Dispatches

Cell Division: The Need for Speed

A bundle of microtubules known as the spindle midzone is rapidly assembled after anaphase onset, recruiting multiple signaling proteins that regulate cytokinesis. A new study reveals that positive feedback driven by clustering of a kinesin-6 motor underlies the explosive assembly of the spindle midzone.

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Biological systems frequently control critical transitions with a combination of relatively slow decision-making and rapid execution. To paraphrase (badly) the Swan of Avon, "If yer gonna do it, best do it quick". This is particularly evident during the cell cycle: the decisions to enter or leave mitosis, for example, can be the result of many minutes or hours worth of cellular deliberation. In contrast, the biochemical and morphological changes that ensue from these decisions occur with such striking abruptness that they are considered switch-like. While an enormous amount is known about how the decision-making process works only a few details are known about how switch-like behavior is achieved once the decision is made. In those cases in which it has been worked out, positive feedback plays a key role. For example, the explosive activation of cyclin B–Cdk1 complexes at the G2–M boundary results from interlocking positive feedback loops. First, cyclin B–Cdk1 directly deactivates its inactivators Wee1 and Myt1, kinases that are responsible for inhibitory phosphorylations on cyclin B–Cdk1, and second, cyclin B–Cdk1 directly activates its activator Cdc25, a dual specificity phosphatase that dephosphorylates the Wee1/Myt1 inhibitory phosphorylation sites [1]. These feedback loops permit the cell to rapidly amplify a small amount of active Cdk1 to ensure complete commitment to mitosis. Similarly, anaphase A — the process whereby sister chromatids are segregated to opposite poles of the spindle — is made abrupt by a positive feedback loop. Cyclin B–Cdk1 phosphorylates securin, a protein that delays anaphase in response to perturbations, such as DNA damage, by inhibiting separase, a protease that disrupts sister-chromatid cohesion. Phosphorylation reduces the rate at which securin is ubiquitinated by the anaphase-promoting complex (APC) ubiquitin ligase, resulting in securin stabilization. When high levels of APC activity accumulate, some securin is ubiquitinated and destroyed, thus releasing some separase. The liberated protease then activates Cdc14, a phosphatase which dephosphorylates securin, making securin more susceptible to ubiquitination by the APC and thereby completing a positive feedback loop [2]. This positive feedback mechanism leads to the abrupt, synchronous, and irreversible dissolution of sister-chromatid attachments at the metaphase–anaphase transition.

In this issue of *Current Biology*, Hutterer *et al.*[3] provide evidence that positive feedback underlies the rapid assembly of the spindle midzone, a bundle of microtubules that forms during anaphase and serves as a signaling platform that controls cytokinesis, the final step in the cell cycle. The midzone, which assembles between the spindle poles, is the product of at least two complementary systems. The first is oligomerization of the microtubule-associated protein (MAP) PRC1/SPD-1/Ase1, a microtubule-bundling protein [4]. The second is activation of centralspindlin, a heterotetramer of MKLP1/ZEN-4/Klp9p, a microtubule plus-end-directed kinesin-6, and MgcRacGAP/CYK-4, a GTPase-activating protein (GAP) [5]. MgcRacGAP interacts with several key regulators of cytokinesis, including the guanine nucleotide exchange factor (GEF) Ect2 [6], the contractile ring component Anillin [7], and the aforementioned microtubule-bundling protein PRC1 [8]. The ability of PRC1 and centralspindlin to direct spindle midzone assembly is regulated by cyclin B–Cdk1 phosphorylation. PRC1 oligomerization is negatively regulated by cyclin B–Cdk1 phosphorylation in early mitosis [9], and centralspindlin's ability to bind microtubules and function as a microtubule motor is suppressed by cyclin B–Cdk1 phosphorylation of the MKLP1 motor domain [10]. Importantly, cyclin B–Cdk1 activity falls abruptly at the metaphase–anaphase transition, thus explaining why PRC1 and centralspindlin assemble the spindle midzone specifically at anaphase onset.

However, the means by which centralspindlin is concentrated on the midzone microtubules remained unclear. Hutterer *et al.* [3] shed light on this question by showing that centralspindlin rapidly accumulates at the spindle midzone after anaphase onset in a pattern characteristic of positive feedback, in which CYK-4 recruitment follows a sigmoidal pattern, switching rapidly from minimal to maximal accumulation (Figure 1).

What is the basis of this positive feedback? Hutterer *et al.* [3] show that centralspindlin undergoes oligomerization due to the higher-order clustering of the kinesin component, ZEN-4. While polymerization is a common feature of cytoskeletal proteins, it has not commonly been observed for microtubule motors. Hutterer *et al.* [3] used a series of clever experimental manipulations to show that the biochemical upshot of ZEN-4 clustering is an increase in the capacity of centralspindlin to drive microtubule bundling and an increase in processivity, the ability of a motor to maintain association with microtubules during the nucleotide hydrolysis cycle. The authors identified amino acids 585–601 of ZEN-4 as being responsible for clustering and found that a clustering-deficient mutant, Z585GFP, had significantly reduced microtubule-bundling activity and greatly reduced processivity. In contrast, a clustering-competent construct, Z601GFP, showed strong microtubule-bundling activity and high processivity. Further, the authors showed that artificial oligomerization of the clustering-deficient mutant Z585GFP was sufficient to restore processivity.

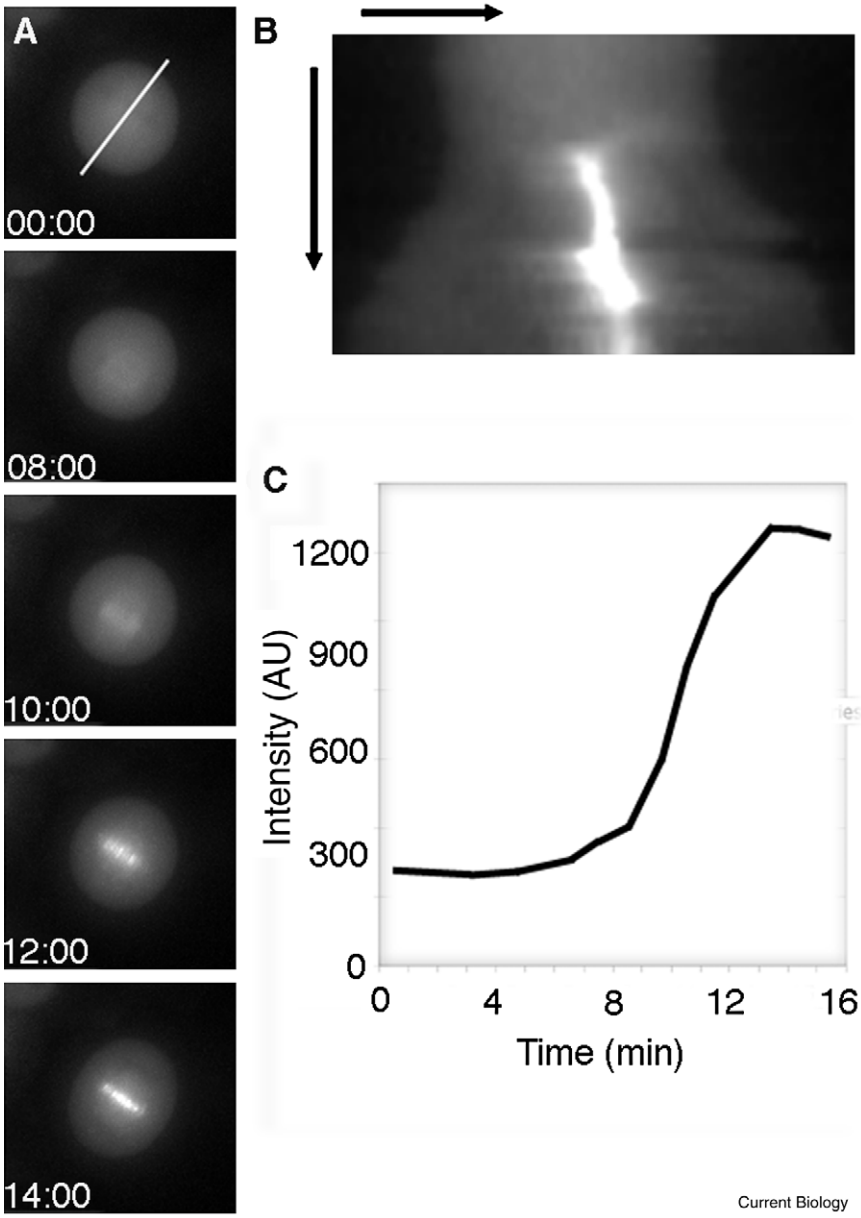

Figure 1. Switch-like recruitment of MKLP to the midzone in anaphase.

(A) Frames from a time-lapse movie showing recruitment of eGFP–MKLP to the midzone during anaphase. The white line runs perpendicular to the long axis of the spindle and is used to generate a kymograph shown in (B). (B) Kymograph of eGFP–MKLP recruitment based on white line shown in (A) (horizontal axis, distance; vertical axis, time). Note the abrupt rise in fluorescence. (C) Plot of spindle midzone eGFP–MKLP fluorescence intensity (arbitrary units) over time. The signal rises abruptly, beginning at about 7 minutes, and peaks by 13 minutes. All data are based on a time-lapse movie kindly provided by M. Mishima.

Intriguingly, oligomerization of centralspindlin has an additional consequence that may be important for cytokinesis. That is, ZEN-4 clustering is essential for accumulation of centralspindlin at the plus ends of microtubules. Why is this significant? While centralspindlin is thought to contribute to cytokinesis by its involvement in midzone formation, it is also thought to promote formation of the cytokinetic apparatus via localization to astral microtubules that approach the cell equator, well away from the midzone [11]. Further, in a recent modeling study, successful stimulation of cytokinesis required accumulation of MKLP1 at the plus ends of microtubules near the cortical equator [12].

The results presented in Hutterer *et al.* [3] suggest several complementary positive feedback mechanisms could rapidly accelerate recruitment of centralspindlin and spindle midzone formation. Clustering of ZEN-4 leads to increased microtubule bundling, which contributes to midzone formation directly and also provides more substrate for centralspindlin to bind, which, in turn, could promote further bundling. Oligomerization of ZEN-4 also leads to increased processivity, which promotes accumulation of ZEN-4 at microtubule plus ends and ensures that centralspindlin has a greater opportunity to snare other microtubules, further increasing bundling and thereby providing more substrate for centralspindlin recruitment. Further positive feedback could be provided by the interaction of MgcRacGAP with PRC1 [8]. This point is underscored by recent work in fission yeast, which demonstrates that the MKLP1 homolog, klp9p, binds directly to the PRC1 homolog, ase1p, in the midzone. The conformation induced by binding of the MAP to the motor enables proper orientation for efficient antiparallel microtubule sliding [13]. Additionally, the interaction of PRC1 with MgcRacGAP may help facilitate the phosphorylation of MgcRacGAP by Polo-like kinase 1 (Plk1), which is necessary for the recruitment of the GEF Ect2 to the spindle midzone where it can activate Rho [14]. Taken together, it appears that PRC1 and centralspindlin play interdependent roles in spindle midzone assembly.

The study by Hutterer *et al.* [3] reveals the importance of clustering of the kinesin component of centralspindlin for microtubule bundling, processive motility along microtubules *in vitro*, and successful cytokinesis *in vivo*, and raises new questions. For instance, what is the organization of the kinesin motors within the clusters, and what is it about this geometry that makes clustered centralspindlin preferentially accumulate at microtubule plus ends and bundle antiparallel microtubules? Is clustering of centralspindlin also important for accumulation of the complex at the equatorial cortex where it interacts with the GEF Ect2 to promote a zone of high Rho activity, which in turn promotes formation of the contractile ring? Is clustering itself

regulated in a cell cycle dependent fashion by phosphorylation or by binding of other proteins? Finally, as the clustering region as a whole is not well conserved, is clustering a general feature of MKLP1 (kinesin-6) in other organisms and, if so, how is it controlled?

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Visual Perception: An Orderly Cue for Consciousness

Our subjective experience of the order in which things happens feels secure to us: C follows B follows A. This impression of order is simply that, an impression. A recent study shows that our impressions can sometimes be illusory.

Vincent Walsh

Physicists are lucky, they get to say things like, bring us your initial conditions (a kind of mathematical first born) and our equations will work forwards or backwards in time to show that the "separation between the past, present and the future is only an illusion". Everybody nod at the elegance. Along the way, physicists made an important conceptual leap: time and space are really two sides of the same sheet. Psychologists are less lucky, they never sound as cool as Einstein and are only beginning to see the connections between space and time.

But the connection, though different of course, is as fundamental to cognition as it is to cosmology. Although space and time, through no more than historical accident, have been studied as separate subjects in psychology, they are inseparable in behaviour. To shake hands, kiss, speak, catch and even to look at something means to get some body part — hands, lips, eyes — to the right place at the right time. In real behaviour there is no such thing as the right place at the wrong time: a kiss becomes a head butt, speech a mumble, a catch becomes an empty clap and what you were moving your eyes towards may no longer be there.

The neural underpinnings of cognitive space-time for action have been set out with many predictions [1,2] and the correspondence between space-related and time-related behaviours has been seen in several different studies, some of which have shown that our experience of temporal order can be reversed [3–5]. As they report in this issue of *Current Biology*, Wu *et al.* [6] have taken a new approach to the question of temporal perception by using a spatial illusion called motion induced blindness or MIB (see http://www.michaelbach.de/ot/mot_mib/ for a demonstration — the associated home page really is a service to mankind, and a crushing blow to anyone naïve enough to have confidence in their senses). In MIB, subjects view a simple stimulus made up of rotating + signs. The display contains a circle or a number of circles and from time to time one or more of these disappear from your experience, although physically they remain on the screen.

What Wu *et al.* [6] did with this illusion (do look at it — it IS an illusion) was deceptively simple. They introduced a flash presented inside the now invisible circle and this flash reinstated the perception of the circle (see their stimuli at http://www.cerco.upstlse.fr/~rufin/illusoryreversaldemo). The first surprise is this: although the flash caused the subjects to see the circle again the subjects reported the circle as appearing *before* the flash. The second surprise is that the circle was seen approximately 100 milliseconds before the flash that caused it to be seen. These two things need an explanation.

The first thing to note is that there have been other reports of temporal reversals but the magnitude of the effect is not as large as reported here. So, are we looking at something fundamentally new or something that optimizes conditions for obtaining temporal reversals? Bachmann *et al.* [4], for example, reported a 30–50 milliseconds temporal reversal when two spots differing in luminance were presented successively, the dimmer of the two stimuli being reported first. Could it be that Wu *et al.*'s [6] invisible circle, being suppressed, has a weaker representation than the flash and that