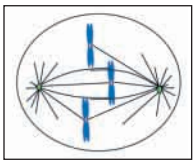
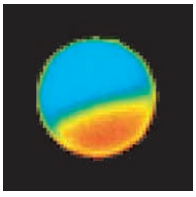


The spindle checkpoint: attachment, tension and signalling

The spindle assembly checkpoint ensures that chromosomes segregate correctly at mitosis, delaying anaphase until they are all attached and aligned at the equatorial plane. Central to this checkpoint are kinetochores – proteinaceous complexes that link chromatids to spindle microtubules and seem somehow able to alert the cell if improperly attached. In a Commentary on p. 3547, Harish Joshi and co-workers discuss how the checkpoint signals and what it actually monitors. Recent studies indicate that the checkpoint component Mad2 dynamically associates with and is activated by unattached kinetochores. As part of a mitotic checkpoint complex (MCC) containing BubR1 and Bub3, Mad2 then appears to interact with another protein, Cdc20, to inhibit the anaphase-promoting complex (APC) that drives sister chromatid separation. Other work has focused on whether it is simply kinetochore attachment or the tension created by binding of kinetochore pairs to microtubules emanating from opposite poles that inactivates checkpoint signalling. Although this issue is by no means settled, Joshi and co-workers believe there is strong evidence that both mechanisms operate and that Mad2 and Bub1/BubR1 monitor attachment and tension, respectively.

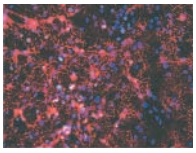


Making waves in eggs

Fertilization of animal and plant eggs triggers one or more Ca^{2+} waves that are propagated across the egg by a network of ER Ca^{2+}-release sites. These waves are initiated at specific pacemaker sites in response to sperm-triggered production of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] from phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. But what controls their dynamics and why do the wave patterns differ in some organisms? In a Commentary on p. 3557, Rémi Dumollard and co-workers review work that is shedding light on how such Ca^{2+} waves are generated. The pacemakers appear to be located in cortical ER-rich domains comprising densely packed sheets and tubes of ER membrane. Studies of mice eggs indicate that these domains contain clustered type I Ins(1,4,5)P_3 receptors (IP_3RIs) that release Ca^{2+} into the cytoplasm; the pacemakers are thus sites that have enhanced sensitivity to Ins(1,4,5)P_3. In ascidians, by contrast, the pacemaker site is defined instead by a localized source of Ins(1,4,5)P_3 production – probably PtdIns(4,5)P_2-rich microvilli – and this explains the different characteristics of the Ca^{2+} waves in these eggs.

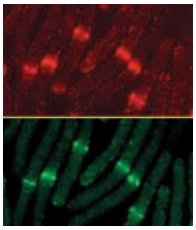
A catalyst for SNARE assembly

Members of the Munc18/Sec1 family, such as Sly1p, play essential roles in membrane trafficking. The proteins bind to t-SNAREs (components of the SNARE complexes that drive membrane fusion); however, their exact role is far from clear, since some experiments suggest that they can inhibit membrane fusion. Koji Yoda and co-workers now demonstrate that Sly1p directly stimulates SNARE assembly (see p. 3683). They show that the interaction between Sly1p and the t-SNARE Sed5p is significantly reduced in a temperature-sensitive *Sly1*^{ts} mutant and that Sly1p^{ts} protein has a lower affinity for Sed5p than does its wild-type counterpart. Most significant, however, is the authors' use of a novel in vitro assay for SNARE complex assembly in *Sly1*^{ts} mutant lysates, in which they show that purified Sly1 protein stimulates the formation of trans-SNARE complexes involving Sed5p and its partner the v-SNARE Bet1p. Since Sly1p-Sed5p binding is unaffected by SNARE complex formation, Yoda and co-workers propose that Sly1p acts prior to complex formation; the Munc18/Sec1 family might thus function by binding to t-SNAREs and inducing a 'pro-v-SNARE' binding state.



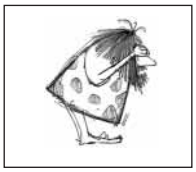
Oligodendrocyte differentiation from ES cells

Oligodendrocytes develop from neuroepithelial cells and myelinate axons in the CNS. Our understanding of their development is limited, since quantities sufficient for biochemical analyses cannot be purified and the stem cells from which they originate have not been isolated. To get around this problem, Nathalie Billon and co-workers have developed a method for generating oligodendrocytes in vitro (see p. 3657). They engineered ES cells to express drug-resistance/susceptibility genes from promoters active in neuroepithelial cells (*βgeo-Sox2*) and undifferentiated ES cells (*tk-Oct4*) and used the drugs (G418 and Ganciclovir) to select for neuroepithelial cells and eliminate undifferentiated ES cells, respectively; they then exposed the cells to signals known to promote oligodendrocyte differentiation (FGF-2, Sonic Hedgehog, PDGF-AA and thyroid hormone). Under these conditions, the cells followed the developmental pathway observed in vivo, adopting the correct morphology and expressing appropriate markers (Olig2, PDGFRα, NG2 proteoglycan and galactocerebroside) at the correct times. The technique indicates that an important developmental lineage can be reconstituted entirely from ES cells. Moreover, it could be adapted to produce human cells and thus be used in treatment of demyelinating diseases such as multiple sclerosis.



Control of cytokinesis by the spindle checkpoint

The separation of daughter cells at mitosis is driven by constriction of a cytokinetic actomyosin ring (CAR) by myosin motors such as fission yeast Myo2. Clearly, this must occur only after the spindle has formed and sister chromatids have separated. How exactly anaphase onset and CAR formation are coordinated has been unclear, however. Daniel Mulvihill and Jeremy Hyams have approached the problem by using a Myo2-GFP fusion protein to monitor the timing and regulation of CAR formation in fission yeast (see p. 3575). They show that recruitment of Myo2 to the CAR at the onset of anaphase A is significantly delayed by microtubule-depolymerizing drugs but that this delay does not occur if cells lack the spindle-assembly checkpoint component Mad2. Mulvihill and Hyams also show that full recruitment of the polo-related kinase (Plo1) to spindle pole bodies (SPBs) is similarly compromised by microtubule depolymerization and, again, this depends on Mad2. The authors conclude that recruitment of Plo1 to SPBs normally provides at least part of the signal for CAR formation but that activation of the spindle checkpoint can block this and thereby delay cytokinesis.



Sticky Wicket – the 'boffin' look

Scientists are usually presented in the media as Einstein-look-alikes with wild, grey hair and ill-fitting clothes. Is this an accurate portrayal? In his column on p. 3541, Caveman argues that cell biologists do not look like this. Indeed he even claims that, unlike his cartoon alter ego, he does not look like a scientist at all!

In the next issue of JCS