REVIEW ARTICLE
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Chemical tools for dissecting cell division

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Components of the cell division machinery typically function at varying cell cycle stages and intracellular locations. To dissect cellular mechanisms during the rapid division process, small-molecule probes act as complementary approaches to genetic manipulations, with advantages of temporal and in some cases spatial control and applicability to multiple model systems. This Review focuses on recent advances in chemical probes and applications to address select questions in cell division. We discuss uses of both enzyme inhibitors and chemical inducers of dimerization, as well as emerging techniques to promote future investigations. Overall, these concepts may open new research directions for applying chemical probes to advance cell biology.

A central process for life is to pass genetic material to offspring through cell division. In eukaryotes, the genetic material is packaged into chromosomes, which replicate and then segregate to daughter cells. For somatic cells or asexual reproduction, daughter cells acquire identical chromosome copies from the parental cell through mitosis. For sexual reproduction, two cycles of meiotic cell division produce haploid gametes from a diploid parent. Multiple molecular machineries are required for successful division in both mitosis and meiosis. Microtubules, consisting of polarized tubulin polymers, organize into a bipolar structure with minus ends anchored to spindle poles. This dynamic spindle interacts with the chromosomes through kinetochore protein complexes to drive chromosome movement to the spindle equator (congression), ensure that sister chromosomes attach correctly to opposite spindle poles (error correction) and segregate the chromosomes in anaphase. In addition, the spindle assembly checkpoint (SAC) regulates cell cycle progression to promote accurate chromosome segregation by delaying anaphase until all kinetochores are attached to spindle microtubules. Defects in any of these processes can lead to aneuploidy and structural rearrangements of chromosomes, which are highly associated with cancer and developmental diseases[1–4]. The underlying spatial and temporal regulation of cell division is a long-standing focus of cell, developmental and reproductive biology studies.

A common cell biology paradigm is to draw inferences by contrasting how cells respond to different perturbations. Genetic perturbations, such as knockout, overexpression or RNA interference (RNAi), have led to many advances linking molecular functions to phenotypic observations. However, the lack of temporal control (typically on the order of days) limits studies of dynamic processes in cell division, which is completed within minutes to hours. Moreover, components of the cell division machinery can target different substrates with distinct functions at different intracellular locations, which are difficult to dissect by genetic perturbations. As a complementary approach, small-molecule probes provide seconds-to-minutes temporal control and can also be designed for spatial control and reversibility. Using enzymatic inhibitors, for example, acute effects can be observed immediately after adding a small molecule, avoiding potential secondary effects (including lethality in some cases) that can accumulate over time after genetic perturbations. Furthermore, as a single protein can have multiple functions, enzymatic inhibition can help uncover non-enzymatic mechanisms. With the continual development of new small-molecule inhibitors, mechanistic studies have taken advantage of these probes to address a variety of questions in cell division.

In recent years, cell biologists have increasingly exploited chemical inducers of dimerization, which bring multiple proteins together (Fig. 1a) and provide experimental control over the local concentrations of a specific protein target and its associated partners. Among a variety of dimerizers, established platforms used in cell division studies include rapamycin and the trimethoprim-halo ligand (TMP-Halo, TH) system. Rapamycin dimerizes FK506-binding protein (FKBP) and the FKBP–rapamycin-binding (FRB) protein[5], and TH dimerizes the *Escherichia coli* dihydrofolate reductase (eDHFR) and the Haloenzyme (Fig. 1b)[6]. By fusing these protein tags to a desired anchor and effector pair, dimerization can be accomplished with minute-scale kinetics upon chemical addition. Moreover, these probes have been engineered to be light responsive, allowing recruitment with spatial control. For instance, TH dimerizers have been derived to three platforms: a coumarin cage for light-induced recruitment (CTH; Fig. 1c), an NVOC (6-nitroveratryl oxycarbonyl) insertion for light-induced cleavage (TNH; Fig. 1d) and a combination of the two (CTNH)[7,8]. These photoinducible methods can respond to light in a few seconds on top of the gained spatial control and thus provide a powerful toolkit to study cell division events occurring in a short time window at defined intracellular sites.

Photocaged chemical dimerizers such as CTH and NTH are typically irreversible upon light exposure or reversible for a single cycle of uncaging followed by photocleavage in the case of CTNH. In contrast, the protein conformational changes underlying genetically encoded light-inducible dimerization systems are generally reversible[9], which enables the system to be perturbed repetitively but can be a limitation if continuous light exposure is required to maintain dimerization over time. Photocaged dimerizers remain active after uncaging by a single pulse of light, which can have advantages for long-term perturbations[10] or if a subcellular structure such as a randomly oscillating kinetochore is difficult to target repeatedly[11]. Thus, genetically encoded and small-molecule-based approaches have complementary advantages and limitations.

In this Review, we summarize recent advances using small-molecule probes to dissect cell division mechanisms (Tables 1 and 2) and envision future techniques and potential strategies for probe design. Instead of a comprehensive review of chemical approaches, we focus on chemical probes and strategies leading to advances that would otherwise be difficult to achieve using only genetic perturbations. Topics covered here focus on prometaphase

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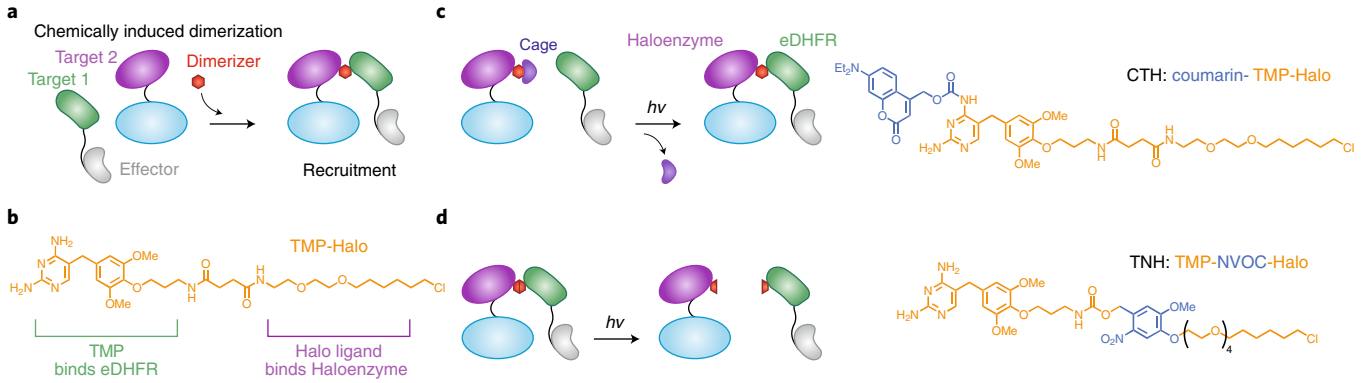

Fig. 1 | Chemically induced dimerization recruits effector proteins to anchors. a, A small-molecule dimerizer binds to a pair of protein targets, each of which is fused to an effector or anchor protein. If the anchor is localized to an intracellular structure, dimerizer addition recruits the effector to that structure. **b**, TMP-Halo dimerizer. The bifunctional linker includes a TMP moiety and a Halo ligand to dimerize proteins fused to eDHFR and a Haloenzyme. **c**, Schematic of light-induced dimerization using CTH. A coumarin-caged dimerizer initially binds to the Halo-tagged anchor protein. Upon light-mediated uncaging, the exposed TMP moiety recruits eDHFR-tagged effectors to anchors. **d**, Schematic of light-induced cleavage using TNH. The photocleavable moiety NVOC, inserted between TMP and Halo, releases the bound effectors from anchors upon light activation.

Table 1 | Enzymatic inhibitors and their applications in cell division studies

Inhibitor	Function	Applications in cell division
5-Iodotubercidin	Haspin inhibitor	Depletion of centromeric Aurora B pool[56]
Apcin	APC/C inhibitor	Prolongment or shortening of metaphase at low or high SAC activity[70,71]
Centrinone	PIK4 inhibitor	Modulation of centriole numbers[18], generation of asymmetric spindles[19,20]
FCPT, BRD9876	Eg5 rigor inhibitor	Spindle cross-linking[40], block of poleward microtubule flux[38,39], reduction of inter-kinetochore tension[11]
GSK923925	CENP-E rigor inhibitor	Generation of pole-proximal chromosomes[7,61]
GW108X	KIF15 inhibitor	Depletion of spindle-bound KIF15 (ref. [42])
KIF15-IN-1	KIF15 rigor inhibitor	Reduction of inter-kinetochore tension[11]
MLN8054, MLN8237	Aurora A inhibitor	Inhibition of Aurora A activity after mitotic entry[61,62]
proTAME	APC/C inhibitor	Prolongment of metaphase[69]
Reversine	MPS1 inhibitor	Shortening of metaphase and generation of segregation errors[65]

Table 2 | Chemical dimerizers and their applications in cell division studies

Dimerizer	Function	Applications in cell division
AP20187	FKBP homodimerization	Dimerization of MPS1 for transactivation[75]
Auxin/IAA	Degradation of AID-tagged protein by recruiting ubiquitin ligases	Rapid depletion of proteins without known inhibitors[82]
CTH	Light-inducible dimerization of eDHFR and Haloenzyme	Recruitment of kinesin motors[7] or Aurora B[11] to kinetochores
LoKI	Inhibition of kinases proximal to the SNAP-tagged protein	Inhibition of pole- or kinetochore-localized pools of Aurora A and Plk1 (ref. [87])
Rapamycin	FKBP and FRB dimerization	Recruitment of Aurora B to centromeres[58] or MAD1 to kinetochores[72,73], testing of MPS1 function at different locations[76,89]
TNH	Photocleavable dimerizer linking eDHFR and Haloenzyme	Initiation of mitotic exit by releasing MAD1 from kinetochores[7]

and metaphase events in both mammalian and yeast mitosis, but the tools and underlying concepts should be transferrable to other cell stages, meiotic studies and other model systems.

PLK4 and centrosome duplication

Setting up a dynamic spindle requires microtubules to nucleate and polymerize from their organizing centers. One major pathway is through centrosomes[12,13] (Fig. 2a). The interior centriole component organizes pericentriolar material to regulate centrosome maturation[14] and duplicates under the regulation of Polo-like kinase 4 (PLK4) during interphase[15–17]. PLK4 activity controls centriole numbers and microtubule density, making it an attractive target to address how centrosomes regulate cell proliferation signaling and spindle dynamics. The PLK4 inhibitor centrinone can block centriole duplication and thus has been used to generate cells lacking centrosomes[18] (Fig. 2b) or to generate asymmetric spindles[19,20] (Fig. 2c). These studies exploited several properties of centrinone, as discussed below, that are typical of small-molecule inhibitors but difficult to achieve by genetic approaches: applicability across different cell lines and species, acute inhibition and reversibility after inhibitor washout.

Cells containing supernumerary centrosomes are highly associated with chromosome instability and tumorigenesis[21,22], leading to the hypothesis that transformed cells gain a fitness advantage by centrosome over-replication. To test whether proliferation of transformed cells depends on supernumerary centrosomes, centrinone

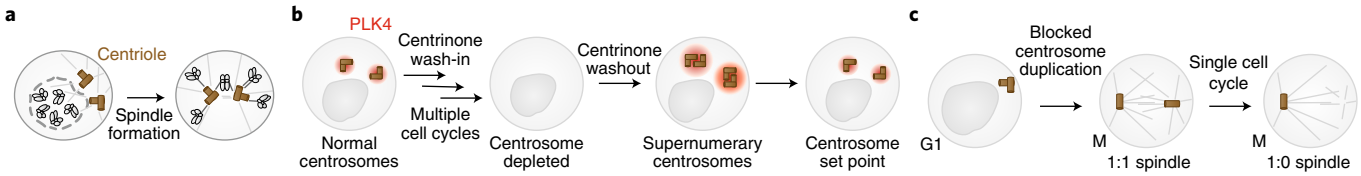

Fig. 2 | Centrinone inhibits PLK4 activity. a, Mitotic spindle formation. After nuclear envelope breakdown (dashed line), centrosomes act as microtubule-organizing centers to shape the spindle. Dynamic spindle microtubules capture chromosomes for later congression and segregation. **b**, Centrinone prevents centriole duplication by inhibition of PLK4. Because PLK4 has dual activities of autoactivation and phosphoregulated proteolysis, centrinone washout induces a wave of PLK4 activity that generates overduplicated centrioles in cancer cells. After several cell cycles, the centriole number eventually returns to the initial 'set point'. **c**, Centrinone generates asymmetric spindles. By blocking centriole duplication in S phase, the cell contains halved centriole numbers in mitosis (M; 1:1 spindle). In the next cycle, the daughter cells contain only one isolated centriole (1:0 spindle), resulting in asymmetric spindles.

was used to deplete centrioles. Multiple human cancer cell lines are more resistant to centriole loss following centrinone addition than normal cells[18], but overexpression of TRIM37, which degrades pericentriolar materials, abolishes this resistance[23,24]. Thus, transformed cells with amplifications of a region containing the *TRIM37* gene are 'addicted' to supernumerary centrosomes and vulnerable to PLK4 inhibition. To test whether centrosomes can recover after loss, centrinone was washed out to reactivate PLK4, resulting in a return of centrosome numbers to the initial set point in cancer cells (Fig. 2b). In contrast, normal cells lacking centrosomes prolong mitosis and arrest in G1 phase by a p53-mediated pathway, preventing new centriole assembly that would occur in S phase[25–27].

As centrosomes increase mitotic spindle dynamics, centrinone can be used to modulate spindle architecture by partially depleting centrosomes. When cells with a single centrosome enter mitosis, the spindle is asymmetric because only one pole includes a centrosome, providing an internal control between the two halves of the spindle. This approach was used to show that centrosomes not only nucleate microtubules and regulate minus-end dynamics at spindle poles but also control plus-end dynamics at kinetochores, leading to a shorter half-spindle on the acentrosomal side[19]. The astral microtubule density is also asymmetric in spindles with a single centrosome, providing a system to probe the role of these microtubules in spindle elongation. Experiments with *Potorous tridactylus* (rat-kangaroo) cells under physical confinement showed that the centrosome-depleted half-spindle elongates more slowly than its counterpart, supporting a model in which compression forces tighten the cortex–microtubule coupling so that cortical force generators exert more force to slide spindle poles apart[20]. These studies highlight the use of centrinone to control centrosome numbers, without the need for low-throughput laser ablation approaches that have also been used to remove centrosomes[28].

Kinesin motors on spindle microtubules

Bipolar spindles require a group of molecular motors to organize microtubules in a polarized structure, such that the microtubules from opposite poles act as tracks for chromosome congression (Fig. 3a). Eg5 (kinesin-5) and KIF15 (kinesin-12) motors work cooperatively to cross-link microtubules and determine pole-to-pole distances. Because of their unique homotetrameric structure, Eg5 motors can both slide interpolar microtubules apart and cross-link parallel microtubule arrays[29–31]. As an auxiliary mechanism of spindle assembly, KIF15 maintains spindle bipolarity in the absence of Eg5 (ref. [32]). Additionally, KIF15 acts predominantly on kinetochore microtubules (k-fibers)[33,34], suggesting that it may regulate chromosome dynamics. After a bipolar spindle has been established, chromosomes near the poles require the kinetochore motor protein CENP-E (kinesin-7) for congression, which powers movement toward the metaphase plate[35]. Failures in these various motor proteins arrest cells in mitosis through SAC activation. Inhibitors of these motors can have different mechanisms of action, such as by locking motors on microtubules (rigor inhibition) or weakening microtubule interactions, which has been valuable for dissecting key kinesin functions as illustrated in the examples below.

Spindle microtubules have long been thought to couple mechanically[36]. K-fiber mechanics, in particular, have been studied because of the established roles of these microtubules in chromosome segregation[37]. To test how microtubule cross-linking contributes to k-fiber load-bearing, the Eg5 rigor inhibitor FCPT provides a useful tool (Fig. 3b,c). Rigor inhibition increases microtubule cross-linking by trapping Eg5 in a strong binding rigor state while diminishing the powerstroke (as indicated by diminished poleward microtubule flux[38,39]), switching Eg5 from an active force generator to a passive cross-linker[40]. Movements of non-sister kinetochores are more highly correlated upon FCPT treatment, suggesting a coupled network across k-fibers[41]. To determine the effects of cross-linking on k-fiber load-bearing, the relaxation of centromere stretch upon k-fiber ablation serves as an experimental readout, where strong relaxation amplitude corresponds to weak load-bearing activity (Fig. 3d). FCPT treatment decreases the mechanical relaxation amplitude, indicating that induced cross-linking activity increases k-fiber load-bearing[39]. Thus, mitotic spindles act as a connected network of cross-linked microtubules, suggesting that spindle function depends on integration of mechanics from each microtubule and motor.

Comparisons between different inhibitors, or between inhibitors and RNAi, can dissect which activity mediates a biological process. This approach was used to determine how KIF15 motors organize k-fibers, using the splaying of microtubules within a k-fiber after severing by laser ablation as an assay[42]. One inhibitor, GW108X, reduces KIF15 microtubule cross-linking activity by weakening microtubule binding, whereas the rigor inhibitor KIF15-IN-1 only diminishes the powerstroke. GW108X promotes k-fiber splaying upon ablation but KIF15-IN-1 does not, suggesting that the remaining microtubule cross-linking upon rigor inhibition is sufficient to hold k-fibers together. Similarly, to dissect how CENP-E contributes to SAC signaling, phenotypes with the CENP-E rigor inhibitor GSK923925 were compared to those with genetic knockdown[43]. If SAC silencing results directly from CENP-E binding microtubules, as suggested previously[44,45], then inhibiting CENP-E in a rigor state should be sufficient to resume mitotic progression. The finding that CENP-E rigor inhibition mirrors the phenotype of genetic knockdown suggests that SAC silencing requires the motor activity of CENP-E for chromosome congression and that microtubule binding is not sufficient.

CENP-E drives congression by transporting chromosomes from the spindle pole toward the equator. This activity requires the motor to selectively walk on k-fibers, which face toward the equator, rather

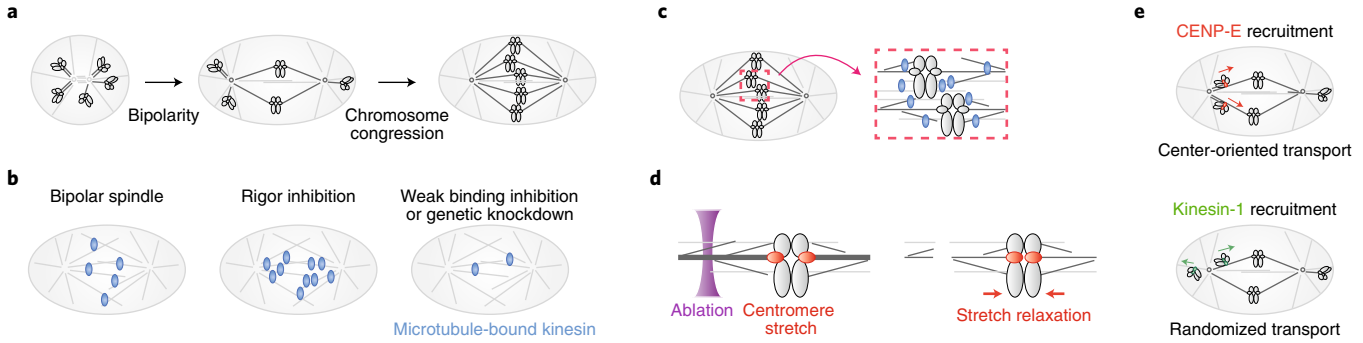

Fig. 3 | Kinesin motors on spindle microtubules. a, Chromosome congression upon establishing spindle bipolarity. Eg5 and KIF15 motors cross-link and powerstroke on microtubule arrays to establish spindle bipolarity. CENP-E motors translocate uncongressed chromosomes from spindle poles to the equator. **b**, Inhibition modes of kinesin motors. Rigor inhibition traps kinesin in a strong binding state on microtubules, whereas inhibitors inducing a weak binding state or genetic knockdown decreases the number of microtubule-bound motors. **c**, K-fibers are mechanically coupled in the spindle. Kinesin motors cross-link interpolar microtubules, k-fibers and bridging fibers. **d**, Centromere relaxation assay. Centromeres are stretched by k-fibers pulling bioriented sister kinetochores in opposite directions and relax immediately following k-fiber ablation a few micrometers away from one kinetochore. Strong relaxation indicates weak load-bearing on k-fibers. **e**, Chromosome congression assay. CENP-E recruitment to the kinetochores of pole-proximal chromosomes drives movement toward the metaphase plate, whereas kinesin-1 transports chromosomes away from the pole in all directions.

than astral microtubules facing the cortex. A post-translational modification of tubulin, detyrosination, differentiates these microtubule populations, with astral microtubules more tyrosinated and k-fibers more detyrosinated[46]. To test whether CENP-E can read this 'tubulin code', endogenous CENP-E was depleted by short interfering RNA (siRNA) to generate pole-proximal chromosomes and the CENP-E motor domain was recruited to the kinetochores of chromosomes near one pole using the photocaged dimerizer CTH (Fig. 1c)[7]. This spatially defined recruitment leaves the opposite pole unaffected as an internal control. CENP-E motor recruitment triggered selective chromosome movement toward the metaphase plate, whereas the kinesin-1 motor domain did not show this selectivity (Fig. 3e). This finding illustrates the use of photocaged dimerizers for spatial control and shows that CENP-E biochemistry optimizes chromosome congression, supporting the tubulin code hypothesis[47].

Chromosome error correction

Correct chromosome segregation requires robust error correction mechanisms, which selectively stabilize correct 'biorientation' in which sister kinetochores are attached to opposite spindle poles while destabilizing incorrect attachments (Fig. 4a). The interactions between kinetochores and spindle microtubules are regulated by a combination of mechanical and biochemical factors[37,48–50]. Mechanically, tension generated at kinetochores by pulling forces from opposite spindle poles indicates biorientation, whereas low tension signals an error to be corrected. Biochemically, Aurora B kinase phosphorylates kinetochore substrates such as NDC80 to destabilize their interactions with microtubules. One mechanism to bridge mechanics and biochemistry is for tension to locally inhibit Aurora B activity at kinetochores to stabilize correct attachments. The observed inner-centromere localization of Aurora B suggests a model in which error correction depends on this centromeric pool. Because tension pulls sister kinetochores apart, away from the inner centromere, this model predicts low Aurora B activity at bioriented kinetochores, which would selectively stabilize these attachments. To test this model, small-molecule probes have enabled complementary strategies in different model systems to either decrease or increase enrichment of Aurora B at centromeres while preserving its global kinase activity.

First, in mammalian cells, Aurora B enrichment at centromeres requires phosphorylation by haspin and BUB1 kinases of histones H3 and H2A, respectively[51–55]. The haspin kinase inhibitor 5-iodotubercidin[56] reduces centromeric Aurora B levels and

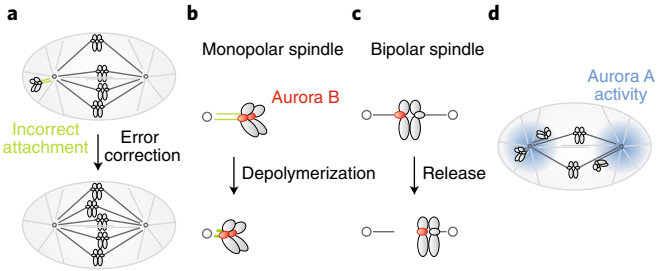

Fig. 4 | Aurora kinases regulate kinetochore–microtubule interactions. **a**, Lack of tension across a sister kinetochore pair provides a signal to identify incorrect attachments, which are destabilized to allow new attachments to form. This process continues until all attachment errors are resolved. **b**,**c**, Effects of recruiting Aurora B to kinetochores to increase local kinase activity. **b**, On monopolar spindles, kinetochore recruitment primarily triggers k-fiber depolymerization to move the chromosome toward the attached pole. **c**, Conversely, recruiting Aurora B to a single kinetochore of a bioriented sister pair primarily induces k-fiber release, with the targeted kinetochore moving away from the pole to which it was initially attached. **d**, Aurora A localizes to spindle poles and phosphorylates kinetochores near the pole to destabilize microtubule attachments.

was used to test whether tension sensing depends on this pool of kinase. A live-cell assay using fluorescence lifetime imaging to measure Förster resonance energy transfer (FLIM-FRET) showed that binding of the NDC80 complex to microtubules is regulated by tension and by Aurora B[57]. Furthermore, the tension dependence of NDC80 binding to microtubules was lost after treatment with 5-iodotubercidin, indicating that centromeric Aurora B is a part of the tension-sensing mechanism. Conversely, other experiments showed minimal effects on phosphorylation of Aurora B substrates at kinetochores after reducing centromeric Aurora B levels, suggesting that a non-centromere pool of Aurora B can phosphorylate kinetochore substrates[51,52].

Second, rapamycin-induced dimerization has been used to enrich centromeric Aurora B (IPL1) in budding yeast. By fusing the IPL1 activator SLI15 (INCENP ortholog) to FRB and the centromeric protein MIF2 (CENP-C ortholog) to FKBP, rapamycin addition recruits SLI15 and IPL1 to centromeres[58]. Centromere recruitment rescues chromosome biorientation when other

pathways for localizing IPL1 to centromeres are inhibited, indicating that biorientation depends on centromeric Aurora B in yeast. In these experiments, chemically induced dimerization enables acute gain of function (~10 minutes) after chromosomes are successfully aligned.

In addition to regulating local Aurora B activity at kinetochores, tension could dictate the outcome of Aurora B activation. Aurora B can destabilize kinetochore–microtubule interactions by two mechanisms, induction of either microtubule release from kinetochores or depolymerization while maintaining attachment[48,49]. To determine whether these mechanisms depend on tension, Aurora B was recruited to kinetochores using the photocaged chemical dimerizer CTH[11] (Fig. 1c). Because microtubule release reduces pulling forces exerted by k-fibers, whereas depolymerization generates pulling forces, the two mechanisms predict chromosome movement in opposite directions. Aurora B recruitment to an individual kinetochore from a sister pair induces microtubule release on bipolar spindles with high tension. In contrast, recruitment induces microtubule depolymerization while maintaining attachment on monopolar spindles, where tension is lower (Fig. 4b,c). Furthermore, decreasing tension using Eg5 or KIF15 rigor inhibitors (BRD9876 and KIF15-IN-1, respectively) slows the microtubule release rate. Thus, phosphorylation converts a catch bond, in which tension stabilizes attachments[59], to a more conventional slip bond, in which kinetochores release microtubules under tension. In these experiments, photocaged dimerizers provide control over local kinase activity with second and sub-micrometer spatiotemporal precision to probe the biological processes underlying error correction.

The tension-dependent response to Aurora B activity suggests different correction pathways for two distinct attachment errors. A merotelic error, in which a single kinetochore attaches to both spindle poles, is under tension due to microtubules pulling in opposite directions. Phosphorylation in this case would facilitate microtubule release, providing an opportunity to make a new attachment. Conversely, for a syntelic kinetochore pair, in which both sisters attach to the same pole with lower tension, phosphorylation induces k-fiber depolymerization to move the chromosomes toward the spindle poles[60]. To determine how this poleward chromosome movement leads to error correction, the CENP-E rigor inhibitor GSK923925 was used to examine kinetochore phosphorylation on both pole-proximal and aligned chromosomes in mitotic cells[61] (Fig. 4d). The microtubule tether NDC80 was more highly phosphorylated near spindle poles, and this spatial dependence was diminished by treatment with the Aurora A kinase inhibitor MLN8237. In line with these results, kinetochores close to spindle poles are more likely to detach from microtubules in mouse oocytes in meiosis I, and this spatial dependence also decreases with Aurora A inhibition using MLN8054 (ref. [62]) Together, these findings indicate that Aurora A at spindle poles phosphorylates kinetochores near the pole to promote microtubule detachment and allow the formation of new attachments. For these experiments, Aurora A inhibitors enabled acute inhibition after establishing spindle bipolarity, without perturbing earlier Aurora A functions in spindle assembly.

Metaphase duration and the SAC

To ensure accurate chromosome segregation, SAC signaling delays anaphase onset in response to kinetochores lacking microtubule attachment (Fig. 5a). SAC activation, as typically measured by kinetochore localization of checkpoint proteins such as MAD1 and MAD2, activates the mitotic checkpoint complex (MCC) and inhibits the E3 ubiquitin ligase APC/C^{CDC20} to prevent cyclin B and securin degradation[63]. Attachments are stabilized by chromosome biorientation, which silences SAC signaling and leads to APC/C activation. Failure to maintain SAC activity results in premature anaphase onset and chromosome segregation errors. Small-molecule probes have been used to examine mechanisms of SAC activation and silencing, through both direct inhibition of various components and induced dimerization to manipulate their localization.

Because low levels of SAC proteins are sufficient to catalyze downstream signaling, RNAi experiments can reach different conclusions even with undetectable SAC protein expression[64]. Furthermore, SAC kinases may have diverse functions beyond their kinase activities. Chemical inhibitors provide an alternative approach to probe interactions between SAC components complementary to RNAi experiments. For example, Aurora B inhibition prevents MPS1 kinase localization at kinetochores, whereas MPS1 inhibition using reversine does not change Aurora B localization but prevents MAD1 localization, suggesting that MPS1 acts downstream of Aurora B to regulate SAC signaling[65]. Furthermore, inhibition of MPS1 kinase activity generates a high frequency of unaligned chromosomes in comparison to genetic knockdown[66], suggesting that inactive MPS1 competes with microtubules for binding to NDC80 at kinetochores and thereby impedes chromosome alignment[67,68]. In another example, APC/C inhibition using proTAME prevents the APC/C from loading its activator CDC20 and prolongs metaphase for hours, but only for approximately 20 minutes in *MAD2*-knockdown cells. This finding indicates cooperative APC/C inhibition by proTAME and SAC activity, suggesting a positive feedback loop between SAC silencing and APC/C activation[69]. Another inhibitor, apcin, blocks binding of APC/C^{CDC20} to its substrates and further prolongs metaphase arrest in combination with proTAME[70]. When cells are arrested with high SAC activity by the MCC inhibiting APC/C, however, apcin can paradoxically induce mitotic slippage by outcompeting the MCC[71]. These examples illustrate the use of small-molecule inhibitors to test interactions between a target and its regulating factors.

Chemically induced dimerization has been used to probe SAC signaling in greater detail. For example, MAD1, MAD2, MPS1 and several other checkpoint proteins localize to unattached kinetochores, raising the question of which are sufficient for APC/C inhibition. MAD1 recruitment to kinetochores in human cells at metaphase, after the checkpoint has been silenced, reactivated the SAC without an increase in kinetochore MPS1 levels, highlighting the importance of MAD1 localization[72,73]. Furthermore, as several SAC kinases can phosphorylate themselves (autophosphorylation), dimerization strategies can manipulate this reaction. For instance, chemical dimerization, using AP20187 to dimerize two FKBP-F36V domains[74], enhances MPS1 kinase activity, suggesting that recruitment to kinetochores activates the kinase by increasing local concentration[75]. Another question is how the SAC senses microtubule attachments so that it is selectively activated by unattached kinetochores and silenced at each kinetochore by end-on microtubule binding. To determine whether this process depends on the spatial relationship between signaling components, MPS1 and its protein substrate were recruited to varying positions in budding yeast kinetochores[76] (Fig. 5b,c). Recruiting MPS1 to the inner kinetochore successfully activated the SAC, whereas recruitment to the outer kinetochore (farther from the centromeric nucleosome) did not. These findings indicate that physical proximity between MPS1 and inner-kinetochore components activates the SAC on unattached kinetochores. In line with this interpretation, the SAC was also activated by recruiting the key MPS1 substrate, a domain of the kinetochore protein SPC105 (mammalian KNL1 ortholog), to the outer kinetochore. Together, these findings suggest a model in which a physical barrier blocks the interaction between MPS1 and SPC105 upon microtubule attachment, silencing the SAC by preventing SPC105 phosphorylation. A likely candidate for this barrier is the DAM1 complex, which localizes to yeast kinetochores after end-on microtubule binding and is positioned in between MPS1 and SPC105. Overall, these experiments illustrate applications of rapamycin-induced dimerization to multiple proteins in different model systems and the ability of this approach to recapitulate

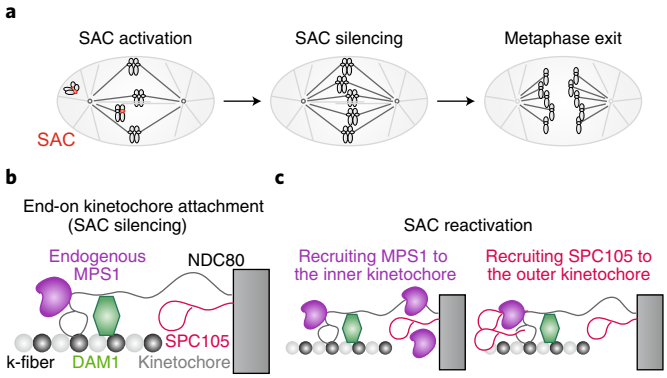

Fig. 5 | Activating and silencing the SAC. a, The SAC is activated by unattached kinetochores to delay anaphase onset until all chromosomes are attached to spindle microtubules (SAC silencing). **b**, End-on microtubule attachment at kinetochores silences the SAC. Microtubule-bound DAM1 complex blocks the interaction between MPS1 at the outer kinetochore and SPC105 at the inner kinetochore. **c**, SAC reactivation by rapamycin-induced dimerization. Recruiting exogenous MPS1 close to SPC105 or recruiting a domain of SPC105 close to MPS1 is sufficient to reactivate the SAC.

physiological features by ectopic protein recruitment to probe a complex biological process.

Leading technology and optogenetics

In this section, we appraise several unexplored strategies for cell division studies. We anticipate that chemically induced dimerization and optogenetics can further broaden the applicability of these strategies by providing spatiotemporal control. For example, chemical probes that induce proteasome-assisted proteolysis have been developed to regulate intracellular protein levels by modifying the targeted proteins with multiple ubiquitin proteins on surface-accessible lysine residues. This 'ubiquitination' process consists of a series of reactions catalyzed by multiple enzymes, including an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin ligase. To target a protein for degradation, one approach is to recruit such ubiquitination systems proximally to the target. Proteolysis-targeting chimeras (PROTACs), in which a designed bifunctional probe bridges ubiquitin ligases and the target to induce degradation, provide a complementary strategy to small-molecule inhibition[77]. However, the slow degradation rate of PROTACs (on the order of hours) limits the application for studying more rapid mitotic processes. To resolve this temporal limitation, one solution is to genetically fuse the target protein to a peptide that is recognized by the ubiquitination system as a 'degron'. The auxin-inducible degron (AID) system[78] provides efficient degradation and high specificity by directly recruiting an E3 ligase (the SCF^{TIR1} complex) to an AID peptide upon addition of auxin/IAA (Fig. 6a). The targeted protein can be degraded within minutes of auxin addition, as shown for a variety of proteins involved in cell division[79–81]. This promising degradation activity supports assays requiring acute depletion of target proteins and is especially useful for those lacking known inhibitors. For example, this strategy was applied to target PLK4 before the discovery of centrinone[82]. Furthermore, because the AID strategy depletes the target protein whereas enzymatic inhibitors do not, phenotypic comparison can uncover non-enzymatic roles of the target protein. However, the AID system lacks spatial control. As an alternative, a photosensitive degron system adopts optogenetics to spatially control degron activity and can degrade target proteins within an hour[83,84] (Fig. 6b). Using a similar strategy, the AID degron could be engineered to gain spatial control by adding a photocage moiety to auxin derivatives. This chemical approach has the potential advantage of requiring genetic fusion of only a peptide degron rather than a larger light-responsive protein, which can cause steric constraints for large protein complexes.

Another limitation of degron systems is that degradation efficacy relies on high activity of the degron and a low expression level of the target protein. In comparison to degrons, more acute inhibition can be achieved by directly blocking substrate accessibility using synthetic proteins or substrate-mimetic molecules (nanobodies or monobodies; Fig. 6c). This strategy is especially suitable for highly expressed targets. For instance, designed monobodies rapidly block binding of Aurora A kinase to its activator TPX2 and thereby reduce Aurora A activity in vitro[85]. However, this method offers limited intracellular control, as monobodies block their targets immediately after expression. One potential strategy is to combine a low-affinity monobody with other manipulation tools to tune substrate affinity or local concentrations. For instance, light-responsive optobodies have been engineered from a pair of split nanobody fragments with low target affinity fused to optogenetic dimerization domains. Light exposure immediately increase inhibition affinity by an order of magnitude[86] and can provide spatial control. Increasing local concentrations by recruiting low-affinity monobodies can also allow the monobody to outcompete the original substrate. Here chemically induced dimerizers are candidates for tuning substrate affinity or local concentrations.

Similarly, enzymatic inhibitors can be linked to protein anchors to gain spatial control. One strategy, termed local kinase inhibition (LoKI), is to genetically fuse the protein anchor to a SNAP tag and conjugate a kinase inhibitor with the SNAP-binding ligand chloropyrimidine (CLP) to locally concentrate inhibitors around SNAP-tagged anchors[87] (Fig. 6d). In cells expressing an anchor localized to kinetochores or spindle poles, the inhibitor conjugate successfully targeted local Aurora A or Polo-like kinase 1 activity. Kinetochore-localized Aurora A inhibition generated more unphosphorylated kinetochores than centrosome-localized inhibition, supporting the idea of a kinetochore-localized pool of Aurora A[88]. LoKI provides a powerful approach to dissect localized kinase functions during cell division, with the possibility of incorporating light-sensitive moieties for improved temporal control.

Finally, we propose a strategy to improve the magnitude of recruitment for chemically induced dimerization studies. Tandem repeats of dimerization tags or effector proteins have been used successfully[7,11,58,76,87,89,90], but these genetic manipulations have limitations associated with increased protein size. Alternatively, peptides inducing liquid droplet formation could be used to enrich recruitment. These peptides commonly consist of multivalent domains forming low-affinity interactions, which enable binding to multiple partners at critical concentrations[91]. Manipulating the local concentration of the peptide around its critical concentration

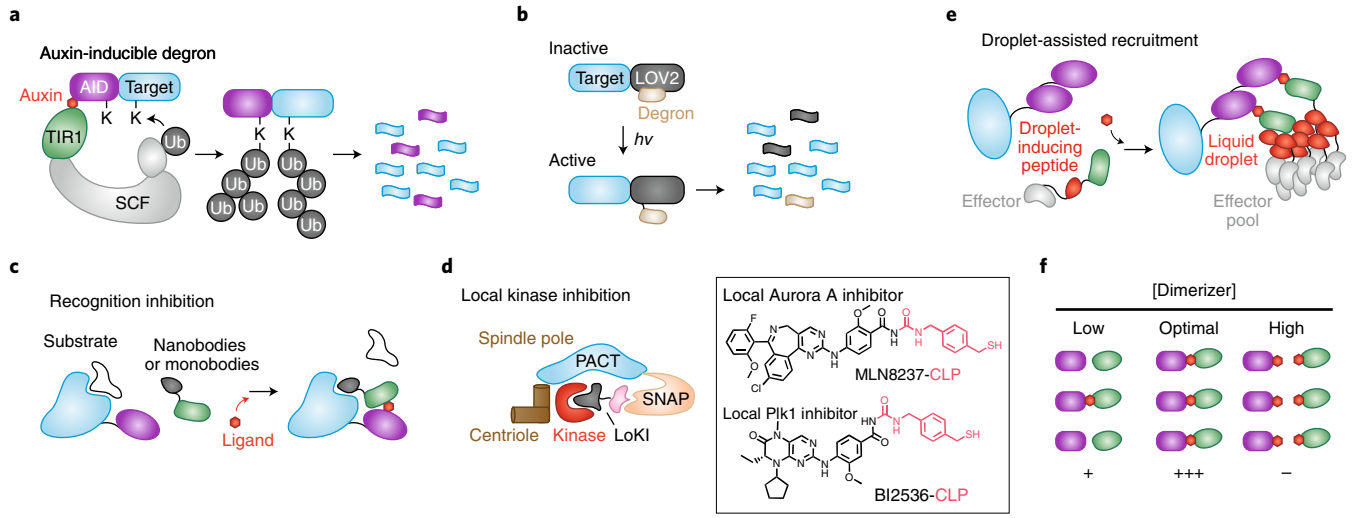

Fig. 6 | Prospective strategies for modulating enzymatic activities. a, Inducible protein degradation. Auxin/IAA addition recruits AID-tagged target proteins to the AID-recognition component SCF^{TIR1}, which catalyzes polyubiquitination to degrade the target. **b**, Photosensitive degron. Light triggers a conformational change in the LOV2 domain, which exposes the degron to recruit E3 ligases and leads to degradation of the target. **c**, Recognition inhibition strategy. A low-affinity inhibitory protein displaces the original high-affinity substrate by increasing its local concentration through recruitment to the target. Possible candidates for inhibitory proteins include user-designed nanobodies and monobodies. **d**, LoKI strategy. Kinase inhibitors conjugated with the SNAP tag ligand CLP can be locally concentrated proximally to SNAP-tagged proteins. In this example, by fusing a SNAP tag to the centrosome-localized PACT domain[98], inhibitors are concentrated at spindle poles. **e**, Droplet-assisted recruitment. A peptide induces droplet formation upon surpassing its critical concentration, thereby increasing the recruitment of effectors. **f**, Hook effect of chemically induced dimerization. Scenarios of limiting or excessive dimerizers reduce dimerization efficacy.

can thus efficiently modulate droplet formation using optogenetics[92,93] or chemically induced dimerization[94]. Cargo fused to these peptides can thus recruit additional cargos to enrich recruitment after surpassing the critical concentration (Fig. 6e). Moreover, multiple repeats of droplet-inducing peptides are structurally compatible with most proteins, as these peptides are typically short and disordered.

Closing remarks

The studies described in this Review demonstrate how small-molecule probes have been designed and applied to address biological questions related to cell division. Enzymatic inhibitors provide several advantages over conventional genetic manipulations: applicability across multiple model systems, temporal control and, in many cases, reversibility after inhibitor washout. Dimerization-inducing probes provide another level of control over genetically tagged proteins, in some cases with light-responsive modifications for spatial and temporal precision that is especially applicable to rapid and localized processes in cell division. Furthermore, chemical and genetic perturbations can work together to modulate multiple targets at the same time, as these modulations are typically bioorthogonal.

Although small-molecule probes can provide clear advantages, there are also limitations. Designing enzymatic inhibitors with high specificity remains a challenging problem for structural and chemical biologists. One way to minimize potential off-target effects is to limit the dose and timing of inhibitor addition using assays focused on a narrowly defined biological process. Furthermore, two orthogonal approaches targeting the same enzyme or process are advantageous, as their potential artifacts are likely to be different. Chemically induced dimerization strategies avoid some drawbacks of enzymatic inhibitors, as their designed ligands are often bioorthogonal to the chosen model system. However, dimerization can cause steric hindrance that disrupts the functions of the anchor or the recruited effector. Structural orientation, the interaction range of the recruited protein and linker lengths can be considered in the design, although optimization often relies on trial and error. Moreover, chemical dimerizers often show a 'hook effect' with a three-body system: reduced recruitment efficacy at high concentrations of probes[95] (Fig. 6f). The excessive bridging probes outnumber the two proteins, such that the two proteins bind to different populations of probes and never come together. The hook effect can be avoided by using 'molecular glue' probes, such as rapamycin[96,97], or caged molecules[6]. Molecular glue probes have a strong binding cooperativity when the two proteins dimerize, whereas their binding affinities for the individual proteins are weak. With caged molecules, a wash-in step can saturate the first receptor, followed by a washout step to remove unbound molecules, thus avoiding the hook effect. Understanding the advantages and limitations of various approaches will help bridge the gap between chemical probe design and experimental needs for biological studies.

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References

1. Levine, M. S. & Holland, A. J. The impact of mitotic errors on cell proliferation and tumorigenesis. *Genes Dev.* **32**, 620–638 (2018).
2. Sansregret, L., Vanhaesebroeck, B. & Swanton, C. Determinants and clinical implications of chromosomal instability in cancer. *Nat. Rev. Clin. Oncol.* **15**, 139–150 (2018).
3. Leibowitz, M. L., Zhang, C.-Z. & Pellman, D. Chromothripsis: a new mechanism for rapid karyotype evolution. *Annu. Rev. Genet.* **49**, 183–211 (2015).
4. Knouse, K. A., Davoli, T., Elledge, S. J. & Amon, A. Aneuploidy in cancer: seq-ing answers to old questions. *Annu. Rev. Cancer Biol.* **1**, 335–354 (2017).
5. Choi, J., Chen, J., Schreiber, S. L. & Clardy, J. Structure of the FKBP12–rapamycin complex interacting with the binding domain of human FRAP. *Science* **273**, 239–242 (1996).
6. Ballister, E. R., Aonbangkhen, C., Mayo, A. M., Lampson, M. A. & Chenoweth, D. M. Localized light-induced protein dimerization in living cells using a photocaged dimerizer. *Nat. Commun.* **5**, 5475 (2014).
7. Zhang, H. et al. Optogenetic control of kinetochore function. *Nat. Chem. Biol.* **13**, 1096–1101 (2017).

This article reports photocaged and photocleavable chemical optogenetic probes and their use for kinetochore studies. For example, recruitment of the motor protein CENP-E to kinetochores near spindle poles demonstrates selective transport toward the spindle equator.
8. Aonbangkhen, C., Zhang, H., Wu, D. Z., Lampson, M. A. & Chenoweth, D. M. Reversible control of protein localization in living cells using a photocaged-photocleavable chemical dimerizer. *J. Am. Chem. Soc.* **140**, 11926–11930 (2018).
9. Rost, B. R., Schneider-Warme, F., Schmitz, D. & Hegemann, P. Optogenetic tools for subcellular applications in neuroscience. *Neuron* **96**, 572–603 (2017).
10. Ballister, E. R., Ayloo, S., Chenoweth, D. M., Lampson, M. A. & Holzbaur, E. L. F. Optogenetic control of organelle transport using a photocaged chemical inducer of dimerization. *Curr. Biol.* **25**, R407–R408 (2015).
11. Chen, G.-Y. et al. Tension promotes kinetochore–microtubule release by Aurora B kinase. *J. Cell Biol*. https://doi.org/10.1083/jcb.202007030 (2021).
Using chemical optogenetics to recruit Aurora B to an individual kinetochore, this report demonstrates that tension dictates contrasting chromosome error correction pathways: microtubules release at high tension or depolymerize at low tension. Furthermore, decreased inter-kinetochore tension using rigor inhibitors of mitotic kinesin motors slows microtubule release.
12. Nigg, E. A. & Stearns, T. The centrosome cycle: centriole biogenesis, duplication and inherent asymmetries. *Nat. Cell Biol.* **13**, 1154–1160 (2011).
13. Heald, R. & Khodjakov, A. Thirty years of search and capture: the complex simplicity of mitotic spindle assembly. *J. Cell Biol.* **211**, 1103–1111 (2015).
14. Kirkham, M., Müller-Reichert, T., Oegema, K., Grill, S. & Hyman, A. A. SAS-4 is a *C. elegans* centriolar protein that controls centrosome size. *Cell* **112**, 575–587 (2003).
15. Habedanck, R., Stierhof, Y. D., Wilkinson, C. J. & Nigg, E. A. The Polo kinase Plk4 functions in centriole duplication. *Nat. Cell Biol.* **7**, 1140–1146 (2005).
16. Bettencourt-Dias, M. et al. SAK/PLK4 is required for centriole duplication and flagella development. *Curr. Biol.* **15**, 2199–2207 (2005).
17. Aydogan, M. G. et al. An autonomous oscillation times and executes centriole biogenesis. *Cell* **181**, 1566–1581 (2020).
18. Wong, Y. L. et al. Reversible centriole depletion with an inhibitor of Polo-like kinase 4. *Science* **348**, 1155–1160 (2015).
This paper reports development of the PLK4 inhibitor centrinone and applications in depleting centrosomes. In comparison to other approaches, centrinone offers advantages such as acute inhibition, reversibility and applicability across different model systems.
19. Dudka, D., Castrogiovanni, C., Liaudet, N., Vassal, H. & Meraldi, P. Spindle-length-dependent HURP localization allows centrosomes to control kinetochore-fiber plus-end dynamics. *Curr. Biol.* **29**, 3563–3578 (2019).
This study used centrinone to deplete a centrosome and generate an asymmetric mitotic spindle. Comparing the spindle-bound molecules on the two halves of the spindle revealed an increase in the microtubule stabilizer HURP on the shorter half-spindle.
20. Guild, J., Ginzberg, M. B., Hueschen, C. L., Mitchison, T. J. & Dumont, S. Increased lateral microtubule contact at the cell cortex is sufficient to drive mammalian spindle elongation. *Mol. Biol. Cell* **28**, 1975–1983 (2017).
21. Ganem, N. J., Godinho, S. A. & Pellman, D. A mechanism linking extra centrosomes to chromosomal instability. *Nature* **460**, 278–282 (2009).
22. Silkworth, W. T., Nardi, I. K., Scholl, L. M. & Cimini, D. Multipolar spindle pole coalescence is a major source of kinetochore mis-attachment and chromosome mis-segregation in cancer cells. *PLoS ONE* **4**, e6564 (2009).
23. Yeow, Z. Y. et al. Targeting TRIM37-driven centrosome dysfunction in 17q23-amplified breast cancer. *Nature* **585**, 447–452 (2020).
24. Meitinger, F. et al. TRIM37 controls cancer-specific vulnerability to PLK4 inhibition. *Nature* **585**, 440–446 (2020).
25. Lambrus, B. G. et al. A USP28–53BP1–p53–p21 signaling axis arrests growth after centrosome loss or prolonged mitosis. *J. Cell Biol.* **214**, 143–153 (2016).
26. Meitinger, F. et al. 53BP1 and USP28 mediate p53 activation and G1 arrest after centrosome loss or extended mitotic duration. *J. Cell Biol.* **214**, 155–166 (2016).
27. Fong, C. S. et al. 53BP1 and USP28 mediate p53-dependent cell cycle arrest in response to centrosome loss and prolonged mitosis. *eLife* **5**, 1–18 (2016).
28. Magidson, V., Lončarek, J., Hergert, P., Rieder, C. L. & Khodjakov, A. Laser microsurgery in the GFP era: a cell biologist's perspective. *Methods Cell. Biol.* **82**, 239–266 (2007).
29. Kapitein, L. C. et al. The bipolar mitotic kinesin Eg5 moves on both microtubules that it crosslinks. *Nature* **435**, 114–118 (2005).
30. Uteng, M., Hentrich, C., Miura, K., Bieling, P. & Surrey, T. Poleward transport of Eg5 by dynein–dynactin in *Xenopus laevis* egg extract spindles. *J. Cell Biol.* **182**, 715–726 (2008).
31. Shimamoto, Y., Forth, S. & Kapoor, T. M. Measuring pushing and braking forces generated by ensembles of kinesin-5 crosslinking two microtubules. *Dev. Cell* **34**, 669–681 (2015).
32. Sturgill, E. G., Norris, S. R., Guo, Y. & Ohi, R. Kinesin-5 inhibitor resistance is driven by kinesin-12. *J. Cell Biol.* **213**, jcb.201507036 (2016).
33. Sturgill, E. G. & Ohi, R. Kinesin-12 differentially affects spindle assembly depending on its microtubule substrate. *Curr. Biol.* **23**, 1280–1290 (2013).
34. Drechsler, H., McHugh, T., Singleton, M. R., Carter, N. J. & McAinsh, A. D. The kinesin-12 Kif15 is a processive track-switching tetramer. *eLife* **3**, e01724 (2014).
35. Kapoor, T. M. et al. Chromosomes can congress to the metaphase plate before biorientation. *Science* **311**, 388–391 (2006).
36. Tolić, I. M. Mitotic spindle: kinetochore fibers hold on tight to interpolar bundles. *Eur. Biophys. J.* **47**, 191–203 (2018).
37. Nicklas, R. B. How cells get the right chromosomes. *Science* **275**, 632–637 (1997).
38. Needleman, D. J. et al. Fast microtubule dynamics in meiotic spindles measured by single molecule imaging: evidence that the spindle environment does not stabilize microtubules. *Mol. Biol. Cell* **21**, 323–333 (2010).
39. Elting, M. W., Prakash, M., Udy, D. B. & Dumont, S. Mapping load-bearing in the mammalian spindle reveals local kinetochore fiber anchorage that provides mechanical isolation and redundancy. *Curr. Biol.* **27**, 2112–2122 (2017).
40. Groen, A. C. et al. A novel small-molecule inhibitor reveals a possible role of kinesin-5 in anastral spindle-pole assembly. *J. Cell Sci.* **121**, 2293–2300 (2008).
41. Vladimirou, E. et al. Nonautonomous movement of chromosomes in mitosis. *Dev. Cell* **27**, 411–424 (2013).
42. Begley, M. A. et al. K-fiber bundles in the mitotic spindle are mechanically reinforced by Kif15. Preprint at *bioRxiv* https://doi.org/10.1101/2020.05.19 (2020).
43. Wood, K. W. et al. Antitumor activity of an allosteric inhibitor of centromere-associated protein-E. *Proc. Natl Acad. Sci. USA* **107**, 5839–5844 (2010).
This paper reports the CENP-E rigor inhibitor GSK923925 and its use to prevent chromosome congression and activate the SAC.
44. Yao, X., Abrieu, A., Zheng, Y., Sullivan, K. F. & Cleveland, D. W. CENP-E forms a link between attachment of spindle microtubules to kinetochores and the mitotic checkpoint. *Nat. Cell Biol.* **2**, 484–491 (2000).
45. Tanudji, M. et al. Gene silencing of CENP-E by small interfering RNA in HeLa cells leads to missegregation of chromosomes after a mitotic delay. *Mol. Biol. Cell* **15**, 3771–3781 (2004).
46. Barisic, M. et al. Microtubule detyrosination guides chromosomes during mitosis. *Science* **348**, 799–803 (2015).
47. Barisic, M. & Maiato, H. The tubulin code: a navigation system for chromosomes during mitosis. *Trends Cell Biol.* **26**, 766–775 (2016).
48. Sarangapani, K. K. & Asbury, C. L. Catch and release: how do kinetochores hook the right microtubules during mitosis? *Trends Genet.* **30**, 150–159 (2014).
49. Lampson, M. A. & Grishchuk, E. L. Mechanisms to avoid and correct erroneous kinetochore–microtubule attachments. *Biology* **6**, 1 (2017).
50. Broad, A. J. & Deluca, J. G. The right place at the right time: Aurora B kinase localization to centromeres and kinetochores. *Essays Biochem*. **64**, 299–311 (2020).
51. Broad, A. J., DeLuca, K. F. & DeLuca, J. G. Aurora B kinase is recruited to multiple discrete kinetochore and centromere regions in human cells. *J. Cell Biol.* **219**, e201905144 (2020).
52. Hadders, M. A. et al. Untangling the contribution of haspin and Bub1 to Aurora B function during mitosis. *J. Cell Biol.* **219**, e201907087 (2020).
53. Wang, F. et al. Haspin inhibitors reveal centromeric functions of Aurora B in chromosome segregation. *J. Cell Biol.* **199**, 251–268 (2012).
54. De Antoni, A., Maffini, S., Knapp, S., Musacchio, A. & Santaguida, S. A small-molecule inhibitor of haspin alters the kinetochore functions of Aurora B. *J. Cell Biol.* **199**, 269–284 (2012).
55. Liang, C. et al. Centromere-localized Aurora B kinase is required for the fidelity of chromosome segregation. *J. Cell Biol.* **219**, e201907092 (2020).
56. Eswaran, J. et al. Structure and functional characterization of the atypical human kinase haspin. *Proc. Natl Acad. Sci. USA* **106**, 20198–20203 (2009).
57. Yoo, T. Y. et al. Measuring NDC80 binding reveals the molecular basis of tension-dependent kinetochore–microtubule attachments. *eLife* **7**, 1–34 (2018).
A method using fluorescence lifetime microscopy and FRET was developed to measure binding between the NDC80 complex and microtubules in live cells. Combining this method with 5-Iodotubercidin to inhibit haspin kinase shows that the tension dependence of NDC80 binding requires centromere-localized Aurora B.
58. García-Rodríguez, L. J., Kasciukovic, T., Denninger, V. & Tanaka, T. U. Aurora B–INCENP localization at centromeres/inner kinetochores is required for chromosome bi-orientation in budding yeast. *Curr. Biol.* **29**, 1536–1544 (2019).
Centromeric enrichment of Aurora B using rapamycin-induced dimerization rescues chromosome segregation fidelity when other pathways for localizing IPL1 to centromeres are inhibited.
59. Akiyoshi, B. et al. Tension directly stabilizes reconstituted kinetochore–microtubule attachments. *Nature* **468**, 576–579 (2010).

60. Lampson, M. A., Renduchitala, K., Khodjakov, A. & Kapoor, T. M. Correcting improper chromosome–spindle attachments during cell division. *Nat. Cell Biol.* **6**, 232–237 (2004).
61. Ye, A. A. et al. Aurora A kinase contributes to a pole-based error correction pathway. *Curr. Biol.* **25**, 1842–1851 (2015).
62. Chmátal, L., Yang, K., Schultz, R. M. & Lampson, M. A. Spatial regulation of kinetochore microtubule attachments by destabilization at spindle poles in meiosis I. *Curr. Biol.* **25**, 1835–1841 (2015).
63. Lara-Gonzalez, P., Westhorpe, F. G. & Taylor, S. S. The spindle assembly checkpoint. *Curr. Biol.* **22**, R966–R980 (2012).
64. Musacchio, A. & Salmon, E. D. The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* **8**, 379–393 (2007).
65. Santaguida, S., Tighe, A., D'Alise, A. M., Taylor, S. S. & Musacchio, A. Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine. *J. Cell Biol.* **190**, 73–87 (2010).
66. Dou, Z. et al. Dynamic localization of Mps1 kinase to kinetochores is essential for accurate spindle microtubule attachment. *Proc. Natl Acad. Sci. USA* **112**, E4546–E4555 (2015).
67. Ji, Z., Gao, H. & Yu, H. Kinetochore attachment sensed by competitive Mps1 and microtubule binding to Ndc80C. *Science* **348**, 1260–1264 (2015).
68. Hiruma, Y. et al. Competition between MPS1 and microtubules at kinetochores regulates spindle checkpoint signaling. *Science* **348**, 1264–1267 (2015).
69. Zeng, X. et al. Pharmacologic inhibition of the anaphase-promoting complex induces a spindle checkpoint-dependent mitotic arrest in the absence of spindle damage. *Cancer Cell* **18**, 382–395 (2010).
70. Sackton, K. L. et al. Synergistic blockade of mitotic exit by two chemical inhibitors of the APC/C. *Nature* **514**, 646–649 (2014).
71. Richeson, K. V. et al. Paradoxical mitotic exit induced by a small molecule inhibitor of APC/C^{Cdc20}. *Nat. Chem. Biol.* **16**, 546–555 (2020).
72. Ballister, E. R., Riegman, M. & Lampson, M. A. Recruitment of Mad1 to metaphase kinetochores is sufficient to reactivate the mitotic checkpoint. *J. Cell Biol.* **204**, 901–908 (2014).
73. Kuijt, T. E. F., Omerzu, M., Saurin, A. T. & Kops, G. J. P. L. Conditional targeting of MAD1 to kinetochores is sufficient to reactivate the spindle assembly checkpoint in metaphase. *Chromosoma* **123**, 471–480 (2014).
74. Clackson, T. et al. Redesigning an FKBP–ligand interface to generate chemical dimerizers with novel specificity. *Proc. Natl Acad. Sci. USA* **95**, 10437–10442 (1998).
75. Kang, J., Chen, Y., Zhao, Y. & Yu, H. Autophosphorylation-dependent activation of human Mps1 is required for the spindle checkpoint. *Proc. Natl Acad. Sci. USA* **104**, 20232–20237 (2007).
76. Aravamudhan, P., Goldfarb, A. A. & Joglekar, A. P. The kinetochore encodes a mechanical switch to disrupt spindle assembly checkpoint signalling. *Nat. Cell Biol.* **17**, 868–879 (2015).
Using rapamycin-induced dimerization, the SAC can be reactivated by recruiting MPS1 kinase close to its substrate, SPC105. This study proposes that a physical barrier between MPS1 and SPC105 silences the SAC when kinetochores achieve end-on microtubule attachment.
77. Burslem, G. M. & Crews, C. M. Proteolysis-targeting chimeras as therapeutics and tools for biological discovery. *Cell* **181**, 102–114 (2020).
78. Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T. & Kanemaki, M. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat. Methods* **6**, 917–922 (2009).
79. Holland, A. J., Fachinetti, D., Han, J. S. & Cleveland, D. W. Inducible, reversible system for the rapid and complete degradation of proteins in mammalian cells. *Proc. Natl Acad. Sci. USA* **109**, E3350–E3357 (2012).
80. Natsume, T., Kiyomitsu, T., Saga, Y. & Kanemaki, M. T. Rapid protein depletion in human cells by auxin-inducible degron tagging with short homology donors. *Cell Rep.* **15**, 210–218 (2016).
81. Daniel, K. et al. Conditional control of fluorescent protein degradation by an auxin-dependent nanobody. *Nat. Commun.* **9**, 3297 (2018).
82. Lambrus, B. G. et al. p53 protects against genome instability following centriole duplication failure. *J. Cell Biol.* **210**, 63–77 (2015).
83. Renicke, C., Schuster, D., Usherenko, S., Essen, L. O. & Taxis, C. A LOV2 domain-based optogenetic tool to control protein degradation and cellular function. *Chem. Biol.* **20**, 619–626 (2013).
84. Hermann, A., Liewald, J. F. & Gottschalk, A. A photosensitive degron enables acute light-induced protein degradation in the nervous system. *Curr. Biol.* **25**, R749–R750 (2015).
85. Zorba, A. et al. Allosteric modulation of a human protein kinase with monobodies. *Proc. Natl Acad. Sci. USA* **116**, 13937–13942 (2019).
86. Yu, D. et al. Optogenetic activation of intracellular antibodies for direct modulation of endogenous proteins. *Nat. Methods* **16**, 1095–1100 (2019).
87. Bucko, P. J. et al. Subcellular drug targeting illuminates local kinase action. *eLife* **8**, e52220 (2019).
This article reports a strategy to inhibit kinase activity locally by conjugating kinase inhibitors to the SNAP-binding ligand CLP and fusing a SNAP tag to proteins that localize to specific intracellular structures. The bifunctional probe inhibits kinase activity proximal to the SNAP-tagged proteins.
88. DeLuca, K. F. et al. Aurora A kinase phosphorylates Hec1 to regulate metaphase kinetochore–microtubule dynamics. *J. Cell Biol.* **217**, 163–177 (2018).
89. Aravamudhan, P., Chen, R., Roy, B., Sim, J. & Joglekar, A. P. Dual mechanisms regulate the recruitment of spindle assembly checkpoint proteins to the budding yeast kinetochore. *Mol. Biol. Cell* **27**, 3405–3417 (2016).
90. Chen, C. et al. Ectopic activation of the spindle assembly checkpoint signaling cascade reveals its biochemical design. *Curr. Biol.* **29**, 104–119 (2019).
91. Hyman, A. A., Weber, C. A. & Jülicher, F. Liquid–liquid phase separation in biology. *Annu. Rev. Cell Dev. Biol.* **30**, 39–58 (2014).
92. Shin, Y. et al. Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets. *Cell* **168**, 159–171 (2017).
93. Bracha, D. et al. Mapping local and global liquid phase behavior in living cells using photo-oligomerizable seeds. *Cell* **175**, 1467–1480 (2018).
94. Zhang, H. et al. Nuclear body phase separation drives telomere clustering in ALT cancer cells. *Mol. Biol. Cell* **31**, 2048–2056 (2020).
95. Douglass, E. F., Miller, C. J., Sparer, G., Shapiro, H. & Spiegel, D. A. A comprehensive mathematical model for three-body binding equilibria. *J. Am. Chem. Soc.* **135**, 6092–6099 (2013).
96. Schreiber, S. L. Immunophilin-sensitive protein phosphatase action in cell signaling pathways. *Cell* **70**, 365–368 (1992).
97. Banaszynski, L. A., Liu, C. W. & Wandless, T. J. Characterization of the FKBP–rapamycin–FRB ternary complex. *J. Am. Chem. Soc.* **127**, 4715–4721 (2005).
98. Gillingham, A. K. & Munro, S. The PACT domain, a conserved centrosomal targeting motif in the coiled-coil proteins AKAP450 and pericentrin. *EMBO Rep.* **1**, 524–529 (2000).

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Competing interests

The authors declare no competing interests.

Additional information

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