueled by endocytosis of cadherin complexes (Le et al., 1999; de Beco et al., 2009). Endocytosis can be stimulated by proteins that associate with cadherin–catenin complexes, including proteases that shed the cadherin ectodomains, and the ubiquitin ligase Hakai (Fujita et al., 2002). Cadherin internalization can be regulated by different pathways depending on the cellular context, involving clathrin-dependent and clathrin-independent mechanisms. These endocytic processes must be carefully regulated, as an untimely destabilization of cadherin-mediated adhesion can lead to alterations in tissue architecture and growth, features of several diseases, including cancers (Mosesson et al., 2008).

In the past decade, p120 catenins (p120, ARVCF, δ-catenin, and p0071) have emerged as critical regulators of cadherin-mediated adhesion (Reynolds, 2007). p120, the founding family member, is a component of cadherin complexes (Reynolds et al., 1994), and its association with the cadherin JMD is important for retaining cadherins at the membrane (Ireton et al., 2002). Moreover, p120 loss causes rapid internalization of cadherins, followed by proteasomal and/or lysosomal-mediated degradation (Davis et al., 2003; Xiao et al., 2003a,b, 2005; Miyashita and Ozawa, 2007).

Although these studies expose p120 as a master regulator of cadherin levels at the membrane, exactly how p120 governs cadherin endocytosis rates has remained unclear. Based upon experiments in which endocytic machinery components (clathrin, dynamin, and AP2) have been impaired (Chiasson et al., 2009) or cadherin endocytic motifs have been mutated (Hong et al., 2010; Troyanovsky et al., 2007), researchers have posited that p120 binding to cadherins may in some way prevent junctional complex endocytosis. In this issue, Nanes et al. add new molecular insights into the mechanism. The authors show that the VE-cadherin JMD functions as a bimodal platform for either p120 binding or endocytic signaling. Moreover, they identify a key conserved amino acid residue within the JMD, which, when mutated, blocks endocytosis without the need for p120.

Recently, the cocrystallization of p120 bound to E-cadherin's JMD has yielded insights into the essential residues of this binding interface (Ishiyama et al., 2010). Previous studies had attributed the core function of p120-cadherin to its ability to bind and mask a dileucine endocytic motif present in the JMD (Miyashita and Ozawa, 2007; Hong et al., 2010). The crystal structure showed that interactions between p120 and the JMD domain might be sufficient to sterically prevent accessibility of the dileucine cadherin endocytic motif to endocytic adaptors such as the AP2-clathrin adaptor, thereby placing this motif at the crux of the bimodal switch controlling the mutually exclusive binding of either p120 or the endocytic machinery.

The affinity of p120 and AP2 for the JMD dileucine motif is similar, pointing toward the existence of a balanced regulation

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Figure 1. **Model of VE-cadherin stabilization at the cell membrane.** (A) VE-cadherin binds to p120 and β-catenin. p120 associates with the juxtamembrane (JMD) domain of the cadherin cytoplasmic tail, whereas β-catenin binds to the more distal portion (catenin binding domain, CBD). Cadherin internalization is triggered by p120 dissociation, exposing a conserved endocytic factor recognition motif (DEE; 646–648) within the JMD. (B) When this motif is mutated in VE-cadherin, adherens junctions are resistant to endocytosis independent of p120 binding.
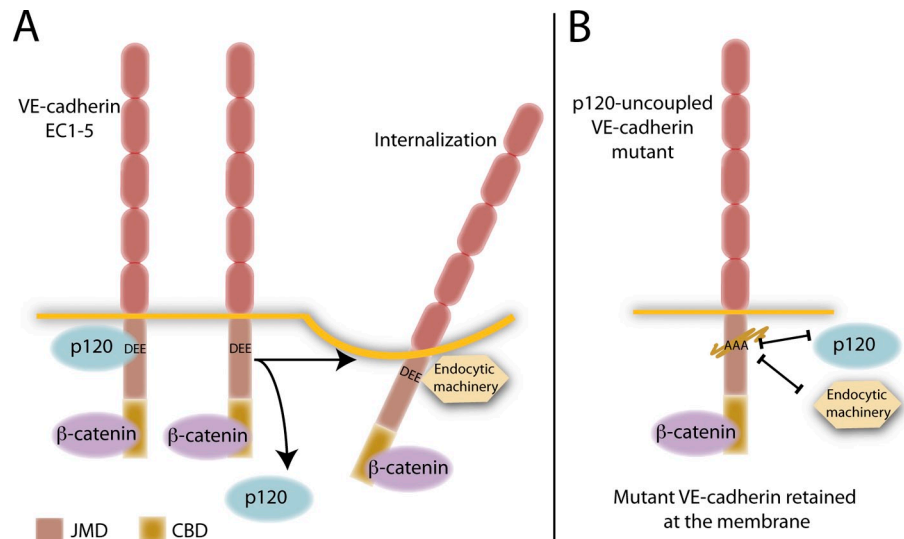
of cadherin endocytic rates and cadherin retention at the membrane. However, evaluating this balance in cellular contexts has not been possible because of the inability to uncouple p120 binding to the JMD and endocytosis. Nanes et al. (2012) have now overcome this hurdle. They first used a simulated model of the p120–E-cadherin crystal structure, which highlighted a conserved p120-binding region that is present in the JMD of both VE- and E-cadherin. However, the VE-cadherin JMD lacked endocytic dileucine and tyrosine residues present in E-cadherin, which are involved in clathrin internalization and Hakai-dependent ubiquitination, respectively.

Because both types of adherens junctions undergo dynamic endocytic-based remodeling, the authors astutely realized that they might be able to exploit VE- and E-cadherin differences to unearth novel endocytic signals within the sequence that might be conserved among cadherins. To this end, the author first used mutant VE-cadherin chimeric proteins, consisting of the cytoplasmic domain of VE-cadherin fused to the extracellular domain of the IL-2 receptor, and internalization assays. They discovered that the core p120-binding region on its own was endocytosed, in a fashion similar to the full VE-cadherin cytoplasmic tail. This occurred in a clathrin-dependent manner, as previously observed in Kowalzcyk's laboratory (Chiasson et al., 2009). Point mutagenesis identified some mutants no longer able to bind p120, which is consistent with previous findings (Thoreson et al., 2000). But the authors made an interesting finding: mutations in a conserved acidic motif (DEE) within the p120-core binding region of the JMD displayed loss of p120 binding and also blocked cadherin internalization (Fig. 1). Moreover, DEE mutant VE-cadherins localized stably at the membrane even in the absence of p120, although with an increased diffusion within the membrane. This increase in mobility suggests a reduction in cadherin lateral clustering, a process modulated by the binding of p120 to the JMD (Yap et al., 1998). Interestingly, in crystal structures, the E-cadherin JMD binding to p120 induced oligomerization of the complex (Ishiyama et al., 2010).

These new tools now allow uncoupling of p120 binding from cadherin endocytosis, which will be instrumental in unraveling new p120 cadherin roles in cell adhesion. The VE-cadherin mutant that fails to bind to p-120 still coimmunoprecipitates with β-catenin. These findings are intriguing, given that overexpression of p120 can rescue the otherwise poor adhesive properties of cadherins mutant for β-catenin binding (Ohkubo and Ozawa, 1999). In addition, interactions between p120 and α-catenin at adherens junctions seem to contribute in preventing cadherin endocytosis (Troyanovsky et al., 2011). Given these collective results, it will be interesting in the future to measure the binding affinities of endocytosis-uncoupled VE-cadherin mutants for its binding partners.

Overall, these data provide strong evidence that the JMD landing pad provides the nuts and bolts of the decision of whether an adherens junction remains at the cell surface or whether it is internalized. But who makes the decision? Recent results from Gumbiner's group provide a possible clue. They show that cadherin activation stimulates the dephosphorylation of specific Ser/Thr residues within the N-terminal domain of p120, and this in turn stabilizes intercellular adhesion (Petrova et al., 2012).

The new tools developed by Kowalczyk's group (Nanes et al., 2012) will pave the way for researchers to dig further into the mechanism. In the current study, the authors use their newfound tools to analyze the consequences to cell migration when p120-JMD binding is uncoupled from endocytosis. In scratched monolayers of endothelial cells, cell migration was decreased. Importantly, when they examined the VE-cadherin mutant in which p120 binding was blocked but cadherin internalization could proceed normally, cell migration was largely normal. These findings indicate that the migration defects seen in the cells expressing the E-cadherin mutant are rooted in inhibition of endocytosis, rather than lack of p120 recruitment to junctions. They further suggest that endocytic trafficking of cadherins is necessary to transiently destabilize cell–cell contacts that otherwise impede migration. This notion is particularly intriguing given that when E-cadherins are stabilized at intercellular junctions, they can sequester proteins that are required for integrin-based migration (Livshits et al., 2012). Kowalczyk's findings (Nanes et al., 2012) now suggest a means

by which dynamic changes in intercellular adhesion can be achieved to trigger such downstream events.

Although less well characterized, there are other regulatory circuits that might also be affected by transiently liberating p120 from intercellular junctions. Thus, for example, p120 enhances cadherin stability through its ability to interact with afadin and Rap1, thereby bridging connections with nectin intercellular junctions (Hoshino et al., 2005). Other direct and indirect p120 associates that might affect cadherin internalization include the endocytic adaptor Numb (Sato et al., 2011) and the signaling enzyme γ-secretase (Kiss et al., 2008). Additionally, p120 can also regulate Rac1 activity, which influences cadherin endocytosis in a clathrin-independent way (Akhtar and Hotchin, 2001). Thus, removing p120 or devising additional mutations to uncouple these interactions may be needed to fully unravel all the mysteries underlying p120's power in governing intercellular adhesion in tissue development and maintenance (Davis and Reynolds, 2006; Elia et al., 2006; Perez-Moreno et al., 2006; Smalley-Freed et al., 2010; Marciano et al., 2011; Stairs et al., 2011; Chacon-Heszele et al., 2012; Kurley et al., 2012). That said, by dissecting p120's web at the crossroads between intercellular junction stabilization and endocytosis, Kowalczyk and coworkers (Nanes et al., 2012) now illustrate the power of their approach and provide new insights into how similar strategies might ultimately enable this molecular crossword puzzle to be solved.

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