Current Biology
Review

Evolutionary Dynamics of the Spindle Assembly Checkpoint in Eukaryotes

Geert J.P.L. Kops[1], Berend Snel[2], and Eelco C. Tromer[3]
[1]Oncode Institute, Hubrecht Institute – KNAW (Royal Netherlands Academy of Arts and Sciences) and University Medical Centre Utrecht, Utrecht, The Netherlands
[2]Theoretical Biology and Bioinformatics, Department of Biology, Science Faculty, Utrecht University, Utrecht, The Netherlands
[3]Department of Biochemistry, University of Cambridge, Cambridge, UK
Correspondence: g.kops@hubrecht.eu (G.J.P.L.K.), b.snel@uu.nl (B.S.), ecet2@cam.ac.uk (E.C.T.)
https://doi.org/10.1016/j.cub.2020.02.021

The tremendous diversity in eukaryotic life forms can ultimately be traced back to evolutionary modifications at the level of molecular networks. Deep understanding of these modifications will not only explain cellular diversity, but will also uncover different ways to execute similar processes and expose the evolutionary 'rules' that shape the molecular networks. Here, we review the evolutionary dynamics of the spindle assembly checkpoint (SAC), a signaling network that guards fidelity of chromosome segregation. We illustrate how the interpretation of divergent SAC systems in eukaryotic species is facilitated by combining detailed molecular knowledge of the SAC and extensive comparative genome analyses. Ultimately, expanding this to other core cellular systems and experimentally interrogating such systems in organisms from all major lineages may start outlining the routes to and eventual manifestation of the cellular diversity of eukaryotic life.

Introduction

Eukaryotes share many cellular structures and functions, yet their cellular morphology and behavior are extremely diverse. They range from multiciliated predators of prokaryotes to photosynthetic algae and rapidly dividing cocci (see Figure 1 for a simplified view of major eukaryotic lineages). The diversity in cellular morphology and behavior can ultimately be traced back to the molecular networks that govern cellular processes. A major question in biology is how the molecular networks that underlie these huge phenotypic differences arose, evolved, and diverged. The increasing availability of draft genome and transcriptome sequences of diverse microbial eukaryotes and detailed molecular descriptions of pathways and their components from various model organisms have sparked a renewed interest in this question. These developments, coming together in the field of evolutionary cell biology [1], are driving new insights into how evolution of molecular networks enables innovations and changes in cellular functions. In a striking example, comparative genomics and detailed knowledge of cell motility predicted the capacity of amoeboid motility in an early branching fungus, which was then experimentally verified [2].

A pathway uniquely suited for discovery of the molecular details of evolutionary change is the spindle assembly checkpoint (SAC), a mitotic signaling pathway that safeguards chromosome segregation fidelity (Figure 2). The SAC is *a priori* expected to be universal given the eukaryotic dependency on microtubule-based spindles for cell division. And, importantly, its function can be directly assessed across the tree of life by the ability of species to delay cell division in response to microtubule depolymerization. Finally, its relevance for human cell division and its relation to various pathologies have resulted in detailed knowledge of the molecular components and networks underlying the SAC response. Such knowledge greatly helps to detect divergent homologs, as well as to interpret functional changes in predicted homologs as compared with less well studied systems. Here, we discuss surprising observations on the evolution and diversity of the SAC in eukaryotes, and contextualize them in relation to the evolution of other cellular pathways.

Molecular Wiring of the Core SAC Response

The SAC translates the lack of interaction of chromosomes with microtubules to the cell cycle machinery. Our extensive knowledge of the underlying molecular mechanisms during this process is largely derived from studies in metazoan and yeast model organisms. The following summary (and the one in the next paragraph) is therefore one of a 'canonical' SAC, operating in those organisms. Chromosomes are segregated by microtubule-based spindles to which they connect via protein complexes known as kinetochores that are assembled on centromeric chromatin [3]. In brief, when not bound by microtubules, kinetochores produce an inhibitor of the anaphase promoting complex/cyclosome (APC/C), thereby preventing the APC/C and its co-activator Cdc20 (APC/C^{Cdc20}) from ubiquitylating and hence orchestrating destruction of substrates such as B-type cyclins (Figure 2) [4–6]. This buys the cell time to get all chromosomes attached and ready for segregation. The inhibitor of APC/C^{Cdc20} is known as the mitotic checkpoint complex (MCC). The MCC consists of four subunits — BubR1/Mad3, Bub3, Mad2 and Cdc20 — that are assembled into one diffusible complex by unattached kinetochores [7–11]. Inhibition of APC/C^{Cdc20} occurs predominantly by the MCC subunit BubR1/Mad3 (MadBub in many lineages; see 'A tale of two MadBubs' below). BubR1/Mad3 operates as a pseudosubstrate [7,12–16] by virtue of a number of short linear motifs (SLiMs: KEN-box, D-box, ABBA motif; Figure 3) that in *bona fide* substrates direct their interaction with and ubiquitylation by APC/C^{Cdc20} [17,18]. By binding APC/C^{Cdc20}, the MCC binds and inhibits the APC/C-bound Cdc20, prevents substrate and E2 ubiquitin ligase binding, and re-arranges the catalytic site to disfavor activity [7,19,20].

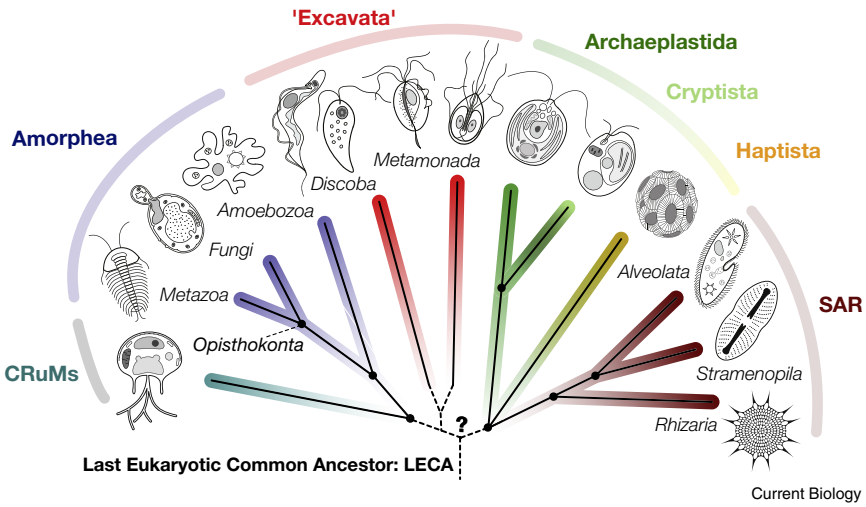


Figure 1. Eukaryotic tree of life.
All current day eukaryotic species stem from a common ancestor known as the LECA (last eukaryotic common ancestor). The molecular diversity of eukaryotic species and their evolutionary relationships have become clearer recently with the addition of a large number of newly sequenced genomes and transcriptomes of mostly single-celled species. These sequences have illuminated parts of the eukaryotic tree of life that were poorly resolved. The figure illustrates a current hypothesis on the eukaryotic tree of life, inspired by a recent study (some lineages are left out to reduce complexity) [163]. It shows the unknown position of the root (dotted lines) of eukaryotes (LECA) and the subsequent diversification of species into seven major (super)groups. Cartoons of various representatives of these groups indicate the extensive cellular diversity found amongst eukaryotes [165]. For some lineages the main sub-lineages are shown, such as for example the metazoan, fungal and amoebozoan lineages forming the Amorphea supergroup, or alveolates, stramenopiles and rhizarians forming the SAR supergroup. Of note: Excavata, a group containing mostly single-celled flagellates (red colour), is now considered to be polyphyletic, meaning that the exact phylogenetic affiliation of sub-lineages within the eukaryotic tree is unclear. For clarity we grouped two major lineages, Metamonads and Discoba, but indicate their uncertain phylogenetic relationship to each other and the LECA (see dotted lines).

Various protein scaffolds and enzymatic activities bring MCC components together on kinetochores and facilitate their assembly into a holo-complex (Figure 3) [15,21–23]. The Bub1 protein (a paralog of BubR1/Mad3; see 'A tale of two MadBubs' below) is central to this. Bub1 directly binds BubR1/Mad3 and Cdc20, and, through binding the scaffold Mad1, also recruits Mad2. These interactions occur through various SLiMs, and are promoted by the kinase Mps1 at multiple levels [18,22,24–27]. Bub1's ABBA motif binds Cdc20, and its CDI motif, upon phosphorylation by Cdk1 and Mps1, connects to an RLK sequence in Mad1 [18,22,24,25,28]. Getting all MCC components together on kinetochores is not enough, however. The eventual assembly of MCC requires the conformational 'closing' of Mad2 [29]. Soluble, 'open' Mad2 molecules adopt a closed conformation upon homodimerization with a Mad1-bound pool of 'closed' Mad2 molecules. The 'closing' of soluble Mad2 enables it to interact with Cdc20 and promote formation of MCC.

Linking the SAC to the Outer Kinetochore

The core SAC network is connected to the kinetochore in a way that allows it to be responsive to microtubule attachments [30,31]. Kinetochores are built on centromeric DNA marked by nucleosomes containing the histone H3 variant CENPA/CenH3 [32,33]. Through various linker complexes, these centromeric nucleosomes connect with a network of proteins that engage microtubules (Figure 3) [34–38]. Collectively known as KMN (Knl1 complex, Mis12 complex, Ndc80 complex), this network connects with microtubules via the two divergent calponin homology (CH) domains of the Ndc80–Nuf2 heterodimer and the positively charged amino terminus of the Knl1 protein (Figure 3) [39–41]. The latter has a disordered region full of SLiMs (MELT (Met-Glu-Leu-Thr) or derivatives thereof). When phosphorylated by Mps1, these MELTs serve as docking sites for Bub3, and therefore by extension for its partner Bub1 [42–48]. Mps1 itself is located close to its kinetochore substrates: it transiently binds the Ndc80–Nuf2 dimer, an interaction that diminishes when Ndc80–Nuf2 engages microtubules [49–51].

Correct kinetochore–microtubule interactions cause termination of SAC signaling. In addition to Mps1 displacement, this requires removal of the other core SAC components from kinetochores. The most conserved mechanism for this appears to be the reversal of SAC-activating phosphorylations by PP1 phosphatases that bind two SLiMs (SILK and RVSF) near the amino terminus of Knl1 (Figure 3) [52–56]. Equally important to allow anaphase initiation is the disassembly of MCC and liberation of APC/C^{Cdc20}. This is achieved predominantly by ubiquitylation of Cdc20 to destabilise the MCC-APC/C^{Cdc20} interaction, involving the APC/C subunit Apc15 [57–61]. Animal cells additionally break up the MCC by remodeling Mad2 via the AAA+ ATPase Trip13 [62–65]. In animals, Cdc20 ubiquitylation and Mad2 remodeling require the ancient Mad2 paralog $p31^{comet}$, which at least for the latter process acts as an adaptor for Trip13 [62,64,66–68]. Without continuous replenishment of 'fresh' MCC, these two mechanisms culminate in unrestricted APC/C^{Cdc20} activity and thus anaphase initiation (Figure 3) [57,59,69].

The Deep Evolutionary Origins of the SAC

The target of the SAC (APC/C^{Cdc20}) and the core SAC components are present in a wide range of eukaryotes and are thus inferred to have originated before the birth of the last common ancestor of all eukaryotes (LECA; Figure 1) [70–72]. But from what systems were they derived? The Archaeal ancestor to eukaryotes is the likely donor of E2–E3-type ubiquitin ligases, from which the APC/C evolved [73]. Interestingly, ubiquitin-dependent control of cell division occurs in at least one Archaeon [74]. As for the core SAC, pre-LECA eukaryotes likely obtained an ancestral Mad2-$p31^{comet}$-Trip13 system from prokaryotes. Mad2 and $p31^{comet}$ belong to the HORMA-domain protein family, and many prokaryotes encode an operon in which one or two HORMA-domain genes co-exist with a Trip13-like AAA+ ATPase [72,75]. In bacteria, this operon is involved in bacteriophage immunity [76]. The pre-LECA origin of the APC/C^{Cdc20} pseudosubstrate inhibitor BubR1/Mad3 is

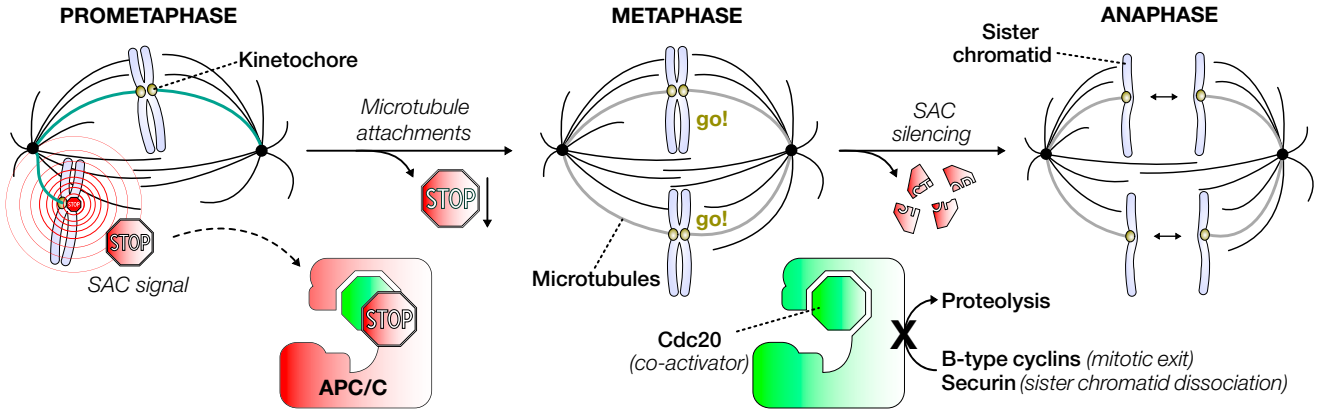


Figure 2. The spindle assembly checkpoint response.
During the final phases of cell division, chromosomes interact with the microtubule-based spindle via kinetochores, large protein complexes bound to centromeric chromatin. During the process of acquiring connections to the spindle (prometaphase), kinetochores signal to the cell cycle machinery whenever they have not established microtubule interactions. This signal is the spindle assembly checkpoint (SAC). The SAC delays the onset of anaphase by inhibiting the E3 ubiquitin ligase Anaphase Promoting Complex/Cyclosome (APC/C). Only when all kinetochores have achieved stable microtubule connections (metaphase) is the SAC silenced, the APC/C activated, its substrates ubiquitylated and anaphase allowed to proceed.

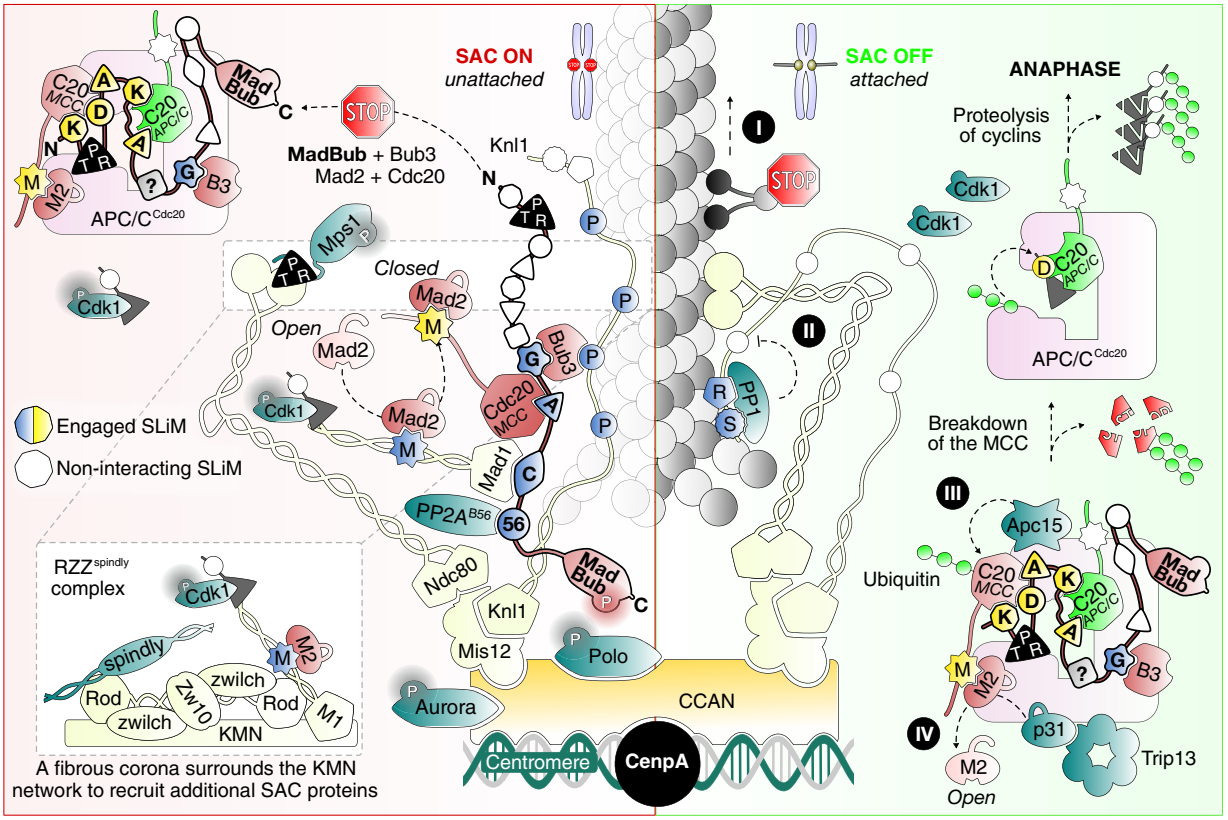
less clear. Its phylogenetically traceable domains are TPR (tetratricopeptide repeat) and kinase, and the latter arose sometime during eukaryogenesis from unknown origins. TPR domains are commonly found in prokaryotes [77]. The one in BubR1/Mad3 (and in its paralog Bub1) is most closely related to the TPR domain in MPS1. Beyond that, our prior analyses indicated second-closest similarity with HAT repeat proteins involved in the core spliceosome [72].

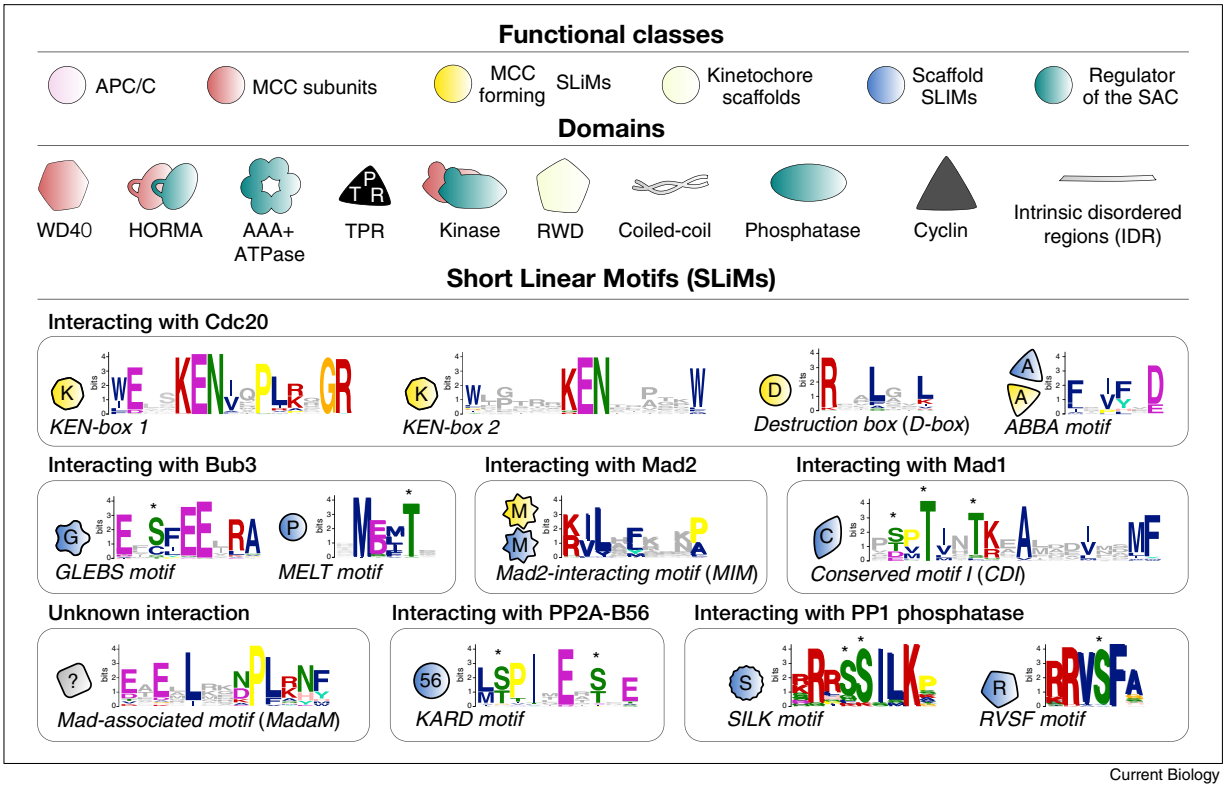
More generally, the kinetochore and the SAC may have deep evolutionary connections with nuclear pore complexes (NPCs). During the closed mitosis of budding yeast, Mad1 cycles between unattached kinetochores and the inner ring of NPCs to modulate transport of a mitotic regulator [78]. Human Mad1 binds the basket of NPCs, where it promotes the production of a pre-mitotic pool of MCC [79,80]. Consistently, other core human SAC components (Mps1, Mad2) bind human NPCs too [81,82]. This SAC–NPC connection has also been observed in, for example, *Arabidopsis thaliana* [83], suggesting deep evolutionary origins and a role for SAC proteins at NPCs in the LECA [83]. The connection may go even further. Bub3 is a pre-LECA paralog of Rae1 [72], an mRNA transport factor. Rae1 binds a channel component through a GLEBS-like motif [84], much like how Bub3 binds Bub1 [85]. In addition, members of the Y-complex of NPCs are among the proteins most closely co-evolving with the core SAC [71], and proteins from various NPC subcomplexes localize to kinetochores in human cells [86–90]. The SAC and NPCs may thus have extensive and potentially ancient shared functions.

How Conserved Is the SAC?

The canonical SAC system was in place when the eukaryotic lineages started to emanate from the LECA. But does that mean it is widely conserved in eukaryotes? This question consists of two parts: do all eukaryotes have a mechanism to delay cell cycle progression in response to lack of chromosome–spindle interactions? And if so, does that mechanism involve production of an MCC-like APC/C^{Cdc20} inhibitor? Many metazoan and fungal model organisms have a SAC response, but several species such as *Giardia intestinalis* (Metamonada), *Trypanosoma brucei* (Kinetoplastida), *Toxoplasma gondii* (Apicomplexa) and the ciliate *Tetrahymena thermophila* appear unable to delay cell cycle progression in response to effective microtubule depolymerization by spindle poisons such as nocodazole [91–94]. Absence of a SAC response in *T. brucei* is in agreement with the absence of a BubR1/Mad3-like homolog and a kinetochore-independent function of the Mad2-like homolog (Kinetoplastida in Figure 4) [95]. *G. intestinalis* and other (non-parasitic) diplomonads such as the *Spironucleus* lineage are unique in that their common ancestor lost the APC/C as a means to control the cell cycle [71,96]. Strikingly, however, they retained core SAC components (Mps1, Mad2, Bub3, Bub1-like), some of which have key but undefined roles in chromosome segregation ([97] and our unpublished data in Figure 4).

More remarkable, perhaps, than loss of a SAC response are species with an intact SAC response but without the canonical SAC pathway. This is of particular interest because such species may have supplanted the ancient SAC with novel, yet to be discovered mechanisms. The critical MCC components Mad2 and BubR1/Mad3 are widely conserved, and BubR1/Mad3 paralogs in virtually all instances retained its essential pseudosubstrate motifs [17]. Surprisingly, however, cells of the flatworm *Schmidtea mediterranea* can respond to nocodazole with a G2/M delay but lack Mad2, BubR1/Mad3, and Bub1 [98] (note that we identified a highly divergent *S. mediterranea* Mad1 ortholog; Figure 4 and sequences in Table S1 and Data S1). The delay was dependent on the Rod–Zw10–Zwilch (RZZ) kinetochore complex and likely mediated by APC/C^{Cdc20}. *S. mediterranea* cells therefore may generate a novel and yet unknown kinetochore-based APC/C^{Cdc20} inhibitor. *Trichomonas vaginalis* (Metamonada) too may have a divergent APC/C^{Cdc20} inhibitor. It responds to nocodazole with a G2/M delay [99], but its genome does not appear to encode Mad2, and its two





Current Biology

(legend on next page)

BubR1/Mad3/Bub1-like paralogs lack the SLiM motifs to inhibit APC/C^{Cdc20} (Figure 4).

How many other lineages may have evolved alternative SAC mechanisms? Most species tested from the Opisthokonta (animals, fungi) and Archaeplastida (plant) supergroups (reviewed in [100,101]), mount a SAC response and express BubR1/Mad3 and Mad2 orthologs. At least conceptually, their SAC thus appears ancestral. We and others have nevertheless identified quite a number of species from various supergroups with no detectable Mad3/BubR1 and Mad2 orthologs (Figure 4). These are often parasites, possibly explaining extreme genome reduction [102,103]. Yet the free-living, non-parasitic ciliate *Paramecium tetraurelia* (SAR supergroup) is also devoid of SAC components [71,104]. In addition, while species with no detectable Mad2 can have a BubR1/Mad3 ortholog, this ortholog then often lacks the key SLiMs that make it an APC/C^{Cdc20} inhibitor (see, for example, *T. thermophila* and other ciliates, and the ancient budding yeast lineage *Hanseniaspora* (Fast Evolving Lineages) in Figure 4 [71,104]). In such species a BubR1/Mad3/Bub1-like ortholog is thus likely retained for functions other than the SAC.

Evolutionary Dynamics of SAC Scaffolds and Catalysts

A Tale of Two MadBubs

Bub1, the vital SAC scaffold, shares ancestry with BubR1/Mad3 in a most striking manner. The LECA had a single Bub1/BubR1/Mad3-like gene (dubbed MadBub) that we envision acted as scaffold and APC/C^{Cdc20} inhibitor. Such a single MadBub protein is present in many eukaryotes, and a *Kluyveromyces polysporus* MadBub gene can indeed replace Bub1 and Mad3 in *Saccharomyces cerevisiae* [105]. On at least 16 independent occasions, a MadBub gene duplicated to give rise to two paralogs [17,106,107]. Remarkably, those paralogous pairs followed similar evolutionary trajectories where one retained only scaffold functionalities (e.g., CDI motif and kinase) while the other retained only MCC functionalities (e.g., KEN, D-box and ABBA motif; Figure 5). Bub1-like genes (and by extension BubR1/Mad3-like genes) are therefore not one-to-one orthologous if they arose after independent gene duplications (e.g., budding yeast Bub1 and vertebrate Bub1). This is not a trivial point — an X-ray structure of the kinase domain of *Drosophila melanogaster* BubR1 was recently used to model the (pseudo)kinase domain of its human namesake using the faulty assumption that they are 1-to-1 orthologous [108], where using the X-ray structure of human BUB1 kinase would have been more relevant and may have yielded different insights [106]. Of note, MadBub duplication in the ancestor of flies (e.g., fruit flies and mosquitos) yielded unique pairs of paralogs, where the Bub1-like protein lost almost all SLiMs, while the BubR1/Mad3-like one retained all [17] (Figure 5B).

What can explain such widespread, parallel evolution of paralogous gene pairs? Duplication of the MadBub gene may have opened ways to resolve an adaptive conflict between the two functions residing in one gene, as was shown for galactose pathway evolution in budding yeasts [109]. Interestingly, Mad3 gene expression in fission yeast is 3–4-fold higher than that of Bub1, and reducing Mad3 expression back to Bub1 levels substantially compromised the SAC [110]. In an alternative but not mutually exclusive scenario, MadBub evolution may be a striking case of the duplication–degeneration–complementation (DDC) model of protein evolution [111]. In this model, MadBub A and B (paralogs directly after duplication) can co-exist neutrally until A loses one of its essential functionalities (e.g., APC/C^{Cdc20} inhibitor), after which B is 'forced' to retain it. If B then loses the other function (scaffold), A is forced to retain it, leading to the paralogous pairs we see today. If A loses scaffold function after it lost APC/C^{Cdc20} inhibitor function, only B will be retained in extant species. Indeed, instances of retention of only one MadBub gene after whole genome duplication have been reported [112], and fitness experiments with budding yeast strains mimicking steps in subfunctionalization did not find evidence for adaptive conflict resolution [105].

Dynamic SLiM Arrays for the Kinetochore–SAC Connection

In Opisthokonta model organisms, Knl1 indirectly recruits all MCC components by virtue of repeats of a MELT motif. When phosphorylated, these motifs bind Bub3–Bub1 dimers and without them, the SAC is inactive [42–46]. While the carboxy-terminal tandem RWD domains and the amino-terminal RVSF motif are well conserved in Knl1 orthologs, the interior MELT motifs diverge extensively across eukaryotes. They undergo recurrent episodes of repeat duplications and deletions, sequence degeneration and sequence alteration to change phospho-signatures (Figure 5) [113]. Such patterns are reminiscent of genetic arms races. Intriguingly, Bub1 levels at kinetochores during mouse meiosis dictate the direction of centromere drive — stronger centromeres recruit more Bub1 to create higher depolymerization capacity and thus higher reorientation probability for retention in the egg [114]. The arms race between 'selfish' centromeres and the host may thus be impacted by the ability of Knl1 motifs to recruit Bub1. Although centromere drive is proposed to be restricted to species with asymmetric meiosis [115], it is conceivable that a similar mechanism accounts for repeat evolution in other eukaryotes. After all, anisogamy

Figure 3. Molecular wirings of the canonical SAC network.
When unbound by microtubules (left), the outer kinetochore recruits a host of SAC proteins to generate a four-component APC/C^{Cdc20} inhibitor (MCC (red): MadBub (TPR + SLiMs + Bub kinase), Bub3 (B3), Mad2 (M2), Cdc20 (C20)), thus preventing anaphase initiation. End-on microtubule binding (right) eliminates SAC signaling by (I) dynein-mediated SAC protein removal; (II) PP1-mediated protein dephosphorylation; and (III,IV) MCC disassembly by Apc15-dependent Cdc20 ubiquitylation (III) and by Trip13-p31^{comet}-mediated Mad2 extraction (IV). SAC proteins and relevant protein domains are color-coded by function, and further annotated in the lower panel: APC/C^{Cdc20} inhibitor (red: MCC components), regulators (dark green: enzymes and co-factors) or scaffold (light yellow: Mad1, RZZ (Rod-Zwilch-Zw10) or KMN network (Knl1-, Mis12- and Ndc80-complexes)). See main text for more details on protein functions. Please note that we here depict the MadBub protein instead of separate Bub1 and BubR1/Mad3 paralogs, as MadBub is the more common situation among eukaryotes (including in Amorphea (containing, among others, all fungal and animal lineages). Various protein–protein interactions required for SAC activity, including those required for APC/C^{Cdc20} inhibition, are mediated by SLiMs (short linear motifs). Their location in a protein sequence and their relevant interactions are depicted in the cartoon. SLiM symbols, consensus sequence logos and main interactors are listed in the bottom panel. Conserved amino acid positions in SLiMs are colored; asterisk indicates phospho-regulated residues. Non-interacting SLiMs are represented as white and engaged SLiMs are shown in colour, to distinguish where each of these motifs is active.

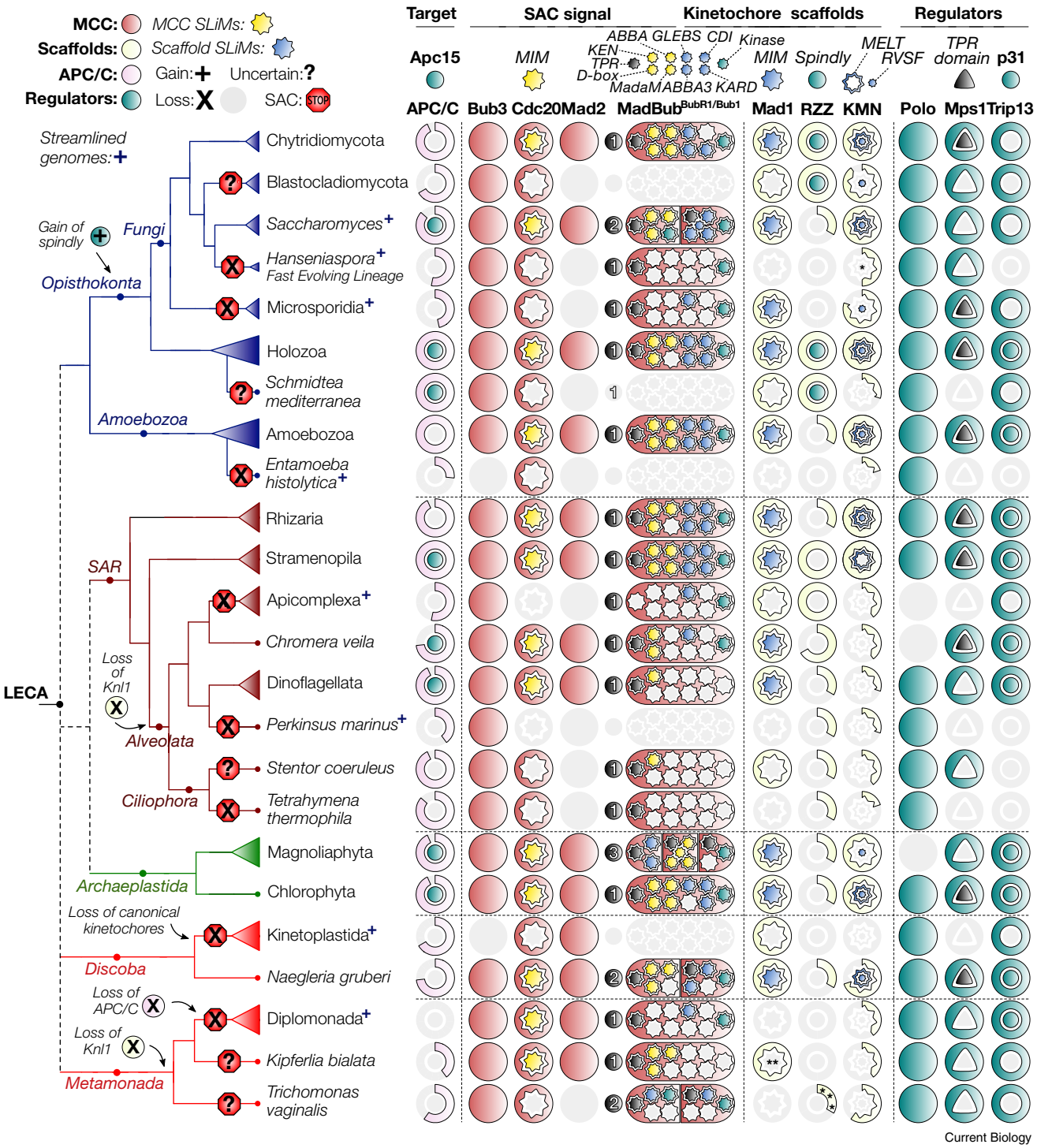


Figure 4. Diversity of the SAC network in eukaryotes.
Evolutionary reconstruction (selected species, topology as in Box1) of the SAC network reveals the diversity of the SAC network and highlights parallel losses of subunits, domains and/or SLiMs of the SAC (see light-yellow shaded taxa). Circle plots indicate the presence (colored) or absence (grey) of subunits and SliMs (8-pointed star) of the SAC network in eukaryotes. The color scheme for these features is the same as in Figure 3. For complexes with multiple subunits (APC/C (15), KMN (10) and RZZ (3)), circles are partially colored to indicate the amount of subunits present in respective lineages. Multiple species belonging to the same phylogenetic groups are collapsed where possible, and SAC protein content of the inferred common ancestor is then shown (e.g., Blastocladiomycota is based on the patterns of *Allomyces macrogynus* and *Catenaria anguillulae*; see Table S1). Projected losses of the SAC are inferred when either most components of the MCC (Mad2, Bub3, MadBub, Cdc20) or key SLiMs to inhibit APC/C^{Cdc20} (Figure 3) are absent. Question marks signify lineages lacking most but not all MCC components/SLiMs or lineages with no MCC components but an experimentally observed SAC-like response (*T. vaginalis, S. mediterranea*). In case of lineages

(legend continued on next page)

(substantial size differences between the gametes) is a widely occurring feature of eukaryotic sexual reproduction. Of note: a single MELT motif suffices for maintaining a robust SAC in human cells, but more are needed for high fidelity chromosome biorientation and this correlates with Bub1 levels at kinetochores [46].

Evolving Kinase Control of the SAC Response

The changes to motif sequence in Knl1 repeats may underlie alterations to the kinetochore kinases that regulate Knl1–Bub1/MadBub interactions. The MELT motifs have an optimal sequence for phosphorylation by the kinase Mps1 [116,117], but this regulatory input has diversified. Mps1 was lost in *Caenorhabditis elegans*, and Plk1 has taken over its role [118]. In other lineages, the motif does not adhere to the Mps1 consensus sequence, or has no phosphorylatable amino acid. The MEED motif in drosophilids, for example, cannot be regulated by phosphorylation [113]. This does not mean that the Knl1–Bub interaction is unregulated in drosophilids: a motif preceding MEED and known to be essential for Bub3-Bub1 recruitment in human cells [46] has a strong Aurora kinase substrates signature [119] (Figure 5B). Likewise, alterations to MELT to include extra S/T residues (*Lepidoptera*, *Dictyostelids*) may accommodate additional kinase input or alterations to substrate preference of the Mps1 kinase [113].

In addition to its conserved, carboxy-terminal dual specificity kinase domain [120], Mps1 contains a TPR domain near its amino terminus that is most closely related to the TPR domains of Bub1 and BubR1/Mad3 (or MadBub) [121]. Their kinase domains are not closely related, suggesting independent fusions of the two kinase domains to the same TPR-domain-containing protein [72,121]. Because Mps1 and MadBub both localize to kinetochores, this ancestral TPR-domain-containing protein was likely already dedicated to kinetochore function. Interestingly, a similar paralog expansion of a protein operating in a primordial kinetochore gave rise to all current kinetochore proteins with RWD domains [72], signaling perhaps a common theme in kinetochore evolution. Although the TPR domain of Mps1 was part of the LECA, it is often absent from current-day Mps1 orthologs (Figure 4) [121]. In human cells, this domain contributes to kinetochore localization of Mps1 and auto-regulation [121,122]. It is conceivable that its role in these events is dispensable or achieved by other means.

Interpreting Variations in SAC Systems

The detailed knowledge of the mechanisms by which SAC components interact with each other and with the kinetochore enables interpretation of divergent protein architectures in specific lineages. Flowering plants such as *A. thaliana* (Magnoliapohyta in Figure 4) have three MadBub-related genes (BMF1–3), obtained by two gene duplication events [17,106]. BMF2 is BubR1/Mad3-like, while BMF1 and BMF3 both are Bub1-like that further subfunctionalized: BMF1 retained the kinase domain but shed all other functional motifs, while BMF3 did the opposite and in fact expanded the ABBA and CDI motifs to three each (see Magnoliophyta in Figure 4 or flowering plants in Figure 5A) [17,106]. Since Bub1 kinase activity is important for chromosome biorientation in human cells [18,25,123–125], BMF1 is likely involved only in chromosome biorientation, leaving SAC scaffold function to BMF3. As predicted by this, BMF1 is dispensable for the *A. thaliana* SAC [83]. Intriguingly, the connection of the SAC to kinetochores also diverged in flowering plants. BMF3 lacks a GLEBS motif that mediates the interaction with Bub3 and thus Knl1 [17]. Furthermore, while Knl1 does have a MELT-like motif (a single, divergent one; Figure 5A) [113], Mps1 does not regulate BMF3 or MAD1 kinetochore localization. Instead, Mps1 appears to directly regulate Mad2 [83,126], a twist to the SAC architecture that is, as far as we know, unique to plants.

In the moss *Physcomitrella patens* (Figures 5A), the BubR1/Mad3-like paralog has expanded its carboxy-terminal ABBA motifs (known to bind Cdc20) 10-fold [17]. The Bub1-like paralog likewise has a four-fold repeat of the CDI motif, which binds to the MAD1 scaffold. Remarkably, arraying CDI motifs in human Bub1 to make it *P. patens*-like increased Mad1 kinetochore levels ~5-fold [127], supporting the hypothesis that *P. patens* evolved to enhance its SAC signaling capacity. This may not be unique to mosses — MadBubs in the Stramenopila lineage (SAR supergroup) can have similar ABBA arrays [17]. Whether this represents a 'super-SAC' or an adaptation to loss of SAC signaling efficiency at other levels is an exciting subject for future study.

Co-evolutionary Patterns in the SAC–Kinetochore Network

As illustrated by the plant SAC network, knowledge of the function and evolution of SAC proteins helps experimental investigation of SAC networks that diverge from the ancestral version. The analysis of co-evolution patterns within the SAC network takes this to the next level. In a recent study of presence/absence patterns of kinetochore and SAC proteins, the Mad1 ortholog in ~20% of species with both Mad1 and Mad2 orthologs had no detectable Mad2-interaction motif (MIM) [71]. This is a strong predictor of absence of an interaction, because the MIM was always absent from Mad1 orthologs in species in which no Mad2 ortholog could be found. (Of note, the Mad1 ortholog we found in the flatworm *S. mediterranea* genome also does not have a MIM; Figure 4)). This was true also for Cdc20, which interacts with Mad2 via a similar MIM [71]. This example illustrates how looking at functional motifs sheds new light on the likelihood of conserved interaction between Mad2 and its binding partners, and how such analysis increased confidence that Mad2 had really been lost in species in which Mad2 could not be found.

One can take such analyses one step further and use presence/absence patterns of proteins or of particular functional modules within proteins to predict novel functions or interactions. The strong co-evolution of some NPC Y-complex members with the core SAC is an example of an unbiased, genome-wide 'screen' for novel functional interactions. Conversely, poor

with MadBub duplications, numbers indicate the number of paralogs and the cartoon shows which paralog has which domains/SLiMs. Cdc20 and Mad1 both harbor a Mad2-interacting motif (MIM), which plays a role in MCC formation and Mad2 kinetochore recruitment, respectively (Figure 3). *Knl1 is present in *Hanseniaspora* FEL, but lacks both RVSF and MELT motifs. **The Mad1 sequence in *Kipferlia bialata* is incomplete; therefore, it is unclear whether the Mad2-interacting motif is present in this Mad1 ortholog. ***Trichomonas vaginalis* has four Rod paralogs.

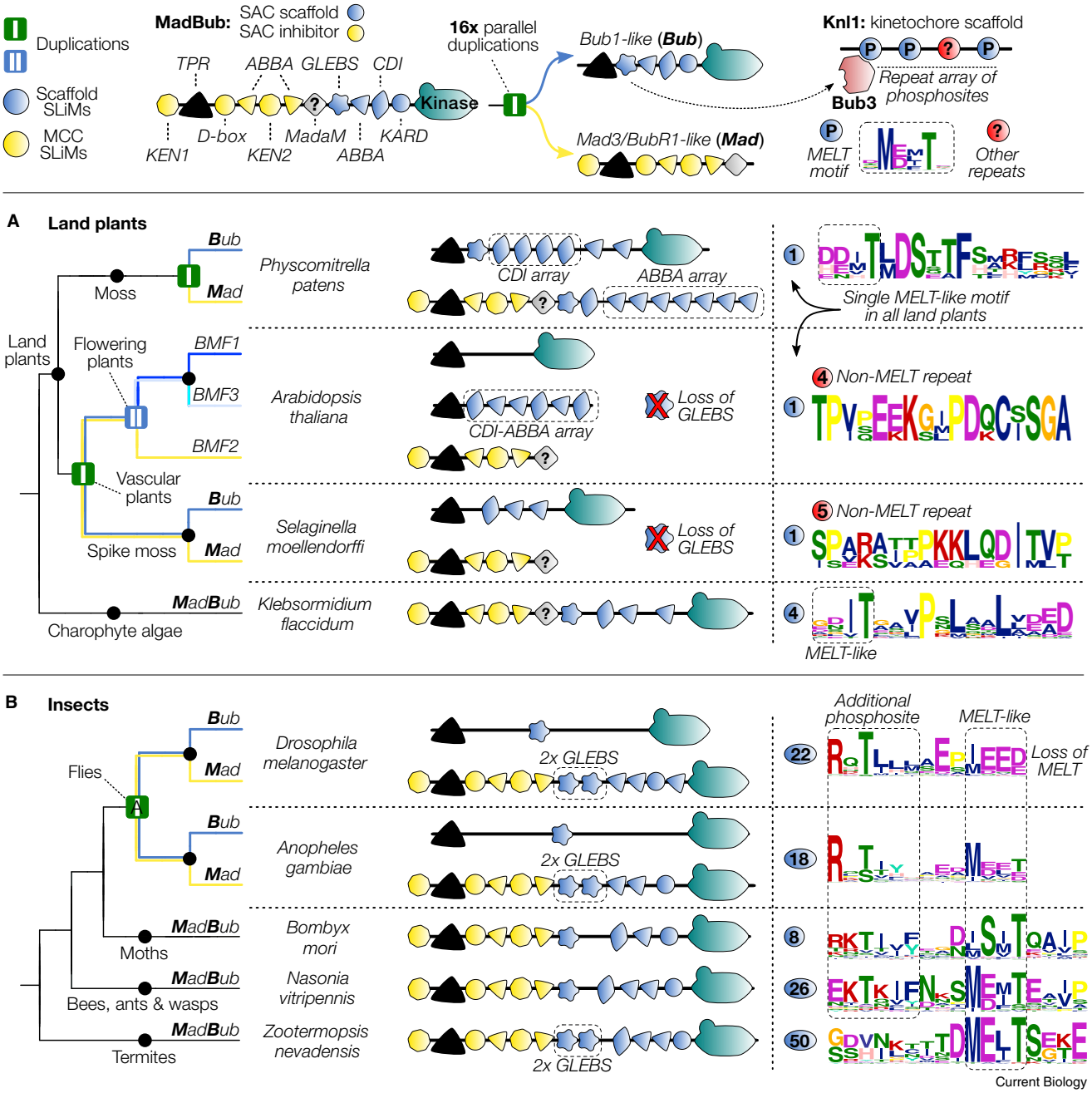


Figure 5. Unique evolutionary fates of MadBub and Knl1 phospho-repeats.

Evolutionary fates of the MadBub gene and its SLiMs (left) and Knl1 repeats (right) in land plants (A) and insects (B). Trajectories of MadBub paralogs are yellow for 'Mad'-like and dark blue for 'Bub'-like. Conserved SLiMs (see top cartoon) are projected onto the linear protein representation and color-coded as in Figure 3. Top cartoon also indicates connection of Knl1 phospho-repeats to GLEBS motif in MadBubs via the Bub3 protein. Gene duplications are indicated by I and II and phylogenetic trees are depicted to show the fate of each paralog after duplication using different colors (yellow/blue blue for the primary MadBub duplication and blue and light blue for the secondary), based on our previously published phylogenetic trees [17]. The consensus of Knl1 repeats is shown as a sequence logo generated using the MEME suite [166] , which, when possible, are aligned to indicate conserved phospho-motifs among the highly divergent repeats of different lineages. Numbers indicate the amount of repeats that were detected. Notable events in (A) Paralogs with variable types and numbers of conserved MadBub SLiMs (e.g., three MadBub paralogs in *A. thaliana* with each a separate function (SAC inhibitor, scaffold and kinase)); loss of GLEBS motif and MELT-like motifs in all vascular plants; ABBA and CDI arrays. Notable events in (B) non-canonical subfunctionalization in insects (Bub paralog contains only GLEBS motif, TPR and kinase domains; Mad paralog contains most MadBub ancestor SLiMs except CDI); gradual but extensive divergence of Knl1 phospho-repeats (MELT motif loss in drosophilids, presence of novel motifs with Aurora-like consensus (RxT) phosphorylation motif.

co-evolution can help dispel notions of conserved functional interactions, as we and others showed for a proposed interaction between the kinetochore protein Zwint-1 and the RZZ complex [71,128]. Moreover, in-depth co-evolution analyses within a network can lead to striking new insights. The unique evolution of MadBub-derived paralogs enabled identification of a cassette of SLiMs (ABBA1–KEN2–ABBA2) that tightly co-segregated with the amino-terminal KEN-box in BubR1/Mad3-like genes [17]. Cell biological and structural analyses verified that, like the KEN box, this cassette is crucial for the ability of BubR1/Mad3 and thus MCC to inhibit APC/C^{Cdc20} [7,17,129].

Expanding on Core SAC Systems

The core of the SAC is highly similar in most fungal and metazoan species [4,71]. But various lineage-specific losses — of which some examples were discussed above — and add-ons have become apparent. The bicaudal-like dynactin adapter Spindly, for example, was invented in the Opisthokonta ancestor, retained in nearly all holozoa (animals and their closest single cell relatives) and lost in many fungal lineages [71]. Together with RZZ, Spindly forms a fibrous corona meshwork at the outer kinetochore [130,131], without which Mad1 cannot efficiently bind or be maintained at unattached kinetochores of animal cells, attenuating the SAC (Figure 3) [132]. RZZ most likely was present in the LECA. Rod and Zwilch were frequently lost in later lineages, but Zw10 was widely retained, likely due to its other role in vesicular trafficking [71,133,134]. RZZ is structurally similar to COP proteins, suggesting it was derived from proteins involved in the formation of the (pre)eukaryotic endomembrane systems [135]. Intriguingly, RZZ is crucial for the SAC response in cells of the flatworm *S. mediterranea*, whose genome does not contain orthologs of Mad2 and BubR1/Mad3 [98]. Some other lineages without MCC components (e.g., *Blastocladiomycota*) or whose MadBub is without MCC SLiMs (e.g., *Apicomplexa, T. vaginalis*) also retained RZZ (Figure 4). It is conceivable, therefore, that its ancestral role in the SAC may have more dimensions than we currently know of.

Spindly has an important second role in the SAC in animals: it recruits the dynein microtubule motor complex to kinetochores [136,137]. Dynein uses kinetochore-bound microtubules to carry away cargo such as Mad1–Mad2 and BubR1. Their removal from kinetochores is a crucial step in shutting off SAC signaling (Figure 3) [138]. Given the limited phylogenetic distribution of Spindly, this is apparently restricted to holozoa and early-branching fungi (Figure 4). Whether other lineages use dynein to silence the SAC is unknown, but at least in the yeast models, no such mechanism appears to operate.

Of the other two SAC silencing pathways in animals, the one driven by PP1-like phosphatases appears very conserved. It operates also in fungi, and the PP1-recruiting SLiM in Knl1 (RVSF) is a hallmark of Knl1 orthologs across the eukaryotic tree of life [6,42,49,50,53,56,71,113]. The MCC disassembly pathway showcases more diversity. The Trip13 ortholog Pch2 does not appear to be important for SAC silencing in budding yeast and p31^{comet} was lost very frequently, leading to its absence in most fungi, excavates and alveolates [6,71,139]. The loss of this mechanism may be related to cell volume in certain species: large but not small *C. elegans* cells require Pch2 to replenish 'open' Mad2 and thereby strengthen the SAC [140]. In budding yeast, Apc15-dependent ubiquitylation of Cdc20 appears to suffice for MCC disassembly. Since Apc15 is ancestral to eukaryotes and is also involved in SAC silencing in human cells, perhaps this is the ancient MCC dismantling mechanism. Whether Trip13-p31^{comet} is an add-on or was part of the LECA SAC response will await experimental interrogation of MCC disassembly in species outside of the Opisthokont lineage [141].

How Unique Are the Evolutionary Dynamics of the SAC?

The extent of lineage-specific innovations is unclear, as they are in principle difficult to predict from comparative genomics. Conversely, because most of the known SAC proteins were present in the LECA, the most obvious differences between species are the result of gene losses that are sometimes extensive. The losses extend to domains and SLiMs. As discussed, such repeated losses can often be corroborated by co-evolutionary loss of interacting proteins or of interaction motifs or domains. They are thus not random, instead revealing that functional modules act as evolutionary modules.

The general pattern of complex ancestors and pervasive secondary loss is observed in many other cellular systems [142–146]. A number of explanations for this pattern have been proposed, including pressures for genomic streamlining (e.g., as a result of a parasitic lifestyle, or of Muller's ratchet [103,147]), or neutral loss ('use it or lose it' [148]). The SAC loss patterns are consistent with the streamlining hypothesis. The SAC is maintained in the majority of lineages (and thus 'used'), and lineages known to have undergone genome streamlining also seem to have lost one or more submodules of the SAC (see blue plus signs in Figure 4). Whatever the process for loss, the question remains if it was a (nearly) neutral process, or may even have been beneficial. For example, when correct chromosome attachment to the spindle is very efficient, the SAC is superfluous in several animal and yeast models [149–152]. In addition, a certain degree of chromosome missegregation may improve adaptability [153–155].

As a group, SAC and kinetochore proteins display relatively high amino acid substitution rates and high dN:dS ratios (as a measure of positive selection) [71]. While some, like Bub3, behave as an average protein, others like p31^{comet} and Zwint-1 are amongst the 20% fastest evolving proteins in human [71]. Moreover, like some other kinetochore proteins in primates [156], KNL1 is under positive selection in modern man [157]. Selection on KNL1 is reasoned to be due to its involvement in brain development [158], and its recently suggested contribution to meiotic drive [114] may also play a role. Whether these are related to KNL1 participation in the SAC, and whether similar functions may explain fast evolution of other SAC/kinetochore proteins remains to be determined. It is of interest to note in this regard that *BUBR1* insufficiency causes various defects in neurodevelopment [159–162].

Outlook: Comparative Cell Biology of the SAC

Deep understanding of the evolution of biochemical networks in cells will be vital for explaining cellular diversity in biology, for uncovering different ways to perform similar processes, and for exposing the evolutionary 'rules' that shape such networks. Here, we outlined evolutionary dynamics of the SAC, a molecular pathway for which functional knowledge and computational

investigations enable detailed interpretation of its evolutionary trajectories and diversity in eukaryotes. But detailed comparative genomics can only take us so far. Experimental interrogation of proteins and molecular pathways in divergent species are needed to, for example, uncover lineage-specific innovations, to test if gene orthologs are also functional orthologs, and to reveal difficult-to-detect orthologs. Such investigations are pivotal to expose and fill fundamental gaps in our overview of SAC function and evolution, especially considering the limited evolutionary diversity represented by animals and fungi, compared to the many other — mostly unicellular — eukaryotes making up the vast eukaryotic tree of life [163]. Experimentation and comparative genomics can really synergise in this way. A global description of kinetochore diversity in eukaryotes [71], for example, inspired the discovery of unique, mosaic point and regional centromeres in early branching fungi [164]. It is exciting to imagine which diversifications to the SAC and other core cellular systems might be uncovered by experiments in other under-studied lineages. In the end, overlaying genomic and experimental analyses with various details on subcellular features and lifestyles may start outlining the routes to and eventual manifestation of cellular diversity of eukaryotes.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2020.02.021.

ACKNOWLEDGEMENTS

We thank Luděk Kořený for kindly sharing cartoons of various eukaryotes used in Figure 1. We thank members of the Snel and Kops labs for critical input. The Kops lab is a member of the Oncode Institute, which is partly financed by the Dutch Cancer Society. Work in the Snel lab is supported by the Netherlands Organisation for Scientific Research (NWO-Vici 016.160.638, to B.S.). E.C.T. is supported by a personal fellowship granted by the Herchel Smith Fund, University of Cambridge, Cambridge, UK.

REFERENCES

1. Lynch, M., Field, M.C., Goodson, H.V., Malik, H.S., Pereira-Leal, J.B., Roos, D.S., Turkewitz, A.P., and Sazer, S. (2014). Evolutionary cell biology: two origins, one objective. Proc. Natl. Acad. Sci. USA *111*, 16990–16994.

2. Fritz-Laylin, L.K., Lord, S.J., and Mullins, R.D. (2017). WASP and SCAR are evolutionarily conserved in actin-filled pseudopod-based motility. J. Cell Biol. *216*, 1673–1688.

3. Cheeseman, I.M. (2014). The kinetochore. Cold Spring Harb. Perspect. Biol. *6*, a015826.

4. London, N., and Biggins, S. (2014). Signalling dynamics in the spindle checkpoint response. Nat. Rev. Mol. Cell Biol. *15*, 736–747.

5. Musacchio, A. (2015). The molecular biology of spindle assembly checkpoint signaling dynamics. Curr. Biol. *25*, R1002–R1018.

6. Vleugel, M., Hoogendoorn, E., Snel, B., and Kops, G.J.P.L. (2012). Evolution and function of the mitotic checkpoint. Dev. Cell *23*, 239–250.

7. Alfieri, C., Chang, L., Zhang, Z., Yang, J., Maslen, S., Skehel, M., and Barford, D. (2016). Molecular basis of APC/C regulation by the spindle assembly checkpoint. Nature *536*, 431–436.

8. Izawa, D., and Pines, J. (2015). The mitotic checkpoint complex binds a second CDC20 to inhibit active APC/C. Nature *517*, 631–634.

9. Sudakin, V., Chan, G.K., and Yen, T.J. (2001). Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. J. Cell Biol. *154*, 925–936.

10. Hardwick, K.G., Johnston, R.C., Smith, D.L., and Murray, A.W. (2000). MAD3 encodes a novel component of the spindle checkpoint which interacts with Bub3p, Cdc20p, and Mad2p. J. Cell Biol. *148*, 871–882.

11. Chao, W.C.H., Kulkarni, K., Zhang, Z., Kong, E.H., and Barford, D. (2012). Structure of the mitotic checkpoint complex. Nature *484*, 208–213.

12. Tang, Z., Bharadwaj, R., Li, B., and Yu, H. (2001). Mad2-Independent inhibition of APCCdc20 by the mitotic checkpoint protein BubR1. Dev. Cell *1*, 227–237.

13. Burton, J.L., and Solomon, M.J. (2007). Mad3p, a pseudosubstrate inhibitor of APCCdc20 in the spindle assembly checkpoint. Genes Dev. *21*, 655–667.

14. King, E.M.J., van der Sar, S.J.A., and Hardwick, K.G. (2007). Mad3 KEN boxes mediate both Cdc20 and Mad3 turnover, and are critical for the spindle checkpoint. PLoS One *2*, e342.

15. Han, J.S., Holland, A.J., Fachinetti, D., Kulukian, A., Cetin, B., and Cleveland, D.W. (2013). Catalytic assembly of the mitotic checkpoint inhibitor BubR1-Cdc20 by a Mad2-induced functional switch in Cdc20. Mol. Cell *51*, 92–104.

16. Lara-Gonzalez, P., Scott, M.I.F., Diez, M., Sen, O., and Taylor, S.S. (2011). BubR1 blocks substrate recruitment to the APC/C in a KEN-box-dependent manner. J. Cell Sci. *124*, 4332–4345.

17. Tromer, E., Bade, D., Snel, B., and Kops, G.J.P.L. (2016). Phylogenomics-guided discovery of a novel conserved cassette of short linear motifs in BubR1 essential for the spindle checkpoint. Open Biol. *6*, 160315.

18. Di Fiore, B., Davey, N.E., Hagting, A., Izawa, D., Mansfeld, J., Gibson, T.J., and Pines, J. (2015). The ABBA motif binds APC/C activators and is shared by APC/C substrates and regulators. Dev. Cell *32*, 358–372.

19. Yamaguchi, M., VanderLinden, R., Weissmann, F., Qiao, R., Dube, P., Brown, N.G., Haselbach, D., Zhang, W., Sidhu, S.S., Peters, J.-M., *et al*. (2016). Cryo-EM of mitotic checkpoint complex-bound APC/C reveals reciprocal and conformational regulation of ubiquitin ligation. Mol. Cell *63*, 593–607.

20. Herzog, F., Primorac, I., Dube, P., Lenart, P., Sander, B., Mechtler, K., Stark, H., and Peters, J.-M. (2009). Structure of the anaphase-promoting complex/cyclosome interacting with a mitotic checkpoint complex. Science *323*, 1477–1481.

21. Faesen, A.C., Thanasoula, M., Maffini, S., Breit, C., Müller, F., van Gerwen, S., Bange, T., and Musacchio, A. (2017). Basis of catalytic assembly of the mitotic checkpoint complex. Nature *542*, 498–502.

22. Ji, Z., Gao, H., Jia, L., Li, B., and Yu, H. (2017). A sequential multi-target Mps1 phosphorylation cascade promotes spindle checkpoint signaling. Elife *6*, e22513.

23. Kulukian, A., Han, J.S., and Cleveland, D.W. (2009). Unattached kinetochores catalyze production of an anaphase inhibitor that requires a Mad2 template to prime Cdc20 for BubR1 binding. Dev. Cell *16*, 105–117.

24. London, N., and Biggins, S. (2014). Mad1 kinetochore recruitment by Mps1-mediated phosphorylation of Bub1 signals the spindle checkpoint. Genes Dev. *28*, 140–152.

25. Zhang, G., Kruse, T., López-Méndez, B., Sylvestersen, K.B., Garvanska, D.H., Schopper, S., Nielsen, M.L., and Nilsson, J. (2017). Bub1 positions Mad1 close to KNL1 MELT repeats to promote checkpoint signalling. Nat. Commun. *8*, 15822.

26. Moyle, M.W., Kim, T., Hattersley, N., Espeut, J., Cheerambathur, D.K., Oegema, K., and Desai, A. (2014). A Bub1-Mad1 interaction targets the Mad1-Mad2 complex to unattached kinetochores to initiate the spindle checkpoint. J. Cell Biol. *204*, 647–657.

27. Overlack, K., Primorac, I., Vleugel, M., Krenn, V., Maffini, S., Hoffmann, I., Kops, G.J.P.L., and Musacchio, A. (2015). A molecular basis for the differential roles of Bub1 and BubR1 in the spindle assembly checkpoint. Elife *4*, e05269.

28. Brady, D.M., and Hardwick, K.G. (2000). Complex formation between Mad1p, Bub1p and Bub3p is crucial for spindle checkpoint function. Curr. Biol. *10*, 675–678.

29. Mapelli, M., and Musacchio, A. (2007). MAD contortions: conformational dimerization boosts spindle checkpoint signaling. Curr. Opin. Struct. Biol. *17*, 716–725.

30. Sacristan, C., and Kops, G.J.P.L. (2015). Joined at the hip: kinetochores, microtubules, and spindle assembly checkpoint signaling. Trends Cell Biol. *25*, 21–28.

31. Foley, E.A., and Kapoor, T.M. (2013). Microtubule attachment and spindle assembly checkpoint signalling at the kinetochore. Nat. Rev. Mol. Cell Biol. *14*, 25–37.

32. Sekulic, N., and Black, B.E. (2012). Molecular underpinnings of centromere identity and maintenance. Trends Biochem. Sci. *37*, 220–229.

33. Black, B.E., and Cleveland, D.W. (2011). Epigenetic centromere propagation and the nature of CENP-a nucleosomes. Cell *144*, 471–479.

34. Foltz, D.R., Jansen, L.E.T., Black, B.E., Bailey, A.O., Yates, J.R., 3rd, and Cleveland, D.W. (2006). The human CENP-A centromeric nucleosome-associated complex. Nat. Cell Biol. *8*, 458–469.

35. Hori, T., Amano, M., Suzuki, A., Backer, C.B., Welburn, J.P., Dong, Y., McEwen, B.F., Shang, W.-H., Suzuki, E., Okawa, K., *et al*. (2008). CCAN makes multiple contacts with centromeric DNA to provide distinct pathways to the outer kinetochore. Cell *135*, 1039–1052.

36. Okada, M., Cheeseman, I.M., Hori, T., Okawa, K., McLeod, I.X., Yates, J.R., 3rd, Desai, A., and Fukagawa, T. (2006). The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. Nat. Cell Biol. *8*, 446–457.

37. Weir, J.R., Faesen, A.C., Klare, K., Petrovic, A., Basilico, F., Fischböck, J., Pentakota, S., Keller, J., Pesenti, M.E., Pan, D., *et al*. (2016). Insights from biochemical reconstitution into the architecture of human kinetochores. Nature *537*, 249–253.

38. Hara, M., and Fukagawa, T. (2017). Critical foundation of the kinetochore: the constitutive centromere-associated network (CCAN). Prog. Mol. Subcell. Biol. *56*, 29–57.

39. Petrovic, A., Mosalaganti, S., Keller, J., Mattiuzzo, M., Overlack, K., Krenn, V., De Antoni, A., Wohlgemuth, S., Cecatiello, V., Pasqualato, S., *et al*. (2014). Modular assembly of RWD domains on the Mis12 complex underlies outer kinetochore organization. Mol. Cell *53*, 591–605.

40. Ciferri, C., Pasqualato, S., Screpanti, E., Varetti, G., Santaguida, S., Dos Reis, G., Maiolica, A., Polka, J., De Luca, J.G., De Wulf, P., *et al*. (2008). Implications for kinetochore-microtubule attachment from the structure of an engineered Ndc80 complex. Cell *133*, 427–439.

41. Cheeseman, I.M., Chappie, J.S., Wilson-Kubalek, E.M., and Desai, A. (2006). The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell *127*, 983–997.

42. London, N., Ceto, S., Ranish, J.A., and Biggins, S. (2012). Phosphoregulation of Spc105 by Mps1 and PP1 regulates Bub1 localization to kinetochores. Curr. Biol. *22*, 900–906.

43. Shepperd, L.A., Meadows, J.C., Sochaj, A.M., Lancaster, T.C., Zou, J., Buttrick, G.J., Rappsilber, J., Hardwick, K.G., and Millar, J.B.A. (2012). Phosphodependent recruitment of Bub1 and Bub3 to Spc7/KNL1 by Mph1 kinase maintains the spindle checkpoint. Curr. Biol. *22*, 891–899.

44. Yamagishi, Y., Yang, C.-H., Tanno, Y., and Watanabe, Y. (2012). MPS1/Mph1 phosphorylates the kinetochore protein KNL1/Spc7 to recruit SAC components. Nat. Cell Biol. *14*, 746–752.

45. Vleugel, M., Omerzu, M., Groenewold, V., Hadders, M.A., Lens, S.M.A., and Kops, G.J.P.L. (2015). Sequential multisite phospho-regulation of KNL1-BUB3 interfaces at mitotic kinetochores. Mol. Cell *57*, 824–835.

46. Vleugel, M., Tromer, E., Omerzu, M., Groenewold, V., Nijenhuis, W., Snel, B., and Kops, G.J.P.L. (2013). Arrayed BUB recruitment modules in the kinetochore scaffold KNL1 promote accurate chromosome segregation. J. Cell Biol. *203*, 943–955.

47. Primorac, I., Weir, J.R., Chiroli, E., Gross, F., Hoffmann, I., van Gerwen, S., Ciliberto, A., and Musacchio, A. (2013). Bub3 reads phosphorylated MELT repeats to promote spindle assembly checkpoint signaling. Elife *2*, e01030.

48. Zhang, G., Lischetti, T., and Nilsson, J. (2014). A minimal number of MELT repeats supports all the functions of KNL1 in chromosome segregation. J. Cell Sci. *127*, 871–884.

49. Ji, Z., Gao, H., and Yu, H. (2015). CELL DIVISION CYCLE. Kinetochore attachment sensed by competitive Mps1 and microtubule binding to Ndc80C. Science *348*, 1260–1264.

50. Hiruma, Y., Sacristan, C., Pachis, S.T., Adamopoulos, A., Kuijt, T., Ubbink, M., von Castelmur, E., Perrakis, A., and Kops, G.J.P.L. (2015). CELL DIVISION CYCLE. Competition between MPS1 and microtubules at kinetochores regulates spindle checkpoint signaling. Science *348*, 1264–1267.

51. Kemmler, S., Stach, M., Knapp, M., Ortiz, J., Pfannstiel, J., Ruppert, T., and Lechner, J. (2009). Mimicking Ndc80 phosphorylation triggers spindle assembly checkpoint signalling. EMBO J. *28*, 1099–1110.

52. Liu, D., Vleugel, M., Backer, C.B., Hori, T., Fukagawa, T., Cheeseman, I.M., and Lampson, M.A. (2010). Regulated targeting of protein phosphatase 1 to the outer kinetochore by KNL1 opposes Aurora B kinase. J. Cell Biol. *188*, 809–820.

53. Rosenberg, J.S., Cross, F.R., and Funabiki, H. (2011). KNL1/Spc105 recruits PP1 to silence the spindle assembly checkpoint. Curr. Biol. *21*, 942–947.

54. Pinsky, B.A., Nelson, C.R., and Biggins, S. (2009). Protein phosphatase 1 regulates exit from the spindle checkpoint in budding yeast. Curr. Biol. *19*, 1182–1187.

55. Vanoosthuyse, V., and Hardwick, K.G. (2009). A novel protein phosphatase 1-dependent spindle checkpoint silencing mechanism. Curr. Biol. *19*, 1176–1181.

56. Nijenhuis, W., Vallardi, G., Teixeira, A., Kops, G.J.P.L., and Saurin, A.T. (2014). Negative feedback at kinetochores underlies a responsive spindle checkpoint signal. Nat. Cell Biol. *16*, 1257–1264.

57. Mansfeld, J., Collin, P., Collins, M.O., Choudhary, J.S., and Pines, J. (2011). APC15 drives the turnover of MCC-CDC20 to make the spindle assembly checkpoint responsive to kinetochore attachment. Nat. Cell Biol. *13*, 1234–1243.

58. Uzunova, K., Dye, B.T., Schutz, H., Ladurner, R., Petzold, G., Toyoda, Y., Jarvis, M.A., Brown, N.G., Poser, I., Novatchkova, M., *et al*. (2012). APC15 mediates CDC20 autoubiquitylation by APC/C(MCC) and disassembly of the mitotic checkpoint complex. Nat. Struct. Mol. Biol. *19*, 1116–1123.

59. Foster, S.A., and Morgan, D.O. (2012). The APC/C subunit Mnd2/Apc15 promotes Cdc20 autoubiquitination and spindle assembly checkpoint inactivation. Mol. Cell *47*, 921–932.

60. Kim, D.H., Han, J.S., Ly, P., Ye, Q., McMahon, M.A., Myung, K., Corbett, K.D., and Cleveland, D.W. (2018). TRIP13 and APC15 drive mitotic exit by turnover of interphase- and unattached kinetochore-produced MCC. Nat. Commun. *9*, 4354.

61. May, K.M., Paldi, F., and Hardwick, K.G. (2017). Fission yeast Apc15 stabilizes MCC-Cdc20-APC/C complexes, ensuring efficient Cdc20 ubiquitination and checkpoint arrest. Curr. Biol. *27*, 1221–1228.

62. Eytan, E., Wang, K., Miniowitz-Shemtov, S., Sitry-Shevah, D., Kaisari, S., Yen, T.J., Liu, S.-T., and Hershko, A. (2014). Disassembly of mitotic checkpoint complexes by the joint action of the AAA-ATPase TRIP13 and p31(comet). Proc. Natl. Acad. Sci. USA *111*, 12019–12024.

63. Wang, K., Sturt-Gillespie, B., Hittle, J.C., Macdonald, D., Chan, G.K., Yen, T.J., and Liu, S.-T. (2014). Thyroid hormone receptor interacting protein 13 (TRIP13) AAA-ATPase is a novel mitotic checkpoint-silencing protein. J. Biol. Chem. *289*, 23928–23937.

64. Ye, Q., Rosenberg, S.C., Moeller, A., Speir, J.A., Su, T.Y., and Corbett, K.D. (2015). TRIP13 is a protein-remodeling AAA+ ATPase that catalyzes MAD2 conformation switching. Elife *4*, e07367.

65. Alfieri, C., Chang, L., and Barford, D. (2018). Mechanism for remodelling of the cell cycle checkpoint protein MAD2 by the ATPase TRIP13. Nature *559*, 274–278.

66. Miniowitz-Shemtov, S., Eytan, E., Kaisari, S., Sitry-Shevah, D., and Hershko, A. (2015). Mode of interaction of TRIP13 AAA-ATPase with the Mad2-binding protein p31comet and with mitotic checkpoint complexes. Proc. Natl. Acad. Sci. USA *112*, 11536–11540.

67. Yang, M., Li, B., Tomchick, D.R., Machius, M., Rizo, J., Yu, H., and Luo, X. (2007). p31comet blocks Mad2 activation through structural mimicry. Cell *131*, 744–755.

68. Westhorpe, F.G., Tighe, A., Lara-Gonzalez, P., and Taylor, S.S. (2011). p31comet-mediated extraction of Mad2 from the MCC promotes efficient mitotic exit. J. Cell Sci. *124*, 3905–3916.

69. Varetti, G., Guida, C., Santaguida, S., Chiroli, E., and Musacchio, A. (2011). Homeostatic control of mitotic arrest. Mol. Cell *44*, 710–720.

70. Eme, L., Trilles, A., Moreira, D., and Brochier-Armanet, C. (2011). The phylogenomic analysis of the anaphase promoting complex and its targets points to complex and modern-like control of the cell cycle in the last common ancestor of eukaryotes. BMC Evol. Biol. *11*, 265.

71. van Hooff, J.J., Tromer, E., van Wijk, L.M., Snel, B., and Kops, G.J. (2017). Evolutionary dynamics of the kinetochore network in eukaryotes as revealed by comparative genomics. EMBO Rep. *18*, 1559–1571.

72. Tromer, E.C., van Hooff, J.J.E., Kops, G.J.P.L., and Snel, B. (2019). Mosaic origin of the eukaryotic kinetochore. Proc. Natl. Acad. Sci. USA *116*, 12873–12882.

73. Hennell James, R., Caceres, E.F., Escasinas, A., Alhasan, H., Howard, J.A., Deery, M.J., Ettema, T.J.G., and Robinson, N.P. (2017). Functional reconstruction of a eukaryotic-like E1/E2/(RING) E3 ubiquitylation cascade from an uncultured archaeon. Nat. Commun. *8*, 1120.

74. Risa, G.T., Hurtig, F., Bray, S., Hafner, A.E., Harker-Kirschneck, L., Faull, P., Davis, C., Papatziamou, D., Mutavchiev, D.R., Fan, C., *et al*. (2019). Proteasome-mediated protein degradation resets the cell division cycle and triggers ESCRT-III-mediated cytokinesis in an archaeon. bioRxiv, 774273.

75. Burroughs, A.M., Zhang, D., Schäffer, D.E., Iyer, L.M., and Aravind, L. (2015). Comparative genomic analyses reveal a vast, novel network of nucleotide-centric systems in biological conflicts, immunity and signaling. Nucleic Acids Res. *43*, 10633–10654.

76. Ye, Q., Lau, R.K., Mathews, I.T., Watrous, J.D., Azimi, C.S., Jain, M., and Corbett, K.D. (2019). HORMA domain proteins and a Pch2-like ATPase regulate bacterial cGAS-like enzymes to mediate bacteriophage immunity. bioRxiv, 694695.

77. D'Andrea, L.D., and Regan, L. (2003). TPR proteins: the versatile helix. Trends Biochem. Sci. *28*, 655–662.

78. Cairo, L.V., Ptak, C., and Wozniak, R.W. (2013). Mitosis-specific regulation of nuclear transport by the spindle assembly checkpoint protein Mad1p. Mol. Cell *49*, 109–120.

79. Rodriguez-Bravo, V., Maciejowski, J., Corona, J., Buch, H.K., Collin, P., Kanemaki, M.T., Shah, J.V., and Jallepalli, P.V. (2014). Nuclear pores protect genome integrity by assembling a premitotic and Mad1-dependent anaphase inhibitor. Cell *156*, 1017–1031.

80. Schweizer, N., Ferrás, C., Kern, D.M., Logarinho, E., Cheeseman, I.M., and Maiato, H. (2013). Spindle assembly checkpoint robustness requires Tpr-mediated regulation of Mad1/Mad2 proteostasis. J. Cell Biol. *203*, 883–893.

81. Liu, S.-T., Chan, G.K.T., Hittle, J.C., Fujii, G., Lees, E., and Yen, T.J. (2003). Human MPS1 kinase is required for mitotic arrest induced by the loss of CENP-E from kinetochores. Mol. Biol. Cell *14*, 1638–1651.

82. Campbell, M.S., Chan, G.K., and Yen, T.J. (2001). Mitotic checkpoint proteins HsMAD1 and HsMAD2 are associated with nuclear pore complexes in interphase. J. Cell Sci. *114*, 953–963.

83. Komaki, S., and Schnittger, A. (2017). The spindle assembly checkpoint in Arabidopsis is rapidly shut off during severe stress. Dev. Cell *43*, 172–185.e5.

84. Pritchard, C.E., Fornerod, M., Kasper, L.H., and van Deursen, J.M. (1999). RAE1 is a shuttling mRNA export factor that binds to a GLEBS-like NUP98 motif at the nuclear pore complex through multiple domains. J. Cell Biol. *145*, 237–254.

85. Wang, X., Babu, J.R., Harden, J.M., Jablonski, S.A., Gazi, M.H., Lingle, W.L., de Groen, P.C., Yen, T.J., and van Deursen, J.M. (2001). The mitotic checkpoint protein hBUB3 and the mRNA export factor hRAE1 interact with GLE2p-binding sequence (GLEBS)-containing proteins. J. Biol. Chem. *276*, 26559–26567.

86. Joseph, J., Liu, S.-T., Jablonski, S.A., Yen, T.J., and Dasso, M. (2004). The RanGAP1-RanBP2 complex is essential for microtubule-kinetochore interactions in vivo. Curr. Biol. *14*, 611–617.

87. Orjalo, A.V., Arnaoutov, A., Shen, Z., Boyarchuk, Y., Zeitlin, S.G., Fontoura, B., Briggs, S., Dasso, M., and Forbes, D.J. (2006). The Nup107-160 nucleoporin complex is required for correct bipolar spindle assembly. Mol. Biol. Cell *17*, 3806–3818.

88. Mishra, R.K., Chakraborty, P., Arnaoutov, A., Fontoura, B.M.A., and Dasso, M. (2010). The Nup107-160 complex and gamma-TuRC regulate microtubule polymerization at kinetochores. Nat. Cell Biol. *12*, 164–169.

89. Dawlaty, M.M., Malureanu, L., Jeganathan, K.B., Kao, E., Sustmann, C., Tahk, S., Shuai, K., Grosschedl, R., and van Deursen, J.M. (2008). Resolution of sister centromeres requires RanBP2-mediated SUMOylation of topoisomerase IIalpha. Cell *133*, 103–115.

90. Salina, D., Enarson, P., Rattner, J.B., and Burke, B. (2003). Nup358 integrates nuclear envelope breakdown with kinetochore assembly. J. Cell Biol. *162*, 991–1001.

91. Ploubidou, A., Robinson, D.R., Docherty, R.C., Ogbadoyi, E.O., and Gull, K. (1999). Evidence for novel cell cycle checkpoints in trypanosomes: kinetoplast segregation and cytokinesis in the absence of mitosis. J. Cell Sci. *112* (Pt 24), 4641–4650.

92. Markova, K., Uzlikova, M., Tumova, P., Jirakova, K., Hagen, G., Kulda, J., and Nohynkova, E. (2016). Absence of a conventional spindle mitotic checkpoint in the binucleated single-celled parasite Giardia intestinalis. Eur. J. Cell Biol. *95*, 355–367.

93. Kaczanowski, A., Gaertig, J., and Kubiak, J. (1985). Effect of the antitubulin drug nocodazole on meiosis and postmeiotic development in Tetrahymena thermophila. Induction of achiasmatic meiosis. Exp. Cell Res. *158*, 244–256.

94. Morrissette, N.S., and Sibley, L.D. (2002). Disruption of microtubules uncouples budding and nuclear division in Toxoplasma gondii. J. Cell Sci. *115*, 1017–1025.

95. Akiyoshi, B., and Gull, K. (2013). Evolutionary cell biology of chromosome segregation: insights from trypanosomes. Open Biol. *3*, 130023.

96. Gourguechon, S., Holt, L.J., and Cande, W.Z. (2013). The Giardia cell cycle progresses independently of the anaphase-promoting complex. J. Cell Sci. *126*, 2246–2255.

97. Vicente, J.-J., and Cande, W.Z. (2014). Mad2, Bub3, and Mps1 regulate chromosome segregation and mitotic synchrony in Giardia intestinalis, a binucleate protist lacking an anaphase-promoting complex. Mol. Biol. Cell *25*, 2774–2787.

98. Grohme, M.A., Schloissnig, S., Rozanski, A., Pippel, M., Young, G.R., Winkler, S., Brandl, H., Henry, I., Dahl, A., Powell, S., *et al*. (2018). The genome of Schmidtea mediterranea and the evolution of core cellular mechanisms. Nature *554*, 56–61.

99. Riley, D.E., Krieger, J.N., Miner, D., and Rabinovitch, P.S. (1994). Trichomonas vaginalis: dominant G2 period and G2 phase arrest in a representative of an early branching eukaryotic lineage. J. Eukaryot. Microbiol. *41*, 408–414.

100. Murray, A.W. (2011). A brief history of error. Nat. Cell Biol. *13*, 1178–1182.

101. Komaki, S., and Schnittger, A. (2016). The spindle checkpoint in plants-a green variation over a conserved theme? Curr. Opin. Plant Biol. *34*, 84–91.

102. Jackson, A.P. (2015). Preface. The evolution of parasite genomes and the origins of parasitism. Parasitology *142* (*Suppl 1*), S1–S5.

103. Wolf, Y.I., and Koonin, E.V. (2013). Genome reduction as the dominant mode of evolution. Bioessays *35*, 829–837.

104. Steenwyk, J.L., Opulente, D.A., Kominek, J., Shen, X.-X., Zhou, X., Labella, A.L., Bradley, N.P., Eichman, B.F., Čadež, N., Libkind, D., *et al*. (2019). Extensive loss of cell-cycle and DNA repair genes in an ancient lineage of bipolar budding yeasts. PLoS Biol. *17*, e3000255.

105. Nguyen Ba, A.N., Strome, B., Osman, S., Legere, E.-A., Zarin, T., and Moses, A.M. (2017). Parallel reorganization of protein function in the spindle checkpoint pathway through evolutionary paths in the fitness landscape that appear neutral in laboratory experiments. PLoS Genet. *13*, e1006735.

106. Suijkerbuijk, S.J.E., van Dam, T.J.P., Karagöz, G.E., von Castelmur, E., Hubner, N.C., Duarte, A.M.S., Vleugel, M., Perrakis, A., Rüdiger, S.G.D., Snel, B., *et al*. (2012). The vertebrate mitotic checkpoint protein BUBR1 is an unusual pseudokinase. Dev. Cell *22*, 1321–1329.

107. Murray, A.W., and Marks, D. (2001). Can sequencing shed light on cell cycling? Nature *409*, 844–846.

108. Huang, Y., Lin, L., Liu, X., Ye, S., Yao, P.Y., Wang, W., Yang, F., Gao, X., Li, J., Zhang, Y., *et al*. (2019). BubR1 phosphorylates CENP-E as a switch enabling the transition from lateral association to end-on capture of spindle microtubules. Cell Res. *29*, 562–578.

109. Hittinger, C.T., and Carroll, S.B. (2007). Gene duplication and the adaptive evolution of a classic genetic switch. Nature *449*, 677–681.

110. Heinrich, S., Geissen, E.-M., Kamenz, J., Trautmann, S., Widmer, C., Drewe, P., Knop, M., Radde, N., Hasenauer, J., and Hauf, S. (2013). Determinants of robustness in spindle assembly checkpoint signalling. Nat. Cell Biol. *15*, 1328.

111. Force, A., Lynch, M., Pickett, F.B., Amores, A., Yan, Y.L., and Postlethwait, J. (1999). Preservation of duplicate genes by complementary, degenerative mutations. Genetics *151*, 1531–1545.

112. Murray, A.W. (2012). Don't make me mad, Bub! Dev. Cell *22*, 1123–1125.

113. Tromer, E., Snel, B., and Kops, G.J.P.L. (2015). Widespread recurrent patterns of rapid repeat evolution in the kinetochore scaffold KNL1. Genome Biol. Evol. *7*, 2383–2393.

114. Akera, T., Trimm, E., and Lampson, M.A. (2019). Molecular strategies of meiotic cheating by selfish centromeres. Cell *178*, 1132–1144.e10.

115. Malik, H.S. (2009). The centromere-drive hypothesis: a simple basis for centromere complexity. Prog. Mol. Subcell. Biol. *48*, 33–52.

116. Hennrich, M.L., Marino, F., Groenewold, V., Kops, G.J.P.L., Mohammed, S., and Heck, A.J.R. (2013). Universal quantitative kinase assay based on diagonal SCX chromatography and stable isotope dimethyl labeling provides high-definition kinase consensus motifs for PKA and human Mps1. J. Proteome Res. *12*, 2214–2224.

117. Dou, Z., von Schubert, C., Körner, R., Santamaria, A., Elowe, S., and Nigg, E.A. (2011). Quantitative mass spectrometry analysis reveals similar substrate consensus motif for human Mps1 kinase and Plk1. PLoS One *6*, e18793.

118. Espeut, J., Lara-Gonzalez, P., Sassine, M., Shiau, A.K., Desai, A., and Abrieu, A. (2015). Natural loss of Mps1 kinase in nematodes uncovers a role for Polo-like kinase 1 in spindle checkpoint initiation. Cell Rep. *12*, 58–65.

119. Alexander, J., Lim, D., Joughin, B.A., Hegemann, B., Hutchins, J.R.A., Ehrenberger, T., Ivins, F., Sessa, F., Hudecz, O., Nigg, E.A., *et al*. (2011). Spatial exclusivity combined with positive and negative selection of phosphorylation motifs is the basis for context-dependent mitotic signaling. Sci. Signal. *4*, ra42.

120. Pachis, S.T., and Kops, G.J.P.L. (2018). Leader of the SAC: molecular mechanisms of Mps1/TTK regulation in mitosis. Open Biol. *8*, 180109, 180109.

121. Nijenhuis, W., von Castelmur, E., Littler, D., De Marco, V., Tromer, E., Vleugel, M., van Osch, M.H.J., Snel, B., Perrakis, A., and Kops, G.J.P.L. (2013). A TPR domain-containing N-terminal module of MPS1 is required for its kinetochore localization by Aurora B. J. Cell Biol. *201*, 217–231.

122. Pachis, S.T., Hiruma, Y., Tromer, E.C., Perrakis, A., and Kops, G.J.P.L. (2019). Interactions between N-terminal modules in MPS1 enable spindle checkpoint silencing. Cell Rep. *26*, 2101–2112.e6.

123. Vleugel, M., Hoek, T.A., Tromer, E., Sliedrecht, T., Groenewold, V., Omerzu, M., and Kops, G.J.P.L. (2015). Dissecting the roles of human BUB1 in the spindle assembly checkpoint. J. Cell Sci. *128*, 2975–2982.

124. Klebig, C., Korinth, D., and Meraldi, P. (2009). Bub1 regulates chromosome segregation in a kinetochore-independent manner. J. Cell Biol. *185*, 841–858.

125. Baron, A.P., von Schubert, C., Cubizolles, F., Siemeister, G., Hitchcock, M., Mengel, A., Schröder, J., Fernández-Montalván, A., von Nussbaum, F., Mumberg, D., *et al*. (2016). Probing the catalytic functions of Bub1 kinase using the small molecule inhibitors BAY-320 and BAY-524. Elife *5*, e12187.

126. de Oliveira, E.A.G., Romeiro, N.C., Ribeiro, E. da S., Santa-Catarina, C., Oliveira, A.E.A., Silveira, V., de Souza Filho, G.A., Venancio, T.M., and Cruz, M.A.L. (2012). Structural and functional characterization of the protein kinase Mps1 in Arabidopsis thaliana. PLoS One *7*, e45707.

127. Zhang, G., Kruse, T., Guasch Boldú, C., Garvanska, D.H., Coscia, F., Mann, M., Barisic, M., and Nilsson, J. (2019). Efficient mitotic checkpoint signaling depends on integrated activities of Bub1 and the RZZ complex. EMBO J. *38*, e100977.

128. Zhang, G., Lischetti, T., Hayward, D.G., and Nilsson, J. (2015). Distinct domains in Bub1 localize RZZ and BubR1 to kinetochores to regulate the checkpoint. Nat. Commun. *6*, 7162.

129. Di Fiore, B., Wurzenberger, C., Davey, N.E., and Pines, J. (2016). The mitotic checkpoint complex requires an evolutionary conserved cassette to bind and inhibit active APC/C. Mol. Cell *64*, 1144–1153.

130. Pereira, C., Reis, R.M., Gama, J.B., Celestino, R., Cheerambathur, D.K., Carvalho, A.X., and Gassmann, R. (2018). Self-assembly of the RZZ complex into filaments drives kinetochore expansion in the absence of microtubule attachment. Curr. Biol. *28*, 3408–3421.e8.

131. Sacristan, C., Ahmad, M.U.D., Keller, J., and Fermie, J. (2018). Dynamic kinetochore size regulation promotes microtubule capture and chromosome biorientation in mitosis. Nat. Cell Biol. *20*, 800–810.

132. Kops, G.J.P.L., Kim, Y., Weaver, B.A.A., Mao, Y., McLeod, I., Yates, J.R., 3rd, Tagaya, M., and Cleveland, D.W. (2005). ZW10 links mitotic checkpoint signaling to the structural kinetochore. J. Cell Biol. *169*, 49–60.

133. Civril, F., Wehenkel, A., Giorgi, F.M., Santaguida, S., Di Fonzo, A., Grigorean, G., Ciccarelli, F.D., and Musacchio, A. (2010). Structural analysis of the RZZ complex reveals common ancestry with multisubunit vesicle tethering machinery. Structure *18*, 616–626.

134. Hirose, H., Arasaki, K., Dohmae, N., Takio, K., Hatsuzawa, K., Nagahama, M., Tani, K., Yamamoto, A., Tohyama, M., and Tagaya, M. (2004). Implication of ZW10 in membrane trafficking between the endoplasmic reticulum and Golgi. EMBO J. *23*, 1267–1278.

135. Mosalaganti, S., Keller, J., Altenfeld, A., Winzker, M., Rombaut, P., Saur, M., Petrovic, A., Wehenkel, A., Wohlgemuth, S., Müller, F., *et al*. (2017). Structure of the RZZ complex and molecular basis of its interaction with Spindly. J. Cell Biol. *216*, 961–981.

136. Gama, J.B., Pereira, C., Simões, P.A., Celestino, R., Reis, R.M., Barbosa, D.J., Pires, H.R., Carvalho, C., Amorim, J., Carvalho, A.X., *et al*. (2017). Molecular mechanism of dynein recruitment to kinetochores by the Rod-Zw10-Zwilch complex and Spindly. J. Cell Biol. *216*, 943–960.

137. Griffis, E.R., Stuurman, N., and Vale, R.D. (2007). Spindly, a novel protein essential for silencing the spindle assembly checkpoint, recruits dynein to the kinetochore. J. Cell Biol. *177*, 1005–1015.

138. Howell, B.J., McEwen, B.F., Canman, J.C., Hoffman, D.B., Farrar, E.M., Rieder, C.L., and Salmon, E.D. (2001). Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. J. Cell Biol. *155*, 1159–1172.

139. Vader, G. (2015). Pch2(TRIP13): controlling cell division through regulation of HORMA domains. Chromosoma *124*, 333–339.

140. Défachelles, L., Russo, A.E., Nelson, C.R., and Bhalla, N. (2019). PCH-2TRIP13 regulates spindle checkpoint strength. bioRxiv, 389080.

141. Ji, J., Tang, D., Shen, Y., Xue, Z., Wang, H., Shi, W., Zhang, C., Du, G., Li, Y., and Cheng, Z. (2016). P31comet, a member of the synaptonemal complex, participates in meiotic DSB formation in rice. Proc. Natl. Acad. Sci. USA *113*, 10577–10582.

142. Huynen, M.A., de Hollander, M., and Szklarczyk, R. (2009). Mitochondrial proteome evolution and genetic disease. Biochim. Biophys. Acta *1792*, 1122–1129.

143. van Dam, T.J.P., Townsend, M.J., Turk, M., Schlessinger, A., Sali, A., Field, M.C., and Huynen, M.A. (2013). Evolution of modular intraflagellar transport from a coatomer-like progenitor. Proc. Natl. Acad. Sci. USA *110*, 6943–6948.

144. Hirst, J., Barlow, L.D., Francisco, G.C., Sahlender, D.A., Seaman, M.N.J., Dacks, J.B., and Robinson, M.S. (2011). The fifth adaptor protein complex. PLoS Biol. *9*, e1001170.

145. Kahn, S.E., Larson, V.G., Beard, J.C., Cain, K.C., Fellingham, G.W., Schwartz, R.S., Veith, R.C., Stratton, J.R., Cerqueira, M.D., and Abrass, I.B. (1990). Effect of exercise on insulin action, glucose tolerance, and insulin secretion in aging. Am. J. Physiol. *258*, E937–E943.

146. Elias, M., Brighouse, A., Gabernet-Castello, C., Field, M.C., and Dacks, J.B. (2012). Sculpting the endomembrane system in deep time: high resolution phylogenetics of Rab GTPases. J. Cell Sci. *125*, 2500–2508.

147. Lynch, M. (2010). Evolution of the mutation rate. Trends Genet. *26*, 345–352.

148. Albalat, R., and Cañestro, C. (2016). Evolution by gene loss. Nat. Rev. Genet. *17*, 379–391.

149. Li, R., and Murray, A.W. (1991). Feedback control of mitosis in budding yeast. Cell *66*, 519–531.

150. Hoyt, M.A., Totis, L., and Roberts, B.T. (1991). S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function. Cell *66*, 507–517.

151. Sansregret, L., Patterson, J.O., Dewhurst, S., López-García, C., Koch, A., McGranahan, N., Chao, W.C.H., Barry, D.J., Rowan, A., Instrell, R., *et al*. (2017). APC/C dysfunction limits excessive cancer chromosomal instability. Cancer Discov. *7*, 218–233.

152. Wild, T., Larsen, M.S.Y., Narita, T., Schou, J., Nilsson, J., and Choudhary, C. (2016). The spindle assembly checkpoint is not essential for viability of human cells with genetically lowered APC/C activity. Cell Rep. *14*, 1829–1840.

153. Pavelka, N., Rancati, G., Zhu, J., Bradford, W.D., Saraf, A., Florens, L., Sanderson, B.W., Hattem, G.L., and Li, R. (2010). Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. Nature *468*, 321–325.

154. Yang, F., Teoh, F., Tan, A.S.M., Cao, Y., Pavelka, N., and Berman, J. (2019). Aneuploidy enables cross-adaptation to unrelated drugs. Mol. Biol. Evol. *36*, 1768–1782.

155. Tsai, H.-J., and Nelliat, A. (2019). A double-edged sword: aneuploidy is a prevalent strategy in fungal adaptation. Genes *10*, e787.

156. van der Lee, R., Wiel, L., van Dam, T.J.P., and Huynen, M.A. (2017). Genome-scale detection of positive selection in nine primates predicts human-virus evolutionary conflicts. Nucleic Acids Res. *45*, 10634–10648.

157. Prüfer, K., Racimo, F., Patterson, N., Jay, F., Sankararaman, S., Sawyer, S., Heinze, A., Renaud, G., Sudmant, P.H., de Filippo, C., *et al*. (2014). The complete genome sequence of a Neanderthal from the Altai Mountains. Nature *505*, 43–49.

158. Omer Javed, A., Li, Y., Muffat, J., Su, K.-C., Cohen, M.A., Lungjangwa, T., Aubourg, P., Cheeseman, I.M., and Jaenisch, R. (2018). Microcephaly modeling of kinetochore mutation reveals a brain-specific phenotype. Cell Rep. *25*, 368–382.e5.

159. Choi, C.-I., Yoo, K.H., Hussaini, S.M.Q., Jeon, B.T., Welby, J., Gan, H., Scarisbrick, I.A., Zhang, Z., Baker, D.J., van Deursen, J.M., *et al*. (2016). The progeroid gene BubR1 regulates axon myelination and motor function. Aging *8*, 2667–2688.

160. Yang, Z., Jun, H., Choi, C.-I., Yoo, K.H., Cho, C.H., Hussaini, S.M.Q., Simmons, A.J., Kim, S., van Deursen, J.M., Baker, D.J., *et al*. (2017). Age-related decline in BubR1 impairs adult hippocampal neurogenesis. Aging Cell *16*, 598–601.

161. Cho, C.H., Yang, Z., Yoo, K.H., Oliveros, A., and Jang, M.H. (2018). BubR1 insufficiency impairs affective behavior and memory function in mice. Int. Neurourol. J. *22*, S122–S130.

162. de Wolf, B., and Kops, G.J.P.L. (2017). Kinetochore malfunction in human pathologies. Adv. Exp. Med. Biol. *1002*, 69–91.

163. Burki, F., Roger, A.J., Brown, M.W., and Simpson, A.G.B. (2019). The new tree of eukaryotes. Trends Ecol. Evol. *45*, 43–55.

164. Navarro-Mendoza, M.I., Pérez-Arques, C., Panchal, S., Nicolás, F.E., Mondo, S.J., Ganguly, P., Pangilinan, J., Grigoriev, I.V., Heitman, J., Sanyal, K., *et al*. (2019). Early diverging fungus mucor circinelloides lacks centromeric histone CENP-A and displays a mosaic of point and regional centromeres. Curr. Biol. *29*, 3791–3802.

165. Koreny, L., and Field, M.C. (2016). Ancient eukaryotic origin and evolutionary plasticity of nuclear lamina. Genome Biol. Evol. *8*, 2663–2671.

166. Bailey, T.L., Boden, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., Ren, J., Li, W.W., and Noble, W.S. (2009). MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res. *37*, W202–W208.