

Cleavage furrow formation and ingression during animal cytokinesis: a microtubule legacy

Pier Paolo D'Avino*, Matthew S. Savoian and David M. Glover

Cancer Research UK Cell Cycle Genetics Research Group, Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK

*Author for correspondence (e-mail: p.davino@gen.cam.ac.uk)

Journal of Cell Science 118, 1549-1558 Published by The Company of Biologists 2005
doi:10.1242/jcs.02335

Summary

Cytokinesis ensures the proper partitioning of the nuclear and cytoplasmic contents into independent daughter cells at the end of cell division. Although the metazoan mitotic spindle has been implicated in the placement and advancement of the cleavage furrow, the molecules responsible for these processes have remained elusive. Recent studies have provided insights into the role of different microtubule structures and associated proteins in cleavage furrow positioning and ingression together with the signalling events that regulate the dynamics of the equatorial cell cortex during cytokinesis. We try to unify

these findings into a general model of cytokinesis in which both astral and central spindle microtubules have the ability to induce furrowing. We further propose that the evolutionarily conserved centralspindlin complex serves as a master controller of cell cleavage in *Drosophila* by promoting both furrow formation and ingression. The same mechanism might be conserved in other organisms.

Key words: Central spindle, Cleavage furrow, Contractile ring, Cytokinesis, Microtubules, Rho GTPases

Introduction

Cytokinesis is the final act of cell division. In a typical animal mitosis, a cleavage furrow forms at the equatorial cortex after anaphase. This furrow then advances inwards to separate the two daughter cells. Since the early studies of Rappaport more than forty years ago (Rappaport, 1961), it has become clear that spindle microtubules play a key role in furrow formation, but the exact nature of this role has been debated. Two opposing models have been proposed and both have very active supporters. In the 'astral relaxation' model, microtubules are supposed to inhibit furrow formation close to the spindle poles, thereby allowing the cortex to contract only at the equator (Fig. 1). The 'astral stimulation' model, by contrast, assumes that a subpopulation of astral microtubules determine the cleavage plane by delivering a 'furrow-inducing signal' at the equatorial cortex (Fig. 1). More recently, this model has been expanded to accommodate the increasing evidence that an array of anti-parallel microtubules released from the spindle poles during anaphase is essential for cytokinesis in most systems (Fig. 1) (reviewed by Gatti et al., 2000). Different terms have been used to denote this structure: 'central spindle', 'spindle midzone' or 'interzonal microtubules', generating some confusion in the field. We use the term 'central spindle' throughout this article to indicate the array of microtubules formed between segregating chromosomes during anaphase. Regardless of whether microtubules play inhibitory or stimulatory roles, it is obvious that they must interact with the cell cortex to promote the formation and ingression of the cleavage furrow. Thus, a full understanding of the mechanisms that control cytokinesis requires the combined analysis of events occurring at the spindle and cell cortex.

Here, we review recent studies of the roles of astral and

central spindle microtubules in concert with the signalling pathways that regulate the dynamics of the equatorial cell cortex during cytokinesis. We also propose a model that tries to accommodate all the evidence and we suggest a possible molecular candidate for the furrow-inducing signal.

Astral versus central spindle microtubules

Genetic and micromanipulation experiments have indicated that chromosomes and centrosomes are not essential for cytokinesis (Bucciarelli et al., 2003; Khodjakov and Rieder, 2001; Megraw et al., 2001; Zhang and Nicklas, 1996). Conversely, the data supporting a key role for astral or central spindle microtubules in control of furrow formation and ingression are overwhelming (Adams et al., 1998; Alsop and Zhang, 2003; Canman et al., 2003; Cao and Wang, 1996; Dechant and Glotzer, 2003; Inoue et al., 2004; Mishima et al., 2002; Raich et al., 1998; Savoian et al., 1999a; Wheatley and Wang, 1996). In perhaps the most conclusive experiment, Alsop and Zhang used micromanipulation experiments in grasshopper spermatocytes to show that, after removal of both asters and chromosomes, the residual spindle microtubules can still self-assemble into organized bundles that promote furrowing (Alsop and Zhang, 2003). This revealed that microtubules and associated proteins are the only internal cellular components required for furrow initiation. Moreover, since these bundles resemble a central spindle, this finding supports a key role for this structure during cytokinesis. By contrast, two recent studies have shown that the central spindle is dispensable for cytokinesis. First, Verbrugghe and White have observed that *Caenorhabditis elegans spd-1* mutant embryos lack a central spindle but can nevertheless

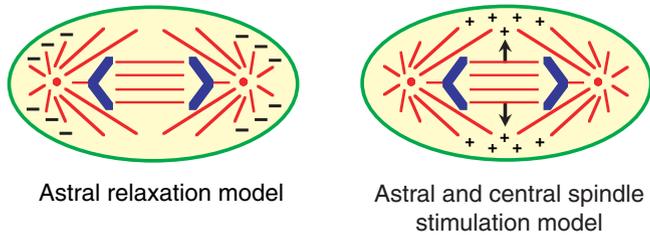


Fig. 1. Schematic representation of the two opposing models proposed to explain the role of microtubules in furrow formation and ingression.

successfully complete the first embryonic divisions, though cytokinesis fails in subsequent cell divisions (Verbrugghe and White, 2004). Second, Canman et al. showed that drug-induced monopolar spindles, which lack central spindle microtubules, in mammalian PtK1 cells initiate and complete cytokinesis (Canman et al., 2003). They did, however, note a particularly stable subpopulation of microtubules that interact with the cell cortex at the cleavage site after contacting or passing by the chromosomes.

The apparent conflict between studies ascribing furrow-inducing properties to astral versus central spindle microtubules can perhaps be reconciled by the observation that, in *Drosophila* primary spermatocytes, two different populations of microtubules contribute to the formation and ingression of the cleavage furrow (Inoue et al., 2004). Time-lapse analysis revealed that a set of 'peripheral' astral microtubules contacts the cortex exactly at the cleavage site and forms overlapping antiparallel bundles that promote furrow ingression. These bundles then merge with a distinct 'interior' population of central spindle microtubules to complete furrow ingression and cytokinesis. Analysis of microtubule dynamics in *Drosophila* S2 cells indicates that a similar process occurs in mitotic cells (Inoue et al., 2004). In this case, astral microtubules, probably equivalent to the stable microtubules observed by Canman et al. in PtK1 cells (Canman et al., 2003), do not appear to form bundles; this implies that contact between the microtubule plus ends and the cell cortex is sufficient for furrow initiation. In *Drosophila* spermatocytes, peripheral and interior microtubules both appear to be able to stimulate furrow formation. Spermatocytes expressing mutant forms of the microtubule-associated protein Orbit specifically lack a robust interior central spindle; nevertheless the peripheral microtubules can initiate furrows that subsequently regress (Inoue et al., 2004). Conversely, spermatocytes lacking the motor protein Klp67A exhibit disorganized, bent interior central spindles that protrude towards the cortex and appear to promote furrow formation in areas containing very few astral microtubules (M. K. Gatt, M.S.S., M. G. Riparbelli, C. Massarelli, G. Callaini and D.M.G., unpublished).

Thus, both astral and central spindle microtubules appear to be able to induce furrowing, and the contribution of each component may vary depending upon the organism and cell type, as previously proposed (Wang, 2001). *Drosophila* spermatocytes and tissue culture cells may exemplify a general mechanism, in which astral microtubules initiate furrowing and then signals from the central spindle are necessary to stabilize and propagate furrow ingression. Different cell types might have adapted this general mechanism to meet specific

requirements, including extreme situations in which only one of the two components, astral arrays or the central spindle, is sufficient to induce both furrow formation and ingression. One important implication of this hypothesis, however, is that both populations of microtubules can deliver the same molecular signal to the cortex to promote furrowing.

Astral relaxation

In contrast to the astral stimulation hypothesis described above, the astral relaxation model postulates that astral microtubules inhibit furrowing at the cortical polar regions. This model originates from the observations that depolymerization of microtubules induces ectopic furrows and uncoordinated cortical contractions in mammalian cells and *C. elegans* embryos. For example, when mammalian cells are exposed to the microtubule-depolymerizing drug nocodazole in prometaphase and then are forced to enter anaphase by inactivation of the spindle assembly checkpoint, they exhibit mostly uncoordinated cortical contractions (Canman et al., 2000). The same drug also induces ectopic furrowing in *C. elegans* embryos, and embryos that have a defective Nedd-8 ubiquitin-like protein pathway display an almost identical phenotype (Kurz et al., 2002). Embryos with a defective Nedd-8 pathway have short astral microtubules probably because this pathway negatively regulates the activity of the microtubule-severing complex katanin. For this reason, these experiments have been interpreted as supporting the astral relaxation theory. However, inactivation of other proteins required for the growth of astral microtubules, such as the microtubule-associated proteins TAC-1 and ZYG-9, cause a different furrowing phenotype. In these mutants, astral microtubules are very short and the spindle becomes transversely oriented with respect to the antero-posterior axis and positioned abnormally close to the posterior pole of the embryo. As expected, one furrow forms at the posterior pole bisecting the spindle, whereas another one, parallel to the spindle axis, appears at the anterior pole (Bellanger and Gonczy, 2003; Le Bot et al., 2003; Matthews et al., 1998; Srayko et al., 2003). Thus, it seems unlikely that the reduction or absence of astral microtubules can, by itself, account for the multiple ectopic furrows observed after nocodazole treatment and inhibition of the Nedd-8 pathway. Rather, both treatments presumably have pleiotropic effects on spindle dynamics and cortex activity that ultimately lead to the uncoordinated cortical contractions.

More recently, Dechant and Glotzer proposed an alternative hypothesis that includes both relaxation and stimulation mechanisms (Dechant and Glotzer, 2003). They provided genetic evidence that, in *C. elegans*, centrosome separation is important for cleavage furrow formation and signals from the central spindle are only required subsequently for furrow ingression. Measurements of the density of microtubules in different compromised genetic backgrounds indicated an inverse correlation between microtubule density and furrow formation. This led them to propose that low microtubule density, generated by a critical degree of centrosome separation, could be the signal that triggers cleavage furrow formation. Subsequent signals from the central spindle would then be required for furrow ingression. In this scenario, if the spindle does not elongate sufficiently to create a 'local minimum' of microtubule density, then cleavage furrow

formation should be prevented. In this study, however, microtubule density was analysed in fixed preparations, which may not detect dynamic interactions between microtubules and the cortex. Time-lapse microscopy is needed to confirm these results and monitor microtubule behaviour during anaphase. In addition, this model cannot explain the furrowing phenotype observed in the *zyg-9* and *tac-1* mutants described above. Finally, if this hypothesis is correct, then one would expect that, if the centrosomes separate farther, multiple or ectopic furrows should form, as observed in microtubule depolymerization experiments. This does not seem to be the case. For example, in embryos that have mutant *air-2*, *zen-4* and *spd-1* genes, which respectively encode a kinase, a kinesin and a microtubule-binding protein required for cytokinesis (see below and Table 1), the spindle length is increased, but only a single furrow forms and does so at the proper location (Dechant and Glotzer, 2003; Verbrugghe and White, 2004). Intriguingly, whereas all three mutants lack a central spindle, cytokinesis is completed in *spd-1* mutants and fails in *zen-4* and *air-2* mutant embryos. Therefore, in *C. elegans*, cytokinesis can be successfully accomplished in the absence of a central spindle, but not without ZEN-4 and AIR-2 (see also below).

Molecules that regulate central spindle assembly and microtubule dynamics during cytokinesis

The centralspindlin complex

Despite the findings in *C. elegans*, the central spindle is crucial in most systems for successful completion of cytokinesis. Its assembly in all metazoans studied to date requires a highly conserved complex: centralspindlin. This comprises two components: an MKLP1 subfamily kinesin [ZEN-4 in *C. elegans*, Pavarotti (PAV) in *Drosophila* and MKLP1/2 in humans; Table 1] and a Rho-family GTPase-activating protein (CYK-4 in worms, RacGAP50C in flies and MgcRacGAP in mammals) (Mishima et al., 2002; Somers and Saint, 2003). Loss of either typically results in a diminished or absent central spindle. For example, inactivation of PAV or RacGAP50C in *Drosophila* dramatically affects central spindle assembly and prevents furrow formation (Adams et al., 1998; Goshima and Vale, 2003; Somers and Saint, 2003; Somma et al., 2002). Similarly, inactivation of either ZEN-4 or CYK-4 leads to failure of central spindle microtubule bundling and cytokinesis; in this case, the cleavage furrow initiates but then regresses (Jantsch-Plunger et al., 2000; Mishima et al., 2002; Powers et al., 1998; Raich et al., 1998). A similar phenotype is seen in mammalian cells when CHO1/MKLP1 is depleted; the central spindle is poorly formed and the furrow fails to ingress completely (Kuriyama et al., 2002; Liu et al., 2004; Matulienė and Kuriyama, 2002).

In contrast to lower metazoans, mammals have a second MKLP1-like protein, MKLP2. This motor was originally thought to be involved in vesicle transport and was named RAB6-kinesin (Echard et al., 1998), but subsequent studies indicated that, like MKLP1, the protein concentrates on the central spindle following chromosome disjunction (Fontijn et al., 2001; Gruneberg et al., 2004; Hill et al., 2000; Neef et al., 2003). Moreover, microinjection of antibodies and RNAi directed against MKLP2 phenocopy some aspects of MKLP1 inactivation, such as the formation of disorganized central spindles and furrows that briefly ingress (Fontijn et al., 2001;

Table 1. Names used in different organisms for the factors involved in central spindle and contractile ring assembly/dynamics during cytokinesis

Mammals	<i>Drosophila</i>	<i>Caenorhabditis elegans</i>	Function
Aurora B	Aurora B	AIR-2	Kinase
CIT-K	STI/DCK	Absent	Kinase
CLASP	ORBIT/MAST	CLS-2	MAP
ECT-2	PBL	LET-21	GEF
INCENP	INCENP	ICP-1	Centromeric protein
HKIF-4	KLP3A	KLP19	KLP
KIF18	KLP67A	Unknown	KLP
MgcRacGAP	RacGAP50C	CYK-4	GAP
MKLP1/2	PAV	ZEN-4	KLP
CHO1			
Plk1	Polo	PLC1/2	Kinase
PRC1	FEO	SPD-1 (?)*	MAP
ROCK	ROK	LET-502	Kinase

Abbreviations: GEF, GTP guanine nucleotide exchange factor; GAP, GTPase-activating protein; KLP, kinesin-like protein; MAP, microtubule-associated protein.

*The question mark indicates that the functional correspondence between SPD-1 and PRC-1 is still uncertain.

Hill et al., 2000; Neef et al., 2003). MKLP1 and MKLP2 might therefore have overlapping or redundant roles during cytokinesis, but distinct MKLP2 functions have been identified (see below).

Aurora B and Polo kinases

Recruitment of centralspindlin to the central spindle requires the chromosomal passenger proteins INCENP and Aurora B kinase, both of which are also part of the evolutionarily conserved Aurora B or chromosomal passenger protein complex (Adams et al., 2000; Adams et al., 2001a; Adams et al., 2001b; Kaitna et al., 2000). In *C. elegans*, disruption of this complex prevents stable ZEN-4 localization and furrows initiate but ultimately regress (Kaitna et al., 2000; Schumacher et al., 1998; Severson et al., 2000). Similarly, depletion of Aurora B in *Drosophila* or mammalian cells results in lack of a prominent central spindle and failed cytokinesis (Giet and Glover, 2001; Terada et al., 1998). How Aurora B exerts this effect is not fully understood. The *C. elegans* Aurora B orthologue, AIR-2, forms a complex with ZEN-4 in vitro (Severson et al., 2000). Moreover, in mammalian cells, the bundling of central spindle microtubules requires Aurora B kinase activity (Murata-Hori et al., 2002). Thus, one possibility is that Aurora B phosphorylates and somehow activates MKLP1. However, MKLP1 does not seem to be an Aurora B substrate (Neef et al., 2003). Furthermore, MKLP2 forms a complex with Aurora B and is required for the relocation of the passenger protein complex from the centromeres to the central spindle. Intriguingly, MKLP2-depleted cells still recruit MKLP1 to the central spindle. This indicates that, unlike in flies and worms, the chromosomal passenger protein complex is not required for MKLP1 localization in mammals (Gruneberg et al., 2004). Recent studies indicate that the RacGAP member of the centralspindlin complex is a target of Aurora B, but the exact role of this phosphorylation event is still unclear and controversial: whereas one study reported that Aurora B phosphorylation converts MgcRacGAP into a

RhoGAP (Minoshima et al., 2003), another showed that phosphorylation of different residues is actually needed to promote its GTPase activity towards Rac and Cdc42 (Ban et al., 2004).

Polo-like kinases (Plks) are also involved in central spindle formation. In *Drosophila*, PAV and Polo kinase are mutually dependent for localization on the central spindle (Adams et al., 1998; Carmena et al., 1998). This co-dependence reflects the formation of a conserved complex between MKLP1 members and Plks (Adams et al., 1998; Lee et al., 1995). In mammals, Plks can phosphorylate MKLP1 and this is necessary for its targeting to the central spindle (Lee et al., 1995; Liu et al., 2004). Likewise, MKLP2 is both a binding partner and a substrate of Plk1. Phosphorylation is not required for MKLP2 localization, because non-phosphorylatable mutants are still targeted to the central spindle; however, Plk1 no longer is (Neef et al., 2003). Thus, phosphorylation appears to regulate the interaction of Plk1 and MKLP2. In addition, the microtubule-bundling activity of MKLP2 is negatively regulated by Plk1 in vitro and, although it is unclear whether the same occurs in vivo, microinjection of antibodies against the phosphorylated domains of MKLP2 causes cytokinetic failure (Hill et al., 2000; Neef et al., 2003).

Kinesins recycled during cytokinesis

Two kinesin-like proteins (KLPs) that play a role in chromosome congression and segregation are also required for central spindle formation. The first of these, Klp67A in *Drosophila*, is a Kip3 subfamily microtubule catastrophe factor that redistributes from kinetochores to the central spindle at anaphase (Savoian et al., 2004). Prior to anaphase, Klp67A depolymerizes microtubules (Goshima and Vale, 2003; Gandhi et al., 2004; Savoian et al., 2004). Surprisingly, fixed cell studies revealed that *klp67A* mutant primary spermatocytes have poorly defined or absent central spindles (Gandhi et al., 2004; Savoian et al., 2004) (M. K. Gatt, M.S.S., M. G. Riparbelli, C. Massarelli, G. Callaini and D.M.G., unpublished). Time-lapse imaging of mutant embryos and primary spermatocytes indicated that central spindles often fail to form and, in the latter cell type, the few microtubule bundles present are highly unstable and rapidly degraded (Gandhi et al., 2004) (M. K. Gatt, M.S.S., M. G. Riparbelli, C. Massarelli, G. Callaini and D.M.G., unpublished). This suggests that Klp67A changes function after the metaphase-to-anaphase transition from a depolymerase to a microtubule-stabilizing factor.

The second KLP is the chromatin-associated chromokinesin KLP3A/KIF4 (Table 1). In *Drosophila*, mutation of *klp3A* results in greatly reduced or absent central spindles in spermatocytes and a similar phenotype is observed after microinjection of anti-KLP3A antibodies in embryos (Kwon et al., 2004; Williams et al., 1995). This is in striking contrast to RNAi studies in S2 cells, which suggested that KLP3A is not essential for central spindle formation (Kwon et al., 2004; Somma et al., 2002). However, this difference between cell types is likely to represent the exception rather than the rule, because the human orthologue of KLP3A, KIF4, is also needed for central spindle formation (Kurasawa et al., 2004; Mazumdar et al., 2004). Depletion of KIF4 disrupts the central spindle localization of MKLP1 and the chromosomal passengers, which become broadly distributed at the equator of

the cell (Kurasawa et al., 2004). KIF4 requirement in central spindle formation might also result from the perturbation of the activity of one of its binding partners, the microtubule-bundling protein PRC1 (see below). KIF4 silencing does not prevent targeting of PRC1 to the central spindle, but its localization becomes more diffuse (Kurasawa et al., 2004)

Microtubule-associated proteins (MAPs)

PRC1 was originally identified in a screen for CDK substrates and subsequently shown to be a central spindle component required for cytokinesis (Jiang et al., 1998). RNAi-mediated silencing of PRC1 in tissue culture cells prevents central spindle assembly because the two half spindles fail to interdigitate with one another (Mollinari et al., 2002). PRC1 depletion also phenocopies the mislocalization of the chromosomal passenger proteins and MKLP1 observed following RNAi-mediated knock down of KIF4. Interestingly, loss of PRC1 prevents the redistribution of KIF4 and CENP-E from the chromatin to the equator. Consistent with these phenotypes, PRC1 interacts with both MKLP1 and CENP-E (Kurasawa et al., 2004). Loss of the *Drosophila* PRC1 orthologue Fascetto (Feo) also leads to cytokinetic failure in larval neuroblasts and S2 cells (Verni et al., 2004). However, in these cells, interdigitation of the two half spindles does not appear to be affected. Nevertheless, the central spindle is highly disorganized, with both PAV and the microtubule minus-end-associating protein ASP (for 'abnormal spindle protein') becoming broadly distributed across the length of the central spindle microtubules.

Recent studies in *Drosophila* primary spermatocytes have identified a role for the microtubule plus-end-stabilizing protein Orbit/Mast in cytokinesis (Inoue et al., 2004). This protein associates with kinetochores and the spindle matrix and then redistributes to the central spindle following anaphase onset. Orbit, however, is unique because it specifically localizes to the interior central spindle and not to the peripheral astral microtubules. *orbit* mutants consistently lack a robust interior central spindle while maintaining stable bundles of peripheral astral microtubules. As a result, cleavage furrows initiate but subsequently fail (Inoue et al., 2004).

Temporal regulation of central spindle assembly

Two recent reports indicate that the assembly of the central spindle is regulated by the cyclin-dependent kinase CDK-1 (Mishima et al., 2004; Zhu and Jiang, 2005). In mammals, phosphorylation of PRC-1 by CDK-1 prevents its interaction with KIF-4 and consequent translocation to the plus ends of the central spindle microtubules. It is only after the inactivation of CDK-1 by the anaphase-promoting complex (APC) that PRC1 is dephosphorylated and binds KIF-4 (Zhu and Jiang, 2005).

A similar mechanism also regulates the activation of the centralspindlin complex at anaphase onset (Mishima et al., 2004). Mishima et al. presented evidence that phosphorylation of ZEN-4 by CDK-1 inhibits its microtubule-binding activity. Inactivation of CDK-1 by APC and subsequent dephosphorylation of ZEN-4 would then allow the centralspindlin complex to bind microtubules. Mishima et al. have suggested that the CDC-14 phosphatase may be the

enzyme responsible for ZEN-4 dephosphorylation, but this has been challenged by results showing that *cdc-14* mutants are viable and do not present cytokinesis defects (Saito et al., 2004). This is further supported by observations in *Drosophila* indicating that RNAi-mediated depletion of CDC-14 does not impair cytokinesis (Eggert et al., 2004; L. Capalbo and D.M.G., unpublished). Thus, another, unidentified, phosphatase is required to activate the centralspindlin complex at anaphase onset, possibly in conjunction with CDC-14.

Regulation of cortical activity during cytokinesis

The mitotic spindle must be able to influence the equatorial cortex to promote furrow formation and ingression. Structural studies have revealed that an actomyosin contractile ring forms at the equatorial cortex during cytokinesis (Satterwhite and Pollard, 1992). This ring is organized on a scaffold of cytoskeletal proteins such as the septins and the actin-binding protein anillin, which might also regulate its interaction with the cell membrane (Field and Alberts, 1995; Giansanti et al., 1999; Kinoshita and Noda, 2001; Neufeld and Rubin, 1994). For many years it has been suggested that a stable actomyosin ring constricts towards the centre through a 'purse-string'-like mechanism. However, this model has been challenged by evidence that the contractile ring is a highly dynamic structure that probably has multiple independent furrowing units whose components are continuously assembled and disassembled (Savoian et al., 1999b). Indeed, many proteins that regulate actin dynamics, such as cofilin, profilin and members of the diaphanous (Dia) family, localize to the cleavage furrow and are required for cytokinesis (Castrillon and Wasserman, 1994; Giansanti et al., 1998; Gunsalus et al., 1995; Somma et al., 2002; Swan et al., 1998).

Not surprisingly, members of the small Rho GTPase family have been implicated as key regulators of the actomyosin ring. Among these well-known cytoskeletal regulators, RhoA appears to play a crucial role, because its inactivation by mutation, drug treatment or RNAi impairs cytokinesis in many systems. Moreover, the RhoGEF PEBBLE/ECT2 (Table 1) is required for cleavage furrow formation, which supports the idea that Rho activation is crucial for cytokinesis (Prokopenko et al., 1999; Tatsumoto et al., 1999). RhoA has been proposed to regulate actomyosin ring dynamics through several signalling pathways (Fig. 2). By binding members of the Formin-homology (FH) protein family, it could activate profilin and promote polymerization of G-actin into F-actin (Fig. 2) (Watanabe et al., 1997). In addition, by acting through two distinct kinase effectors, citron kinase (CIT-K) and Rho kinase (ROK), this GTPase could also control the organization and contractility of the ring (Fig. 2) (D'Avino et al., 2004; Kosako et al., 2000; Madaule et al., 1998). Genetic and pharmacological evidence indicates that ROK is involved in cytokinesis in worms, flies and mammalian cells, although it appears to play an ancillary and nonessential role in ring constriction (Bettencourt-Dias et al., 2004; Eggert et al., 2004; Kosako et al., 2000; Piekny and Mains, 2002; Winter et al., 2001).

The precise role of CIT-K during cytokinesis is still controversial. Expression of truncated variants in mammalian cells induces cytokinesis defects and a failure in ring contraction, results that led to the proposal that CIT-K is important for actomyosin contractility (Madaule et al., 1998;

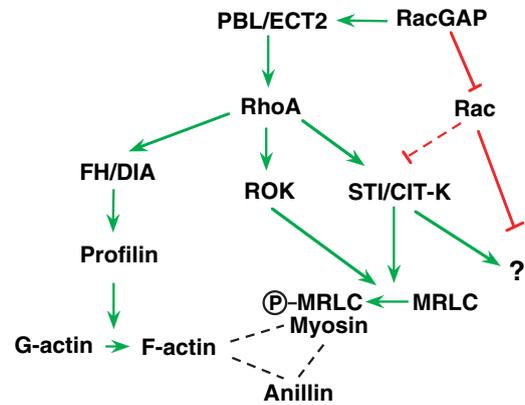


Fig. 2. The signalling pathways that control cortical activity during cytokinesis. The green arrows indicate activation and the red lines denote inhibition. The dotted red line suggests the possibility that Rac directly suppresses STI/CIT-K activity. The question mark indicates putative STI targets that have yet to be identified. The dotted black lines indicate the interactions of anillin with actin and myosin.

Madaule et al., 2000). However, CIT-K-knockout mice display cytokinesis defects only in some cell populations of the central nervous system and during spermatogenesis, indicating that CIT-K may be a tissue-specific Rho effector during cytokinesis (Di Cunto et al., 2000; Di Cunto et al., 2002). By contrast, the *Drosophila* CIT-K orthologue, Sticky (STI), is essential for cell division in all tissues, and its inactivation by mutation or RNAi leads to late cytokinesis failure accompanied by the formation of abnormal actin blebs at the equatorial cortex (D'Avino et al., 2004; Echard et al., 2004; Naim et al., 2004; Shandala et al., 2004). CIT-K in flies might thus be important for contractile ring organization rather than contractility (D'Avino et al., 2004). Cells depleted of the contractile ring component anillin exhibit an almost identical defect (Somma et al., 2002). This led to the hypothesis that STI/CIT-K regulates anillin activity (D'Avino et al., 2004; Naim et al., 2004). Straight et al. have now shown that anillin can also bind the non-muscle myosin II and that this interaction is regulated through phosphorylation of its regulatory light chain (MRLC) (Straight et al., 2005). Since biochemical and genetic evidence indicated that MRLC is a target of STI/CIT-K (D'Avino et al., 2004; Yamashiro et al., 2003), we can infer that this kinase controls the organization of the actomyosin ring by regulating myosin-anillin-actin interactions (Fig. 2).

Notably, although both CIT-K and ROK can phosphorylate MRLC, the effects of these two phosphorylation events are completely different: ROK controls the contraction, whereas CIT-K regulates the organization of the ring. A potential explanation comes from studies by Yamashiro et al., who showed that CIT-K induces di-phosphorylation, rather than mono-phosphorylation, of MRLC and that di-phosphorylated MRLC has a more constrained localization at the cleavage furrow than the mono-phosphorylated form (Yamashiro et al., 2003). These results, together with previous observations that MRLC di-phosphorylation can affect filament assembly (Ikebe and Hartshorne, 1985), led to the proposal that di-phosphorylated MRLC plays a role in crosslinking of actin filaments rather than stimulation of motor activity.

The Rac GTPases, members of the Rho GTPase family, are also emerging as important regulators of the behaviour of the cortex during cytokinesis (D'Avino et al., 2004; Muris et al., 2002; Yoshizaki et al., 2003; Yoshizaki et al., 2004). Studies in *Drosophila* and mammals indicate that Rac activity needs to be repressed during furrow ingression and that this inhibition is mediated by the RacGAP component of the centralspindlin complex (D'Avino et al., 2004; Yoshizaki et al., 2004) (Fig. 2). Genetic evidence in flies suggests that Rac antagonizes Rho signalling by inhibiting, directly or indirectly, STI/CIT-K activity and that RacGAP50C functions as a Rac repressor (D'Avino et al., 2004). Expression of a constitutively active form of Rac induces the formation of multinucleate cells in several mammalian cell types (Muris et al., 2002; Yoshizaki et al., 2004). Consistent with these findings, fluorescence resonance energy transfer (FRET) analysis has indicated that Rac activity decreases at the cleavage furrow during cytokinesis and that this inactivation is abolished by the expression of a dominant-negative form of MgcRacGAP (Yoshizaki et al., 2003; Yoshizaki et al., 2004). Interestingly, in some cell types, Rac inhibition appears to be sufficient for cleavage, because cytokinesis can be successfully achieved in the presence of the Rho-inactivating drug C3 (Yoshizaki et al., 2004).

Why do Rac GTPases need to be repressed during cytokinesis? The success of cytokinesis depends not only on the contraction of the actomyosin-based machinery (activated by Rho) but also on a reduced stiffness of the cell cortex (Robinson and Spudich, 2000). This, together with observations of the organization of cortical actin filaments (Fishkind and Wang, 1993), led Wang to propose that cleavage furrow ingression could be primarily triggered by a global cortical contraction associated with a localized collapse at the equatorial cortex rather than by enhanced contractility along the equator (Wang, 2001). The first element of this model is that actin filaments extend over a range of different angles along the cortex and their interaction with myosin during cytokinesis generates both inward and lateral forces. The second assumption is that the signal delivered by astral or central spindle microtubules induces local weakening of the equatorial cortical region by altering the organization of the actin filaments without affecting their interaction with myosin. The combination of this local weakening with cortical contraction forces then triggers furrow ingression. In such a scenario, inhibition of Rac activity could be the triggering event that causes localized equatorial cortical collapse. Unfortunately, changes in the activity of members of the Rho GTPase family during mitosis are not completely understood. RhoA activity appears to be essential for the increase in cortical rigidity associated with the cell rounding observed in many cultured cells upon entering mitosis (Maddox and BurrIDGE, 2003). This rigidity is likely to be the result of a specific equilibrium between Rho and Rac signalling pathways at the cortex, because these two GTPases antagonize each other in several biological processes (BurrIDGE and Wennerberg, 2004). The outcome of this antagonism is a rigid meshwork of cortical actomyosin filaments whose contractility and dynamics are low because of the inhibitory activity of Rac (Fig. 3). In metaphase, the filaments at the equatorial cortex are preferentially aligned along the spindle axis (Fishkind and Wang, 1993) (Fig. 3). During cytokinesis, Rac suppression at the equator could

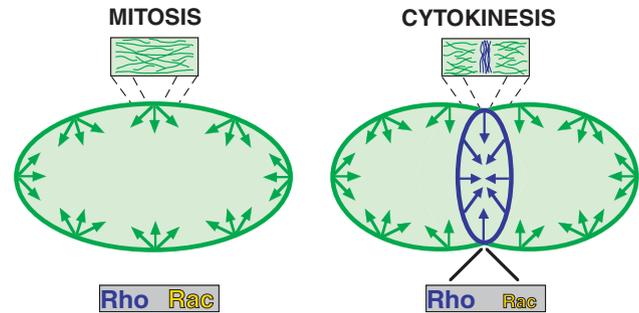


Fig. 3. A model depicting the role of the antagonism between RhoA and Rac GTPases in regulating cortical activity during mitosis and cytokinesis. The arrows denote the cortical forces that mediate cell rounding during mitosis and cleavage ingression during cytokinesis. Equilibrium between RhoA (blue) and Rac (yellow) activities generates a 'green' rigid cortex and the relative forces necessary for cell rounding during mitosis. The 'blue' ring indicates the high RhoA activity at the equatorial cortex required for contractile ring formation and furrow ingression. The rectangles illustrate a view from the top of the actomyosin filaments at the equatorial cortex.

release cortical stiffness by depolymerizing actomyosin filaments or inactivating crosslinking factors. This would allow Rho to rearrange the filaments in dense bundles oriented parallel to the equator to promote furrowing (Fig. 3). Interestingly, FRET analysis has also indicated that, during cytokinesis, Rac activity increases at the polar cortical regions (Yoshizaki et al., 2003), possibly to allow a certain degree of cortical expansion that complements furrow constriction.

This model can explain at least two puzzling observations. First, in some cell types, the contractile ring is not easily detected or Rho activation seems dispensable (Cao and Wang, 1990; Yoshizaki et al., 2004). One possibility is that, in small round cells, equatorial collapse (i.e. Rac inactivation) is sufficient for complete ingression of the furrow whereas large cells require both equatorial collapse and Rho-mediated reorganization and contraction of the actomyosin ring. Second, in many systems, ROK-mediated contractility appears to play a facilitating rather than essential role (Bettencourt-Dias et al., 2004; Eggert et al., 2004; Kosako et al., 2000; Piekny and Mains, 2002; Winter et al., 2001). In this case, equatorial collapse and Rho-mediated reorganization of actomyosin filaments at the equatorial cortex could be sufficient to promote and propagate furrow ingression even in the absence of myosin-mediated contractility.

The furrow-inducing signal

The nature of the signal that promotes the formation and position of the cleavage furrow is probably the most important unanswered question in cytokinesis. In principle, this signal, if it exists, should possess at least three major properties: (1) it must be necessary and possibly sufficient for furrow formation; (2) it must be either activated or delivered to the cortex just before furrow ingression; and (3) it must be able to regulate the signalling pathways that control cleavage furrow formation and ingression (Fig. 2). Several observations in *Drosophila* indicate that the centralspindlin complex has all of these properties. First, inactivation of either member of the complex,

PAV or RacGAP50C, prevents furrow formation (Adams et al., 1998; Goshima and Vale, 2003; Somers and Saint, 2003; Somma et al., 2002). Moreover, expression of a PAV variant that lacks motor activity prevents furrow formation and results in the abnormal accumulation of both the mutated protein and actin at the spindle poles; this indicates that the complex recruits at least one component of the contractile ring (Minestrini et al., 2003). Second, both members of the complex localize and concentrate at the equatorial cortex after anaphase onset in embryos and S2 cells (Adams et al., 1998; Minestrini et al., 2003; Somers and Saint, 2003; Somma et al., 2002). In addition, time-lapse analysis of primary spermatocytes has revealed that green fluorescent protein (GFP)-tagged PAV decorates the plus ends of the ‘peripheral astral’ microtubules that contact the cortex at the cleavage site (M.S.S. and D.M.G., unpublished). Third, the studies described above indicate that, in both flies and mammals, the RacGAP member of the complex inhibits Rac activity at the cleavage furrow to allow its constriction (D’Avino et al., 2004; Yoshizaki et al., 2004). In addition, Somers and Saint have reported that RacGAP50C physically interacts with the RhoGEF Pebble (PBL), and genetic data suggest that this promotes Rho activation (Somers and Saint, 2003; D’Avino et al., 2004). Thus, the RacGAP member of the centralspindlin complex appears to be able to regulate the two major signalling pathways that control actomyosin dynamics during cytokinesis (Fig. 2).

One can therefore propose a comprehensive model for cytokinesis in which the motor component of the centralspindlin complex delivers RacGAP to the equatorial cortex to promote cleavage furrow formation and contractile ring assembly (Fig. 4). In this model, the complex also mediates, along with other MAPs and KLPs, microtubule bundling to ensure the stability of the central spindle and provide a continuous source of furrow-promoting activity. In addition, Aurora B and Polo phosphorylation should play an important role regulating the activity of the complex. This model, also known as the ‘double-ring’ hypothesis (Saint and Somers, 2003), can explain several observations. For example, the presence of centralspindlin on both astral and central spindle microtubules would explain why both populations of microtubules have the ability to promote furrowing (see above). In situations in which the central spindle is compromised, cleavage furrow ingression usually begins and then aborts. This indicates that the complex localized to the astral microtubule ends is sufficient to initiate furrowing, but probably requires the continuous supply of active complex from the central spindle to promote furrow progression.

Although several studies in *Drosophila* indicate that the centralspindlin complex represents the long sought-after furrow-inducing signal, it is unclear whether this mechanism

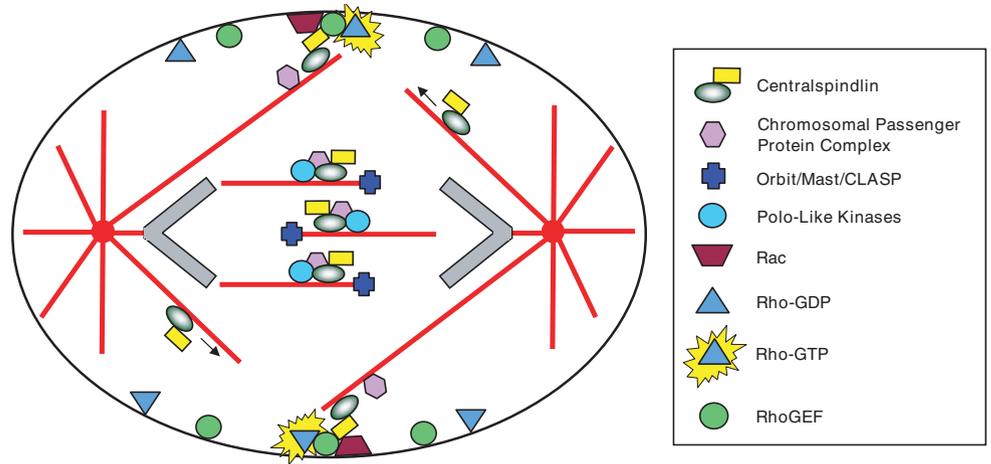


Fig. 4. A schematic diagram depicting the role of the centralspindlin complex and other factors involved in furrow formation and ingression. The centralspindlin comprises a motor component (green oval) and a RacGAP protein (yellow rectangle). Other MAPs and KLPs important for central spindle formation such as PRC1, KLP3A/KIF4 and KLP67A are not indicated.

has been conserved in other organisms. In mammals, the scenario is complicated by the evolutionary duplication of the motor component of the complex to give MKLP1 and MKLP2, which appear to have redundant and specific functions (see above). RNAi-mediated depletion of CHO1/MKLP1 causes late failure in cytokinesis whereas, in MKLP2-silenced cells, the furrow briefly ingresses and then relaxes (Matuliene and Kuriyama, 2002; Neef et al., 2003). Unfortunately, the cytokinesis phenotype of cells depleted of both kinesins has not been reported, although it is likely to be more severe than that of the single RNAi knockdowns. As for MgcRacGAP, no detailed loss-of-function analysis of furrow formation has been documented, and most studies report the effects of a ‘dominant-negative’ variant containing a mutated GAP domain (Hirose et al., 2001; Lee, J. S. et al., 2004). In this mutant, however, the domains able to interact with either MKLP1/2 or the PBL counterpart ECT2 are unaffected, and therefore not all MgcRacGAP functions are eliminated. Thus, the precise roles of the centralspindlin complex in mammals remain unclear.

In *C. elegans*, the centralspindlin complex does not appear to be required for the early stages of cytokinesis because inactivation of either member of the complex (encoded by *zen-4* and *cyk-4*, see above) causes late cytokinesis failure (Jantsch-Plunger et al., 2000; Mishima et al., 2002; Powers et al., 1998; Raich et al., 1998). Moreover, in this organism, the role of the complex in furrow ingression appears to be independent of its function in central spindle formation because, in *spd-1* mutants, which lack a central spindle, cytokinesis completes successfully (Verbrugghe and White, 2004). Consistent with this is the observation that ZEN-4 localizes to the cleavage furrow even in the absence of a central spindle (Verbrugghe and White, 2004). However, most of the studies of cytokinesis in *C. elegans* have been performed during the first, asymmetric, embryonic division. At this stage, the embryonic cortex is strongly polarized along the anterior-posterior (AP) axis and the cortical properties of the anterior and posterior regions are markedly different, as reflected by the different cortical morphology and actin localization (reviewed by Schneider and Bowerman, 2003). Thus, additional cortical cues might

establish the proper asymmetric position of the cleavage furrow. Such an hypothesis has been discarded by Dechant and Glotzer on the basis that furrow initiation is prevented only in embryos lacking the ZEN-4 kinesin and some of the AP asymmetry determinants, such as the PAR-2 and G α proteins (Dechant and Glotzer, 2003). By contrast, embryos depleted of ZEN-4 and another AP determinant, PAR-3, display a furrowing activity similar to that of animals lacking only ZEN-4. This led the authors to suggest that spindle elongation/centrosome separation, rather than AP asymmetry, plays a key role in furrow positioning by regulating microtubule density at the cortex (see above). However, direct evidence that high microtubule density can indeed inhibit furrowing is still lacking. Moreover, cortical pulling forces are responsible for centrosome separation in *C. elegans*, and these forces are weak in *par-2* and *G α* mutants but strong in *par-3* mutants (Colombo et al., 2003; Grill et al., 2001). Thus, an alternative explanation is that strong cortical pulling forces promote furrow initiation in the absence of the centralspindlin complex, perhaps through a mechanism similar to equatorial collapse (Wang, 2001). In this respect, it would be informative to know whether the centralspindlin complex is necessary for furrow formation during symmetric divisions in *C. elegans*.

Concluding remarks and future perspectives

Research in the field of cytokinesis has taken a considerable leap forward in recent years. Genetic analyses in model organisms such as *C. elegans* and *Drosophila* have given a significant boost to our understanding of the mechanisms that control this finely orchestrated process. We are on the verge of solving the puzzle of what controls the position and the ingression of the cleavage furrow. A growing body of evidence indicate that the contact of astral microtubules with the equatorial cortex promote furrow formation, supporting the astral stimulation model. However, the possibility that astral microtubules influence the cortical activity of polar regions cannot be discarded. The key mechanism that triggers furrow formation in most systems seems to be the local reorganization of the actomyosin cytoskeleton at the equator. Here, we propose that this reorganization is mediated by the RacGAP member of the centralspindlin complex, which can inhibit Rac GTPases and activate RhoA through its interaction with the RhoGEF PBL/ECT2 (Fig. 4). Thus, the crucial event for furrow formation and ingression would be the microtubule-mediated delivery of the centralspindlin complex to the cortex, where it acts as a master regulator of both processes. If this model is correct, then several other questions need to be answered. For example, what controls the stability of the astral microtubules that contact the equatorial cortex at the cleavage site and how does centralspindlin selectively localize to the plus ends of these microtubules? We also need to elucidate the exact roles of Polo and Aurora B kinases in controlling the activity of the complex during cytokinesis. Finally, how is membrane insertion co-ordinated with furrow ingression? The identification of the pathways and molecules that mediate the addition of new membrane at the cleavage site is emerging as one of the most challenging and interesting research areas in cytokinesis (Albertson et al., 2005; Strickland and Burgess, 2004). Thanks to the application of genomics and proteomics, most of the players involved in cell cleavage have been

identified (Eggert et al., 2004; Skop et al., 2004), and the next challenge will be to understand the interplay between these molecules and the signalling pathways they regulate.

We thank two anonymous referees for helpful suggestions. Work in the D.M.G. laboratory is supported by Cancer Research UK, MRC and BBSRC.

References

- Adams, R. R., Tavares, A. A., Salzberg, A., Bellen, H. J. and Glover, D. M. (1998). *pavarotti* encodes a kinesin-like protein required to organize the central spindle and contractile ring for cytokinesis. *Genes. Dev.* **12**, 1483-1494.
- Adams, R. R., Wheatley, S. P., Gouldsworthy, A. M., Kandels-Lewis, S. E., Carmena, M., Smythe, C., Gerloff, D. L. and Earnshaw, W. C. (2000). INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. *Curr. Biol.* **10**, 1075-1078.
- Adams, R. R., Eckley, D. M., Vagnarelli, P., Wheatley, S. P., Gerloff, D. L., Mackay, A. M., Svingen, P. A., Kaufmann, S. H. and Earnshaw, W. C. (2001a). Human INCENP colocalizes with the Aurora-B/AIRK2 kinase on chromosomes and is overexpressed in tumour cells. *Chromosoma* **110**, 65-74.
- Adams, R. R., Maiato, H., Earnshaw, W. C. and Carmena, M. (2001b). Essential roles of *Drosophila* inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. *J. Cell Biol.* **153**, 865-880.
- Albertson, R., Riggs, B. and Sullivan, W. (2005). Membrane traffic: a driving force in cytokinesis. *Trends. Cell Biol.* **15**, 92-101.
- Alsop, G. B. and Zhang, D. (2003). Microtubules are the only structural constituent of the spindle apparatus required for induction of cell cleavage. *J. Cell Biol.* **162**, 383-390.
- Ban, R., Irino, Y., Fukami, K. and Tanaka, H. (2004). Human mitotic spindle-associated protein PRC1 inhibits MgcRacGAP activity toward Cdc42 during the metaphase. *J. Biol. Chem.* **279**, 16394-16402.
- Bellanger, J. M. and Gonczy, P. (2003). TAC-1 and ZYG-9 form a complex that promotes microtubule assembly in *C. elegans* embryos. *Curr. Biol.* **13**, 1488-1498.
- Bettencourt-Dias, M., Giet, R., Sinka, R., Mazumdar, A., Lock, W. G., Balloux, F., Zafiroopoulos, P. J., Yamaguchi, S., Winter, S., Carthew, R. W. et al. (2004). Genome-wide survey of protein kinases required for cell cycle progression. *Nature* **432**, 980-987.
- Bucciarelli, E., Giansanti, M. G., Bonaccorsi, S. and Gatti, M. (2003). Spindle assembly and cytokinesis in the absence of chromosomes during *Drosophila* male meiosis. *J. Cell Biol.* **160**, 993-999.
- Burridge, K. and Wennerberg, K. (2004). Rho and rac take center stage. *Cell* **116**, 167-179.
- Canman, J. C., Hoffman, D. B. and Salmon, E. D. (2000). The role of pre- and post-anaphase microtubules in the cytokinesis phase of the cell cycle. *Curr. Biol.* **10**, 611-614.
- Canman, J. C., Cameron, L. A., Maddox, P. S., Straight, A., Tirnauer, J. S., Mitchison, T. J., Fang, G., Kapoor, T. M. and Salmon, E. D. (2003). Determining the position of the cell division plane. *Nature* **424**, 1074-1078.
- Cao, L. G. and Wang, Y. L. (1990). Mechanism of the formation of contractile ring in dividing cultured animal cells. I. Recruitment of preexisting actin filaments into the cleavage furrow. *J. Cell Biol.* **110**, 1089-1095.
- Cao, L. G. and Wang, Y. L. (1996). Signals from the spindle midzone are required for the stimulation of cytokinesis in cultured epithelial cells. *Mol. Biol. Cell* **7**, 225-232.
- Carmena, M., Riparbelli, M. G., Minestrini, G., Tavares, A. M., Adams, R., Callaini, G. and Glover, D. M. (1998). *Drosophila* polo kinase is required for cytokinesis. *J. Cell Biol.* **143**, 659-671.
- Castrillon, D. H. and Wasserman, S. A. (1994). Diaphanous is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the limb deformity gene. *Development* **120**, 3367-3377.
- Colombo, K., Grill, S. W., Kimple, R. J., Willard, F. S., Siderovski, D. P. and Gonczy, P. (2003). Translation of polarity cues into asymmetric spindle positioning in *Caenorhabditis elegans* embryos. *Science* **300**, 1957-1961.
- D'Avino, P. P., Savoian, M. S. and Glover, D. M. (2004). Mutations in sticky

- lead to defective organization of the contractile ring during cytokinesis and are enhanced by Rho and suppressed by Rac. *J. Cell Biol.* **166**, 61-71.
- Dechant, R. and Glotzer, M.** (2003). Centrosome separation and central spindle assembly act in redundant pathways that regulate microtubule density and trigger cleavage furrow formation. *Dev. Cell* **4**, 333-344.
- Di Cunto, F., Imarisio, S., Hirsch, E., Broccoli, V., Bulfone, A., Migheli, A., Atzori, C., Turco, E., Triolo, R., Dotto, G. P. et al.** (2000). Defective neurogenesis in citron kinase knockout mice by altered cytokinesis and massive apoptosis. *Neuron* **28**, 115-127.
- Di Cunto, F. D., Imarisio, S., Camera, P., Boitani, C., Altruda, F. and Silengo, L.** (2002). Essential role of citron kinase in cytokinesis of spermatogenic precursors. *J. Cell Sci.* **115**, 4819-4826.
- Echard, A., Jollivet, F., Martinez, O., Lacapere, J. J., Rousselet, A., Janoueix-Lerosey, I. and Goud, B.** (1998). Interaction of a Golgi-associated kinesin-like protein with Rab6. *Science* **279**, 580-585.
- Echard, A., Hickson, G. R., Foley, E. and O'Farrell, P. H.** (2004). Terminal cytokinesis events uncovered after an RNAi screen. *Curr. Biol.* **14**, 1685-1693.
- Eggert, U. S., Kiger, A. A., Richter, C., Perlman, Z. E., Perrimon, N., Mitchison, T. J. and Field, C. M.** (2004). Parallel chemical genetic and genome-wide RNAi screens identify cytokinesis inhibitors and targets. *PLoS Biol.* **2**, e379.
- Field, C. M. and Alberts, B. M.** (1995). Anillin, a contractile ring protein that cycles from the nucleus to the cell cortex. *J. Cell Biol.* **131**, 165-178.
- Fishkind, D. J. and Wang, Y. L.** (1993). Orientation and three-dimensional organization of actin filaments in dividing cultured cells. *J. Cell Biol.* **123**, 837-848.
- Fontijn, R. D., Goud, B., Echard, A., Jollivet, F., van Marle, J., Pannekoek, H. and Horrevoets, A. J.** (2001). The human kinesin-like protein RB6K is under tight cell cycle control and is essential for cytokinesis. *Mol. Cell. Biol.* **21**, 2944-2955.
- Gandhi, R., Bonaccorsi, S., Wentworth, D., Doxsey, S., Gatti, M. and Pereira, A.** (2004). The *Drosophila* kinesin-like protein KLP67A is essential for mitotic and male meiotic spindle assembly. *Mol. Biol. Cell* **15**, 121-131.
- Gatti, M., Giansanti, M. G. and Bonaccorsi, S.** (2000). Relationships between the central spindle and the contractile ring during cytokinesis in animal cells. *Microsc. Res. Tech.* **49**, 202-208.
- Giansanti, M. G., Bonaccorsi, S., Williams, B., Williams, E. V., Santolamazza, C., Goldberg, M. L. and Gatti, M.** (1998). Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis. *Genes. Dev.* **12**, 396-410.
- Giansanti, M. G., Bonaccorsi, S. and Gatti, M.** (1999). The role of anillin in meiotic cytokinesis of *Drosophila* males. *J. Cell Sci.* **112**, 2323-2334.
- Giet, R. and Glover, D. M.** (2001). *Drosophila* Aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. *J. Cell Biol.* **152**, 669-682.
- Goshima, G. and Vale, R. D.** (2003). The roles of microtubule-based motor proteins in mitosis: comprehensive RNAi analysis in the *Drosophila* S2 cell line. *J. Cell Biol.* **162**, 1003-1016.
- Grill, S. W., Gonczy, P., Stelzer, E. H. and Hyman, A. A.** (2001). Polarity controls forces governing asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. *Nature* **409**, 630-633.
- Gruneberg, U., Neef, R., Honda, R., Nigg, E. A. and Barr, F. A.** (2004). Relocation of Aurora B from centromeres to the central spindle at the metaphase to anaphase transition requires MKlp2. *J. Cell Biol.* **166**, 167-172.
- Gunsalus, K. C., Bonaccorsi, S., Williams, E., Verni, F., Gatti, M. and Goldberg, M. L.** (1995). Mutations in twinstar, a *Drosophila* gene encoding a cofilin/ADF homologue, result in defects in centrosome migration and cytokinesis. *J. Cell Biol.* **131**, 1243-1259.
- Hill, E., Clarke, M. and Barr, F. A.** (2000). The Rab6-binding kinesin, Rab6-KIFL, is required for cytokinesis. *EMBO J.* **19**, 5711-5719.
- Hirose, K., Kawashima, T., Iwamoto, I., Nosaka, T. and Kitamura, T.** (2001). MgcRacGAP is involved in cytokinesis through associating with mitotic spindle and midbody. *J. Biol. Chem.* **276**, 5821-5828.
- Ikebe, M. and Hartshorne, D. J.** (1985). Phosphorylation of smooth muscle myosin at two distinct sites by myosin light chain kinase. *J. Biol. Chem.* **260**, 10027-10031.
- Inoue, Y. H., Savoian, M. S., Suzuki, T., Mathe, E., Yamamoto, M. T. and Glover, D. M.** (2004). Mutations in orbit/mast reveal that the central spindle is comprised of two microtubule populations, those that initiate cleavage and those that propagate furrow ingression. *J. Cell Biol.* **166**, 49-60.
- Jantsch-Plunger, V., Gonczy, P., Romano, A., Schnabel, H., Hamill, D., Schnabel, R., Hyman, A. A. and Glotzer, M.** (2000). CYK-4: a Rho family gtpase activating protein (GAP) required for central spindle formation and cytokinesis. *J. Cell Biol.* **149**, 1391-1404.
- Jiang, W., Jimenez, G., Wells, N. J., Hope, T. J., Wahl, G. M., Hunter, T. and Fukunaga, R.** (1998). PRC1: a human mitotic spindle-associated CDK substrate protein required for cytokinesis. *Mol. Cell* **2**, 877-885.
- Kaitna, S., Mendoza, M., Jantsch-Plunger, V. and Glotzer, M.** (2000). Incenp and an aurora-like kinase form a complex essential for chromosome segregation and efficient completion of cytokinesis. *Curr. Biol.* **10**, 1172-1181.
- Khodjakov, A. and Rieder, C. L.** (2001). Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. *J. Cell Biol.* **153**, 237-242.
- Kinoshita, M. and Noda, M.** (2001). Roles of septins in the mammalian cytokinesis machinery. *Cell Struct. Funct.* **26**, 667-670.
- Kosako, H., Yoshida, T., Matsumura, F., Ishizaki, T., Narumiya, S. and Inagaki, M.** (2000). Rho-kinase/ROCK is involved in cytokinesis through the phosphorylation of myosin light chain and not ezrin/radixin/moesin proteins at the cleavage furrow. *Oncogene* **19**, 6059-6064.
- Kurasawa, Y., Earnshaw, W. C., Mochizuki, Y., Dohmae, N. and Todokoro, K.** (2004). Essential roles of KIF4 and its binding partner PRC1 in organized central spindle midzone formation. *EMBO J.* **23**, 3237-3248.
- Kuriyama, R., Gustus, C., Terada, Y., Uetake, Y. and Matulieni, E.** (2002). CHO1, a mammalian kinesin-like protein, interacts with F-actin and is involved in the terminal phase of cytokinesis. *J. Cell Biol.* **156**, 783-790.
- Kurz, T., Pintard, L., Willis, J. H., Hamill, D. R., Gonczy, P., Peter, M. and Bowerman, B.** (2002). Cytoskeletal regulation by the Nedd8 ubiquitin-like protein modification pathway. *Science* **295**, 1294-1298.
- Kwon, M., Morales-Mulia, S., Brust-Mascher, I., Rogers, G. C., Sharp, D. J. and Scholey, J. M.** (2004). The chromokinesin, KLP3A, dives mitotic spindle pole separation during prometaphase and anaphase and facilitates chromatid motility. *Mol. Biol. Cell* **15**, 219-233.
- Le Bot, N., Tsai, M. C., Andrews, R. K. and Ahringer, J.** (2003). TAC-1, a regulator of microtubule length in the *C. elegans* embryo. *Curr. Biol.* **13**, 1499-1505.
- Lee, J. S., Kamijo, K., Ohara, N., Kitamura, T. and Miki, T.** (2004). MgcRacGAP regulates cortical activity through RhoA during cytokinesis. *Exp. Cell Res.* **293**, 275-282.
- Lee, K. S., Yuan, Y. L., Kuriyama, R. and Erikson, R. L.** (1995). Plk is an M-phase-specific protein kinase and interacts with a kinesin-like protein, CHO1/MKLP-1. *Mol. Cell. Biol.* **15**, 7143-7151.
- Liu, X., Zhou, T., Kuriyama, R. and Erikson, R. L.** (2004). Molecular interactions of Polo-like-kinase 1 with the mitotic kinesin-like protein CHO1/MKLP-1. *J. Cell Sci.* **117**, 3233-3246.
- Madaule, P., Eda, M., Watanabe, N., Fujisawa, K., Matsuoka, T., Bito, H., Ishizaki, T. and Narumiya, S.** (1998). Role of citron kinase as a target of the small GTPase Rho in cytokinesis. *Nature* **394**, 491-494.
- Madaule, P., Furuhashiki, T., Eda, M., Bito, H., Ishizaki, T. and Narumiya, S.** (2000). Citron, a Rho target that affects contractility during cytokinesis. *Microsc. Res. Tech.* **49**, 123-126.
- Maddox, A. S. and Burridge, K.** (2003). RhoA is required for cortical retraction and rigidity during mitotic cell rounding. *J. Cell Biol.* **160**, 255-265.
- Matthews, L. R., Carter, P., Thierry-Mieg, D. and Kemphues, K.** (1998). ZYG-9, a *Caenorhabditis elegans* protein required for microtubule organization and function, is a component of meiotic and mitotic spindle poles. *J. Cell Biol.* **141**, 1159-1168.
- Matulieni, J. and Kuriyama, R.** (2002). Kinesin-like protein CHO1 is required for the formation of midbody matrix and the completion of cytokinesis in mammalian cells. *Mol. Biol. Cell* **13**, 1832-1845.
- Mazumdar, M., Sundareshan, S. and Misteli, T.** (2004). Human chromokinesin KIF4A functions in chromosome condensation and segregation. *J. Cell Biol.* **166**, 613-620.
- Megraw, T. L., Kao, L. R. and Kaufman, T. C.** (2001). Zygotic development without functional mitotic centrosomes. *Curr. Biol.* **11**, 116-120.
- Minestrini, G., Harley, A. S. and Glover, D. M.** (2003). Localization of Pavarotti-KLP in living *Drosophila* embryos suggests roles in reorganizing the cortical cytoskeleton during the mitotic cycle. *Mol. Biol. Cell* **14**, 4028-4038.
- Minoshima, Y., Kawashima, T., Hirose, K., Tonozuka, Y., Kawajiri, A., Bao, Y. C., Deng, X., Tatsuka, M., Narumiya, S., May, W. S., Jr et al.** (2003). Phosphorylation by aurora B converts MgcRacGAP to a RhoGAP during cytokinesis. *Dev. Cell* **4**, 549-560.

- Mishima, M., Kaitna, S. and Glotzer, M. (2002). Central spindle assembly and cytokinesis require a kinesin-like protein/RhoGAP complex with microtubule bundling activity. *Dev. Cell* **2**, 41-54.
- Mishima, M., Pavicic, V., Gruneberg, U., Nigg, E. A. and Glotzer, M. (2004). Cell cycle regulation of central spindle assembly. *Nature* **430**, 908-913.
- Mollinari, C., Kleman, J. P., Jiang, W., Schoehn, G., Hunter, T. and Margolis, R. L. (2002). PRC1 is a microtubule binding and bundling protein essential to maintain the mitotic spindle midzone. *J. Cell Biol.* **157**, 1175-1186.
- Murata-Hori, M., Tatsuka, M. and Wang, Y. L. (2002). Probing the dynamics and functions of aurora B kinase in living cells during mitosis and cytokinesis. *Mol. Biol. Cell* **13**, 1099-1108.
- Muris, D. F., Verschoor, T., Divecha, N. and Michalides, R. J. (2002). Constitutive active GTPases Rac and Cdc42 are associated with endoreplication in PAE cells. *Eur. J. Cancer* **38**, 1775-1782.
- Naim, V., Imarisio, S., di Cunto, F., Gatti, M. and Bonaccorsi, S. (2004). *Drosophila* citron kinase is required for the final steps of cytokinesis. *Mol. Biol. Cell* **15**, 5053-5063.
- Neef, R., Preisinger, C., Sutcliffe, J., Kopajtic, R., Nigg, E. A., Mayer, T. U. and Barr, F. A. (2003). Phosphorylation of mitotic kinesin-like protein 2 by polo-like kinase 1 is required for cytokinesis. *J. Cell Biol.* **162**, 863-875.
- Neufeld, T. P. and Rubin, G. M. (1994). The *Drosophila* peanut gene is required for cytokinesis and encodes a protein similar to yeast putative bud neck filament proteins. *Cell* **77**, 371-379.
- Piekny, A. J. and Mains, P. E. (2002). Rho-binding kinase (LET-502) and myosin phosphatase (MEL-11) regulate cytokinesis in the early *Caenorhabditis elegans* embryo. *J. Cell Sci.* **115**, 2271-2282.
- Powers, J., Bossinger, O., Rose, D., Strome, S. and Saxton, W. (1998). A nematode kinesin required for cleavage furrow advancement. *Curr. Biol.* **8**, 1133-1136.
- Prokopenko, S. N., Brumby, A., O'Keefe, L., Prior, L., He, Y., Saint, R. and Bellen, H. J. (1999). A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. *Genes Dev.* **13**, 2301-2314.
- Raich, W. B., Moran, A. N., Rothman, J. H. and Hardin, J. (1998). Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein ZEN-4. *Mol. Biol. Cell* **9**, 2037-2049.
- Rappaport, R. (1961). Experiments concerning the cleavage stimulus in sand dollar eggs. *J. Exp. Zool.* **148**, 81-89.
- Robinson, D. N. and Spudich, J. A. (2000). Towards a molecular understanding of cytokinesis. *Trends. Cell Biol.* **10**, 228-237.
- Saint, R. and Somers, W. G. (2003). Animal cell division: a fellowship of the double ring? *J. Cell Sci.* **116**, 4277-4281.
- Saito, R. M., Perreault, A., Peach, B., Satterlee, J. S. and van den Heuvel, S. (2004). The CDC-14 phosphatase controls developmental cell-cycle arrest in *C. elegans*. *Nat. Cell Biol.* **6**, 777-783.
- Satterwhite, L. L. and Pollard, T. D. (1992). Cytokinesis. *Curr. Opin. Cell Biol.* **4**, 43-52.
- Savoian, M. S., Earnshaw, W. C., Khodjakov, A. and Rieder, C. L. (1999a). Cleavage furrows formed between centrosomes lacking an intervening spindle and chromosomes contain microtubule bundles, INCENP, and CHO1 but not CENP-E. *Mol. Biol. Cell* **10**, 297-311.
- Savoian, M. S., Khodjakov, A. and Rieder, C. L. (1999b). Unilateral and wandering furrows during mitosis in vertebrates: implications for the mechanism of cytokinesis. *Cell Biol. Int.* **23**, 805-812.
- Savoian, M. S., Gatt, M. K., Riparbelli, M. G., Callaini, G. and Glover, D. M. (2004). *Drosophila* Klp67A is required for proper chromosome congression and segregation during meiosis I. *J. Cell Sci.* **117**, 3669-3677.
- Schneider, S. Q. and Bowerman, B. (2003). Cell polarity and the cytoskeleton in the *Caenorhabditis elegans* zygote. *Annu. Rev. Genet.* **37**, 221-249.
- Schumacher, J. M., Golden, A. and Donovan, P. J. (1998). AIR-2: an Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in *Caenorhabditis elegans* embryos. *J. Cell Biol.* **143**, 1635-1646.
- Severson, A. F., Hamill, D. R., Carter, J. C., Schumacher, J. and Bowerman, B. (2000). The aurora-related kinase AIR-2 recruits ZEN-4/CeMKLP1 to the mitotic spindle at metaphase and is required for cytokinesis. *Curr. Biol.* **10**, 1162-1171.
- Shandala, T., Gregory, S. L., Dalton, H. E., Smallhorn, M. and Saint, R. (2004). Citron kinase is an essential effector of the Pbl-activated Rho signalling pathway in *Drosophila melanogaster*. *Development* **131**, 5053-5063.
- Skop, A. R., Liu, H., Yates, J., III, Meyer, B. J. and Heald, R. (2004). Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. *Science* **305**, 61-66.
- Somers, W. G. and Saint, R. (2003). A RhoGEF and Rho family GTPase-activating protein complex links the contractile ring to cortical microtubules at the onset of cytokinesis. *Dev. Cell* **4**, 29-39.
- Somma, M. P., Fasulo, B., Cenci, G., Cundari, E. and Gatti, M. (2002). Molecular dissection of cytokinesis by RNA interference in *Drosophila* cultured cells. *Mol. Biol. Cell* **13**, 2448-2460.
- Srayko, M., Quintin, S., Schwager, A. and Hyman, A. A. (2003). *Caenorhabditis elegans* TAC-1 and ZYG-9 form a complex that is essential for long astral and spindle microtubules. *Curr. Biol.* **13**, 1506-1511.
- Straight, A. F., Field, C. M. and Mitchison, T. J. (2005). Anillin binds nonmuscle myosin II and regulates the contractile ring. *Mol. Biol. Cell* **16**, 193-201.
- Strickland, L. I. and Burgess, D. R. (2004). Pathways for membrane trafficking during cytokinesis. *Trends. Cell Biol.* **14**, 115-118.
- Swan, K. A., Severson, A. F., Carter, J. C., Martin, P. R., Schnabel, H., Schnabel, R. and Bowerman, B. (1998). *cyk-1*: a *C. elegans* FH gene required for a late step in embryonic cytokinesis. *J. Cell Sci.* **111**, 2017-2027.
- Tatsumoto, T., Xie, X., Blumenthal, R., Okamoto, I. and Miki, T. (1999). Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G2/M phases, and involved in cytokinesis. *J. Cell Biol.* **147**, 921-928.
- Terada, Y., Tatsuka, M., Suzuki, F., Yasuda, Y., Fujita, S. and Otsu, M. (1998). AIM-1: a mammalian midbody-associated protein required for cytokinesis. *EMBO J.* **17**, 667-676.
- Verbrugghe, K. J. and White, J. G. (2004). SPD-1 is required for the formation of the spindle midzone but is not essential for the completion of cytokinesis in *C. elegans* embryos. *Curr. Biol.* **14**, 1755-1760.
- Verni, F., Somma, M. P., Gunsalus, K. C., Bonaccorsi, S., Belloni, G., Goldberg, M. L. and Gatti, M. (2004). Feo, the *Drosophila* homolog of PRC1, is required for central-spindle formation and cytokinesis. *Curr. Biol.* **14**, 1569-1575.
- Wang, Y. L. (2001). The mechanism of cytokinesis: reconsideration and reconciliation. *Cell Struct. Funct.* **26**, 633-638.
- Watanabe, N., Madaule, P., Reid, T., Ishizaki, T., Watanabe, G., Kakizuka, A., Saito, Y., Nakao, K., Jockusch, B. M. and Narumiya, S. (1997). p140mDia, a mammalian homolog of *Drosophila* diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. *EMBO J.* **16**, 3044-3056.
- Wheatley, S. P. and Wang, Y. (1996). Midzone microtubule bundles are continuously required for cytokinesis in cultured epithelial cells. *J. Cell Biol.* **135**, 981-989.
- Williams, B. C., Riedy, M. F., Williams, E. V., Gatti, M. and Goldberg, M. L. (1995). The *Drosophila* kinesin-like protein KLP3A is a midbody component required for central spindle assembly and initiation of cytokinesis. *J. Cell Biol.* **129**, 709-723.
- Winter, C. G., Wang, B., Ballew, A., Royou, A., Karess, R., Axelrod, J. D. and Luo, L. (2001). *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. *Cell* **105**, 81-91.
- Yamashiro, S., Totsukawa, G., Yamakita, Y., Sasaki, Y., Madaule, P., Ishizaki, T., Narumiya, S. and Matsumura, F. (2003). Citron kinase, a Rho-dependent kinase, induces di-phosphorylation of regulatory light chain of myosin II. *Mol. Biol. Cell* **14**, 1745-1756.
- Yoshizaki, H., Ohba, Y., Kurokawa, K., Itoh, R. E., Nakamura, T., Mochizuki, N., Nagashima, K. and Matsuda, M. (2003). Activity of Rho-family GTPases during cell division as visualized with FRET-based probes. *J. Cell Biol.* **162**, 223-232.
- Yoshizaki, H., Ohba, Y., Parrini, M. C., Dulyaninova, N. G., Bresnick, A. R., Mochizuki, N. and Matsuda, M. (2004). Cell type-specific regulation of RhoA activity during cytokinesis. *J. Biol. Chem.* **279**, 44756-44762.
- Zhang, D. and Nicklas, R. B. (1996). 'Anaphase' and cytokinesis in the absence of chromosomes. *Nature* **382**, 466-468.
- Zhu, C. and Jiang, W. (2005). Cell cycle-dependent translocation of PRC1 on the spindle by Kif4 is essential for midzone formation and cytokinesis. *Proc. Natl. Acad. Sci. USA* **102**, 343-348.