

CELL CYCLE

Catching on

Replicated chromosomes bind to the mitotic spindle through specialized structures — kinetochores — that assemble on centromeres. Kinetochores must be captured by spindle microtubules and transported to spindle poles for accurate chromosome segregation. By visualizing individual kinetochore–microtubule interactions in budding yeast, Tomoyuki Tanaka and colleagues have started to unravel the molecular mechanisms underlying kinetochore capture.

In this study, centromere *CEN3*, on chromosome III, and microtubules were marked with green and yellow fluorescent protein, respectively. Metaphase arrest was induced and, simultaneously, *CEN3* was inactivated, allowing its detection at a distance from the spindle pole. Once cells had arrested, *CEN3* was reactivated and its movement followed in metaphase. The authors observed that centromeres were captured laterally by microtubules that extended from the spindle pole, and moved poleward along the microtubules. After *CEN3* reached the spindle pole, the green fluorescent signal split, indicating that sister centromeres bi-orient on the spindle pole.

Using mutational analysis, the authors dissected the functional roles of the different kinetochore components. Mutants of the microtubule plus-end tracking proteins Bim1, Bik1 and Stu2 and the Ran GDP/GTP exchange factor showed reduced numbers of nuclear microtubules, indicating that these factors are necessary for nuclear microtubule extension from spindle poles.

By studying the kinetics of kinetochore capture in different mutants, Tanaka and co-workers identified the CBF3, Ndc80, Mtw1 and Ctf19 complexes, but not the Dam1 or Ipl1 complexes, as being required for kinetochore capture by microtubules.

As the rate of microtubule shrinkage is faster than that of poleward kinetochore transport, the plus ends of the shrinking microtubules are expected to meet the kinetochores, causing them to slide off. However, this never happens — the reason being, as Tanaka and colleagues observed, that microtubules associated with *CEN3* can be ‘rescued’ (that is, microtubule shrinkage is converted to growth). Stu2 that is located at the plus ends of microtubules, and originates from *CEN3*, is believed to have a mediating role in this process.

The authors postulate that kinetochore transport along the microtubule might be mediated by an ATP-driven motor protein. Indeed, a mutant of the kinesin-14 family member Kar3 showed defective kinetochore transport. But, kinetochore transport continued in most mutant cells, indicating that other regulators are probably involved.

Finally, Tanaka and colleagues note that, even though the current study highlights some important differences between yeast and vertebrate cells, kinetochore capture is such a crucial event that the mechanisms are probably conserved.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Tanaka, K. *et al.* Molecular mechanisms of kinetochore capture by spindle microtubules. *Nature* **434**, 987–994 (2005)

STRUCTURE WATCH

Two become three

Two domains are known to mediate protein–protein interactions by binding to phosphotyrosine (pTyr) residues in target proteins: Src-homology-2 (SH2) and pTyr-binding (PTB) domains.

However, in *Cell*, Soltoff and colleagues have now discovered that a C2 domain (conserved domain 2) can bind to specific pTyr-containing peptides. This is an unusual finding, as these domains are known to mediate Ca²⁺-dependent lipid binding.

The authors focused on protein kinase C δ (PKC δ), which contains a C2 domain that does not bind Ca²⁺ or phospholipids and which is regulated by the kinase Src. To understand this regulation, they first identified a protein that associates with both PKC δ and Src, CDCP1. They then showed that Tyr phosphorylation of CDCP1 correlates with an association between CDCP1 and PKC δ . This interaction was found to be mediated by the C2 domain of PKC δ binding to a pTyr residue in CDCP1, and a 1.7-Å-resolution crystal structure of the PKC δ C2 domain bound to an optimal phosphopeptide highlighted a new mode of pTyr binding. Although the phosphate is coordinated by an Arg residue, as in SH2 and PTB domains, Tyr binding involves a ring-stacking interaction with a His residue of the PKC δ C2 domain. PKC δ is the first example of a serine/threonine kinase that contains a pTyr-binding domain. Furthermore, data from this study indicate that Src phosphorylates and binds to CDCP1 through its SH2 domain, which promotes further CDCP1 phosphorylation and PKC δ recruitment to form a protein complex that contains two important kinases.

REFERENCE Benes, C. H. *et al.* The C2 domain of PKC δ is a phosphotyrosine binding domain. *Cell* **121**, 271–280 (2005)

Forced to let go

Nuclear transport is coordinated by nuclear Ran•GTP, which releases cargo from importin proteins and promotes cargo binding to exportin proteins. In *Nature*, Stewart and co-workers provide the basis for understanding the important cargo-release step of nuclear import by presenting the 2.7-Å-resolution crystal structure of full-length yeast importin- β , Kap95, bound to Ran•GTP.

Kap95 is composed of two C-shaped arches that are linked together to form a flexible helicoidal structure. A previous structure that included only the N-terminal arch of importin- β highlighted two important interaction sites with Ran, one of which involved the switch II region (the conformations of the Ran switch I and II regions differ between the GTP- and GDP-bound states). However, this structure containing full-length Kap95 highlights the importance of a third interaction site between the C-terminal arch of Kap95 and the switch I region of Ran•GTP. By exploiting its flexibility and by using different binding sites, importin- β /Kap95 can bind to a range of different cargoes. So, how does Ran•GTP binding release all of these cargoes? On the basis of their structure, the authors propose that, by binding to sites in both the C- and N-terminal arches, Ran•GTP actively displaces cargo and locks importin- β /Kap95 “...in a conformation in which it is unable to exploit its flexibility to bind to different partners”.

REFERENCE Lee, S. J. *et al.* Structural basis for nuclear import complex dissociation by RanGTP. *Nature* 1 May 2005 (doi:10.1038/nature03578)

