# RESEARCH HIGHLIGHTS

## Cytoskeleton links to ciliary organisation

Individual cilia in multiciliated cells display coordinated beating, which generates anterior–posterior flow and is essential for normal development. The cytoskeleton has been implicated in ciliogenesis and in cilia orientation, but its precise role in ciliary organization has not been fully elucidated. Mitchell and colleagues now reveal how actin and microtubules coordinately regulate this process (*J. Cell Biol.* **195,** 19–26; 2011).

The authors identified a previously unrecognized pool of actin that connects neighbouring cilia. Disrupting this actin framework blocked coordinated ciliary polarity and caused irregular basal body spacing. Intriguingly, clusters of neighbouring cilia were still able to orient together, suggesting that actin is only required for long-distance coordination. However, blocking microtubule function with nocadazole inhibited even the local coordination of cilia. Thus, actin and microtubules are both required for ciliary organization but have discrete roles.

In addition to orientation, ciliary beating must also be coordinated to generate flow. This process, called metachronal synchrony, was also dependent on the actin network. Together, these results suggest that the cytoskeleton is essential for coordination of ciliary organization and beating, and raise the possibility that mechanical force is transmitted through cytoskeletal links to synchronize these processes. EJC

## Ubiquitinomics

Two studies — Emanuele *et al.* (*Cell* http://dx.doi.org/10.1016/j.cell.2011.09.019; 2011) and Kim *et al.* (*Mol. Cell* http://dx.doi.org/10.1016/j.molcel.2011.08.025; 2011) — describe global approaches to identify ubiquitylated or degraded substrates.

Most cullin–RING ligases (CRLs) depend on neddylation of the cullin subunit for activity. Emanuele *et al.* used an improved version of a method called global protein stability profiling (GPS) to identify proteins accumulating after addition of a neddylation inhibitor. The results from this screen were then combined with the outcome of a mass-spectroscopy-based proteomics analysis on peptides recognized by an antibody specific to 'GG remnants' — Gly–Gly residues that remain from ubiquitin or ubiquitin-like (UBL) protein modification after tryptic cleavage of ubiquitin. The authors identified 108 common CRL substrates, 58% of which were previously associated with this ligase family.

Using similar proteomic techniques, Kim *et al.* compared the GG-modified proteome of cells treated with the proteasome inhibitor Bortezomib (Btz) and untreated cells, identifying 10,634 unique sites in 3,662 proteins, most of which were quantified. They found that the majority of GG modifications were remnants of ubiquitin rather than other UBLs. Time-course analyses allowed the authors to segregate these into proteins normally subjected to rapid regulatory proteolysis, and those only accumulating after longer Btz exposure, which probably represent quality-control substrates. Interestingly, a large fraction of the detected modifications are dependent on ongoing translation. Kim *et al.* also used the neddylation inhibitor to characterize CRL substrates.

Both studies demonstrate the power of large-scale analyses to obtain an overview of the ubiquitin landscape and to identify new ligase-specific substrates and ubiquitylation sites. CKR

## Mammary gland stem cells

The mammary epithelium is formed by luminal cells, which produce and transport milk and myoepithelial cells. Previous transplantation studies suggested that the several lineages that constitute and regenerate the mammary epithelium arise from multipotent mammary stem cells. Blanpain and colleagues (http://dx.doi.org/10.1038/nature10573) use a lineage-tracing approach in the mouse to show that although multipotent stem cells exist in the neonatal mammary gland, the postnatal mammary gland is maintained by long-lived unipotent stem cells that are able to expand the gland at puberty and renew it during pregnancy. They induced the expression of fluorescently labelled markers previously associated with gland stem cells and followed their contribution to the gland. Multipotent embryonic K14-expressing progenitors were confirmed to exist at birth, but it was found that later in life K14-expressing cells contribute only to the myoepithelial lineage. K8-labelled cells instead defined the luminal stem cells. To reconcile their results with previous studies indicating the presence of bipotent progenitors within the adult mammary gland, the authors transplanted isolated fluorescently labelled K14 or K8 cells, singly or in combination. They found that in conditions where luminal cells are few or absent, rare K14 cells adopt a luminal fate.

Uncovering these distinct long-lived stem cells in the mammary gland should help defining the cells at the origin of breast tumours. NLB

## Regulating metastasis: The two faces of miR-200

The miR-200 microRNA family suppresses the epithelial–mesenchymal transition (EMT) to promote the reverse process, MET; however, there are conflicting reports on its role in metastasis. Korpal *et al.* (*Nature Med.* **17,** 1101–1108; 2011) now show that miR-200 microRNAs promote metastatic colonization by regulating the tumour cell secretome.

Analysis of human breast tumour and lung metastasis samples as well as cancer cell lines correlated miR-200 expression with metastatic potential and poor distant relapse-free survival. miR-200 overexpression reduced entry of tumour cells into the circulation of mice, potentially by inhibiting EMT, but increased the lung-colonization ability of poorly metastatic cancer cell lines. This was not phenocopied by overexpresion of E-cadherin, a known MET-mediator upregulated by miR-200. Gene expression and mass-spectrometry analyses identified Sec23a, a secretory pathway component, as a miR-200 target, and Sec23a depletion, similarly to miR-200 overexpression, suppressed metastatic colonization. Mass-spectrometry analysis of conditioned media from Sec23a-depleted tumour cells revealed reduced secretion of a protein set, the low expression of which correlated with low relapse-free survival. Depletion of two such factors, Igfbp4 and Tinagl1, increased metastatic colonization implicating them in metastasis suppression.

These findings indicate that although miR-200 microRNAs may suppress EMT and early tumour cell dissemination, they promote metastatic colonization of cells that successfully enter the circulation by inhibiting secretion of metastasis suppressors. AIZ

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