Doing cell biology in embryos: regulated membrane traffic and its implications for cadherin biology

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Abstract

Regulated trafficking of cadherin adhesion molecules is often invoked as a mechanism to generate dynamic adhesive cell-cell contacts for tissue modeling and morphogenesis. The past 2-3 years have seen several important papers that tackle the cell biology of cadherin trafficking in organismal systems to provide new insights into both mechanism and morphogenetic impact.

Introduction and context

Adhesive interactions between cells and their environment (other cells and the extracellular matrix) are key regulators of tissue organization. In the past several years, we have come to appreciate that these interactions are intrinsically dynamic, and it has been attractive to postulate that adhesion dynamics may contribute to the remodeling of cell-cell and cell-matrix interactions associated with tissue morphogenesis. In particular, membrane traffic of adhesion molecules has been intensively studied as a cellular mechanism for dynamic turnover of both adhesive contacts and cellular interactions. An important recent advance has been the application of organismal systems (notably *Drosophila* and zebrafish) to study adhesion receptor trafficking. These efforts to 'do cell biology in organisms' carry the advantage of allowing genetic approaches to be used to identify new molecular regulators of receptor trafficking. They also provide the opportunity to test the potential morphogenetic impact of these cellular processes. Although a range of adhesion receptors undergo regulated trafficking, we focus here on recent studies that characterized the molecular regulation of cadherin transport and its functional implications in a variety of developmental systems.

Major recent advances

Characterizing cadherin trafficking and its molecular regulation

Cadherin trafficking and its molecular characterization were originally performed using a variety of cultured mammalian epithelial cells (reviewed in [1,2]). An important stimulus in this field was the demonstration that E-cadherin undergoes endocytic recycling in cultured MDCK epithelial cells [3]. It is now clear that this pathway operates also in *Drosophila* and zebrafish. For example, the Peifer lab [4] recently demonstrated that DE-cadherin in the embryonic ectoderm and amnioserosa is trafficked through both Rab5- and Rab11-positive endosomes, as is also observed in mammalian cells [3,5,6]. Moreover, recycling can be affected at several points in the trafficking itinerary to perturb adherens junction integrity. Disruption of the early endosomal regulator Rab5 as well as Rab11, the latter of which controls traffic through recycling endosomes, both perturbed cadherin junction integrity [4]. Furthermore, cells in the pupal notum from *Drosophila* embryos mutant for exocyst components accumulated DE-cadherin in recycling endosomes and displayed abnormal adherens junctions [7,8]. This implies that the integrity of adherens junctions in these developing tissues

depends on a continuous flux of DE-cadherin through a membrane recycling pathway. Interestingly, the cortical localization of the exocyst in the *Drosophila* retina has itself been observed to depend on the integrity of adherens junctions [9]. This suggests that what we perceive as junctional integrity may arise as an emergent property from positive feedback between membrane recycling, exocyst localization, and junctions themselves.

The genetic amenability of *Drosophila* has also been instrumental in identifying new regulators of cadherin trafficking. Recently, the laboratories of Buzz Baum [10] and Yohann Bellaiche [11] independently identified a novel impact of Cdc42 on DE-cadherin trafficking. Both groups reported that loss of Cdc42 function perturbed adherens junction integrity and also blocked endocytosis of cadherin. Cdc42 was genetically linked to signaling through the Par6/atypical protein kinase C (PKC) pathway, thereby identifying a link between cadherin traffic and a well-described regulator of epithelial polarity. Furthermore, both groups defined downstream genetic interactions with the cytoskeletal regulators WASP and Arp2/3, as well as with dynamin itself [10,11]. This suggested that the Cdc42/Par6/aPKC pathway promotes cadherin internalization, perhaps by regulating the molecular machinery responsible for scission of endocytic precursors to generate vesicles [11]. Consistent with this, Warner and Longmore [12] reported that depletion of Rho in the fly eye increased DE-cadherin internalization in a Cdc42-dependent manner.

One tacit attraction of studying organismal systems is the hope that development in the embryo might provide a robustness that avoids the variability that can be associated with experiments that use cultured cell systems. However, even here, the complexity of biology reminds us that this is not necessarily so. This is exemplified by another recent study in which Harris and Tepass [13] also demonstrate a genetic requirement for Cdc42 to stabilize adherens junctions in the dynamic remodeling tissue of the ventral neuroectoderm. However, they show that here Cdc42 serves to inhibit, rather than promote, endocytosis and recycling of apical membrane components, such as Crumbs and Patj. Furthermore, they provide evidence that the junctional phenotype may be a secondary effect of disrupting the apical localization of Crumbs, which is a known regulator of junctional integrity [14]. This reminds us that there are limits to the extent to which molecular mechanism can be inferred from genetic interactions. Finally, they demonstrate that Cdc42 is preferentially required in the most dynamic tissues, emphasizing that biological context may condition the impact of these signaling pathways. Similarly, Roeth *et al*. [4] showed

that the morphogenetic impact of disrupting Rab5 or Rab11 is also tissue-specific.

Morphogenetic impacts of cadherin trafficking

The identification of key regulators of cadherin traffic provides the convenient opportunity to test their morphogenetic impact in developing embryos, beyond their consequences for adherens junction integrity. This is illustrated during zebrafish gastrulation, in which formation of the prechordal plate requires that its progenitors migrate as a cohesive group of cells from the blastoderm margin in a process that depends on the planar polarity signal, Wnt11. Strikingly, Ulrich *et al*. [15] demonstrated that blocking Rab5C function with antisense morpholinos prevented Wnt11-induced prechordal plate formation. Interestingly, Wnt11 also induces E-cadherin internalization through a Rab5c-dependent pathway. This suggests that induced cadherin internalization might contribute to morphogenetic cellular rearrangements in response to Wnt11.

Cadherin recycling has also been implicated in a variety of tissue rearrangements in the fly. In the *Drosophila* wing, epithelial cells undergo a striking rearrangement during development, being irregularly packed during the larval and prepupal stages, then becoming reorganized into hexagonal arrays shortly before hair formation occurs. Interestingly, E-cadherin is actively endocytosed and recycled through Rab11 endosomes during the process of hexagonal rearrangement. Disruption of endocytosis with dynamin mutants blocks this cellular reorganization [16], suggesting that cadherin recycling may contribute to turnover of junctions necessary for cell-upon-cell rearrangements. Again, however, tissue and cellular context can significantly affect the impact of membrane traffic on cellular rearrangement. Another form of cell-cell movement is cellular intercalation, which has been implicated in tissue elongation events during development [17]. However, in contrast to the *Drosophila* wing, Rab11-mediated recycling appeared to inhibit cellular intercalation in the dorsal trunk of the tracheal system [18]. This implies that membrane trafficking may have quite different morphogenetic impacts depending on the type of morphogenetic movement involved and the tissues being studied.

Future directions

We have focused here on how recent analysis of organismal models has provided new insights into both the molecular regulation and morphogenetic impact of cadherin trafficking. In the latter case, we have also concentrated on cellular rearrangements, but it is likely that cadherin trafficking will have increasingly wide-ranging consequences. For instance, during *Drosophila*

oogenesis, germline stem cells (GSCs) are attached to anterior niche cap cells via adherens junctions and this physical attachment helps to maintain the balance between self-renewing and differentiating cells. Rab11 regulates the connections between the GSCs and the niche cells through polarized trafficking of E-cadherin and other apical proteins [19], implying that Rab11 could indirectly affect self-renewal and proliferation in the stem cell niche.

Permit us to finish, though, with a challenge to the field. Ultimately, detailed progress in understanding the biological impact of cadherin trafficking will require tools that selectively perturb cadherin traffic. To date, the functional impact of cadherin trafficking has been analyzed using techniques that target key regulators of membrane traffic generally, but not necessarily cadherin traffic specifically. Although these have yielded exciting results, such as those reviewed here, there is a very real possibility that some changes in cadherin biology result as indirect effects of perturbing other trafficking events, such as apical localization of Crumbs [13]. For example, clathrin and Rab5 are reported to control cortical signaling of Rac [20], which can affect E-cadherin adhesion and junctional organization [21,22]. And dynamin, best understood for its impact on membrane trafficking, was recently identified as regulating global acto-myosin contractility [23], which also supports junctional integrity [24]. Ongoing efforts to elucidate specific regulation of cadherin trafficking, such as recent efforts to understand the mechanism of cadherin internalization [25], will then be essential if we are to specifically ablate cadherin trafficking. The combination of those tools, combined with the range of organismal systems now available to test their impact, will portend heady times in our efforts to understand the cellular basis of morphogenesis.

Abbreviations
GSC, germline stem cell; MDCK, Madin-Darby canine kidney; PKC, protein kinase C; WASP, Wiskott-Aldrich syndrome protein.

Competing interests
The authors declare that they have no competing interests.

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