New CRIME plots

Ran and transport factors regulate mitosis

In the mid-1990s, the chromosome region maintenance 1 (Crm1) protein was identified in yeast as the product of a gene that is essential for proliferation and chromosome stability (Nishi *et al*, 1994). Its human homologue, CRM1, was subsequently shown to interact with the nucleoporin CAN, which is a component of oncogenic fusion proteins found in leukaemia cells (Fornerod *et al*, 1997). These authors also noticed a homologous domain at the amino-terminus of CRM1, importin β and other proteins—now known to interact with the GTPase Ran—and named it the CRIME domain for ‘CRM1, importin β etc’. Several laboratories soon discovered that CRM1 is the export vector for proteins from the nucleus to the cytoplasm and that it recognizes the nuclear export signal (NES) in these proteins. Export requires RanGTP to stabilize the complexes that are formed by CRM1 and NES-containing proteins. These data shifted the focus towards the export function of CRM1 and, since then, its involvement in the cell cycle has been somewhat neglected. However, recent findings now put CRM1 at the front of the mitotic stage and contribute to a more general reappraisal of the role of transport factors in mitosis.

In the August issue of *Nature Cell Biology*, Wang *et al* (2005) have addressed the role of CRM1 in the control of centrosome duplication. Centrosomes are major microtubule (MT)-organizing centres in mammalian somatic cells and form the spindle poles at mitosis. More than a century ago, Boveri observed that tumour cells typically contain many centrosomes compared with only one or two in normal cells. Whether supernumerary centrosomes are a cause or a consequence of aneuploidy is still a matter of debate, but it has been suggested that their presence can facilitate the propagation of aneuploidy, particularly in certain cancer types (D’Assoro *et al*, 2002). A complex regulatory network is in place in normal cells to ensure that these organelles duplicate only once per cell cycle. Wang and colleagues were alerted to a role for CRM1 in preventing centrosome overduplication by the finding that the HBx oncoprotein, encoded by the hepatitis B virus (HBV), harbours an NES motif that interacts with CRM1. CRM1 is mainly nuclear, but when soluble proteins are extracted from cells, a small fraction remains attached to centrosomes. In HBV-infected cells, HBx sequesters CRM1 to the cytoplasm and concomitantly induces supernumerary centrosomes and multipolar spindles (Forgues *et al*, 2003). Centrosomes also contain a small amount of Ran GTPase stably associated with the anchoring protein AKAP450 (Keryer *et al*, 2003), and RanBP1, which is a regulator of the Ran GTPase cycle and also carries an NES

(Di Fiore *et al*, 2003). Reasoning that centrosomes might be a site of regulated interactions between Ran, RanBP1 and CRM1, Wang and co-workers set out to search for factors that control centrosome duplication and that might be regulated by the Ran–CRM1 pathway. They focused on nucleophosmin (NPM), a nucleolar protein thought to work as a ‘licensing’ factor (Okuda, 2002): NPM associates with duplicated centrosomes in mitosis and prevents their reduplication. In the S phase of the next cell cycle, NPM dissociates from centrosomes and duplication begins.

The authors show that NPM is a shuttling protein that has NES, nuclear localization signal (NLS) and nucleolar localization signal (NoLS) motifs. To test the importance of CRM1 interaction with the NES of NPM, they used several strategies to inhibit CRM1 function—addition of the CRM1-specific inhibitor leptomycin B (LMB), expression of HBx or RanBP1 overexpression—and found that NPM consistently delocalized from centrosomes. Remarkably, LMB also displaced NPM from the purified centrosomal fraction. The authors conclude that, when NPM is released from the nucleolus after nuclear envelope (NE) breakdown, the NES directs it to interact with centrosomal CRM1, which prevents centrosome reduplication in that cell cycle. In support of this idea, only wild-type, but not NES-defective, NPM restored control of centrosome duplication to cells that lack NPM owing to RNA interference (RNAi). The localization of NPM at centrosomes is established in mitosis and is lost in late G1/early S phases of the next cell cycle following phosphorylation by G1 cyclin–cdk2 complexes (Fig 1). The NES of NPM contains a phosphorylation site that could modulate its interaction with CRM1. Wang and colleagues have shown that NPM mutants, the NES of which cannot be phosphorylated, localize at centrosomes. By contrast, mutations that mimic constitutive phosphorylation in the NES impair both the centrosomal localization of NPM and its ability to control overduplication negatively. Thus, NPM must interact with CRM1 to ensure control of centrosome duplication.

Ran has been implicated in the control of centrosome duplication by the finding that centrosomes undergo several rounds of replication in the presence of the Adenovirus E1A oncoprotein, which interacts with Ran and interferes with RanGTP formation catalysed by the RCC1 exchange factor (De Luca *et al*, 2003). Now the involvement of CRM1, first hinted at by the studies on HBx, suggests that classical trimeric export complexes, RanGTP–CRM1–NES-protein, restrict centrosome duplication to once per cell cycle (Fig 1). Interestingly, these findings imply that the unrelated oncogenic Adenovirus and HBV seem to use a common strategy to inactivate the RanGTP–CRM1-dependent pathway that controls centrosome duplication.

Some gaps remain to be filled in the overall picture. Several studies in somatic cells indicate that altered expression of Ran network components predominantly generate spindle abnormalities in mitosis, whereas centrosome duplication is an S-phase phenomenon. This suggests that control of NES substrates by centrosomal CRM1 is only part of the story. Also of relevance are

NLS-containing spindle assembly factors: these factors, exemplified by TPX2, are released from nuclei at NE breakdown, reach centrosomes and organize spindle poles by regulating the assembly of mitotic MT arrays that will eventually interact with chromosomes. Their activity is inhibited by another transport factor, importin β , in a manner that is counteracted by RanGTP (reviewed by Harel & Forbes, 2004). Thus, regulators of the nucleotide-bound state of Ran can influence the assembly of a functional bipolar spindle by modulating RanGTP–importin- β -dependent pathways, regardless of the number of centrosomes. The interaction between the control of centrosome number through CRM1 and of the spindle-organizing activity of centrosomes through importins requires further clarification. Nevertheless, the study by Wang and colleagues marks a significant step forwards in linking the Ran–CRM1 pathway to the control of centrosome duplication.

The ability of CRM1 to control centrosome duplication is a new, unexpected function of this export factor, which can be added to an emerging range of CRM1 functions in the mitotic apparatus. Indeed, a role for CRM1 has also been recently identified at kinetochores (Arnaoutov *et al*, 2005). Kinetochores provide not only a 'structural platform' for anchoring chromosomes to MTs that emanate from each pole, but also a regulatory platform for spindle checkpoint factors. These factors monitor the attachment of spindle MTs and transduce signals that prevent the separation and segregation of sister chromatids as long as unattached kinetochores are present. When all chromosomes are attached and kinetochores are under tension at metaphase, the checkpoint is inactivated and segregation begins. It was noticed some time ago that a complex containing RanGAP1 and RanBP2 localizes at mitotic kinetochores (Joseph *et al*, 2002). RanGAP1 is the GTP-hydrolysing factor on Ran, and RanBP2 acts as a SUMO ligase on several substrates, including RanGAP1, and indeed only the sumoylated form of RanGAP1 localizes at kinetochores. Both RanGAP1 and RanBP2 are cytoplasmic factors, with a significant enrichment at the nuclear pore complexes in interphase, and so their localization at crucial mitotic structures was unexpected. In fact, further work showed that an imbalance between RanGAP1 and RCC1 impaired spindle checkpoint function in cycling *Xenopus* extracts (Arnaoutov & Dasso, 2003). Moreover, the inactivation of RanBP2 by RNAi in human cells caused abnormal chromosome congression, mislocalization of kinetochore factors and altered centromere structure (Salina *et al*, 2003). The kinetochore localization of RanGAP1/RanBP2 is dependent on factors required for MT attachment (for example, Hec1 and Nuf2) and is mutually exclusive with that of a genuine checkpoint factor, Mad1: whereas Mad1 is present on unattached kinetochores, RanGAP1/RanBP2 are only recruited to attached kinetochores, which suggests that MT attachment induces a conformational change that causes Mad1 release and RanGAP1/RanBP2 loading on kinetochores (Joseph *et al*, 2004).

It has been shown recently that, besides the centrosomal fraction, a kinetochore-associated fraction of CRM1 is also visible in human mitotic cells. Indeed, CRM1 inactivation by LMB results in a failure to recruit RanGAP1/RanBP2 at kinetochores and generates mitotic defects that resemble in part those observed in the absence of RanBP2 function (Arnaoutov *et al*, 2005). Do RanGTP–CRM1 complexes therefore regulate the spindle checkpoint? Mechanistically, there is no direct answer as yet. RCC1

Fig 1 | Control of centrosome duplication by the RanGTP–CRM1–NPM complex. Centrosomes are represented as a pair of orthogonal centrioles at each pole of the mitotic spindle. In the magnification, nucleophosmin (NPM) binds centrosomal CRM1 through its nuclear export signal (NES) and prevents reduplication. RanGTP (GTP) localizes at centrosomes together with AKAP450 and RanBP1 (BP1). NPM is phosphorylated in the next cell cycle by the cdk2 kinase: this displaces NPM from centrosomes and allows duplication to take place. CRM1 inhibition by leptomycin B (LMB), and NES inactivation in NPM, yield supernumerary centrosomes.

associates with mitotic chromatin and generates RanGTP. RanGTP and CRM1 are required for RanGAP1/RanBP2 loading onto kinetochores, which eventually lowers the local RanGTP concentration. Thus, it is plausible that CRM1 modulates RanGTP levels in a spatially restricted manner at kinetochores, and that this in turn regulates the inactivation of the spindle checkpoint in response to MT attachment.

The newly discovered functions of CRM1 at crucial mitotic structures indicate that transport factors do not only regulate the subcellular localization of proteins. They are also, after NE breakdown, 'functionally' recycled to operate actively in mitotic control, largely by imparting spatial control to pathways of spindle formation and function. Specific and spatially restricted modes of Ran control are beginning to emerge in mitosis, although there are still many open questions. For example, it is apparent that the RanGTP–CRM1 complex targets different substrates depending on where it localizes, as indicated by the efficient targeting of the NPM-specific NES by centrosome-associated, but not kinetochore-associated, CRM1. A specific mode of action of Ran regulators is also evident. In interphase, RanGAP1 mediates GTP hydrolysis on Ran by acting in concert with RanBP1, which increases RanGAP1

activity tenfold. However, in mitosis, these regulators are targeted to different sites. RanBP1 is found predominantly at MTs and centrosomes, and regulates spindle pole integrity. RanGAP1 also associates with MTs but is excluded from poles and is recruited to kinetochores: therefore, RanGAP1 operates independently of RanBP1 at these sites, but dependently on RanBP2-mediated sumoylation. Unravelling how Ran-dependent signals operate spatially is an important challenge for understanding the regulatory processes that underlie faithful mitotic division. In summary, transport factors and the components of the Ran GTPase network with which they operate are not mere vectors of biologically relevant factors, but can be regarded as a special class of regulators of mitosis and, ultimately, of genomic stability through cell division in their own right.

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