New views on the neural crest epithelial-mesenchymal transition and neuroepithelial interkinetic nuclear migration

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By developing a technique for imaging the avian neural crest epithelial–mesenchymal transition (EMT), we have discovered cellular behaviors that challenge current thinking on this important developmental event, including the probability that complete disassembly of the adherens junctions may not control whether or not a neural epithelial cell undergoes an EMT. Further, neural crest cells can adopt multiple modes of cell motility in order to emigrate from the neuroepithelium. We also gained insights into interkinetic nuclear migration (INM). For example, the movement of the nucleus from the basal to apical domain may not require microtubule motors nor an intact nuclear envelope, and the nucleus does not always need to reach the apical surface in order for cytokinesis to occur. These studies illustrate the value of live-cell imaging to elucidate cellular processes.

During an epithelial-mesenchymal transition (EMT), epithelial cells lose high-affinity adherens junctions and apical-basolateral polarity, and gain mesenchymal cell characteristics such as motility and invasiveness.1,2 A classic example of an EMT is the emigration of neural crest cells from the dorsal neural tube of amniotes.3,4 By imaging the avian neural crest EMT directly at high-resolution, we have gained several valuable insights about the cellular mechanisms of this EMT.3

**Complete Disassembly of Adherens Junctions is Neither Always Necessary nor Sufficient for an EMT**

Currently, the most popular model for the trunk neural crest EMT is that the disassembly of adherens junction-mediated adhesion controls the emigration of neural crest cells from the neural tube.5,6 It has also been proposed that the separation of neural tube cells from the luminal adherens junctions by the plane of cell division might be sufficient to produce neural crest cells.7 By imaging the neural crest EMT directly, we provide evidence that challenges these hypotheses. For example, as neural crest cells emigrate from the neuroepithelium, a small percentage rupture their cell tail and leave behind bits of cellular debris, including components of the adherens junctions, at the lumen.8 Other times the adherens junction components move along with the retracting tail, which is compatible with electron microscopic data showing intact adherens junctions at non-apical positions in other emigrating cell types.8,9 Live-cell analysis of the ingression of zebrafish retinal ganglion cells also reveals that adhesion complexes are not completely disassembled prior to cell tail retraction, and also slide along with the retracting cell tail.10 Therefore, based on our studies and others, the complete disassembly of adhesion complexes may not be necessary in order for cells to depart from an epithelium.

We also found that when mitotic neuroepithelial cells are separated from the luminal adhesions by the plane of cell division, most daughter cells re-integrate into...
During an EMT, epithelial cells acquire the ability to emigrate out of the epithelium. Time-lapse analysis of the neural crest EMT reveals that, pre-neural crest cells adopted a range of migration phenotypes, forming filopodia, blebs and polarity protein Vangl2/Stabismus is mutated. Therefore, although cell adhesion complexes are important for epithelial integrity, it seems likely that other molecular mechanisms, such as those that govern cell polarity, also control whether individual cells retain epithelial identity or not.

The neuroepithelium3 (Fig. 1, see movie 1 in the Suppl. material). Hence, separation of cells from the adherens junctions is not always sufficient to generate neural crest cells. Zebrafish neural keel cells also re-integrate into the neural epithelium following cell division. Interestingly, this process is disrupted if the planar cell polarity protein Vangl2/Stabismus is mutated. Therefore, although cell adhesion complexes are important for epithelial integrity, it seems likely that other molecular mechanisms, such as those that govern cell polarity, also control whether individual cells retain epithelial identity or not.

**Modes of Neural Crest Motility**

During an EMT, epithelial cells acquire the ability to emigrate out of the epithelium. Time-lapse analysis of the neural crest EMT reveals that, pre-neural crest cells adopted a range of migration phenotypes, forming filopodia, blebs and
Other insights into INM were acquired when we used mCherry-tubulin as a cytoskeletal marker for live-cell imaging. The biophysical forces that position the nucleus could involve myosin motors interacting with actin, or microtubule motors such as dynein or kinesin moving on microtubule tracks, both of which link to the nuclear envelope by KASH and SUN proteins.26 Our imaging revealed that just prior to movement of the nucleus from a basal position to the apical domain and mitosis, the mCherry-tubulin signal in between the nucleus and the apical surface is reduced, which suggests that the microtubule track is degraded. Therefore nuclear movement in the apical direction may be microtubule independent (Fig. 3A’–C’, see also Suppl. Movies 3–5). Instead, the shuttling of the nucleus apically prior to mitosis may involve actin/myosin, just as nuclear translocation in migratory neural precursor cells requires myosin II.27 Also of note, the velocity of nuclear migration is faster in the apical direction prior to mitosis, as opposed to the basal direction following cell division,3,28 suggesting distinct

**Insights into INM**

A characteristic of the pseudostratified neuroepithelium is the occurrence of interkinetic-nuclear migration (INM).23 During this event the nucleus migrates from a basal position to the apical surface where cytokinesis occurs, and then the daughter-cell nuclei are transported basally again. One of our novel observations was that the centrosome leaves the apical domain (where the cilium is nucleated24) just prior to apical nuclear migration and cytokinesis at the lumen3 (Fig. 1). After the centrosome is released from the apical surface it may be pulled towards the nucleus by Lis1/dynein on microtubules.25 The role of RhoB during the neural crest EMT could potentially promote blebbing motility or to create changes in cortical tension. Finally, it is likely that a combination of mechanical properties (protrusiveness, contractility and cell-matrix adhesions) contribute to the ability of neural crest cells to emigrate from the neural tube.22

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The occasional “lobopodium”12 (Fig. 2, see Suppl. Movie 2). What is the mechanism of neural crest emigration? The classic model for cellular motility in vitro involves the formation of a lamellipodium, attachment to a substratum, directional contractile forces and release of the trailing end,13,14 although cells migrating in three-dimensional environments often prefer “blebbing” motility.15,16 It is likely that these cell processes are molecularly similar since they have been shown to be interconvertable in Fundulus deep cells.12 Alternatively, because most neural crest cells undergo a tail retraction during the EMT,3 it may be that actin contraction at the apical surface is important.17,18 Further still, the forces that drive the “sorting out” of germ layers19 (likely regulated by changes in cortical tension)20 could also be responsible for the segregation of neural crest cells from the neuroepithelium. Interestingly, the neural crest EMT can be stimulated ectopically by expression of constitutively active RhoB (together with Sox9 to prevent apoptosis).21 The role of RhoB during

Figure 2. Neural crest emigration accompanied by filopodia and blebs. (A) The mem-EGFP-labeled neural epithelial cell indicated within the white box is shown in the time-lapse series below. (A’) Note that there are two different mem-EGFP-labeled cells close together within the field of view, a dorsal neural tube cell (red dot) and a neural crest cell (no dot). The neural epithelial cell (red dot) detaches from the lumen and migrates out of the neural tube. The formation of filopodia are indicated with red arrowheads and membrane blebs with red arrows. Scale bars are 10 μm.
Figure 3. Examples of neural epithelial cells undergoing INM. (A–C) Neuroepithelial cells were labeled with GFP-α-catenin (green) and mCherry-tubulin (red). Cells within the white boxes are shown in the time-lapse series below. (A') Prior to nuclear migration in an apical direction, the mCherry-tubulin signal in the cell tail is reduced (white arrowheads) and mCherry-tubulin enters the nucleus (white arrow, t = 0:21). The nucleus is then transported from a basal position to the apical surface, where the mitotic spindle forms (bright mCherry-tubulin signal) and cytokinesis occurs. The nuclei of the daughter cells (white and blue dots) are then transported basally. (B') Before nuclear migration from the basal to apical surface, the mCherry-tubulin signal in the cell tail is reduced (white arrowheads) and mCherry-tubulin enters the nucleus (white arrow, t = 0:28). Mitotic spindle formation also occurs while the nucleus is in a basal position (bright mCherry-tubulin signal, t = 0:28). The nucleus is then transported to the apical surface where cytokinesis occurs. The daughter cell nuclei (white and blue dots) are subsequently shuttled to a basal position. (C') Note the presence of two different mCherry-tubulin cell tails in the viewing field. Prior to nuclear migration from the basal to apical surface, the mCherry-tubulin signal in one cell tail is reduced (white arrowheads) and mCherry-tubulin enters the nucleus (white arrow, t = 0:28). Nuclear migration in the apical direction and spindle formation occurs at t = 0:35, but the nucleus does not make it all the way to the apical surface before cell division occurs (t = 0:56). After cytokinesis, the nuclei of the daughter cells (white and blue dots) are transported in a basal direction. Scale bars are 10 μm.
mechanisms for moving the nucleus in opposite directions.

We also recorded a variety of behaviors suggesting that mitosis does not always occur at the apical surface. For example, there was an increase in the mCherry-tubulin signal in the nuclear region prior to apical nuclear migration, indicating that cells can enter pro-metaphase (as evidenced by nuclear envelope breakdown) before the nucleus arrives at the apical surface (Fig. 3A, see Suppl. Movie 3). Additionally, we recorded one example of mitotic spindle formation when the nucleus was still at a basal position prior to apical nuclear migration (Fig. 3B, see Suppl. Movie 4), further indicating that the nucleus does not have to reach the lumen in order to initiate mitosis. Because the cytoskeleton-linking SUN and KASH proteins integrate into the nuclear envelope, it is not known how the nucleus is transported apically following nuclear-envelope breakdown, although the nuclear matrix may not be completely dissolved after mitotic spindle formation.29 Finally, we also discovered that cytokinesis can occur even if the nucleus does not reach the apical domain (Fig. 3C, see Suppl. Movie 5).

In summary, our data show that the centrosome leaves the lumen prior to mitosis, that the movement of the nucleus apically prior to mitosis may be microtubule independent, and that nuclear-envelope breakdown, spindle formation and cytokinesis do not always have to occur at the apical surface.

These insights into the nuclear crest EMT and INM illustrate the value of live-cell imaging and direct observation. In the future, the combination of molecular perturbations with live-cell imaging will be a valuable tool to further unravel the mechanisms of these events.

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Note

Supplementary materials can be found at: www.landesbioscience.com/supplement/AhlstromCIB2-6-Sup01.mov www.landesbioscience.com/supplement/AhlstromCIB2-6-Sup02.mov www.landesbioscience.com/supplement/AhlstromCIB2-6-Sup03.mov www.landesbioscience.com/supplement/AhlstromCIB2-6-Sup04.mov www.landesbioscience.com/supplement/AhlstromCIB2-6-Sup05.mov

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