Federica Arbizzani, Sergio A Rincon, Anne Paoletti (2019 Jun 21)

Increasing ergosterol levels delays formin-dependent assembly of F-actin cables and disrupts division plane positioning.

*Journal of cell science* : [DOI : jcs.227447](https://doi.org/jcs.227447)

**Résumé**

In most eukaryotes, cytokinesis is mediated by the constriction of a contractile acto-myosin ring (CR) which promotes the ingress of the cleavage furrow. Many components of the CR interact with plasma membrane lipids suggesting that lipids may regulate CR assembly and function. Although there is clear evidence that phospho-inositides play an important role in cytokinesis, much less is known about the role of sterols in this process. Here we studied how sterols influence division plane positioning and CR assembly in fission yeast. We show that increasing ergosterol levels on the plasma membrane blocks the assembly of F-actin cables from cytokinetic precursor nodes, preventing their compaction into a ring. Abnormal F-actin cables form after a delay, leading to randomly placed septa. Since the formin Cdc12 was detected on cytokinetic precursors and the phenotype can be partially rescued by inhibiting the Arp2/3 complex, which competes with formins for F-actin nucleation, we propose that ergosterol may inhibit formin dependent assembly of F-actin cables from cytokinetic precursors.

Isabelle Loiodice, Marcel E Janson, Penny Tavormina, Sebastien Schaub, Divya Bhatt, Ryan Cochran, Julie Czupryna, Chuanhai Fu, Phong T Tran (2019 Mar 7)

Quantifying Tubulin Concentration and Microtubule Number Throughout the Fission Yeast Cell Cycle.

*Biomolecules* : [DOI : E86](https://doi.org/E86)

**Résumé**

The fission yeast serves as a good genetic model organism for the molecular dissection of the microtubule (MT) cytoskeleton. However, analysis of the number and distribution of individual MTs throughout the cell cycle, particularly during mitosis, in living cells is still lacking, making quantitative modelling imprecise. We use quantitative fluorescent imaging and analysis to measure the changes in tubulin concentration and MT number and distribution throughout the cell cycle at a single MT resolution in living cells. In the wild-type cell, both mother and daughter spindle pole body (SPB) nucleate a maximum of 23 ± 6 MTs at the onset of mitosis, which decreases to a minimum of 4 ± 1 MTs at spindle break down. Interphase MT bundles, astral MT bundles, and the post anaphase array (PAA) microtubules are composed primarily of 1 ± 1 individual MT along their lengths. We measure the cellular concentration of αβ-tubulin subunits to be ~5 µM throughout the cell cycle, of which one-third is in polymer form during interphase and one-quarter is in polymer form during mitosis. This analysis provides a definitive characterization of αβ-tubulin concentration and MT number and distribution in fission yeast and establishes a foundation for future quantitative...
Lara Katharina Krüger, Jérémie-Luc Sanchez, Anne Paoletti, Phong Thanh Tran (2019 Feb 27)  
**Kinesin-6 regulates cell-size-dependent spindle elongation velocity to keep mitosis duration constant in fission yeast.**  
eLife : DOI : 10.7554/eLife.42182

**Résumé**

The length of the mitotic spindle scales with cell size in a wide range of organisms during embryonic development. Interestingly, in embryos, this goes along with temporal regulation: larger cells speed up spindle assembly and elongation. We demonstrate that, similarly in fission yeast, spindle length and spindle dynamics adjust to cell size, which allows to keep mitosis duration constant. Since prolongation of mitosis was shown to affect cell viability, this may resemble a mechanism to regulate mitosis duration. We further reveal how the velocity of spindle elongation is regulated: coupled to cell size, the amount of kinesin-6 Klp9 molecules increases, resulting in an acceleration of spindle elongation in anaphase B. In addition, the number of Klp9 binding sites to microtubules increases overproportionally to Klp9 molecules, suggesting that molecular crowding inversely correlates to cell size and might have an impact on spindle elongation velocity control.

**Année de publication : 2017**

Ishutesh Jain, Phong T Tran (2017 Jun 8)  
**Multiple Motifs Compete for EB-Dependent Microtubule Plus End Binding.**  

**Résumé**

Microtubule (MT) dynamics are regulated by a plethora of microtubule-associated proteins (MAPs). An important MT regulator is the end binding protein EB, which serves as a scaffold to recruit other MAPs to MT plus ends. In this issue of Structure, Kumar et al. (2017) describe LxxPTPh, a new linear sequence motif that can bind EBs. The finding opens up the possibility of discovering new MT regulators.

**Kinesin-5-independent mitotic spindle assembly requires the antiparallel microtubule crosslinker Ase1 in fission yeast.**  
Nature communications : 15286 : DOI : 10.1038/ncomms15286
Résumé

Bipolar spindle assembly requires a balance of forces where kinesin-5 produces outward pushing forces to antagonize the inward pulling forces from kinesin-14 or dynein. Accordingly, Kinesin-5 inactivation results in force imbalance leading to monopolar spindle and chromosome segregation failure. In fission yeast, force balance is restored when both kinesin-5 Cut7 and kinesin-14 Pkl1 are deleted, restoring spindle bipolarity. Here we show that the cut7Δpkl1Δ spindle is fully competent for chromosome segregation independently of motor activity, except for kinesin-6 Klp9, which is required for anaphase spindle elongation. We demonstrate that cut7Δpkl1Δ spindle bipolarity requires the microtubule antiparallel bundler PRC1/Ase1 to recruit CLASP/Cls1 to stabilize microtubules. Brownian dynamics-kinetic Monte Carlo simulations show that Ase1 and Cls1 activity are sufficient for initial bipolar spindle formation. We conclude that pushing forces generated by microtubule polymerization are sufficient to promote spindle pole separation and the assembly of bipolar spindle in the absence of molecular motors.

Sergio A Rincon, Miguel Estravis, Florent Dingli, Damarys Loew, Phong T Tran, Anne Paoletti (2017 Feb 7)
SIN-Dependent Dissociation of the SAD Kinase Cdr2 from the Cell Cortex Resets the Division Plane.

Résumé

Proper division plane positioning is crucial for faithful chromosome segregation but also influences cell size, position, or fate [1]. In fission yeast, medial division is controlled through negative signaling by the cell tips during interphase and positive signaling by the centrally placed nucleus at mitotic entry [2-4]: the cell geometry network (CGN), controlled by the inhibitory cortical gradient of the DYRK kinase Pom1 emanating from the cell tips, first promotes the medial localization of cytokinetic ring precursors organized by the SAD kinase Cdr2 to pre-define the division plane [5-8]; then, massive nuclear export of the anillin-like protein Mid1 at mitosis entry confirms or readjusts the division plane according to nuclear position and triggers the assembly of a medial contractile ring [5, 9-11]. Strikingly, the Hippo-like septation initiation network (SIN) induces Cdr2 dissociation from cytokinetic precursors at this stage [12-14]. We show here that SIN-dependent phosphorylation of Cdr2 promotes its interaction with the 14-3-3 protein Rad24 that sequesters it in the cytoplasm during cell division. If this interaction is compromised, cytokinetic precursors are asymmetrically distributed in the cortex of newborn cells, leading to asymmetrical division if nuclear signaling is abolished. We conclude that, through this new function, the SIN resets the division plane in newborn cells to ensure medial division.

Année de publication : 2016

Sergio A Rincon, Anne Paoletti (2016 Jan 26)
Molecular control of fission yeast cytokinesis.
Seminars in cell & developmental biology : 28-38 : DOI : 10.1016/j.semcdb.2016.01.007

Résumé

Cytokinesis gives rise to two independent daughter cells at the end of the cell division cycle. The fission yeast Schizosaccharomyces pombe has emerged as one of the most powerful systems to understand how cytokinesis is controlled molecularly. Like in most eukaryotes, fission yeast cytokinesis depends on an acto-myosin based contractile ring that assembles at the division site under the control of spatial cues that integrate information on cell geometry and the position of the mitotic apparatus. Cytokinetic events are also tightly coordinated with nuclear division by the cell cycle machinery. These spatial and temporal regulations ensure an equal cleavage of the cytoplasm and an accurate segregation of the genetic material in daughter cells. Although this model system has specificities, the basic mechanisms of contractile ring assembly and function deciphered in fission yeast are highly valuable to understand how cytokinesis is controlled in other organisms that rely on a contractile ring for cell division.

Sergio A Rincon, Anne Paoletti (2016 Jan 11)
From Structure to Function: A Comprehensive Compendium of Tools to Unveil Protein Domains and Understand Their Role in Cytokinesis.

Résumé

Unveiling the function of a novel protein is a challenging task that requires careful experimental design. Yeast cytokinesis is a conserved process that involves modular structural and regulatory proteins. For such proteins, an important step is to identify their domains and structural organization. Here we briefly discuss a collection of methods commonly used for sequence alignment and prediction of protein structure that represent powerful tools for the identification homologous domains and design of structure-function approaches to test experimentally the function of multi-domain proteins such as those implicated in yeast cytokinesis.

Année de publication : 2015

Imène B Bouhlel, Kathleen Scheffler, Phong T Tran, Anne Paoletti (2015 May 27)
Monitoring SPB biogenesis in fission yeast with high resolution and quantitative fluorescent microscopy.
Methods in cell biology : 383-92 : DOI : 10.1016/bs.mcb.2015.03.005

Résumé

Like centrosomes, yeast spindle pole bodies (SPBs) undergo a tightly controlled duplication
cycle in order to restrict their number to one or two per cell and promote the assembly of a bipolar spindle at mitotic entry. This conservative duplication cycle is tightly coordinated with cell cycle progression although the mechanisms that ensure this coordination remain largely unknown. In this chapter, we describe simple high resolution microscopy- and quantitative light microscopy-based methods that allow to monitor SPB biogenesis in fission yeast and may be useful to study the molecular pathways controlling the successive phases of the duplication cycle.

Mercè Guzmán-Vendrell, Sergio A Rincon, Florent Dingli, Damarys Loew, Anne Paoletti (2015 Apr 17)
Molecular control of the Wee1 regulatory pathway by the SAD kinase Cdr2.

Résumé

Cell growth and division are tightly coordinated to maintain cell size constant during successive cell cycles. In Schizosaccharomyces pombe, the SAD kinase Cdr2 regulates the cell size at division and the positioning of the division plane. Cdr2 forms nodes on the medial cortex containing factors that constitute an inhibitory pathway for Wee1. This pathway is regulated by polar gradients of the DYRK kinase Pom1, and involves a direct inhibitor of Wee1, the SAD kinase Cdr1. Cdr2 also interacts with the anillin Mid1, which defines the division plane, and with additional components of the medial cortical nodes, including Blt1, which participate in the mitotic-promoting and cytokinetic functions of nodes. Here, we show that the interaction of Cdr2 with Wee1 and Mid1 requires the UBA domain of Cdr2, which is necessary for its kinase activity. In contrast, Cdr1 associates with the C-terminus of Cdr2, which is composed of basic and KA-1 lipid-binding domains. Mid1 also interacts with the C-terminus of Cdr2 and might bridge the N- and C-terminal domains, whereas Blt1 associates with the central spacer region. We propose that the association of Cdr2 effectors with different domains might constrain Cdr1 and Wee1 spatially to promote Wee1 inhibition upon Cdr2 kinase activation.

Kathleen Scheffler, Refael Minnes, Vincent Fraisier, Anne Paoletti, Phong T Tran (2015 Apr 15)
Microtubule minus end motors kinesin-14 and dynein drive nuclear congression in parallel pathways.
The Journal of cell biology : 47-58 : DOI : 10.1083/jcb.201409087

Résumé

Microtubules (MTs) and associated motors play a central role in nuclear migration, which is crucial for diverse biological functions including cell division, polarity, and sexual reproduction. In this paper, we report a dual mechanism underlying nuclear congression during fission yeast karyogamy upon mating of haploid cells. Using microfluidic chambers for long-term imaging, we captured the precise timing of nuclear congression and identified two minus end-directed motors operating in parallel in this process. Kinesin-14 Klp2 associated...
with MTs may cross-link and slide antiparallel MTs emanating from the two nuclei, whereas dynein accumulating at spindle pole bodies (SPBs) may pull MTs nucleated from the opposite SPB. Klp2-dependent nuclear congression proceeds at constant speed, whereas dynein accumulation results in an increase of nuclear velocity over time. Surprisingly, the light intermediate chain Dli1, but not dynactin, is required for this previously unknown function of dynein. We conclude that efficient nuclear congression depends on the cooperation of two minus end-directed motors.

Imène B Bouhlel, Midori Ohta, Adeline Mayeux, Nicole Bordes, Florent Dingli, Jérôme Boulanger, Guilhem Velve Casquillas, Damarys Loew, Phong T Tran, Masamitsu Sato, Anne Paoletti (2015 Mar 3)

**Cell cycle control of spindle pole body duplication and splitting by Sfi1 and Cdc31 in fission yeast.**

*Journal of cell science* : 1481-93 : [DOI : 10.1242/jcs.159657]

**Résumé**

Spindle pole biogenesis and segregation are tightly coordinated to produce a bipolar mitotic spindle. In yeasts, the spindle pole body (SPB) half-bridge composed of Sfi1 and Cdc31 duplicates to promote the biogenesis of a second SPB. Sfi1 accumulates at the half-bridge in two phases in Schizosaccharomyces pombe, from anaphase to early septation and throughout G2 phase. We found that the function of Sfi1-Cdc31 in SPB duplication is accomplished before septation ends and G2 accumulation starts. Thus, Sfi1 early accumulation at mitotic exit might correspond to half-bridge duplication. We further show that Cdc31 phosphorylation on serine 15 in a Cdk1 (encoded by cdc2) consensus site is required for the dissociation of a significant pool of Sfi1 from the bridge and timely segregation of SPBs at mitotic onset. This suggests that the Cdc31 N-terminus modulates the stability of Sfi1-Cdc31 arrays in fission yeast, and impacts on the timing of establishment of spindle bipolarity.

**Année de publication : 2014**

Kathleen Scheffler, Pierre Recouveux, Anne Paoletti, Phong T Tran (2014 Nov 24)

**Oscillatory AAA+ ATPase Knk1 constitutes a novel morphogenetic pathway in fission yeast.**

*Proceedings of the National Academy of Sciences of the United States of America* : 17899-904 : [DOI : 10.1073/pnas.1407226111]

**Résumé**

Cellular morphogenesis relies partly on cell polarization by the cytoskeleton. In the fission yeast Schizosaccharomyces pombe, it is well established that microtubules (MTs) deliver the spatial cue Tea1, a kelch repeat protein, to the tip regions to direct the growth machinery at the cell tips driving the linear extension of the rod-shaped organism to maintain a straight
long axis. Here, we report the characterization of Knk1 (kink), a previously unidentified member of the superfamily of ATPases associated with various cellular activities (AAA(+)), whose deletion causes a unique morphological defect characterized by the formation of kinks close to cell tips. Through genetic analysis, we place Knk1 into a novel pathway controlling cell shape independently of MTs and Tea1. Knk1 localizes at cell tips. Its localization is mediated by the Knk1 N terminus and is enhanced upon ATP binding to the C-terminal ATPase domain. Furthermore, Knk1 tip recruitment is regulated by SRC-like adaptor 2 (Sla2) and cell division cycle 42 (Cdc42) independently of Sla2’s role in endocytosis. Finally, we discovered that Knk1 shows an anticorrelated oscillatory behavior between the two cell tips at a periodicity that is different from the reported oscillatory Cdc42 dynamics.

Judite Costa, Chuanhai Fu, V Mohini Khare, Phong T Tran (2014 Sep 26)

csi2p modulates microtubule dynamics and organizes the bipolar spindle for chromosome segregation.

Molecular biology of the cell : 3900-8 : DOI : 10.1091/mbc.E14-09-1370

Résumé

Proper chromosome segregation is of paramount importance for proper genetic inheritance. Defects in chromosome segregation can lead to aneuploidy, which is a hallmark of cancer cells. Eukaryotic chromosome segregation is accomplished by the bipolar spindle. Additional mechanisms, such as the spindle assembly checkpoint and centromere positioning, further help to ensure complete segregation fidelity. Here we present the fission yeast csi2+. csi2p localizes to the spindle poles, where it regulates mitotic microtubule dynamics, bipolar spindle formation, and subsequent chromosome segregation. csi2 deletion (csi2Δ) results in abnormally long mitotic microtubules, high rate of transient monopolar spindles, and subsequent high rate of chromosome segregation defects. Because csi2Δ has multiple phenotypes, it enables estimates of the relative contribution of the different mechanisms to the overall chromosome segregation process. Centromere positioning, microtubule dynamics, and bipolar spindle formation can all contribute to chromosome segregation. However, the major determinant of chromosome segregation defects in fission yeast may be microtubule dynamic defects.

Viktoriya Syrovatkina, Phong T Tran (2014 Sep 24)

Loss of kinesin-14 results in aneuploidy via kinesin-5-dependent microtubule protrusions leading to chromosome cut.

Nature communications : 7322 : DOI : 10.1038/ncomms8322

Résumé

Aneuploidy-chromosome instability leading to incorrect chromosome number in dividing cells can arise from defects in centrosome duplication, bipolar spindle formation, kinetochore-microtubule attachment, chromatid cohesion, mitotic checkpoint monitoring or cytokinesis. As most tumours show some degree of aneuploidy, mechanistic understanding
of these pathways has been an intense area of research, to provide potential therapeutics. Here we present a mechanism for aneuploidy in fission yeast based on spindle pole microtubule defocusing by loss of kinesin-14 Pkl1, leading to kinesin-5 Cut7-dependent aberrant long spindle microtubule minus-end protrusions that push the properly segregated chromosomes to the site of cell division, resulting in chromosome cut at cytokinesis. Pkl1 localization and function at the spindle pole is mutually dependent on spindle pole-associated protein Msd1. This mechanism of aneuploidy bypasses the known spindle assembly checkpoint that monitors chromosome segregation.