

Shedding a little light on light chains

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Myosin II regulatory light chains have an important role in the organization and function of the contractile machinery at cytokinesis. Two recent reports provide new insights into these important proteins.

Given that it has been the subject of active investigation for more than half a century, it comes as a bit of a shock to discover that myosin II, the venerable patriarch of the myosin superfamily, still has some surprises left in its locker. Best known for its role in muscle contraction, myosin II also has an important function in non-muscle cells as a component of the contractile ring that constricts the cell surface at cytokinesis (Fig. 1). Although the detailed structure of the contractile ring is still not fully resolved, it is thought to consist primarily of actin filaments interspersed with bipolar filaments of myosin II. Powered by the 'motor' activity of myosin II, the actin filaments slide over one another, drawing the ring tighter until two daughter cells are created. Such a mechanism demands not only that myosin II is brought to the right place in the cell at the right time, but also that the assembly of the contractile ring is coordinated with spindle formation. The ATPase activity of myosin II must also be activated on cue so that the contracting ring passes between the two sets of chromosomes as they make their anaphase journey to the opposing spindle poles. Two recent papers^{1,2} not only shed a little light on how this remarkable precision is achieved through the binding and regulatory effect of light chains, but also illustrate very nicely the fact that the essential features of cytokinesis have been conserved throughout evolution.

A series of papers from several laboratories have exploited the powerful genetics of the fission yeast *Schizosaccharomyces pombe*, coupled to the almost completely sequenced genome of this organism, to identify the genes that are involved in the establishment of the cleavage plane and the mechanisms of contractile-ring function (reviewed in refs 3, 4). Despite the fact that it is surrounded by an inflexible cell wall, fission yeast uses a cytokinetic actomyosin ring (CAR) much like that found in animal cells, the only difference being that a new cell wall, or septum, must be formed behind the constricting ring as the final barrier between the two daughter cells (Fig. 1). The CAR actually contains two myosin II iso-

forms, encoded by the *myo2*⁺ (refs 5, 6) and *myp2*⁺ (refs 7, 8) genes. Myo2 is the principal component of the CAR, whereas Myp2 seems to serve an auxiliary function⁹. The functional myosin II motor protein is typically a hexamer consisting of two heavy chains and four light chains; of the light chains, two are essential (ELCs) and two are regulatory (RLCs). The light chains bind to regions containing IQ motifs in the myosin II 'neck' (Fig. 2).

Previous work had shown that the Cdc4 protein is the ELC for both Myo2 and Myp2 (refs 10, 11), although the identity of the RLC remained elusive. However, in the November 2000 issue of *Nature Cell Biology*, Naqvi *et al.*¹ finally identified this subunit, called Rlc1, using an *in silico* screen of the fission-yeast genome. Rlc1 was also independently identified by Le Goff *et al.*² in a genetic screen for cytokinesis mutants. Each group showed that Rlc1 localizes to the CAR by means of its interaction with

both Myo2 and Myp2, and that this interaction depends on the IQ motif. Naqvi and colleagues also showed that although cells lacking Rlc1 are able to grow at higher temperatures, they exhibit cytokinetic defects at lower temperatures. Surprisingly, these defects can be almost totally suppressed by removing the Rlc1-binding site on Myo2. The authors concluded that the fission-yeast RLC, by binding to the Myo2 heavy chain, relieves an auto-inhibitory effect of this domain, a conclusion that has been supported by studies using the slime mould *Dictyostelium discoideum*¹².

It is unknown at this stage whether post-translational modifications are required for the regulation of Rlc1 in yeast, but in most non-muscle cells (as well as in smooth muscle) the phosphorylation of the RLC at serine 19 by myosin-light-chain kinase (MLCK) both stimulates the assembly of myosin II into filaments and activates its ATPase activity¹³. These effects are counterbalanced by phosphorylation of serines 1 and 2 by protein kinase C (PKC). As cells enter metaphase, the RLC is phosphorylated at serines 1 and 2 but, at the onset of anaphase, these residues are dephosphorylated and serine 19 is phosphorylated. It is this latter event that seems to trigger contraction of the CAR. The localization and activity of two isoforms of mammalian MLCK were examined recently by Poperechnaya *et al.*¹⁴. They tagged the short and long forms of MLCK (splice variants from the single MLCK gene) with green fluorescent protein. Although the shorter form was found to be cytoplasmic throughout the cell cycle, the longer form localized to stress fibres during interphase and relocalized to

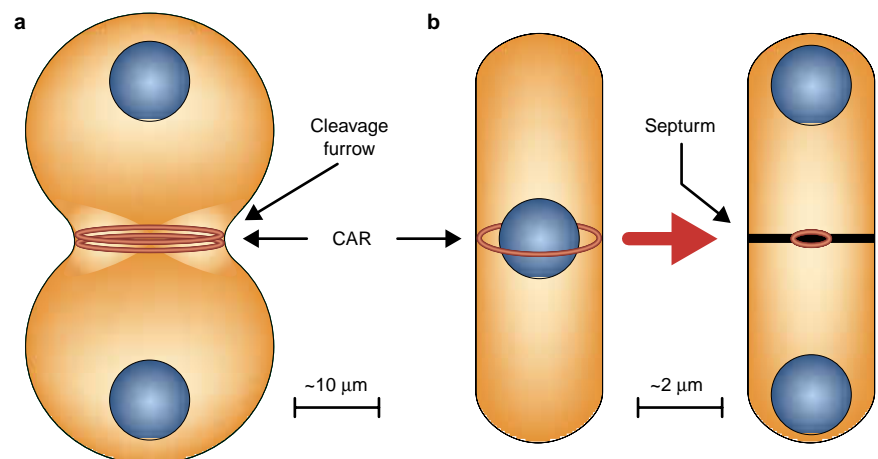


Figure 1 The mechanism of cytokinesis is conserved from yeast to mammals. **a**, In higher eukaryotic cells, at the end of nuclear division, contraction of the CAR (red) results in invagination of the cell membrane to form the cleavage furrow. **b**, In *S. pombe* cells, the CAR forms during metaphase and remains uncontracted until the completion of telophase, when it contracts, marking the site for deposition of a new cell wall or septum.

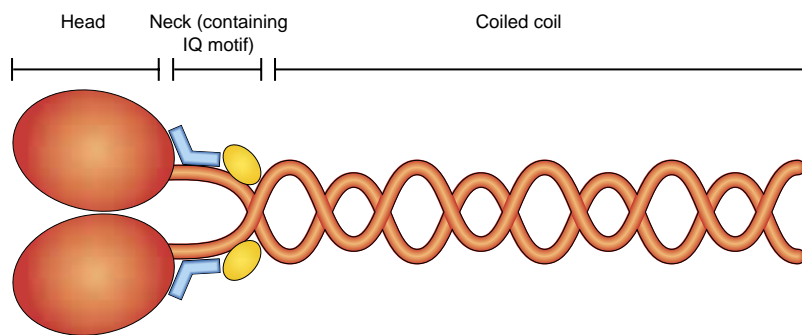


Figure 2 Subunit structure of myosin II. Myosin II molecules are hexamers consisting of two heavy chains (red), which are dimerized through their coiled-coil tails. Each heavy chain is associated with an essential light chain (blue), which is required for filament assembly, and a regulatory light chain (yellow), which is involved in the regulation of filament assembly and the ATPase activity of the heavy chains.

the cleavage furrow at metaphase, where it persisted throughout cytokinesis. The difference in localization of the two forms was a function of several DXRXXL motifs at the site at which the short form is truncated. Whereas three motifs are present in both forms, the long form has an amino-terminal extension of 934 amino acids, which contains a further two DXRXXL motifs. Poperechnaya and colleagues demonstrated that although adding the two extra motifs to the short form confers the ability to localize to stress fibres, the long form is required in its entirety for localization to the cleavage furrow.

As well as variation in localization, the activity of the long form varies throughout the cell cycle, although there is no detectable change in its overall level. The ability of this MLCK to phosphorylate the RLC is reduced

until metaphase but rises in anaphase. By the time cells have completed cytokinesis, its activity is high again; these alterations in activity seem to depend on the phosphorylation state of the kinase. Interestingly, the peaks of the MLCK activity correspond to the timing of its localization to the cleavage furrow and of CAR contraction. This demonstrates that the timing of MLCK activity peaks with the peak of myosin II activity.

Is the RLC in fission yeast also regulated by phosphorylation? The sequence of Rlc1 does not contain serine residues at positions 1, 2 or 9, and the fission-yeast genome project has yet to reveal an MLCK. However, there are several other potential phosphorylation sites and other candidate kinases. Mammalian RLCs are known also to be phosphorylated by both Rho kinase and

p21-activated kinase (PAK). Cdc4 is phosphoprotein, but mutating its conserved serine residues has no obvious effect on cytokinesis¹⁵. In addition, myosins can also be regulated by phosphorylation of their heavy chains¹⁶. What all these observations point to is the fact that several signal-transduction pathways converge during the process of cytokinesis^{13,17}. This makes perfect sense, as the cell has to ensure that the preceding steps of cell division (such as correct segregation of chromosomes) have been completed successfully before the onset of cytokinesis. We certainly have not heard the last of this topic, and the two papers reviewed here represent a small but significant step in writing the complete workshop manual for the CAR.

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