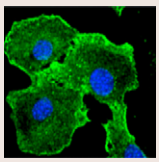
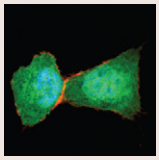
In this issue



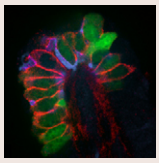
Deadly duo: α- and β-arrestins mediate Notch degradation

The Notch signalling pathway is fundamental to the control of cell communication and fate. The Notch receptor has a limited half-life and, in mammals, inactivated receptor is frequently internalised, ubiquitylated by the E3 ubiquitin ligase ITCH and degraded in the lysosomes; there is, however, no evidence for a direct interaction between ITCH and Notch in mammals. The arrestin protein family have an important role in signal transduction, and both α-arrestins and β-arrestins have been shown to act as adaptor proteins for the recruitment of cargo to the E3 ubiquitin ligases. Now (p. 4457), Christel Brou and colleagues ask whether arrestins could be involved in Notch regulation in mammals. First demonstrating that β-arrestins are required for the ITCH–Notch interaction, the authors then show that β-arrestins are necessary for ITCH-mediated Notch ubiquitylation and degradation. The authors next find that, rather than interacting directly with ITCH, β-arrestins heterodimerise with a member of another subfamily of arrestins called ARRDC1 (arrestin-domain-containing 1; also known as α-arrestin 1), which contains PPxY motifs, permitting a direct interaction with ITCH. By transfecting cells with a form of ARRDC1 that has mutated PPxY motifs, the authors demonstrate reduced ITCH-mediated Notch ubiquitylation and impaired lysosomal degradation of Notch, which was also observed in cells depleted of β-arrestin or ITCH. This study shows that α- and β-arrestins act as key negative regulators of Notch signalling by forming heterodimers that recruit ITCH to its Notch substrate, allowing ubiquitylation and degradation of the inactivated receptor.



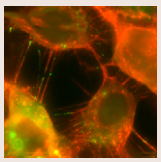
Ligands leave their mark: fingerprinting on TrkA diffusion

The predominant ligand for neurotrophin receptor tropomyosin-related kinase A (TrkA; also known as NTRK1) is nerve growth factor, but other ligands exist, each of which exerts a different cellular outcome. Antonino Cattaneo and colleagues (p. 4445) address how a receptor transduces different ligand-specific signals by studying the dynamics of TrkA at the single-molecule level in the membrane of living cells. Using a new strategy to label TrkA, the authors imaged and tracked single receptor molecules in the absence and presence of a set of diverse biologically relevant ligands. They show that, in the absence of ligands, most of the TrkA receptors are fast-diffusing monomers, with ~20% moving at least an order of magnitude slower, and ~4% that were almost immobile within a small area. In the presence of ligands, the populations of slow and/or immobile TrkA receptors are increased, non-immobile trajectories are slower and confinement areas are restricted. The authors next use improved data analysis to investigate ligand-induced TrkA lateral diffusion. Each ligand induced particular changes in receptor diffusion, as well as in the fraction and type of immobile trajectories, with a specific redistribution of TrkA diffusion subpopulations, thus showing a ligand 'fingerprinting' effect. This signature depends on the specific ligand-binding affinity for TrkA, the ligand-specific intracellular effectors recruited in the signalling platforms and the ligand-specific formation of signalling and/or recycling endosome precursors. Thus, the dynamics of a receptor in the plasma membrane carries a signature that can be traced back to its specific activation event.



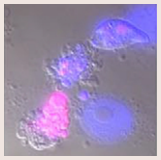
The CaSR in lung neuroendocrine cells

The extracellular Ca^{2+}-sensing receptor (CaSR) is a cell-surface protein that acts as a master regulator of extracellular free ionized Ca^{2+} concentration ($[Ca^{2+}]_o$), but has also been implicated in other cellular functions, and is expressed in many regions outside the $[Ca^{2+}]_o$ homeostasis system, including developing mouse lungs. Here (p. 4490), Dirk Adriaensen and colleagues investigate CaSR expression in postnatal mouse pulmonary neuroepithelial bodies (NEBs). The NEB microenvironment comprises densely innervated, highly specialised clusters of pulmonary neuroendocrine epithelial cells that represent complex sensory receptors in the airways, and harbours a stem cell niche. Using lung slices from GAD67–GFP-expressing mice, functional live-cell imaging, laser microdissection, quantitative real-time RT-PCR and immunohistochemical staining techniques, the authors show CaSR expression in the NEB microenvironment from postnatal day 14 onwards. NEB cells responded to an increase of $[Ca^{2+}]_o$ with a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), and this effect was mimicked by several membrane-impermeant CaSR agonists. Moreover, blocking TRPC channels inhibited the CaSR-dependent rise of $[Ca^{2+}]_i$, suggesting, say the authors, that Ca^{2+} influx through these channels contributes to the total $[Ca^{2+}]_i$ in NEBs mediated by the CaSR. Finally, the authors show that the CaSR regulates baseline $[Ca^{2+}]_i$ in NEBs and coordinates intercellular communication in the NEB microenvironment through paracrine signalling. These data present a pivotal role for the CaSR in integrating multiple signals in the mouse pulmonary NEB microenvironment.



TNT formation requires MYO10 in neuronal cells

Tunnelling nanotubes (TNTs) are long, transient actin-rich projections that mediate the intercellular transfer of various signals, organelles and pathogens. Although recent studies have highlighted some early steps in TNT formation, the molecular basis for their formation remains unclear. On page 4424, Chiara Zurzolo and colleagues investigate how TNTs are formed in neuronal CAD cells. Because neuronal cells are mostly immobile, the authors hypothesise that TNTs arise from dorsal filopodia, and analyse known inducers of this specific subset of filopodia. They show that overexpression of the unconventional actin-based motor protein MYO10 increases the number of TNTs, as well as the unidirectional transfer of vesicles between co-cultured cells. The authors next analyse deletion mutants of MYO10, and find that the full-length protein is necessary for the formation of functional TNTs. Moreover, both the motor and tail domains of MYO10 are required for TNT formation. The authors further examine the role of the tail domain, and identify the F2 lobe of the band 4.1, ezrin, radixin, moesin (FERM) domain within the MYO10 tail as being required for the formation and function of TNTs in the neuronal cells. Finally, the authors rule out activation of the Akt signalling pathway as having a role in the formation of TNTs under stress conditions, in contrast to what has been found in astrocytes. On the basis of these results, the authors propose that a specific subset of MYO10-dependent dorsal filopodia is the precursor of TNTs in neuronal cells.



Chk1–Lats2–p21: a DNA-damage-fighting trio

Cellular responses to DNA damage are coordinated primarily by two distinct kinases: ATM and ATR. These kinases act to induce cell cycle arrest for DNA repair, or apoptosis for the removal of damaged cells. The ATR–Chk1 signalling pathway is activated by ultraviolet (UV) irradiation or DNA replication stress. The kinase large tumour suppressor 2 (Lats2) has a key role in regulating organ size and cell growth in vertebrates, and has been shown to interact with the ATR–Chk1 pathway in the DNA damage response (DDR) and following oncogenic stress, although the details of its physiological functions in the DDR remain poorly understood. p21 (CDKN1A) is a cyclin-dependent kinase (CDK) inhibitor that functions to inhibit the cell cycle, but also inhibits apoptosis by binding to pro-caspase-3 in the cytoplasm. Hiroshi Nojima and colleagues recently reported that Chk1 phosphorylates Lats2 in response to oncogenic stress and UV damage, and here (p. 4358) they investigate the Lats2-mediated regulation of p21 downstream of Chk1 following UV irradiation. They show that, following UV irradiation, Lats2 is phosphorylated by Chk1 at S835, which enhances the kinase activity of Lats2 in an auto-feedback manner. Next, they show that Lats2 phosphorylates p21 at S146, which induces degradation of p21 and promotes apoptosis. Accordingly, overexpression of Lats2 induces p21 degradation, activation of caspase-3 and caspase-9, and apoptosis. The authors propose that the Chk1–Lats2–p21 axis is a novel DDR pathway that facilitates apoptosis following high levels of UV-induced damage, thereby eliminating damaged cells.

From *Development*

Plant germ cells count on chromatin

Unlike animals, plants do not set aside germ cells during embryogenesis. Instead, the precursors of these cells, called spore mother cells, are generated through a somatic-to-reproductive transition that occurs later in life. Whereas epigenetic remodelling has been largely studied in the post-meiotic phase of germline development, it is unknown whether pre-meiotic events contribute to cellular reprogramming in the reproductive lineage. In *Development*, Célia Baroux and colleagues investigate these dynamic changes and uncover widespread chromatin reprogramming during the slow meiotic S-phase that accompanies specification of the female *Arabidopsis* spore mother cell. As in animal primordial germ cells, the authors observe increased nuclear size, a reduction in heterochromatin, depletion of linker histones, chromatin decondensation, and changes in histone modifications and core histone variants in the female spore mother cells. The authors propose a bi-phasic chromatin reprogramming process that is necessary for proper somatic-to-reproductive cell fate transition and, thus, competency to establish the pluripotent, female gametophyte.

She, W., Grimanelli, D., Rutowicz, K., Whitehead, M. W. J., Puzio, M., Kotliński, M., Jerzmanowski, A. and Baroux, C. (2013). Chromatin reprogramming during the somatic-to-reproductive cell fate transition in plants. *Development* **140**, 4008-4019.