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The current view for the silencing of the spindle assembly checkpoint

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Chromosome bipolar attachment is achieved when sister kinetochores are attached by microtubules emanating from opposite spindle poles, and this process is essential for faithful chromosome segregation during anaphase. A fundamental question in cell biology is how cells ensure that chromosome segregation only occurs after bipolar attachment. It is well documented that unattached kinetochores activate the spindle assembly checkpoint (SAC) to delay chromosome segregation. Therefore, the silencing of the SAC is thought to trigger anaphase onset, but how correct chromosome attachment is coupled with SAC silencing and the subsequent anaphase onset is poorly understood. The establishment of chromosome bipolar attachment not only results in the occupancy of kinetochores by microtubules but also applies tension on sister kinetochores. A long-standing debate is whether the kinetochore attachment (occupancy) or the tension silences the SAC. Recent work in budding yeast reveals the SAC silencing network SSN that prevents SAC silencing prior to tension generation at kinetochores. Therefore, this signaling pathway ensures that SAC silencing and the subsequent anaphase onset occur only after chromosome bipolar attachment applies tension on chromosomes. This review will summarize the recent advances in the understanding of the SAC silencing process.

The Checkpoints that Monitor Kinetochore Attachments

The discovery of the spindle assembly checkpoint SAC

During mitosis, sister kinetochores are attached by microtubules emanating from opposite spindle poles, and the spindle elongation during anaphase pulls sister chromatids into daughter cells. Some microtubule poisons bind to tubulin subunits and cause microtubule depolymerization. Because treatment with these poisons disrupts the spindle structure in human cells and blocks mitosis, this group of compounds can inhibit cell growth, and they are widely used for cancer treatment. Yeast cells are also sensitive to these spindle poisons and arrest in metaphase after exposure. Two independent genetic screens were performed for the isolation of yeast mutants that fail to stop the cell cycle in response to the treatment with microtubule poisons, such as nocodazole and benomyl. One group of mutants *mad1*, *mad2*, and *mad3* (mitotic arrest-deficient) was identified as they continue to grow on benomyl-containing plates and lose viability because of the failure to arrest in metaphase.[1] Another group of mutants *bub1*, *bub2*, and *bub3* (budding uninhibited by benzimidazole) was isolated as they recover poorly after exposure to a high concentration of benomyl (70 μg/ml) and rebud on benomyl plates.[2] Another SAC component, Mps1 kinase, was subsequently identified as *mps1* mutants fail to arrest in metaphase in response to dysfunctional spindle pole bodies or a disrupted spindle.[3] Interestingly, overexpression of *MPS1* causes Mad1 protein phosphorylation and arrests wild-type cells in metaphase without any noticeable spindle defect.[4] Because these genes are essential for cell cycle arrest in response to spindle disruption, they were collectively named as the spindle assembly checkpoint (SAC).

In addition to the response to spindle disruption, these SAC proteins are also required for the cell cycle delay induced by dysfunctional kinetochores or mutated centromeric DNA, indicating that the SAC actually monitors kinetochore–microtubule interaction.[5,6] A temperature-sensitive kinetochore mutant *ctf13* arrests in metaphase with a short spindle structure when incubated at 37 °C, but the introduction of *mad1, mad2, bub1*, or *bub3*, but not *bub2*, allows the mutant cells to elongate the spindle for anaphase onset. Bub2 protein was later demonstrated to be a component of another checkpoint pathway that monitors the spindle position and delays mitotic exit.[7-11] Therefore, the SAC includes Mad1, Mad2, Mad3, Bub1, Bub3, and Mps1, and these components are well-conserved in all eukaryotes.[12]

The activation of the spindle assembly checkpoint and its consequence

Studies from both yeast and mammalian cells indicate that some checkpoint proteins are recruited to unattached kinetochores to generate a signal for anaphase entry delay.[13-15] The kinetochore protein Spc105/Knl1 was found to be the docking site for the SAC protein Bub1.[16] The phosphorylation of multiple Met-Glu-Leu-Thr (MELT) motifs in Spc105/Knl1 by Mps1 kinase enables the recruitment of the Bub1–Bub3 complex.[17-20] Recent work from the Biggins lab demonstrates that the phosphorylation of Bub1 by Mps1 leads to Bub1–Mad1 interaction in budding yeast. The interaction of Mad1 with Bub1 and kinetochores can be reconstituted in the presence of Mps1 and Mad2.[21] These observations indicate that the kinetochore acts as a platform

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essential for SAC activation, and some protein phosphorylation events play a key role in SAC activation.

Once Mad1 binds to unattached kinetochores, it further recruits Mad2 and causes the conformational change of Mad2 from "open" to "closed" forms.[22,23] The "closed" Mad2 proteins sequester Cdc20, the activator of APC/C (anaphase promoting complex or cyclosome), thereby preventing APC^{Cdc20} activation.[24] In addition to Mad2, other checkpoint proteins Mad3/BubR1 and Bub3 also bind to Cdc20 to form the mitotic checkpoint complex (MCC).[25-27] Because APC^{Cdc20} mediates the degradation of securin Pds1, the anaphase inhibitor, active SAC delays anaphase onset by stabilizing Pds1.[28] Compared with the understanding of SAC activation and its role in cell cycle control, the SAC silencing process is much less clear. Although a reasonable speculation is that chromosome bipolar attachment silences the SAC to allow anaphase onset, the link between chromosome attachment and SAC silencing is still missing at the molecular level.

Current SAC Silencing Models

The stripping of SAC proteins from kinetochores by the dynein module

It is well documented that SAC proteins localize at unattached kinetochores. Results from higher eukaryotic cells show that anaphase onset occurs several minutes after SAC protein Mad2 dissociates from the last kinetochore, indicating that SAC silencing occurs prior to anaphase onset.[29] The minus-end-directed motor protein dynein also localizes to unattached kinetochores and dissociates upon microtubule attachment.[30] The kinetochore localization of dynein depends on the Rod/Zw10/Zwilch (RZZ) complex, which directly binds to kinetochore protein Ndc80.[31,32] Another protein, termed Spindly, mediates the interaction between dynein and the RZZ complex.[33,34] Earlier data showed that the dynein module removes SAC proteins Mad1 and Mad2 from the kinetochore upon microtubule attachment, and depletion of dynein blocks cells in metaphase and leads to partial retention of Mad2 at bioriented kinetochores.[29,35] Thus, the dynein module was proposed to silence the SAC by stripping the SAC proteins from kinetochores. In addition to its proposed role in SAC silencing, dynein also mediates the rapid poleward chromosome motion and stabilizes kinetochore microtubules. Cells with dysfunctional dynein show mis-orientated kinetochore pairs and destabilized kinetochore microtubule bundles.[36,37] These observations indicate the function of the dynein motor in the stabilization of kinetochore–microtubule attachment.

Unlike direct dynein inhibition, depletion of Spindly in human cells does not block the removal of Mad2 from kinetochores, although this depletion abolishes dynein recruitment onto kinetochores.[38] This observation raises doubts about the role of kinetochore dynein in SAC silencing. Further investigation suggests that kinetochore dynein is essential for SAC silencing in the presence of Spindly.[39] More recent work demonstrates that the kinetochore dynein mediates the initial microtubule capture, which promotes the Ndc80-mediated end-on kinetochore attachment.[31] Therefore, the metaphase block in dynein-depleted cells could be attributed to erroneous kinetochore attachment and/or the failure of SAC silencing. Nevertheless, the successful SAC silencing in Spindly-depleted cells suggests additional mechanisms for SAC silencing. Moreover, dynein-dependent removal of checkpoint proteins from kinetochores does not appear to be a conserved mechanism for SAC silencing, as no obvious Spindly/RZZ homologs are present in lower eukaryotes such as yeast.

The disassembly of the mitotic checkpoint complex MCC

The association of SAC proteins Mad2, Bub3, and Mad3 with the APC/C activator Cdc20 forms the MCC that prevents anaphase onset by inhibiting APC/C^{Cdc20}. Another SAC silencing mechanism involves MCC disassembly. $p31^{comet}$ protein was identified as a Mad2 interactor.[40] This interaction stimulates MCC disassembly, and overexpression of $p31^{comet}$ protein results in less Mad2 bound to Cdc20.[41,42] In addition, APC15/Mnd2 promotes Cdc20 auto-ubiquitination and the subsequent MCC disassembly in yeast and mammalian cells.[43,44] A substrate of mitotic CDK, CEUDC2, also binds to Cdc20 once phosphorylated, and this binding promotes the release of Mad2 from Cdc20 and the subsequent activation of APC/C^{Cdc20}.[45] However, no evidence indicates the direct link between these mechanisms and kinetochore attachment. It is likely that these mechanisms facilitate the robustness of SAC silencing once cells have initiated the SAC silencing process.

The role of protein phosphatase 1 (PP1) in SAC silencing

Among the SAC components, Mps1 is a protein kinase that phosphorylates the kinetochore protein Spc105/Knl1 to promote kinetochore recruitment of SAC proteins.[17-20] If Mps1 kinase activates the SAC by phosphorylating some proteins at the kinetochore, the reversal of these phosphorylation events is likely required to silence/inactivate the checkpoint. Recent work shows that the kinetochore protein Spc105 also recruits protein phosphatase 1 (PP1) through a conserved RVSF motif, and this interaction is required to dephosphorylate the substrates of Ipl1/Aurora B at kinetochores to stabilize microtubule attachment.[46] Results from budding yeast indicate that the binding of PP1 to Spc105 is essential for SAC silencing, but this binding plays a nonessential role for physical chromosome segregation.[47] Consistently, high levels of PP1 promote SAC silencing in fission and budding yeast cells.[48,49] Therefore, the balance of kinase/phosphatase activity at the kinetochore is likely the key to regulate SAC silencing. We speculate that either decreased kinase activity or increased PP1 activity at the kinetochore could trigger SAC silencing. One open question is how this balance is regulated during the cell cycle. Moreover, it is important to know which substrate of PP1 plays a key role in modulating SAC silencing.

Recent Evidence for Tension-Dependent SAC Silencing

A fundamental question in cell cycle control is which event triggers anaphase onset. It has been speculated that the silencing of the SAC allows anaphase onset, but a long-standing debate is whether kinetochore attachment (occupancy) or the tension on sister kinetochores silences the SAC to trigger anaphase entry.[50] Recent evidence in budding yeast favors the tension model.

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The checkpoint response to tension defects in higher eukaryotic cells

Bipolar attachment generates tension on chromosomes. The observation that applying tension on a mis-attached chromosome in grasshopper spermatocytes triggers anaphase onset suggests the role of tension in cell cycle progression.[51] The 3F3/2 antibody detects phosphorylated kinetochore proteins. Interestingly, tension, whether from a micromanipulation needle or from normal mitotic forces, causes dephosphorylation of the kinetochore proteins recognized by 3F3, suggesting that tension controls the phosphorylation status at the kinetochore.[52] The Salmon group further examined the 3F3/2 signal as well as the kinetochore localization of SAC protein Mad2 in PtK1 cells treated with taxol, which stabilizes microtubules and causes tension loss. Although the phosphoepitope 3F3/2 becomes phosphorylated in all the kinetochores after tension is reduced by taxol, very few kinetochores exhibit Mad2 localization, thereby arguing against the role of tension in checkpoint regulation.[53] Further experiments show that tension promotes further kinetochore attachment by microtubules, which may cause the complete loss of Mad2 localization at the kinetochore.[54] Therefore, the role of tension in SAC silencing remains controversial, as it may silence the SAC indirectly by strengthening kinetochore–microtubule interaction.

The checkpoint response to tension defects in budding yeast

The pulling force from the opposite spindle poles as well as cohesion between sister chromatids is necessary for tension generation. Cohesion loss or a complete block of DNA replication results in tensionless chromosomes. When incubated at 37 °C, temperature-sensitive yeast cohesin mutants (*scc1/mcd1*) show delayed anaphase onset as evidenced by the persistent protein levels of anaphase inhibitor Pds1, although the mutant cells can elongate spindles because of the lack of cohesion.[55] Complete block of DNA replication in *cdc6–1* temperature-sensitive mutants also delays anaphase onset due to the lack of sister chromatids.[56] In addition to the SAC proteins, the kinetochore-associated Ipl1 kinase and a pericentromeric protein Sgo1 are required for the anaphase entry delay induced by these tension defects.[55,57] In contrast, Ipl1 and Sgo1 are dispensable for the cell cycle arrest induced by unattached kinetochores, indicating their specific role in the response to tension defects.

In addition to the role in the response to tension defects, Ipl1 promotes the conversion of tensionless chromosomes to unattached ones.[58,59] Thus, one explanation is that Ipl1-dependent destabilization of kinetochore attachment activates the SAC indirectly. However, this speculation is unable to explain the role of Sgo1 in the checkpoint response to tension defects, because Sgo1 does not promote the generation of unattached kinetochores.[58] Therefore, additional mechanisms should also contribute to the anaphase entry delay induced by tension defects.

The checkpoint response to syntelic attachments in budding yeast

Computer-aided reconstruction from electron micrographs of mitotic yeast cells suggest that each kinetochore is attached by a single microtubule,[60] which makes budding yeast an ideal organism to study the regulation of kinetochore–microtubule interaction. Syntelic attachment establishes when 2 sister kinetochores are attached by microtubules from the same spindle pole. Obviously, tension will be absent from chromosomes with syntelic attachment. Recently, our lab developed a genetic approach to induce syntelic attachment in budding yeast, which is a very useful tool to study the response to tension defects.

Cik1 and Kar3 form a motor complex that moves chromosomes along microtubules toward the minus end.[61] In a genome-wide screen for yeast deletion mutants that are sensitive to stressful DNA replication, both *cik1*Δ and *kar3*Δ mutants were isolated, presumably due to the defect in chromosome bipolar attachment.[62] Our further analysis indicates that the loss of function of Cik1/Kar3 increases the chance of syntelic attachment, although the mechanism for this incorrect attachment remains to be determined. Moreover, we found that overexpression of the coiled-coil domain of Cik1 (Cik1-CC) disrupts Cik1–Kar3 interaction, which allows us to conditionally inactivate the Cik1/Kar3 motor complex and induce syntelic attachments.[63]

While analyzing the checkpoint response to syntelic attachments, we found that dysfunctional SAC abolished the anaphase entry delay induced by *CIK1-CC* overexpression, resulting in sister chromatid co-segregation and viability loss. Similarly, *ipl1* and *sgo1*Δ mutants also eliminate the *CIK1-CC*-induced anaphase entry delay, leading to chromosome missegregation.[63] One important question is how a syntelic attachment delays anaphase onset. One possibility is that the sister kinetochores with syntelic attachment become unattached ones with the assistance of

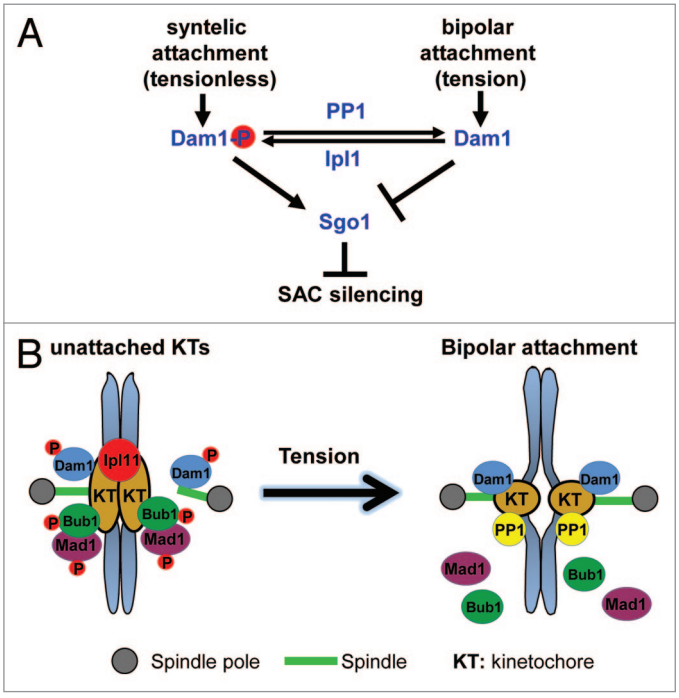

Figure 1. (**A**) The SAC silencing network SSN. Ipl1, PP1, Dam1, and Sgo1 constitute the SSN in budding yeast, which senses the tension at kinetochores and coordinates chromosome bipolar attachment and anaphase onset. (**B**) The working model for tension-induced SAC silencing. Chromosome bipolar attachment alters the balance of kinase/phosphatase at the kinetochore and triggers the dephosphorylation of Dam1, which induces the dephosphorylation of SAC proteins Mad1 and Bub1 to silence the SAC.

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Ipl1 kinase, which subsequently activates the SAC. The second possibility is that the tension defect activates the SAC to delay anaphase onset through a specific signaling pathway (the tension checkpoint). Third, the absence of tension prevents SAC silencing, thereby maintaining the active status of the SAC prior to tension generation.

The absence of tension prevents SAC silencing

Sgo1 is essential for the anaphase entry delay induced by loss of cohesion or syntelic attachments, but it is not involved in generating unattached kinetochores when they are not under tension.[58] To assess if Sgo1 activates the SAC or prevents SAC silencing in the absence of tension, we examined the SAC activation and silencing processes in *sgo1*Δ mutant cells in the absence of tension by analyzing Mad1 phosphorylation, as this modification indicates SAC activation.[64,65] The absence of tension leads to sustained Mad1 phosphorylation, indicating SAC activation. Interestingly, *sgo1*Δ mutants show efficient Mad1 phosphorylation but fail to maintain this phosphorylation in the absence of tension. This result indicates that Sgo1 is dispensable for SAC activation but prevents SAC silencing in cells lacking tension. Therefore, Sgo1 is likely a component of the signaling pathway that prevents SAC silencing when tension is absent.

Ipl1 kinase is also required for the anaphase entry delay in response to tension defects,[63] but the fact that Ipl1 destabilizes kinetochore attachment makes it difficult to define the role of Ipl1 in preventing SAC silencing.[58,59] Before addressing this question, we first determined which Ipl1-dependent phosphorylation event is involved in the response to tension defects. One of the well-characterized Ipl1 substrates in budding yeast is the kinetochore protein Dam1, a subunit of the Dam1/DASH kinetochore complex.[66] Substitution of 3 of the 4 Ipl1 consensus sites with alanine or aspartic acid generates viable nonphosphorylatable (*dam1-3A*) and phosphomimetic (*dam1-3D*) mutants.[67] Strikingly, the *dam1-3A* mutant can also eliminate the anaphase entry delay induced by syntelic attachments. In addition, Mad1 is phosphorylated efficiently in *dam1-3A* cells lacking tension, but this phosphorylation disappears prematurely, a phenotype similar to *sgo1*Δ mutant.[68] One possibility is that Dam1 phosphorylation by Ipl1 may also prevent premature SAC silencing. Alternatively, the stabilization of tensionless kinetochore attachment in *dam1-3A* cells may prevent SAC activation.

We further used the phosphomimetic *dam1-3D* mutant to distinguish these possibilities. Since *dam1-3A* mutant cells show premature SAC silencing in the absence of tension, we expect *dam1-3D* mutants to show difficulty in SAC silencing. Indeed, *dam1-3D* cells exhibit an obvious delay in anaphase entry. If the delay is due to destabilized kinetochore attachment, the combination with a SAC mutant will cause chromosome missegregation and viability loss. However, both *dam1-3D mad1*Δ and *dam1-3D mad2*Δ double mutants are viable, although the anaphase entry delay is abolished completely in these double mutants. Thus, the destabilized kinetochore attachment cannot fully explain the anaphase entry delay in *dam1-3D* mutants. To further test if a detachment-independent mechanism contributes to the anaphase entry delay in *dam1-3D* mutant cells, we used live-cell imaging to follow 2 successive cell cycles in *dam1-3D* and *dam1-3D mad1*Δ cells. Among the daughters of the 33 *dam1-3D mad1*Δ cells, 64 could finish the second round of cell division, as indicated by the successful chromosome segregation, suggesting that most of the mutant cells experienced faithful chromosome segregation in the first round of cell cycle.[68] This result strongly supports a detachment-independent mechanism that prevents anaphase onset in *dam1-3D* mutant cells. Although Ipl1 may delay anaphase onset by generating unattached kinetochores that activate the SAC, our data indicate that the phosphorylation of Dam1 by Ipl1 also prevents SAC silencing in a manner independent of the destabilization of kinetochore attachment.

The SAC silencing network (SSN) coordinates anaphase onset with tension generation at kinetochores

Our data suggest that modulation of the phosphorylation of the kinetochore protein Dam1 plays a key role in the SAC silencing process. Previous work shows that Dam1 only becomes dephosphorylated when sister kinetochores are under tension.[69] Therefore, Dam1 is an ideal candidate for the tension sensor. Prior to tension generation, its phosphorylation by Ipl1 kinase may prevent SAC silencing and anaphase onset. Indeed, the phosphomimetic *dam1-3D* mutant abolishes the premature anaphase onset in *ipl1* mutants in response to tension defects, indicating that Ipl1 prevents anaphase onset through Dam1. Previous work also shows that high levels of PP1 induce SAC silencing, and PP1 dephosphorylates Dam1.[70] We found that *dam1-3D* mutant blocks SAC silencing induced by PP1 overexpression, indicating that Dam1 also acts downstream of PP1. These results support the model that the tension at kinetochores can be converted into a biochemical signal through Dam1 phosphorylation, which further regulates SAC silencing. Therefore, the Ipl1 kinase, phosphatase PP1, and their substrate Dam1 constitute the SAC silencing network SSN that links tension generation to anaphase onset.

Then how to fit Sgo1 into this SSN? The comparison of Dam1 phosphorylation kinetics during the cell cycle in wild-type and *sgo1* mutant cells did not reveal any difference. In contrast, deletion of *SGO1* eliminates the cell cycle delay in *dam1-3D* phosphomimetic mutant cells, suggesting that Sgo1 functions downstream of Dam1 (**Fig. 1A**).[68] Nevertheless, we cannot exclude the possibility that Sgo1 and Dam1 act in parallel to regulate SAC silencing. Taken together, recent advances in budding yeast support the tension model for SAC silencing. Prior to tension generation, the sustained phosphorylation of kinetochore protein Dam1 by Ipl1 prevents SAC silencing and anaphase onset. Once chromosome bipolar attachment applies tension on sister kinetochores, tension-induced Dam1 dephosphorylation by PP1 triggers SAC silencing and anaphase entry (**Fig. 1B**). Therefore, the SAC silencing network SSN couples SAC silencing with bipolar attachment-induced tension generation, ensuring that chromosome segregation only occurs after chromosome bipolar attachment.

Further Directions

The occupancy vs. tension model?

An unresolved debate is whether the attachment of kinetochores (occupancy) or the tension silences the SAC. Recent work

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supports the tension model. If that is the case, does kinetochore occupancy play a role in SAC silencing? Although *sgo1*Δ and *dam1-3A* mutant cells can silence the SAC in the absence of tension, these mutant cells show proficient metaphase arrest in response to nocodazole treatment, which generates unattached kinetochores. Thus, we speculate that kinetochore occupancy is necessary but not sufficient for SAC silencing. SAC silencing could be a 2-step process. First, kinetochore attachment may decrease the capacity of SAC activation, for example, by compromising kinetochore binding of SAC protein Mad2.[53] After chromosome bipolar attachment, the tension on sister kinetochores may silence the SAC by triggering the dephosphorylation of kinetochore protein Dam1 as well as some SAC proteins, such as Mad1 and Bub1. It will be informative to analyze the localization of the SAC components to unattached and attached but tensionless kinetochores. The results may reveal different SAC activation status in attached kinetochores with or without tension.

Does tension regulate the activity of kinases or phosphatases at the kinetochore?

The phosphorylation of some kinetochore or SAC proteins is essential for SAC activation. The reversal of these phosphorylation events is likely critical for SAC silencing. If tension at the kinetochore triggers SAC silencing, one important open question is how tension regulates the activity of kinases or phosphatases at the kinetochore. Using fluorescence biosensors to measure localized phosphorylation dynamics in living Hela cells, the Lens group found that phosphorylation of an Aurora B substrate at the kinetochore depends on its distance from the kinase at the inner kinetochore.[71] Thus, one attractive model is that tension-induced kinetochore stretching separates Ipl1/Aurora B kinase from its substrates at the kinetochore, which may compromise phosphorylation and trigger SAC silencing. Recent work shows that deletion of the N terminus of Sli15, an Ipl1 interactor in yeast cells, abolished kinetochore binding of Ipl1, but the *sli15*-Δ*N* cells grow normally.[72] It will be interesting to examine if the elimination of Ipl1 kinase at kinetochores in *sli15*-Δ*N* cells causes premature SAC silencing. Moreover, the checkpoint kinase Mps1 associates with the kinetochore through Ndc80,[73] so tension on chromosomes may also regulate Mps1 kinase activity to control the timing of SAC silencing.

In addition to protein kinases, the kinetochore protein Spc105/Knl1 also recruits PP1 to the kinetochore, and the abolishment of PP1 recruitment in a *spc105* yeast mutant blocks SAC silencing.[46,47] It remains unclear if the Spc105–PP1 interaction is constitutive or regulated during the cell cycle. One possibility is that tension at kinetochores triggers recruitment of PP1 to the kinetochore to induce SAC silencing. Alternatively, tension enables PP1 to dephosphorylate its substrates through tension-induced kinetochore conformation change. Therefore, an important question regarding SAC silencing is how tension alters the kinase/phosphatase balance at the kinetochore.

Our results support the conclusion that the dephosphorylation of Dam1 is essential for SAC silencing. The key result supporting this conclusion is the observation that phosphomimetic *dam1-3D* mutant cells show compromised dephosphorylation of 2 SAC components, Mad1 and Bub1.[68] However, it remains elusive how Dam1 phosphorylation by Ipl1 prevents the dephosphorylation of Mad1 and Bub1. Most of the *dam1-3D* cells are able to perform faithful mitosis, indicating the low frequency of unattached kinetochores.[68] Nevertheless, results from in vitro assays suggest that Dam1 phosphorylation compromises the recruitment of the Ndc80 complex to microtubules.[74,75] It is possible that Dam1 dephosphorylation induces a stronger Dam1–Ndc80 interaction, which may cause kinetochore conformation changes to trigger SAC silencing. This conformational change likely alters the distance between the substrates and its kinase or phosphatase. Alternatively, this change may promote the dissociation of kinases from the kinetochore or induce recruitment of PP1 to the kinetochore. In support of this speculation, mutation in the Ndc80 loop domain compromises Ndc80–Dam1 interaction and delays SAC silencing in yeast.[76] This loop region in Ndc80 also mediates interaction with the Ska complex in mammalian cells.[77] Moreover, results from the Salmon lab confirm the role of the loop domain in the conformational change of the Ndc80 complex.[78] Further studies are needed to verify whether modulation of the phosphorylation of Dam1 or Ska proteins contributes to the conformation change of the Ndc80 complex and define the role of this change in SAC silencing.

Is the SSN a conserved mechanism for SAC silencing?

The Ipl1/Aurora B kinase destabilizes chromosome attachment in yeast and mammalian cells,[58,59,79,80] but its role in checkpoint control remains controversial. Like yeast *ipl1* mutant cells, mammalian cells treated with nocodazole as well as an Aurora B inhibitor arrest in mitosis, indicating proficient SAC function. Interestingly, Aurora B inhibition accelerates checkpoint exit after nocodazole washout.[81,82] In addition, Aurora B inhibition overrides the checkpoint efficiently when cells are treated with taxol that stabilizes microtubules and compromises tension generation.[82] A reasonable explanation is that Aurora B is also required to prevent premature SAC silencing in mammalian cells when tension is compromised by taxol treatment. Aurora B may also regulate SAC activation by promoting kinetochore detachment.

Our data suggest that Ipl1 prevents SAC silencing by phosphorylating Dam1, a subunit of the Dam1/DASH complex in budding yeast. Then what could the substrate of Aurora B kinase important for SAC silencing in mammalian cells be? Recent evidence indicates that the Ska complex in mammalian cells is likely the functional ortholog of the Dam1 complex.[83,84] Unlike the 10-subunit Dam1 complex, only 3 components are present in the Ska complex. Both Dam1 and Ska complexes associate with the spindle microtubules prior to kinetochore–microtubule interaction.[85] Moreover, both complexes contain some components that are phosphorylated by Ipl1/Aurora B, and this phosphorylation destabilizes kinetochore–microtubule interaction.[74,75,86] Therefore, one untested possibility is that phosphorylation of the Ska complex by Aurora B also prevents SAC silencing in mammalian cells.

In summary, recent work reveals the SAC silencing network (SSN) that coordinates chromosome attachment, tension generation, and anaphase onset. In budding yeast, this network includes a kinetochore protein Dam1, its kinase Ipl1, phosphatase PP1, and a downstream component Sgo1, although more components

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remain to be identified. The SSN prevents SAC silencing prior to chromosome bipolar attachment that applies tension on sister kinetochores. Since premature SAC silencing leads to anaphase onset in the presence of incorrect chromosome attachments, deregulation of this pathway will increase the chance of chromosome missegregation and aneuploidy. Much more work is needed to elucidate the molecular details for this signaling pathway.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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