extensive genetic screens did not yield a Pipe target.

Protease cascades represent a powerful mechanism to orchestrate a rapid, amplified activation mechanism as, for instance, needed for effective blood clotting [12]. At the same time, such cascades need to be spatially restricted. While some of the spatial control is provided by both negative feedback as well as negative regulators such as serpins, the initiation of such protease cascades also needs to be highly regulated. At the present time, we still do not exactly know how the sulfonation of the vitelline-membrane components initiates and restricts the dorso-ventral serine protease cascade in the early embryo. However, Zhang and colleagues [3] discuss some defined scenarios how this can be envisaged. One straightforward possibility is that the sulfonated carbon side chains of the target proteins could act as co-factors for one of the proteases, or they might anchor some of the proteases to the vitelline membrane in an active form. Such possibilities can now be tested and will certainly provide insight into the general mechanism of the regulation of protease activities in extracellular environments.

Dorso-ventral pattern formation in *Drosophila* is intriguing because in the course of its establishment, steps involving discrete and long term stable information alternate with the generation of three molecularly distinct gradients of pattern information: The long-term stable location of the oocyte nucleus provides the cue for the gradient of Gurken leading to a gradient of EGFR activity. This gradient is transformed into a sharp on-off expression domain of Pipe which

results in the localized production of long-term, stable sulfonated vitelline-membrane components. After fertilization of the egg, this discrete information gives rise to a new patterning gradient that has hallmarks of self-organization [13–15] and leads to the production of the active form of Spätzle, the ligand for Toll [1,2]. Finally, Toll activity leads to the nuclear gradient of the transcriptional regulator Dorsal which then promotes an exquisite pattern of dorso-ventral gene expression in the early embryo [16].

Information storage over time is likely to be an issue for many organisms that can undergo periods of induced developmental arrest, e.g. at low temperatures. The *Drosophila* solution of inserting pattern information in the egg shell, a very stable proteinaceous structure, allows the information to be stored for extended time periods, which is necessary given that females will often retain their eggs in the ovary for a long time, until they find a suitable medium for egg laying. Whether a similar solution involving anchoring of stably modified factors in extracellular matrix is used in other organisms will be interesting to see in the future.

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Cell Division: Righting the Check

Studies in fission and budding yeast have continuously led the way for analyzing pathways of cell division. Two elegant studies, one from each yeast species, are opening the gates to study one of the final steps of mitosis — silencing the spindle checkpoint.

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and P. Todd Stukenberg

During mitosis, the spindle assembly checkpoint (SAC) restrains the onset of anaphase until all chromosomes are properly attached to a bipolar spindle and develop tension from the pulling forces exerted from either pole [1]. Kinetochores are specialized regions on chromosomes that serve not only as attachment points for spindle microtubules but also as signaling platforms for the transmission of the SAC signal. The SAC is silenced by two independent events that redundantly ensure all chromosomes are properly attached to the mitotic spindle before the irreversible loss of cohesion that triggers anaphase. Under most circumstances, the SAC remains active until all kinetochores are fully occupied by microtubules (occupancy) and

stretched in response to tension from bipolar attachment (tension) (Figure 1).

A conserved group of proteins forms the core of the SAC machinery. These proteins serve as a surveillance mechanism that ultimately inhibits Cdc20, a specificity factor for the anaphase-promoting complex (APC), until proper kinetochore attachment is complete. The APC catalyzes the ubiquitylation of key mitotic regulators such as securin and cyclin B, resulting in their proteosomal degradation, the initiation of anaphase onset, and mitotic exit. The SAC genes were initially identified in yeast through screens for mutants that prevented mitotic arrest when cells were grown in the presence of microtubule inhibitors. The encoded products of these genes include Mad1, Mad2, Mad3/BubR1, Bub1, Bub3, and Mps1. Bub1 and Bub3 localize Mad1 to the kinetochore where it binds Mad2 and catalyzes the subsequent inactivation of Cdc20 by Mad2. In vertebrates, BubR1 may inhibit Cdc20 directly [2].

Although we understand a lot about the proteins required to initiate and propagate the SAC signal, less is known about how the SAC signal is silenced to allow progression into anaphase. This is in part due to the relative ease of identifying mutants, knockdowns, or inhibitors that abrogate the SAC, compared to identifying those that can restore a SAC arrest. It may also be due in part to controversy over the nature of the SAC signal itself. While most authors suggest that unattached kinetochores, or the absence of tension, activate the SAC [3], an alternative view suggests that the SAC signal is constitutive during mitosis until it is inactivated by proper (amphitelic) attachment of all kinetochores to a bipolar spindle (personal communication from C.L. Reider). Thus, a greater emphasis has been placed on identifying activators of the SAC rather than silencers of it.

To date, several mechanisms have been advanced that are believed to contribute to SAC silencing. In vertebrates, dynein-dependent stripping of SAC proteins from properly attached kinetochores has been proposed as a checkpoint silencing mechanism [4], and Cenp-E binding to kinetochore-bound microtubules inhibits BubR1 kinase activity, also leading to checkpoint silencing [5]. Other examples include the inhibition of the Mad2–CDC20 complex by p31
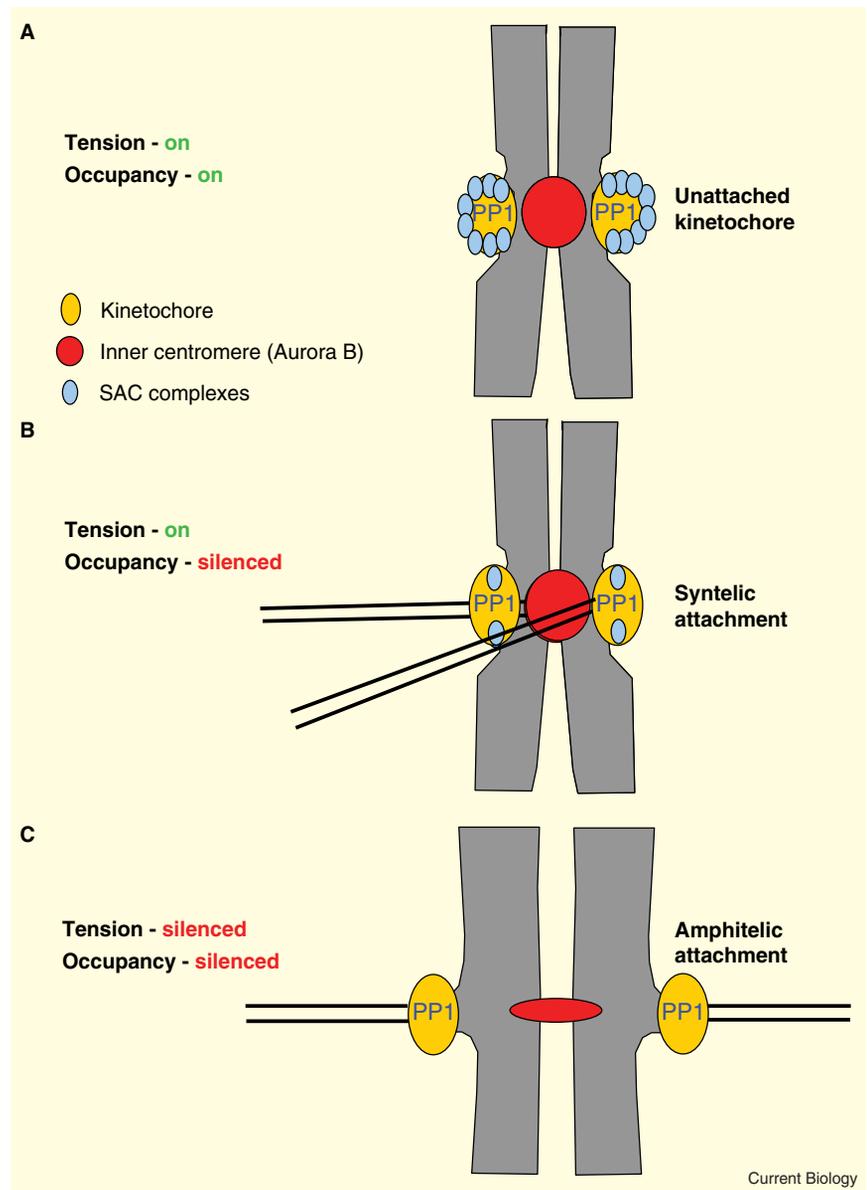
Figure 1. PP1 contributes to SAC regulation at kinetochores.

(A) In the unattached state, SAC proteins are enriched at kinetochores that are in close proximity to the inner centromere and amplify the SAC signal from Aurora B kinase. Kinase signaling predominates over PP1 opposition, and both tension and occupancy branches of the SAC are active. (B) Upon microtubule attachment, some but not all SAC proteins are displaced, resulting in a greater dependence on Aurora kinase activity to maintain the SAC signal in the presence of opposition by PP1. The occupancy branch is silenced and the tension branch remains active. (C) When kinetochores come under tension, they are physically displaced from the inner centromere, allowing PP1 activity to dominate. SAC complexes no longer reside at the kinetochore and both tension and occupancy branches are silenced. Syntelic attachment; sister kinetochores attached to the same spindle pole: amphitelic attachment; sister kinetochores attached to opposite poles (bipolar attachment).

Comet observed in mammalian cells, which can lead to inhibition of the SAC [6]. Also, APC-mediated proteosomal degradation of SAC components has been reported in yeast [7]. This latter mechanism ensures that the checkpoint remains off after cells enter anaphase. However, until now, no specific SAC-silencing gene products have been identified in yeast.

A number of protein kinases have been implicated in SAC signaling. However, their relevant substrates and specific roles remain unclear. Of those kinases conserved from yeast to man, the role of Aurora kinase in SAC

signaling is one of the best characterized [8]. Aurora B kinase is a serine/threonine kinase that forms the catalytic core of the chromosome passenger complex (which includes INCENP, Survivin, and Borealin). Despite a wealth of data demonstrating a highly conserved role for Aurora kinase in SAC signaling, assigning a precise role to Aurora B in the SAC has been a matter of debate. Early results from conditional mutants of *Ipl1*, the Aurora kinase in budding yeast, suggest that its activity is required for signaling a lack of kinetochore tension, but not a loss of microtubule–kinetochore attachment [9]. Similar results are found in mammalian cells when Aurora B activity is inhibited by pharmacological agents or RNA interference [10,11]. This led to the view that Aurora kinase has only an indirect role in SAC signaling through the generation of unattached kinetochores as a result of its well documented ability to correct improper microtubule attachments [12]. However, it has been reported that function-blocking antibodies against Aurora B silence the spindle checkpoint in the presence of unattached kinetochores in both *Xenopus* XTC cells and mitosis-phase *Xenopus* extracts [13]. Similarly, fission yeast require Aurora kinase activity for SAC arrest in the presence of unattached kinetochores [14].

Two papers in this issue of *Current Biology* provide new insights into SAC silencing and the central role of Aurora B kinase activity in the SAC [15,16]. Pinsky, Nelson and Biggins [15] explore the role of protein phosphatase 1 (PP1) activity in SAC silencing. The motivation for studying PP1 is based on the well-defined role of Ipl1 kinase activity in spindle checkpoint signaling in response to lack of tension [1,8,9], and the known role of PP1 in opposing Ipl1 phosphorylation. They hypothesize that reversal of Ipl1-mediated phosphorylation might be required for mitotic exit, and demonstrate that over-expression of Glc7, the PP1 homolog in budding yeast, not only causes chromosome mis-segregation, similar to that seen in *Ipl1* mutants, but also abrogates the SAC in the presence of unattached kinetochores. They also demonstrate that Glc7 expression is required to silence the SAC following an Mps1-induced arrest, suggesting that Glc7 may oppose other kinases in addition to

Ipl1. The beauty of this experiment is that mitotic arrest induced by Mps1 over-expression does not affect kinetochore–microtubule attachments, permitting observations of the role of Glc7 in structurally intact, bi-oriented kinetochores. This suggests that Glc7 is required to silence the SAC in a normal metaphase. In an additional set of elegantly designed experiments, the authors specifically isolate the potential effect of Glc7 loss on kinetochore structure from its effect on SAC signaling to demonstrate a direct role for Glc7 in silencing the SAC in unperturbed mitosis.

These new revelations of PP1's role in SAC silencing imply a more direct role for Aurora kinase in the SAC. This role is specifically addressed in the companion paper by Vanoosthuyse *et al.* [16], also in this issue. They employed a new and powerful assay to define the role of Ark-1 and Dis2 (the Aurora kinase and PP1 homologs in fission yeast, respectively) in SAC signaling. A mutant of Ark-1 was utilized that is specifically inhibited by an ATP analog (1NMPP1) in a real-time single cell assay for checkpoint function, in combination with a temperature-sensitive mutant of β-tubulin that completely depolymerizes microtubules at 18°C. Cooling to the restrictive temperature prevents microtubule–kinetochore attachments and allows propagation of a robust SAC signal from unattached kinetochores. Inhibition of Ark1 in this assay resulted in degradation of GFP-tagged Cdc13, the fission yeast cyclin B homolog, within 15 minutes. This was accompanied by loss of Mad1 and Mad2 from kinetochores, chromosome decondensation, and cell septation. Similar results were obtained at 32°C when Ark1 was inhibited in the presence of the microtubule-depolymerizing drug carbendizim. Therefore, Aurora kinase activity is required for a SAC-induced mitotic arrest produced by lack of microtubule attachment. This occurs as a result of the direct role Aurora kinase plays in SAC signaling, independent of microtubule–kinetochore attachment error correction. These results also suggest that spindle checkpoint signaling in response to lack of tension is not an indirect or secondary effect of Aurora releasing microtubule attachments and triggering the microtubule attachment signal.

Because PP1 opposes Aurora kinase signaling, strains were constructed containing conditional mutants of the kinetochore-localized form of PP1 in fission yeast, Dis2. After demonstrating that loss of Dis2 by itself did not prevent SAC arrest, the authors show that loss of Dis2 activity, but not that of the non-kinetochore localized form of PP1 (SDS2) or other phosphatases, prevented anaphase onset when Ark1 activity is inhibited in the presence of unattached kinetochores. These complementary results clearly demonstrate that PP1 is required to silence the SAC in both yeasts. While it has been previously shown that loss of Glc7 resulted in Pds1 stabilization in budding yeast [17], these current studies provide the most compelling evidence to date linking the silencing of the SAC to a single gene product.

The implications of these studies are far reaching, and a direct role of Aurora kinase activity in both the tension and occupancy arms of SAC is no longer a matter of debate. One of the three catalytic subunits of PP1, PP1γ, specifically localizes to kinetochores in human cells and it will be important to test if it is required to silence the mitotic checkpoint in vertebrates.

Aurora B has now been strongly implicated in the occupancy checkpoint in vertebrates and yeast, arguing for a conserved requirement. This is important since it cannot be explained by an indirect role of Aurora B in releasing microtubule attachments, rather the role must be more direct. A simple model that can resolve the controversy about Aurora B's roles in the tension and occupancy checkpoints is that a small amount of Aurora B activity is sufficient to generate an occupancy signal, while a higher level of Aurora B activity may be required for the tension signal. All experiments that fail to implicate Aurora B in the occupancy branch most likely reflect small amounts of residual Aurora B activity. The temperature-sensitive *ipl1* mutants used in these studies can be suppressed by Glc7 mutants, arguing strongly that *ipl1* mutants retain some function [18]. Furthermore, the two Aurora B inhibitors were used at low concentrations to prevent off-target effects, yet they demonstrate loss of SAC arrest at later time points [10,11]. If there are really two concentration requirements for signal generation, then there should be substrates

that are specific to the tension and occupancy branches of the checkpoint. In an earlier publication, the Hardwick group demonstrated that phosphorylation of Mad3 protein by one of the Aurora kinases is specifically required for the tension checkpoint [19]. Elegant data from these experiments genetically separates the two checkpoint pathways and is the strongest evidence that independent tension and occupancy pathways exist.

Aurora kinases localize to inner centromeres and PP1 localizes to the kinetochores. Many Aurora B substrates are on kinetochores and how Aurora B phosphorylates substrates at a distance is unclear. Current models for tension signaling suggest that the pulling forces of microtubules physically separate kinetochores from inner centromere signals and thus squelch low tension signals. The demonstration that the checkpoint can only be silenced by the Dis2 PPI phosphatase, which localizes to the kinetochore, but not SDS2, which does not, is consistent with this model. In fact, kinetochore localization of Dis2 may indicate that transient localization of the checkpoint proteins to kinetochores is required to silence the checkpoint.

The demonstration that phosphatases are required to silence the checkpoint highlights the importance of phosphorylation to generate the signal and it is only a first clue as to how it is turned off. There are many questions still to address before we understand how kinetochores use PP1 to connect the binding of microtubules, and the resulting tension from bipolar pulling forces, to the silencing of the SAC signal.

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Innate Immunity: Wounds Burst H₂O₂ Signals to Leukocytes

How leukocytes are attracted to wounds is poorly understood. Recent work using zebrafish reveals a novel mechanism of early leukocyte recruitment to wounds through a concentration gradient of hydrogen peroxide.

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Hydrogen peroxide (H_2O_2), an antiseptic, is a typical over-the-counter topical medicine used for minor wounds. In a new study using zebrafish, Niethammer *et al.* [1] suggest that hydrogen peroxide, in addition to killing microbes, may also provide a key signal for leukocyte recruitment to wounds. Tissue wounding induces the rapid recruitment of leukocytes, including neutrophils and macrophages, that function to limit infection by the release of reactive oxygen species, including H_2O_2 [2]. Despite the fundamental importance of this recruitment, our understanding of the mechanisms that recruit leukocytes to wounds has remained fairly limited. The prevailing view is that leukocytes are attracted to wounds by the release of 'danger signals', including ATP, uric acid, lipids, DNA and nuclear proteins [3,4]. But, to date, the key factors that mediate early leukocyte recruitment have not been identified. Niethammer *et al.* [1] now report a surprising finding: tissue wounding induces a rapid concentration gradient of H_2O_2 that provides an essential first step in leukocyte recruitment to injured tissue.

The primary source of reactive oxygen species, including H_2O_2, in