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Apical abscission alters cell polarity and dismantles the primary cilium during neurogenesis

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Abstract

Withdrawal of differentiating cells from proliferative tissue is critical for embryonic development and adult tissue homeostasis; however, the mechanisms that control this cell behaviour are poorly understood. Using high-resolution live-cell imaging in chick neural tube, we uncover a form of cell sub-division, which abscises apical cell-membrane and mediates neuron detachment from the ventricle. This mechanism operates in chick and mouse, is dependent on actin-myosin contraction and results in loss of apical-cell-polarity. Apical abscission also dismantles the primary cilium, known to transduce sonic-hedgehog signals, and is required for expression of cell-cycle-exit gene *p27/Kip1*. We further show that *N-cadherin* levels, regulated by neuronal-differentiation factor, *Neurog2*, determine cilium disassembly and final abscission. This cell-biological mechanism may mediate such cell transitions in other epithelia, in normal and cancerous conditions.

New-born neurons detach an apical cell-process from the ventricular surface and then migrate to the lateral neural tube or to form cortical layers within the brain (1, 2). This step is required for the generation of neuronal and tissue architecture (2, 3) and its' failure leads to human periventricular heterotopia (4). Downregulation of *N-cadherin* is associated with this event (3, 5), as is loss of apical complex proteins (6, 7). The latter may be mediated by down-regulation, protein modification, degradation or re-localisation, or loss of apical membrane.

To investigate cell behaviour underlying neuron birth we labelled membranes of individual cells by mosaic transfection of GFP-GPI (pCAGGS-GFP-GPI) into the chick embryonic spinal cord (8). We then monitored neurogenesis in ex-vivo embryo slice cultures (1) using wide-field time-lapse microscopy (8). New-born neurons have a basally located cell body and extend a long thin cell-process to the apical/ventricular surface. Movies of such cells revealed that shedding of the apical-most cell membrane preceded withdrawal of this cell-process (Fig. 1A). This event, which we name apical abscission, takes ~1 hour (56 mins SD= 18 min, n= 21 cells). It begins with formation of a bulb-like 'bouton', followed by sub-apical constriction, membrane thinning and eventual abscission, after which the apical cell-process withdraws (42 abscising cells, 34 embryos; all stages observed in 21 cells; Fig. 1A, S1, Movie S1 and S2, S3). Abscised particles tracked so far remain at the ventricle.

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Using structured illumination microscopy (8) to generate super-resolution images of abscising cells transfected with membrane localised TagRFP-Farnesyl (TagRFP-Farn) revealed a thin membranous connection between apical cell-process and the abscising particle. This confirmed the existence of abscission events in fixed tissue not subject to culture and imaging regimes ($n = 5$ cells, 3 embryos; Fig. S2, Movie S4). We also observed apical abscission in completely un-manipulated embryos fixed and labelled to reveal the early neuronal marker, Tuj1 (class III beta-tubulin). Some Tuj1⁺ cells with a basally localised nucleus and a ventricle-contacting apical cell-process were found to have a distinct constriction, coincident with sub-apical actin ($n = 31/78$ cells, 5 embryos; Fig. 1B, Movie S5). To characterise the abscised membrane we assessed localisation of endogenous apical Par-complex protein aPKC (9) in such Tuj1⁺ cells; aPKC was confined to the abscising particle ($n = 31/31$ cells, 5 embryos; Fig. 1B; Movie S5). This indicates that differentiating neurons experience rapid loss of apical polarity. It is also consistent with the absence of Par-complex proteins from withdrawing cell-processes (6, 7), which now liberated from apical-junctional complexes extend transient membrane protrusions (18 cells, 9 embryos e.g. see Movies S1 and S2). Similar apical constrictions were visible in Tuj1⁺ ventricle-contacting cells in mouse spinal cord (22/40 cells, 4 embryos; Fig. 1C, Movie S6), with aPKC confined to the abscising particle (22/22 cells). This demonstrates that apical abscission is conserved across species. In chick, we further characterised cells poised to abscise as indicated by a basally located nucleus and ventricle-contacting apical cell-process revealed by TagRFP-Farn labelling and found a similar localisation of actin and aPKC (21/21 cells, 6 embryos; Fig. 1D, Movie S7). Many such TagRFP-Farn-labelled cells with this morphology also express low levels of the early neuronal marker, NeuroM (26/29 cells, 5 embryos, Figs. 1E-G). These NeuroM-positive cells were further found to express the interneuron marker, Lim1/2 (23/23 cells), but not the later neuronal marker HuC/D (0/12 cells) nor the post-mitotic cell marker Cdk-inhibitor p27/Kip1 ((0/11 cells) (10) and see (7)), identifying these cells as immature neurons that have yet to commit to cell cycle exit (Figs. 1E-G).

Neuroepithelial cells contain a sub-apical actin cable that mediates normal cell constriction at the ventricular surface. To investigate whether apical abscission involves actin dynamics we co-transfected GFP-GPI and Actin-TagRFP vectors into chick neural tube and monitored protein localisation. Sub-apical actin was visible in cells poised to abscise and coincided with the region of constriction prior to abscission (Fig. 2A, Movie S8). As abscission commenced, Actin-TagRFP signal intensity increased (8), reaching a maximum shortly before abscission completion (Fig. 2B); actin was then depleted from the withdrawing cell-process tip ($n = 24$ abscising cells, in 18 embryos; Fig. 2A, S3, Movie S8, and S9, S10). This local actin increase raised the possibility that actin-myosin contraction mediates apical abscission.

We therefore next surveyed Myosin localisation using a myosin regulatory light chain 2 GFP construct (MRLC2-GFP); this revealed strong sub-apical localisation and diffuse cytosolic distribution in all cells (Fig. S4). As myosin phosphorylation is essential for actin-mediated apical constriction we next discriminated sites of myosin activity by monitoring MRLC2^{T18DS19D}-GFP, a constitutively active form of MRLC2. To increase the incidence of neuronal differentiation we co-transfected cells with a plasmid encoding the proneural gene

Neurog2 (pCAGGS-*Neurog2*-IRES-nucGFP, (pCIG-*Neurog2*)), which promotes neuronal differentiation (10). In such cells, also co-expressing TagRFP-Farn to label cell membranes, active MRLC2 localised sub-apically until shortly after abscission (8), (n=12 cells, 9 embryos; Figs. 2C, D Movie S11, and S12, S13). Thus actin is localised and myosin is active in the sub-apical region of the abscising neuron.

To test the requirement for myosin activity cells were transfected with GFP-GPI and pCIG-*Neurog2* and slices were cultured in medium containing blebbistatin (inhibitor of myosin motor function), ML-7 (inhibitor of myosin light chain kinase MLCK, which phosphorylates Myosin II) (see Fig. S5) or DMSO control. While few cells in control slices failed to abscise and retract their cell-processes within an 8-hour period (n=4/33 cells; 5 embryos; Fig. 2E, Movie S14 and S15, S16), the majority of cells exposed to blebbistatin (n=33/36 cells, 6 embryos; Fig. 2F, Movies S17 and S18, S19; apical surface definition, Fig. S6) or ML7 (n=68/83 cells, 15 embryos; Fig. 2G, Movies S20 and S21, S22; apical surface definition Fig. S6) remained attached at the ventricle. Furthermore, co-expression of active MRLC2 in the presence of ML-7 rescued its effects, with most cells now abscising within 8-hours (n=14/18 cells, 7 embryos; Fig. 2H, Movies S23 and S24, S25). Mis-expression of active MRLC2 alone, however, did not increase neuron numbers and so the potential to increase actin-myosin contraction is by itself insufficient to promote apical abscission (Fig. S7). These data indicate that myosin activity is required, but not sufficient for apical abscission.

Neuroepithelial cells lining the ventricle possess a primary cilium. This projects from the basal body/centrosome located at the apical pole. While this cilium plays a key role in transducing Sonic-hedgehog (Shh) (and possibly other) signals that maintain neuroepithelial cells in a proliferative state (11), the centrosome is further implicated in positioning axon outgrowth (12). To observe the effect of apical abscission on the primary cilium we transfected GFP-GPI and pCIG-*Neurog2* into the neural tube together with a construct containing a PACT-domain sequence that confers centrosomal localisation fused to TagRFP (PACT-TagRFP). As abscission commenced, the centrosome localised to the withdrawing cell-process (n= 45 cells, 15 embryos; Fig. 3A, Movie S26 and S27, S28). Conversely, the primary cilium, identified with ciliary membrane-associated Arl13b-TagRFP, remained attached to the abscised apical membrane (n = 21 cells, in 7 embryos; Fig. 3B, Movies S29 and S30, S31). During apical abscission the Arl13b-labelled-cilium also shortened (two-fold reduction in cilium length, n = 5 abscising cells,(8)). We further used structured illumination microscopy to confirm the presence of Arl13b-GFP in particles abscised from TagRFP-Farn-labelled cells in tissue not subject to culture and live-imaging (n= 5 cells; Fig. 3C; movie S32).

Active Shh signalling is indicated by accumulation of the Shh transducer Smoothed (Smo) and its key pathway effector Gli2 in the primary cilium (13, 14). Shh signalling is highest in the ventral half of the neural tube and we therefore assessed localisation of endogenous Smo and Gli2 in *TuJ1*⁺ cells with ventricle-contacting cell-processes in this region. This revealed many cells with ciliary accumulation of Smo (n=38/43 cells, 4 chick embryos; Fig. 3D, Movie S33) or Gli2 (n=33/35 cells, 3 mouse embryos, Fig. 3E, Movie S34). This localisation of proteins suggests that cells poised to abscise are responding to Shh signals

and predicts that disjunction of the centrosome and Arl13b-labelled cilium during apical abscission curtails Shh signalling.

Onset of neuronal differentiation is characterised by downregulation of N-cadherin (3, 5), which forms sub-apical adherens junctions between neuroepithelial cells (15) and abnormal persistence of N-cadherin inhibits apical cell-process withdrawal (3). Cadherins are connected intra-cellularly to the contractile actin cable, and this serves to maintain tension at apical junctions and cell-cell adhesion (15). Declining N-cadherin levels within the prospective neuron may therefore trigger apical abscission by loosening cell-cell junctions and connection with the intra-cellular actin-myosin cable. As the centrosome is localised in the withdrawing cell-process it must be released from the cilium prior to final abscission. To determine how persistent N-cadherin affects apical abscission we mis-expressed N-cadherin-YFP together with GFP-GPI and PACT-TagRFP. Increased N-cadherin blocked cell-process withdrawal and the centrosome remained at the apical pole (16 cells, 10 embryos; Fig. 4A, Movie S35 and S36, S37). This indicates that N-Cadherin downregulation is required for centrosome release from the apical surface as well as for final abscission of apical membrane.

One consequence of failure to undergo N-cadherin downregulation and apical abscission might therefore be maintenance of Shh signalling and so inhibition of cell cycle exit. To assess the relationship between cell cycle regulation and apical abscission we next determined the effect of persistent N-cadherin on expression of p27/Kip1, which normally begins after apical-cell process withdrawal (Figs 1G, 4B' and see (7)). N-cadherin mis-expressing cells lacked p27/Kip1 after 24h (NCad-YFP+TagRFP-Farn mis-expressing cells 3% p27/Kip1 positive (25/689 cells, 4 embryos, Fig. 4B); control TagRFP-Farn alone cells 13% p27/Kip1 positive (76/649 cells, 4 embryos, Fig. 4B')). These findings therefore place N-cadherin loss and apical abscission, including cilium disassembly, upstream of cell cycle exit as defined by p27/Kip1 expression. Furthermore, driving premature cell cycle exit by p27/Kip1 mis-expression did not promote apical abscission or neuronal differentiation (Fig S8), consistent with proneural genes promoting expression of Cdk-inhibitors, which then act in concert with other proneural targets to orchestrate neuronal differentiation (16).

N-cadherin downregulation in prospective neurons is mediated by the transcription factor FoxP2/4, expression of which is promoted by the proneural gene *Neurog2* (3, 10). To determine whether *Neurog2* mis-expression is sufficient to overcome excess N-cadherin we co-transfected constructs encoding these two genes into the neural tube. Despite excess N-cadherin, cells with excess *Neurog2* dismantled their cilium and underwent abscission and cell-process withdrawal (16 cells, in 7 embryos; Fig. 4C, Movie S38 and S39, S40). In this context, N-cadherin-TagRFP, was localised to the abscission site and then lost before abscission (n = 19 cells, in 8 embryos; Fig. 4D Movie S41 and S42, S43). This indicates that localisation and regulation of N-cadherin protein (as well as transcriptional downregulation of endogenous *N-cadherin* (3)) is directed by factors downstream of *Neurog2*.

These findings uncover a cell biological mechanism, apical abscission, which takes place downstream of N-cadherin loss and involves actin-myosin dependent cell constriction and dismantling of the primary cilium. This abscission event detaches newborn neurons from the

ventricular surface and results in loss of apical-complex containing cell membrane and therefore apical polarity. By separating centrosome from Arl13b-labelled cilium apical abscission may curtail active Shh signalling, as indicated by ciliary accumulation of Smo and Gli2 in cells poised to abscise. Consistent with a loss of mitogenic Shh, abscission is also required for expression of cell-cycle exit gene p27/Kip1 (Fig. S9). Apical abscission is thus a decisive event in the neuronal differentiation programme, which triggers re-organisation of the new-born neuron and its withdrawal from the ventricular environment. Abscising the apical membrane and leaving this, at least initially, at the apical surface may also help to maintain tissue integrity. During mitosis cilia are resorbed or partially internalised (17) rather than shed and regulated cilium shedding has only been reported in the alga *Chlamydomonas* (18). Loss of apical complex proteins also characterises cells undergoing an epithelial to mesenchymal transition, including tumour cell metastasis (19) and some cancers exhibit cilia loss (20). Investigation of apical abscission in normal and also oncogenic epithelia may therefore provide insight into mechanisms that direct critical cell state transitions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Apical abscission during neuronal differentiation

(A) Time-lapse sequence of a cell undergoing distinct stages of apical abscission, [Movie S1; these are additional frames of a cell shown in supplementary movie 2 in (6)]. (B-D) Maximum intensity projections of constricting abscission site (white arrowheads) visible in Tuj1⁺ ventricle-contacting cells in chick (B) and mouse embryos (C) and TagRFP-Farn-labelled cell in chick (D); the abscising particle is distal to actin and contains the apical Par-complex, marked by aPKC (3D-reconstructions of B, C and D, in Movies S4, S5, S6 respectively). (E-G) Cells poised to abscise express NeuroM, and Lim1/2 (E) but not HuC/D (F) nor p27 (G) (Magenta arrows). Abscission site (white arrowheads), withdrawing apical cell-process (white arrows), abscised particle (yellow arrows), apical surface (white dashed-line) here and in all figures. Scale bars, 10 μ m; enlarged regions, 2 μ m.

Fig. 2. Apical abscission depends on actin-myosin activity

(**A**, **B**) Time-lapse showing actin localisation (**A**)(Movie S8) and (**B**) quantification of Actin-TagRFP intensity during apical abscission (average normalised values for 4 cells, error-bars S.E.M.); (**C**, **D**) active myosin (MRLC2^{T18DS19D}-GFP) (**C**, green at cell-process tip; Movie S11) localises to abscission site (**D**) MRLC2^{T18DS19D}-GFP intensity during apical abscission (average normalised values for 5 cells, error-bars S.E.M.). (**E-H**) Cells exposed to control DMSO undergo abscission (**E**; Movie S14), but not in the presence of blebbistatin (**F**; Movie S17) or ML-7 (**G**; Movie S20). ML-7 abscission inhibition is rescued by expression of, MRLC2^{T18DS19D}-GFP (**H**; Movie S23). For definition of apical surfaces, N-Cadherin and aPKC localisation see Figs. S4, S5. In **B** and **D** Membrane thinning (black arrow), abscission complete (black arrowhead). Scale bars, 10 μm; enlarged regions, 2 μm.

Fig. 3. Apical abscission dismantles the primary cilium

(A, B) Time-lapse sequences showing centrosome release into the apical cell-process (A; Movie S26), while Arl13b-labelled cilium is retained at the apical membrane (B; Movie S29). (C) Widefield and (C') structured illumination imaging (white dotted outline)(Movie S32) of TagRFP-Farn labelled apical cell-process and abscised particle containing Arl13b-GFP-labelled cilium. (D, E) Tuj1⁺ cells with ventricle-contacting apical cell-processes exhibit Smo (D; Movie S33) and Gli2 accumulation (E; Movie S34) (empty arrowheads) in their primary cilium (Identified with Arl13b-GFP or Ift88 (Intraflagellar-transport-protein 88) respectively). Scale bars, 10 μ m; enlarged regions and C, C', 2 μ m.

Fig. 4. N-Cadherin loss promotes apical abscission

(A) Cells mis-expressing NCad-YFP constrict (white arrowheads) but do not abscise and the centrosome (PACT-tagRFP, magenta arrows) remains at the apical cell-pole (Movie S35). (B) N-Cad-YFP mis-expressing cells do not express p27, (B') which is normally detected after apical cell-process detachment (cell nuclei; empty arrowheads); (C) Neurog2 mis-expression rescues centrosome release and abscission in NCad-YFP-expressing cells (Movie S38). (D) Neurog2 mis-expression decreases sub-apical NCad-TagRFP levels (magenta arrows), followed by abscission and cell-process withdrawal, note, a second underlying cell has yet to withdraw (Movie S41); Scale bars, 10 μ m; enlarged regions, 2 μ m.