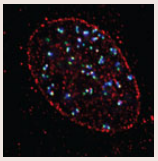
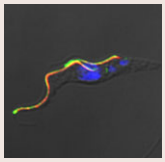
In this issue



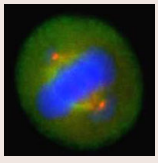
New site of action for p31^{comet}

The spindle assembly checkpoint (SAC) ensures accurate chromosome segregation during mitosis by delaying anaphase until all kinetochores are correctly attached. The mitotic checkpoint complex (MCC) – a protein complex that contains Mad2, Cdc20, BubR1 and BubR3 – is a key SAC component and inhibits APC/C activation. The Mad2-binding protein p31^{comet} antagonises the SAC, but the mechanism behind its action remained poorly understood. A previous model proposed that p31^{comet} creates an 'inhibitory cap' at kinetochores, that prevents the recruitment of additional Mad2 molecules and needs to be removed to allow MCC formation. On page 3905, Stephen Taylor and colleagues now provide evidence that p31^{comet}, instead, exerts its checkpoint inhibitory role downstream of kinetochores. Using a series of immunoprecipitation and RNAi approaches, they show that p31^{comet} binds to Mad2 when it is associated with the MCC components Cdc20 and BubR1, and subsequently extracts Mad2 from this complex. Although the remaining protein complex can still inhibit APC/C, the authors propose that this extraction presents an early step in MCC disassembly that is required for efficient entry into anaphase once all kinetochores are attached. Further, they propose that the constitutive turnover of the MCC presents a mechanism by which the spindle checkpoint can be fine-tuned.



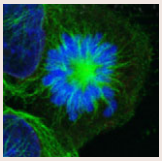
Flagellar attachment shapes *T. brucei*

Trypanosoma brucei is the flagellated protozoan parasite that causes human sleeping sickness. Its flagellum is required for cell motility, division and morphogenesis, and correct flagellar attachment is crucial for these processes. In trypanosomatids, the flagellum is attached to the cell body through the so-called flagellum attachment zone (FAZ), which is also required for the segregation of the basal body. Only two protein components of this unique cytoskeletal structure have been described so far. Here, Cynthia He and colleagues (p. 3848) identify the coiled-coil- and C2-domain-containing protein (CC2D) as a new FAZ component that is essential for FAZ assembly and the determination of cell morphology in *T. brucei*. Using immunofluorescence and immuno-gold labelling, they find that CC2D localises to the FAZ filament, the FAZ-associated ER as well as basal bodies. Depletion of CC2D by using RNAi inhibits FAZ assembly during flagellar duplication and results in detachment of the flagellum. Furthermore, cells that lack CC2D display abnormal basal body segregation during the cell cycle and also morphological defects that include shorter cell length and disruption of the anterior subpellicular microtubule network. Correct FAZ assembly, therefore, is not only important for flagellum attachment but is also essential to determine cell morphology.



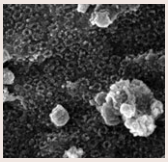
NEK7 doubles up centrioles

Centrosomes not only function as microtubule organising centres, but are also essential for correct mitotic spindle assembly and cell cycle progression. Each cell contains a single centrosome, which is replicated precisely once per cell cycle, and a number of centriolar and PCM proteins have been implicated in the regulation of the centrosome duplication cycle. The nimA-related serine/threonine-protein kinase NEK7 also localises to the centrosome and is required for the correct formation of the mitotic spindle. But is this kinase also involved in centriole duplication? On page 3760, Kunsoo Rhee and colleagues now provide an answer to this question by showing that depletion of NEK7 by using siRNA inhibits centriole duplication. By contrast, the ectopic expression of a centrosome-targeted version of the kinase results in centriole overduplication. Furthermore, NEK7 is required for the specific recruitment of PCM proteins during interphase and depletion of the kinase results in centrosome maturation defects that, in turn, affect correct formation of the bipolar spindle. However, depletion of PLK4, which is known to be essential for centriole duplication, does not affect PCM accumulation. The researchers, therefore, conclude that NEK7 is essential for the recruitment of PCM to the centriole prior to its duplication, which then provides the correct environment for activation of PLK4 and the subsequent initiation of centriole duplication.



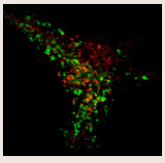
Putting spindles in the right place

Autosomal recessive primary microcephaly (MCPH) is characterised by impaired brain development, which results in a smaller brain and mental retardation. It has been suggested that MCPH arises because symmetrically dividing neural progenitors fail to align their mitotic spindles correctly, which – subsequently – results in defects in the lateral expansion of the developing brain. On page 3884, Pierre Gönczy and co-workers now provide support for this hypothesis by showing that alterations in two microcephaly proteins that are involved in centriole formation result in spindle positioning defects in human cells. They find that expression of centrosome protein 4.1 associated protein (CPAP, also known as CENPJ) that has mutations that mirror those known to be present in patients with MCPH causes aberrant centriole formation. Furthermore, another MCPH protein, SCL-interrupting locus (STIL), is also essential for centriole formation. Using microfabricated chips the authors assess spindle positioning in cells that express CPAP MCPH mutants or lack STIL and find that the centriole defects in these cells are associated with the formation of asymmetric spindles and random spindle positioning. These observations, therefore, provide evidence that centriole formation, spindle positioning and brain development are linked, and might explain how mutations in centrosomal proteins contribute to MCPH pathologies.



Nuclear pores need POM121 twice

Nuclear pore complexes (NPCs) are multi-subunit protein complexes that span the nuclear envelope and mediate transport of macromolecules in and out of the nucleus. In metazoans, NPCs can be formed in two distinct ways: they are either assembled directly into the reforming nuclear envelope at the end of mitosis or inserted into the intact envelope during interphase. Various nucleoporin complexes have been identified as components of the NPC, but the mechanistic details behind NPC assembly have remained a matter of controversy. Amnon Harel and co-workers (p. 3822) now identify the nucleoporin POM121 as an NPC component that is involved in both types of pore complex formation. They show that a soluble, dominant-negative form of POM121 (POM121^{DN}) disrupts both nuclear assembly and NPC formation in a cell-free reconstitution system. It binds to chromatin at sites that are distinct from the previously identified ELYS–Nup107–160 NPC 'seeding' sites. Furthermore, POM121^{DN} prevents nuclear membrane expansion during interphase, which results in the nuclear envelope becoming densely packed with NPCs. On the basis of these observations, the authors conclude that POM121 has important roles in the two assembly mechanisms, and links the formation and expansion of the nuclear membrane with nuclear pore biogenesis.



Integrins WASH up at the plasma membrane

Numerous processes, such as cell motility, cytokinesis and trafficking, are regulated by changes in the actin cytoskeleton. Nucleation promotion factors (NPFs), e.g. members of the Wiskott-Aldrich syndrome protein (WASP) family, together with the Arp2/3 complex, drive actin filament assembly. Previous studies showed that Arp2/3 activity and actin polymerisation are important for the formation and trafficking of endocytic vesicles. On page 3753, Laura Machesky and colleagues now demonstrate that the WASP and SCAR homologue (WASH) and Arp2/3 also regulate retrograde trafficking of α5β1-integrins. WASH is present on early endosomal compartments as well as the multivesicular body or late endosome in a variety of cell types, where it colocalises with the Arp2/3 complex. In cultured ovarian cancer cells, WASH also colocalises with α5β1-integrins and its depletion by siRNA results in integrin accumulation in perinuclear clusters and reduces the recycling rate of integrins to the plasma membrane. In addition, on a cell-derived matrix, WASH depletion results in the formation of shorter pseudopods in response to integrin stimulation, reduces the speed of migration and impairs the ability of cells to invade the matrix. These observations support a new role for WASH in regulation of recycling of α5β1-integrins from internal vesicles to the plasma membrane and, subsequently, in the regulation of invasive motility.