Published in final edited form as:
Dev Cell. 2012 May 15; 22(5): 909–910. doi:10.1016/j.devcel.2012.05.002.

The Art of "Cut and Run": The Role of Rab14 GTPase in Regulating N-cadherin Shedding and Cell Motility

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Abstract

Rab14 and FAM116 define an endocytic recycling pathway that controls proteolytic N-cadherin cleavage by transporting ADAM10 protease to the plasma membrane. When this pathway is disrupted, diminished ADAM10-dependent N-cadherin shedding leads to increased cell-cell adhesion and inhibition of cell motility.

Cell motility is a highly dynamic process that requires the temporal and spatial coordination of many cellular structures, including dynamic changes in the actin cytoskeleton, re-orientation of microtubules and the directional transport of integrins and other adhesion molecules. Endocytic transport, especially endocytic recycling, has emerged as one of the key regulators of cell motility. Indeed, endocytic transport was shown to mediate the plasma membrane insertion of different integrin complexes during cell movement and invasion (Caswell and Norman, 2006). The endocytic recycling of N- and E-cadherins was shown to regulate cell-cell adhesions in tissue culture as well as during embryonic development (Desclozeaux et al., 2008; Kawauchi et al., 2010). Finally, endocytosis and the recycling of signaling receptors is known to control directional cell migration and axonal extensions in response to various chemokines *in vitro* as well as *in vivo*.

Most endocytic recycling flows through compartments known as recycling endosomes. Recycling endosomes were originally considered a sub-compartment of early endosomes that are responsible for protein recycling back to the plasma membrane. Recently, however, it has been recognized that recycling endosomes actually consist of multiple functionally distinct endosomes, which mediate the transport and targeting of different cargoes to varying destinations. The mechanistic understanding of how these cargo proteins are sorted, regulated and targeted during their transit via this very complex recycling endosomal network, is only beginning to emerge.

Rab GTPases are one of the key protein families that define the roles and identity of different endosomes. Indeed, individual Rabs appear to associate with very specific endocytic compartments and were shown to regulate numerous distinct endocytic transport steps, including cargo sorting, transport, tethering and fusion. While some of the Rabs, such as Rab5, Rab11 and Rab21, were shown to regulate the endocytic transport of integrins and E-cadherin, the full extent of the involvement of Rab GTPases in cell motility remains to be

determined (Caswell and Norman, 2006; Lock and Stow, 2005). To that end, in this elegant and data-rich study, Dr. Francis Barr and colleagues systematically analyzed the potential involvement of all Rabs in cell movement. Intriguingly, at least half of the Rabs tested inhibited cell migration in wound healing assays, again demonstrating the importance of membrane transport in cell motility. Cell movement in wound healing assays was most dramatically affected by the knock-down of a subset of Rab GTPases (Rab11, Rab4 and Rab14), all belonging to a closely related Rab GTPase subfamily that is sometimes referred to as Rab11 subfamily GTPases (which also includes Rab25). Rab4, Rab11 and Rab25 are all located at early or recycling endosomes and are known to mediate the recycling of various cargo proteins back to the plasma membrane. The role of Rab14 is less understood, and is a focus of this study. Interestingly, using several elegantly-designed assays, the authors demonstrate that Rab14 is present at a distinct recycling endosome sub-compartment that functions after Rab5 and Rab4, but prior to Rab11. Furthermore, the study provides some evidence suggesting that Rab14 may regulate a separate recycling pathway that transports selected proteins to the plasma membrane independently of the Rab11-mediated recycling pathway. As the result, Rab14 may actually define an independent and functionally distinct endocytic recycling pathway.

All Rab GTPases function by cycling between GDP-bound inactive and GTP-bound active forms (Pfeffer, 2001). The location and timing of Rab activation is determined, at least in part, by specific guanine nucleotide exchange factors (GEFs) that mediate the exchange between GDP and GTP. One of the larger families of known Rab GEFs is defined by the presence of a DENN (differentially expressed in normal and neoplasia) domain (Marat et al., 2011). Since the GEFs that activate the Rab11 subfamily of proteins are not yet determined, authors set out to identify GEFs that activate Rab14 GTPase. True to their style, Dr. Francis Barr and colleagues screened all DENN domain-containing proteins, as well as DENN-related proteins for their effect on cell motility and their ability to activate different Rab GTPases. From this screen, FAM116A has emerged as a member of a DENN-related protein family that had a most dramatic effect on cell motility. Furthermore, FAM116A was identified as a protein that has GEF activity specifically toward Rab14 GTPase. Finally, FAM116A was shown to be required for the recruitment of Rab14 to recycling endosomes. Taken together, these data demonstrate that FAM116A is a physiological GEF for Rab14 and is required for Rab14 activation and function.

How do Rab14 and FAM116A affect cell motility? This is where this study took a very interesting twist. It turned out that the knock-down of Rab14 or FAM116A has no effect on the polarization of the cytoskeleton and Golgi toward the wound edge. Furthermore, E-cadherin and integrin recycling also appeared to be normal, thus eliminating the most obvious mechanisms of Rab14 action. Surprisingly, Rab14 or FAM116A depletion increased the levels of N-cadherin at cell-cell junctions. This suggests that the increase in cell-cell adhesion may be inhibiting the ability of an individual cell to migrate. Consistent with this idea, authors have shown that Rab14 or FAM116A depletion blocked a wound-induced decrease in N-cadherin levels at junctions, and blocked the separation of cells from the edge of the wound. N-cadherin knock-down also completely rescued the effects of Rab14 depletion in wound healing assays. Surprisingly, this increase in N-cadherin mediated cell-cell junctions was not due to N-cadherin recycling defects. Instead, Rab11 and FAM116A appeared to regulate the extracellular cleavage of N-cadherin, a process known as N-cadherin shedding. Since cadherin shedding is mediated by transmembrane proteases of the disintegrin and metalloproteinase domain (ADAM) family, the study next screened for the effect of different ADAM family members on N-cadherin shedding and cell motility. As a result of this screen, ADAM10 was identified as the only ADAM protease whose transport is affected by Rab14 and FAM116A depletion, and which was shown to directly

mediate N-cadherin shedding. ADAM10 depletion also phenocopied defects caused by Rab14 and FAM116A knock-downs.

In summary, this work comprehensively demonstrates that Rab14 and its GEF, FAM116A, regulates the specific endocytic transport of ADAM10 and thereby N-cadherin shedding. The defect in the disassembly of N-cadherin cell-cell junctions, in turn, inhibits cell motility during wound healing. However, many questions remain and no doubt will be the focus of future studies. All Rabs function by recruiting and/or activating specific Rab effector proteins. Only a couple of Rab14 effector proteins have been identified so far, including RUFY1/Rabip4, as well as Kif6B (Ueno et al., 2011; Yamamoto et al., 2010). RUFY1/Rabip4 was shown to be a Rab14 and Rab4 cross-linking effector protein. Since Rab14-mediated ADAM10 transport is independent of Rab4, RUFY1/Rabip4 is probably not involved in Rab14's regulation of cell motility. Thus, Rab14 effector proteins that mediate ADAM10 transport and cell motility remain to be identified. The existence of Rab14-binding proteins that are specific to the ADAM10 targeting pathway may also explain the exquisite specificity of ADAM10 for N-cadehrin in this wound healing assay. ADAM10 was also shown to function as a sheddase for E-cadherin and the EGF receptor (Higashiyama and Nanba, 2005; Solanas et al., 2011), however neither of these proteins were affected by Rab14 or FAM16A depletion. Finally, Rab14 was also implicated in regulating transport from the Golgi to endosomes, endosome and phagosome fusion, as well as insulin-dependent GLUT4 transport. Additional research will be needed to understand how all these Rab14-dependent functions are regulated and integrate with one another.

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