EVOLUTIONARY CONSERVATION BETWEEN BUDDING YEAST AND HUMAN KINETOCHORES

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Accurate chromosome segregation during mitosis requires the correct assembly of kinetochores — complexes of centromeric DNA and proteins that link chromosomes to spindle microtubules. Studies on the kinetochore of the budding yeast *Saccharomyces cerevisiae* have revealed functionally novel components of the kinetochore and its regulatory complexes, some of which are highly conserved in humans.

EUPLOIDY
An entire set of chromosomes is represented in integer increments (haploid, one set; diploid, two sets; triploid, three sets).

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The molecular mechanisms that ensure the accurate segregation of chromosomes during mitosis are fundamental to the conservation of chromosomal EUPLOIDY in eukaryotic organisms. Accurate chromosome segregation requires functional domains within the chromosomal DNA, as well as the coordinated activity of many proteins within the cell cycle.

The kinetochore — centromeric DNA and associated proteins — and its regulating system are essential for the segregation of chromosomes during mitosis. The kinetochore provides the point of attachment to the mitotic spindle, and is the site through which completion of metaphase is sensed by the cell-cycle regulatory machinery, which coordinates the synchronous separation of chromosomes at the onset of anaphase (BOX 1).

The kinetochore of the budding yeast *Saccharomyces cerevisiae* is the best characterized, and in this review we discuss the conservation and diversity of kinetochore function between budding yeast and humans.

Humans versus yeast

Classic cytological studies in 'larger' eukaryotic cells (mammalian cells, amphibian cells and insect cells, for example) have provided a detailed description of spindle dynamics and chromosome movements during mitosis and meiosis. Although these studies have set the stage for our understanding of kinetochore biology, a combination of biochemical and genetic approaches has been required in various organisms to understand the actual mechanisms that underlie chromosome segregation.

Observations of chromosome dynamics in 'smaller' eukaryotes — such as budding yeast — have been more difficult, and are therefore less well documented, owing to the very small sizes of the individual chromosomes and the inherent difficulty of visualizing them under the microscope. Nevertheless, these 'simpler' organisms are excellent model systems for identifying and analysing the molecular components of chromosome segregation, including those required for kinetochore structure and function. An important challenge in the field has been to determine the extent to which the dynamics of chromosome movements in evolutionarily distant organisms, such as budding yeast and humans, are similar. The logical extension of this question is to define the extent to which the molecular mechanisms are conserved.

Kinetochore behaviour

Budding yeast kinetochores. The behaviour of kinetochores during cell-cycle progression in *S. cerevisiae* has been monitored by three groups, either by tagging with the green fluorescent protein (GFP) and/or by fluorescence *in situ* hybridization (FISH)[1–3]. These groups have found that the sister kinetochores separate transiently in the very early spindle at early S phase, whereas the sister arms remain associated (FIG. 1a). (In budding yeast, the centromeric DNA is duplicated in early S phase[4].) Sister

Box 1 | **The spindle checkpoint**

Cell-cycle checkpoints are cellular control systems that sense the completion of a particular event before allowing another event to proceed[106]. The spindle checkpoint is a surveillance system that can delay mitosis in response to either defective spindle organization, or a failure of the chromosomes to attach correctly to the spindle[107].

How does this checkpoint work? The metaphase-to-anaphase transition and exit from mitosis are initiated in cells by a ubiquitin-mediated proteolysis complex called the 'cyclosome' or 'anaphase-promoting complex' (APC; FIG. 4). During mitosis, the APC is thought to ubiquitylate components that are responsible for sister-chromatid cohesion (triggering anaphase), and also cyclin B (causing exit from mitosis as a result of breakdown of the maturation-promoting factor), resulting in rapid degradation of these targets by the proteasome[108,109]. The spindle checkpoint operates by communicating with the APC machinery so that anaphase will not occur.

centromeres repeatedly split and re-associate before finally parting and moving to spindle poles shortly before the onset of anaphase proper, indicating that kinetochores in yeast and humans behave more similarly to one another than had previously been suspected.

These groups also observed that the kinetochores are closely connected with the spindle pole bodies (SPB) — the yeast equivalent of the mammalian microtubule-organizing centre (MTOC) — throughout the cell cycle (FIG. 1a). In budding yeast, the SPB is embedded within the nuclear envelope, and the nuclear envelope does not break down. There seems to be no authentic anaphase-A movement in budding yeast; however, it has been proposed that the decrease in the distance between the kinetochores and the poles (the anaphase-A event) might take place in telophase after full spindle elongation[5,6]. Furthermore, all budding yeast kinetochores cluster together at the spindle poles through interphase[1,7]. The anaphase-B event is very similar in the budding yeast and humans.

Human kinetochores. The behaviour of kinetochores during cell-cycle progression has been observed in mammalian cells, which contain relatively large kinetochore structures[8]. In human cells, the centromeric ALPHOID DNA is duplicated in late S phase (FIG. 1). After nuclear-envelope breakdown, kinetochores associate with kinetochore microtubules that connect chromosomes to the centrosome (the MTOC in humans). Kinetochore–microtubule interactions take place only during mitosis. Bi-orientation of chromosomes (attachment of sister centromeres to opposite poles by means of kinetochore microtubules; BOX 2) is established at metaphase, when transient separation and reassociation of kinetochores occurs (FIG. 1b).

During anaphase, kinetochores move along the spindle microtubules, and sister chromosomes are thereby pulled to opposite poles (anaphase A; FIG. 1b). After this event, elongation of pole-to-pole microtubules increases the distance between the two spindle poles, further separating the sister-chromatid sets (anaphase B; FIG. 1b).

Humans versus yeast. In both yeast and mammalian cells, complete bipolar attachment of all chromosomes must occur before anaphase begins. Before completion of bipolar attachment, or in response to spindle damage, a 'wait anaphase' signal is generated, which is transmitted through the mitotic spindle-checkpoint pathway (BOX 1) to cell-cycle regulators.

Over the past three decades, much has been learned about the DNA sequence elements (determinants in *cis*) and proteins (determinants in *trans*) that comprise the budding yeast and mammalian kinetochores. At the start of these studies, it was unclear how relevant the analysis of budding yeast kinetochore structure and function would be to our understanding of human kinetochore biology. In this review, we compare the molecular components that are known, at present, to be required for kinetochore function in budding yeast and mammals, and consider them as three sets of subcomponents (FIG. 2): first, the chromosomal DNA–inner

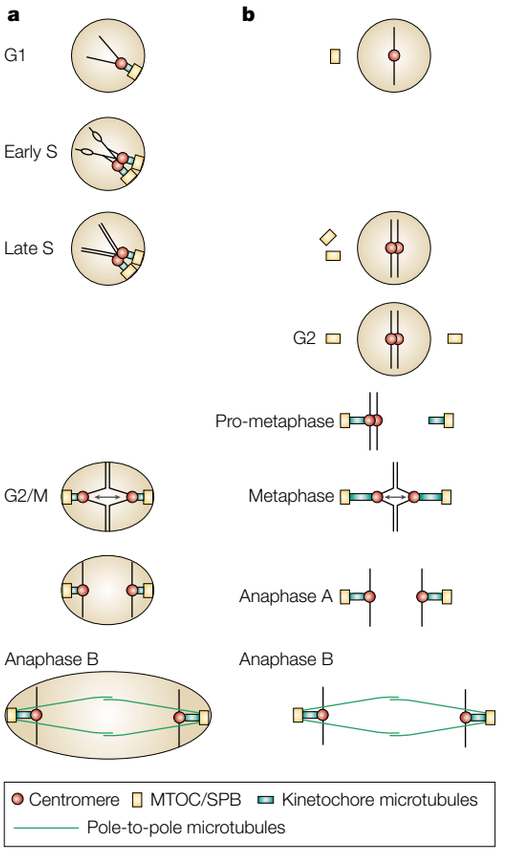

Figure 1 | **Kinetochore function in budding yeast and mammals.** The kinetochore is essential for chromosome segregation in both yeast and mammalian cells. **a** | Budding yeast. The chromosome is attached at its centromere (red) to the spindle pole body (SPB; yellow) through the kinetochore microtubules (aqua). The DNA is replicated in S phase, and the sister chromatids remain together until cohesion is lost and they are pulled to opposite poles during mitosis (M). Elongation of pole-to-pole microtubules (green) increases the distance between the two spindle poles. **b** | Mammalian cells. Kinetochore–microtubule interactions occur only during mitosis. The kinetochores connect the centromeres to the mammalian equivalent of the SPB, the microtubule-organizing centre (MTOC), and remain attached as the sister chromatids are pulled to opposite poles.

ALPHOID DNA
α-satellite DNA; highly repetitive satellite DNA.

SCF UBIQUITIN-LIGASE COMPLEX
An E3 enzyme that targets ubiquitin to cell-cycle-regulatory proteins (for example, Sic1, Clns), using an F-box protein as a specificity factor. SCF refers to 'Skp1/Cul1/F-box protein'.

kinetochore protein interface (FIG. 2a); second, the inner kinetochore–mitotic spindle interface (FIG. 2b); and finally, the kinetochore protein–cell cycle regulatory machinery interface (FIG. 2c). We conclude that the molecular understanding of the less-complex budding yeast kinetochore provides an excellent framework for understanding the more complex kinetochores of humans.

The DNA–inner kinetochore protein interface

Yeast centromeres. In *S. cerevisiae*, the centromeric DNA sequence is a 125-base-pair (bp) region that contains three conserved elements — CDEI, CDEII and CDEIII (REFS 9–11; FIG. 3a). The CDEI 8-bp sequence is bound by the centromere-binding factor 1 (Cbf1 — also known as Cp1, Cpf1 or Cep1), which contains a helix–loop–helix DNA-binding domain, and mediates both transcriptional regulation and chromosome segregation[12,13]. Neither CDEI nor Cbf1 is essential for viability[12,13].

The 78–86-bp region of CDEII is composed of (A+T)-rich DNA, and seems to act as a spacer between the conserved CDEI and CDEIII DNA elements[14]. Mutations in the gene encoding a protein called Cse4 — which is a histone-H3 variant that associates with centromeric DNA *in vivo* — reveal a genetic interaction with an insertion mutation in the CDEII DNA element[15,16]. This indicates that there might be a physical interaction between these components.

Only CDEIII (25 bp) is essential, and point mutations within CDEIII abolish centromere function[17,18]. CDEIII interacts with the multi-protein CBF3 complex[19], which contains four main proteins of 110, 64, 58 and 29 kDa in size. These proteins are encoded by the *NDC10* (also called *CTF14* or *CBF2*), *CEP3* (also called *CBF3B*), *CTF13* and *SKP1* genes, respectively[19–26]. All four proteins are essential for viability, and temperature-sensitive mutations in any one of them abolish the CDEIII-binding activity of the CBF3 complex. In addition, Ndc10–GFP has been found to localize not only at kinetochores, but also along the spindle, indicating that Ndc10 might also function at the spindles[1]. The Skp1 protein and its interaction partner Sgt1 are required for the assembly of the CBF3 complex through activation of Ctf13 (REFS 27,28). Skp1 and Sgt1 also seem to be components of the SCF UBIQUITIN-LIGASE COMPLEX[28–30], although the connection between kinetochore and SCF function is still unclear.

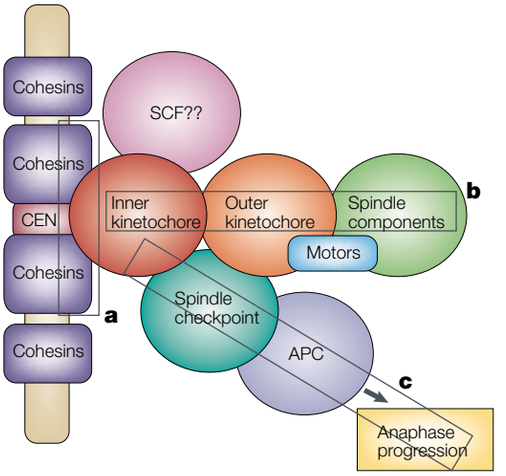

Figure 2 | **Kinetochore organization.** The kinetochore can be thought of as three sets of subcomponents. **a** | The chromosomal DNA–inner kinetochore protein interface. Further details of this interface are shown in FIG. 3. **b** | The inner kinetochore–mitotic spindle interface. **c** | The kinetochore protein–cell cycle machinery interface. APC, anaphase-promoting complex; CEN, centromeric DNA; SCF, SCF ubiquitin-ligase complex.

Box 2 | Kinetochores and cohesion

In eukaryotes, sister chromatids remain connected to one another from S phase until the onset of anaphase. This cohesion is essential for the separation of sister chromatids to opposite poles of the cell at the correct time during mitosis, and it also allows chromosome segregation to take place long after duplication is complete.

A multi-subunit complex called cohesin is essential for connecting the sister chromatids. In budding yeast, the cohesin complex consists of Smc1, Smc3, Scc1 (also known as Mcd1) and Scc3 during mitosis (although Rec8 replaces Scc1 in cells undergoing meiosis). All of these proteins are highly conserved between yeast and humans[110,111]. Several reports show that the protease Esp1 cleaves Scc1 to induce sister-chromatid segregation, indicating that cleavage of cohesin might control sister-chromatid separation (reviewed in REFS 110,111).

Two independent groups have detected the association of Scc1 with centromeres and with discrete sites along chromosome arms. Tanaka and colleagues[112] showed that the association of Scc1 with a centromere depends on Mif2, Ndc10 and Cse4 (but note that Scc1 is not required to maintain the association of Ndc10 with a centromere). Megee and colleagues[113] showed that an ectopically placed centromere directs the binding of Scc1 to 2-kilobase (kb) regions that flank the centromere in a sequence-independent manner. Therefore, the centromere is a *cis*-acting cohesion factor, which is essential for the maintenance as well as the establishment of this cohesion domain. However, the cohesin complex cannot resist the consequent force that leads to sister-centromere splitting and chromosome stretching.

Several reports have shown that the function of cohesin is conserved among eukaryotes; however, in vertebrates, the bulk of cohesin dissociates from chromosome arms during prophase, perhaps as a result of chromosome condensation (note that there is no such condensation in yeast). A small amount of cohesin remains on chromosomes, predominantly around centromeres (reviewed in REF. 110). However, despite the interesting relationship between the kinetochore and cohesin, the molecular link between these complexes has not yet been defined.

Human centromeres. The human kinetochore is the trilaminar proteinaceous structure that interfaces with chromosomes at highly repeated alphoid DNA (171-bp) arrays of 2–4 Mb[31] (see also BOX 3). Several years ago, two independent groups, Harrington *et al.*[32] and Ikeno *et al.*[33], succeeded in reconstituting a functional human centromere from cloned alphoid DNA. This breakthrough is analogous to the classic functional analyses used in the identification of budding yeast centromeric DNA[9–11]. However, in human cells, the established mini-chromosomes became larger than the introduced alphoid DNA. By using FISH with chromosome paint or satellite probes, Ikeno and colleagues[33] suggested that the established mini-chromosomes had not acquired large amounts of non-alphoid DNA sequences from endogenous chromosomes. Nevertheless, the possibility still remains that non-alphoid DNA might be present and could be required for centromere formation.

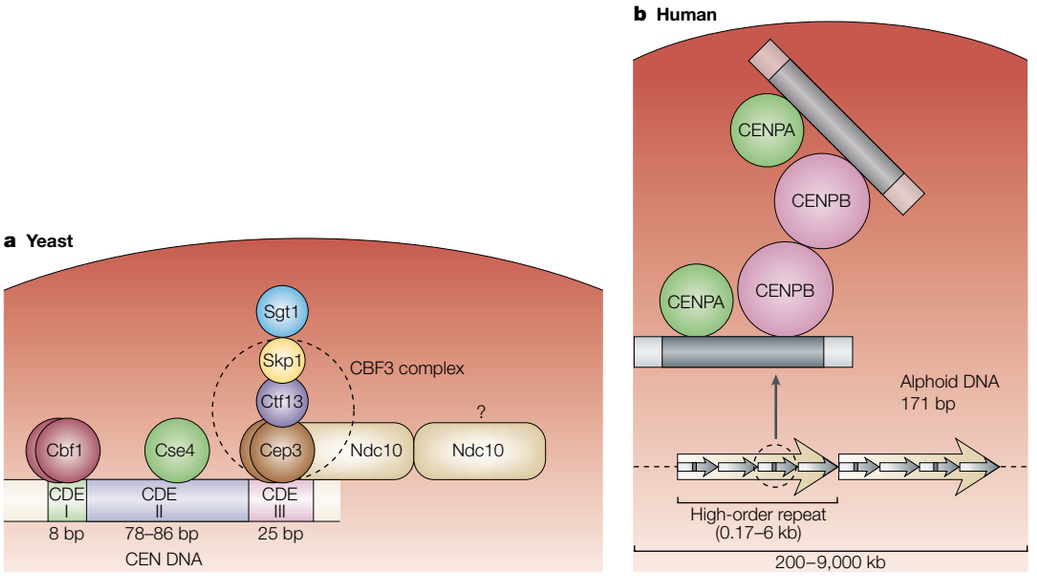

Figure 3 | **The DNA–inner kinetochore protein interface. a** | Yeast. The 125-base-pair (bp) centromeric (CEN) sequence contains three conserved elements. The first is the 8-bp CDEI sequence. This is bound by a protein called Cbf1, which mediates transcriptional regulation and chromosome segregation. The 78–86-bp CDEII sequence is potentially bound by a histone-H3 variant, Cse4. The 25-bp CDEIII sequence is bound by the centromere-binding factor 3 (CBF3) complex, which consists of four proteins: Ndc10, Cep3, Ctf13 and Skp1. **b** | Humans. The human CEN sequence may be bound by the histone-H3 variant CENPA, which is structurally and biochemically similar to yeast Cse4, in addition to the CENPB dimer. The function of CENPB is not yet known, as knockout mice are viable. CENA, centromere protein A; CENB, centromere protein B.

Historically, human centromeres have been located using antibodies (from patients with autoimmune disease) against the centromere-protein (CENP) antigens, which are human kinetochore proteins. CENPA and CENPB (FIG. 3b) are thought to have structural roles in kinetochore formation. CENPA is a histone-H3 variant, and experiments both *in vivo* and *in vitro* indicate that it is probably a centromeric nucleosome component[34–36]. CENPB binds to alphoid DNA and forms a dimer[36–38]; however, the function of CENPB at kinetochores is still obscure because knockout mice are viable and seem to maintain normal centromeres[39–41].

Humans versus yeast. The Cse4 and CENPA proteins are structurally and biochemically similar[15,16,42], and have similar roles in kinetochore function in budding yeast and humans. However, when Stoler and co-workers[15] attempted to rescue either temperature-sensitive or null alleles of *cse4* by high expression of *CENPA*, they were unsuccessful. In addition, transient-expression experiments in mammalian cell lines show that, although an epitope-tagged version of Cse4 (Cse4–haemagglutinin) is transported into the mammalian nucleus, it does not localize to the centromere (REF. 15 and K. F. Sullivan, unpublished data). It is unclear whether these negative results indicate that the two proteins are not orthologues (functionally identical) in their respective organisms. We believe it more likely that they represent true orthologues, but that they cannot complement 'cross-species', owing to the evolution of key protein-interaction surfaces over a long period of time (800–1,000 million years).

Human homologues for various components of the yeast CBF3 complex — Ndc10, Cep3 and Ctf13 — have not been found so far in the public databases. However, both Skp1 and Sgt1 are functionally conserved between budding yeast and humans[28,43]. Human SKP1 can complement the yeast *skp1* temperature-sensitive allele, *skp1-11*, but not the *skp1* null mutant[43] (K.K. *et al.*, unpublished data). However, human SGT1 can rescue the yeast *sgt1* null mutant, indicating that the function of *SGT1* is probably highly conserved[28]. Although it is not yet known whether the human homologues of Skp1 and Sgt1 have a role in kinetochore function, ten Hoopen and colleagues[44] reported that a specific antibody against a barley homologue of yeast Skp1 strongly labelled the centromeres of barley and field-bean metaphase chromosomes.

At the chromosomal DNA–inner kinetochore protein interface (FIG. 2a), we conclude that the *cis* elements (centromeric DNA) and 'direct' *trans* elements (the DNA-binding proteins) do not seem to be highly conserved between budding yeast and humans.

The inner kinetochore–mitotic spindle interface

As shown in FIG. 2b, there must be a protein network from the inner kinetochore to the mitotic spindle. The complete set of proteins that provide this linkage has not been established yet in either humans or budding yeast.

Yeast cells. In budding yeast, *in vivo* crosslinking chromatin-immunoprecipitation (CHIP) methods have recently revealed several new kinetochore proteins that

CHIP
(*in vivo* crosslinking chromatin-immunoprecipitation methods). After live cells are chemically crosslinked, extracted and mechanically sheared, chromatin fragments (crosslinked DNA–protein complexes) are immunoprecipitated.

Box 3 | **Epigenetic effects on kinetochore assembly**

In budding yeast, kinetochores assemble on centromeric DNA only. However, in rare cases in humans and the fruitfly *Drosophila melanogaster*, kinetochores can assemble at positions that lack centromeric DNA. This position is called the 'neocentromere' (reviewed in REF. 114). In humans, du Sart and colleagues[115] analysed the neocentromere in a marker chromosome by restriction and polymerase-chain-reaction mapping, and showed that the neocentromere has the same structure as DNA derived from normal chromosomes. So, a human kinetochore can assemble on DNA that is not normally centromeric.

Drosophila centromeres (420 kb) consist of non-repetitive core sequences and several highly repetitive elements, both of which are necessary for kinetochore formation. Williams and colleagues[116] analysed several mini-chromosomes that lacked the 420-kb centromeric DNA sequence, but were still more stable than acentric chromosome fragments. DNA mini-chromosomes without centromeres seemed to acquire neocentromeres, indicating that functional *Drosophila* kinetochores can assemble on DNA that is not normally centromeric.

How is kinetochore assembly controlled in the absence of a specific centromeric DNA sequence? One hypothesis is that epigenetic control is used. Although there is no direct molecular evidence to indicate that a specific epigenetic event is required for the formation of the neocentromere, there is some evidence in the fission yeast *Schizosaccharomyces pombe* for epigenetic control of the centromere.

The fission yeast centromere (40–100 kb) consists of a central core of 4–7 kb, surrounded by roughly 5 kb of repeated DNA elements. This arrangement is structurally similar to that of the fly centromere, although there is no sequence similarity between human, fly and fission yeast centromeres. Ekwall and colleagues[117] observed that the transient drug treatment that induces histone hyperacetylation induces a heritable hyperacetylated state in centromeric chromatin, and causes chromosome loss. This increased loss persists in daughter cells even after the drug is removed. Assembly of fully functional centromeres might, therefore, be partly imprinted in the underacetylated state of centromeric chromatin.

Centromeric repetitive sequences in these organisms might be needed only for the efficient establishment of kinetochores. In nature, it is rare that kinetochores get assembled on naked DNA. However, it might be important for us to understand the epigenetic mechanism to establish artificial chromosomes more efficiently in humans.

What about the budding yeast? Tanaka and colleagues[112] noted that Scc1, an essential cohesin protein (see BOX 2), associates with authentic centromeres, but not with recently activated centromeres when *ndc10-1*$^{-/-}$ cells are shifted to the restrictive temperature, or when centromeric DNA is mutated. Therefore, the chromatin structure that recruits Scc1 to centromeres might be epigenetically inherited.

associate with centromeric DNA specifically, although not necessarily directly. Meluh and Koshland[45] were the first to use the CHIP assay to show that Mif2 binds to centromeric DNA *in vivo*, possibly through CBF3 components. Hyland and colleagues[46] have used the CHIP assay to show that Ctf19 binds to centromeric DNA *in vivo*. Ortiz and colleagues[47] have also shown that Ctf19, Mcm21 and Okp1 bind to centromeric DNA *in vivo* using the CHIP assay, and that these proteins form a protein complex, which has been called the 'outer-kinetochore complex'. Each component has also been shown to associate with CBF3 components by the two-hybrid system and/or immunoprecipitation[47]. In addition, the Mtw1 protein has been shown to bind centromeric DNA in an Ndc10-dependent manner[1]. Goshima and Yanagida[1] showed that Mtw1 and the sequences that lie 1.8 and 3.8 kb from *CEN3* (the centromere of chromosome III) and *CEN15* (the centromere of chromosome XV), respectively, co-localize near the SPBs (the centromeres and the SPBs are distinguishable), and that Mtw1 co-localizes with Ndc10, except for the extra spindle localization of Ndc10.

Two more proteins — Slk19 and Plc1 — have also separately been shown[48,49] to bind to centromeric DNA *in vivo* by the CHIP assay. *SLK19* genetically interacts with *KAR3* and *CIN8* (both of which encode kinesin-related motor proteins), and a GFP-tagged Slk19 localizes to kinetochores[49]. Plc1 also physically binds Ndc10[48]. Plc1 is the yeast homologue of phospholipase C, although it is not known whether its phospholipase activity mediates kinetochore function[48]. Bir1, a 'baculovirus inhibitor of apoptosis repeat' (BIR MOTIF) protein, was isolated by the two-hybrid system as another protein that interacts with Ndc10, and subsequent genetic analyses of *bir1* mutants support a role in kinetochore function[50].

The recent determination of the close proximity of kinetochores and SPBs has raised a question as to whether some of the previously defined SPB proteins could, in fact, be kinetochore proteins or proteins that link both complexes. Two independent groups, Janke and colleagues[51], and Wigge and Kilmartin[52], have shown that Ndc80, Nuf2, Spc24 and Spc25, which were previously described as components of the SPB, bind to centromeric DNA *in vivo* by the CHIP assay, and function as kinetochore proteins. Very recently, He and colleagues[53] used live-cell imaging and the CHIP assay to show that Spc19 and Spc34 (SPB proteins), Cin8 (a molecular motor), and Dam1, Stu2 and Bik1 (microtubule-associated proteins) are kinetochore subunits.

Human cells. In human cells, CENPC is localized towards the outer centromere, in contrast to the inner-kinetochore localization of CENPB[54]. CENPC might have a role in the connection between centromeric chromatin and the kinetochore. Studies analysing the molecular components associated with centromeres on stable dicentric chromosomes showed that, whereas both active and inactive centromeres contained CENPB, only active centromeres contained CENPC[55,56]. Antibody-injection

BIR MOTIF
A motif found in the 'inhibition of apoptosis' (IAP) proteins. It is essential for interaction of the IAP proteins with pro-apoptotic proteins, including the caspase family of death proteases.

Table 1 | **Budding yeast kinetochore and spindle-checkpoint components**

***Saccharomyces cerevisiae* protein**	**Function/domain**	**Human protein (localization)**	**Homology**	**Conservation**
CBF3 complex				
Ndc10	CBF3 component	-	-	-
Cep3	CBF3 component, zinc-finger domain	-	-	-
Ctf13	CBF3 component, F-box protein	-	-	-
Skp1	CBF3 component, Ctf13 activation, SCF component	p19/SKP1 (centrosome)	3.3e-52	C, RN
CBF3 regulator				
Sgt1	Ctf13 activation, SCF component	SGT1	1e-21	R
Ipl1	Ndc10 kinase	IAK1 (mouse) (spindle pole)	3e-73	S (mouse)*
Glc7	Ndc10 phosphatase?	PP1 (PPP1cc)	1e-162	ND
Other inner kinetochore				
Cse4	Centromeric histone H3	CENPA (centromere)	5e-16	F, CN
Cbf1	CDEI-binding protein, MET-gene regulation	TFE3?	3e-05	ND
Outer kinetochore and others				
Mif2	Inner/outer kinetochore?	CENPC (centromere)	0.31	ND
Ctf19	Outer kinetochore	-	-	-
Mcm21	Outer kinetochore	-	-	-
Okp1	Outer kinetochore	CENPF? (centromere)	0.94	ND
Slk19	Outer kinetochore?	CENPF? (centromere)	3.5e-07	ND
Mtw1	Outer kinetochore?	-	-	-
Plc1	Phospholipase C	PLC-δ1	5.2e-57	R (soybean)
Ndc80	Outer kinetochore?	HEC1 (centromere)	2e-16	R
Nuf2	SPB?/Outer kinetochore?	HNUF2R	2.4	ND
Spc24	SPB?/Outer kinetochore?	-	-	-
Spc25	SPB?/Outer kinetochore?	-	-	-
Spc19	SPB?/Outer kinetochore?	-	-	-
Spc34	SPB?/Outer kinetochore?	-	-	-
Dam1	Microtubule-binding protein	-	-	-
Stu2	Microtubule-binding protein	Ch-TOG	2.6e-27	ND
Bik1	Microtubule-binding protein	CLIP170 (centromere)	1.5e-11	ND
Cin8	Kinesin-related protein	HKSP	1e-85	ND
Spindle checkpoint				
Bub1	Ser/Thr protein kinase	BUB1 (centromere)	1e-42	F
Bub3	WD-repeat protein	BUB3 (centromere)	4e-30	F
Mad1	Coiled–coil domain	MAD1 (centromere/ centrosome?)	0.0081	F
Mad2		MAD2L1‡ (centromere/ centrosome)	6e-39	F, C (*Caenorhabditis elegans*) CN (*Xenopus laevis*)
Mad3	Similar to Bub1, but not to kinase domain	BUBR1 (centromere)§	4e-21	F
Mps1	Ser/Thr protein kinase	PYT/TTK1 (centromere)	9.5e-62	F

* The species in parentheses indicates the result with the homologous protein. For example, C (*Caenorhabditis elegans*) means that the *C. elegans* homologue complements the yeast mutant.
‡ Overproduction of human Mad2 suppresses the thiabendazole sensitivity of *cpf1*-null mutants.
§ BUBR1 is the best hit for the BLAST search with Mad3; however, some people categorize BUBR1 as a Bub1 homologue, because the Bub1 kinase domain is conserved in BUBR1 but not in Mad3[63,118].
C, complementation of the lethality of the mutant; CBF, centromere-binding factor; CN, complementation was negative; F, function is conserved; ND, not done; R, rescue of the lethality of the null mutant; RN, rescue was negative; S, synthetic dosage lethality; SCF, Skp1/Cul1/F-box protein; SPB, spindle pole body.

experiments, analysis of conditional *CENPC* knockouts in chicken cells, and analysis of *CENPC*-knockout mice, have shown that CENPC is essential for mitotic chromosome segregation[57–59].

CENPF assembles onto kinetochores during late G2 and is seen on every chromosome by the onset of prophase[60,61]. During mitosis, CENPF is associated with the outer kinetochore plate and remains there through metaphase[60]. Overexpression of amino-terminally truncated mutant proteins in mammalian cells blocks cell-cycle progression mainly at the G2/M transition[62]. In addition, two-hybrid interaction data show that CENPF interacts with CENPE, a kinetochore-microtubule motor protein[63].

Three microtubule-dependent motors, CENPE, dynein and mitotic-centromere-associated kinesin (MCAK) have been specifically localized to mammalian centromeres[64–67]. Both CENPE and dynein co-localize

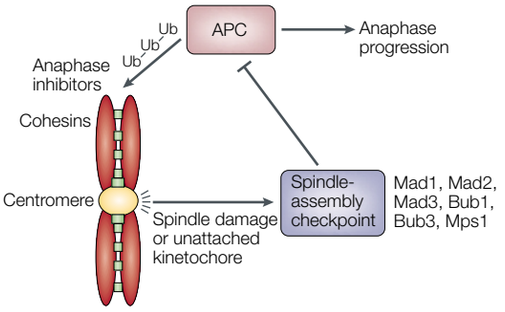

Figure 4 | **Kinetochore function and anaphase progression.** The spindle-assembly checkpoint delays mitosis in response to defective spindle organization or unattached kinetochores. This delay is brought about when components of the spindle-assembly checkpoint are recruited to the unattached kinetochores. These components include the Mad2 protein, which forms a tight complex with Mad1 and inhibits the anaphase-promoting complex (APC), thereby delaying mitosis. One way in which blocking the APC has this effect is by inhibition of the ubiquitin (Ub)-dependent degradation of securin. Destruction of this protein would normally allow cleavage of another protein called separase, which is involved in overcoming the cohesion between sister chromatids.

to the fibrous corona (outside the outer kinetochore)[66,68,69]. By contrast, MCAK partially co-localizes with these motors and extends throughout the centromere region[67,70]. Although extensive *in vitro* and *in vivo* experiments have investigated the motor activities of these three proteins, the precise identities and function of motor activities in mitotic chromosome movement is still unclear (reviewed in REF. 71). The 170 kDa-cytoplasmic linker protein (CLIP170), which is a microtubule-associated protein but not a member of any motor family[72], localizes at kinetochores, and has been shown to be important for proper chromosome congression during pro-metaphase[73].

Humans versus yeast. Is there any homology between the various proteins found at the human and yeast outer kinetochores? Two regions of yeast Mif2 that are essential for its function share homology with the two most highly conserved regions of human CENPC[74,75] (TABLE 1). Interestingly, in human cells, the herpes simplex virus immediate-early protein (Vmw110), a RING-FINGER protein, induces the proteasome-dependent degradation of CENPC, indicating that CENPC might be modified with SUMO-1 or a similar ubiquitin-like protein[76]. In *S. cerevisiae*, temperature-sensitive mutations[75] in *MIF2* can be suppressed by high-level expression of Smt3, the budding yeast homologue of SUMO-1. So, the modification of both proteins might be conserved in budding yeast and humans.

Ortiz and colleagues[47] proposed that four regions of Okp1 are homologous to CENPF. In fact, Slk19 shows higher homology to CENPF than Okp1p does (TABLE 1). It is unclear how comparable CENPF is to these kinetochore proteins of budding yeast on a functional level. Interestingly, Slk19, like Ndc10, is left behind at the spindle midzone when the spindle poles separate in anaphase B[49]. This case is similar to that of CENPF, CENPE, dynein and the inner-centromere proteins (INCENPs), which also associate with the spindle midzone and resulting MIDBODY in human cells[60,64,77,78]. These human proteins are called 'kinetochore passenger proteins' and, although their function in the midzone is unknown, it might be conserved in budding yeast and humans, because these homologous proteins show a similar localization.

Human survivin, a BIR-motif protein, is also a kinetochore-associated passenger protein[79]. Because other BIR-motif proteins act at discrete steps to regulate apoptotic pathways[80], it is possible that survivin links failure of mitotic checkpoint controls to apoptotic activation in human cells[79,81]. The Ndc80, Nuf2 and Bik1 proteins are conserved between budding yeast and humans. Recently[52], the human homologues of Ndc80 and Nuf2 were shown by immunofluorescence to localize to kinetochores of mitotic HeLa cells. In addition, the microtubule-binding region of CLIP170 is highly conserved in the yeast homologue, Bik1 (REF. 82).

We conclude, then, that the structure and function of the inner kinetochore–mitotic spindle interface is highly conserved between budding yeast and humans.

Kinetochore function and anaphase progression

The kinetochore has been linked to the spindle checkpoint (BOX 1), but is there any evidence that components of this checkpoint are associated with kinetochore function? And, if so, is there any conservation between the proteins that are involved in humans and the budding yeast proteins?

Yeast cells. In budding yeast, genetic screens for 'mitotic arrest defective' (*mad*) and 'budding uninhibited by benzimidazole' (*bub*) mutants originally identified six components of the spindle checkpoint[83,84] (FIG. 4). Mad1 and Mad2 form a very tight complex[85]; Mad2, Mad3, Bub3 and Cdc20 form a separate complex[86]. The *BUB1* gene encodes a protein kinase that binds to, and phosphorylates, Bub3 (REF. 87). The Mad1 protein shows a regulated association with Bub1 and Bub3 during the normal cell cycle, and this complex is found at considerably higher levels once the spindle checkpoint is activated[88]. In addition, *MPS1*, which encodes an essential protein kinase, also has a spindle-checkpoint function[89]. Mps1 can phosphorylate Mad1, and overexpression of Mps1 is enough to activate the checkpoint[90].

The kinetochore must somehow send a signal to the spindle checkpoint. Pangilinan and Spencer[91] have shown that *MAD2* and *BUB1* are required for the G2 delay that occurs when there is a problem with the kinetochore, caused either by a *ctf13* mutation, or by the presence of a single-copy centromeric-DNA mutation. Interestingly, *ndc10-1* mutants do not arrest during the G2/M transition at the non-permissive temperature[21], even though another allele, *ndc10-42* (*ctf14-42*), clearly causes a G2/M arrest[20]. Furthermore, NOCODAZOLE does not inhibit cell-cycle progression in *ndc10-1*$^{-/-}$ cells[92].

RING-FINGER
A cysteine-rich zinc-binding domain, which is thought to be required for protein–protein interactions.

MIDBODY
Dense structure formed during cytokinesis at the cleavage furrow. It consists of remnants of spindle fibres and other amorphous material and disappears before cell division is completed.

NOCODAZOLE
A microtubule-depolymerizing drug.

Box 4 | **Relevance to cancer mechanism**

Cancer cells are known for their genetic instability, and ANEUPLOIDY can result when chromosome segregation occurs erroneously. It is thought that mutations leading to increased rates of chromosome mis-segregation in certain classes of cancer might be predisposing factors that accelerate the process of tumorigenesis. By this model, the centromere and its regulatory system, which is essential for genome stability, must have an important role in providing some measure of protection against the development of cancer.

Results from Bert Vogelstein's group[118] indicate that the spindle checkpoint has an important function in human cancer. One of the main mechanisms that contributes to the development of colon cancers is thought to be improper sister-chromosome segregation, which leads to aneuploidy or chromosome instability (a 'CIN tumour'). Two independent cell lines derived from CIN tumours have been shown[118] to carry mutations in the human homologue of *BUB1*. Functional analysis showed that these *BUBR1* mutations had dominant-negative effects: CIN cells that normally retain functional checkpoint controls were severely compromised for checkpoint function when expressing the dominant-negative allele. Complete loss of the mitotic checkpoint control results in embryonic lethality, presumably due to intolerably high rates of chromosome mis-segregation[119–122]. Recently, Michel and colleagues[123] reported that *Mad2*$^{+/-}$ mice develop lung tumours at high rates after long latency periods. These results indicate that all the genes in the detection pathway in FIG. 2b have the potential to be cancer-related targets. So, further studies of kinetochore–spindle-checkpoint biology in both budding yeast and humans could shed light on our understanding of cancer.

These observations indicate that Ndc10 might have an important function in spindle-checkpoint signalling. Recently, Gardner and colleagues[93] showed that the spindle checkpoint is destroyed when the CBF3 components Cep3 or Ndc10 are eliminated from cells using the degron system (which generates a conditional-null mutation by specific proteolysis), indicating that the CBF3 complex is both monitored by the spindle checkpoint and required for checkpoint signalling.

Mammalian cells. The mammalian kinetochore has been proposed as a likely candidate for the site at which the signal is generated that is sensed by the spindle-checkpoint pathway when chromosomes are not attached to the spindle. Laser ablation of both kinetochores of the last unattached chromosome in PtK1 cells (a marsupial cell line) showed that at least one part of the spindle checkpoint is located at the kinetochore, and that the checkpoint is activated by the presence of unattached kinetochores[94].

Vertebrates versus yeast. All of the mitotic checkpoint proteins described above are highly conserved between budding yeast and humans (TABLE 1). Vertebrate homologues of Mad1, Mad2, Mad3, Bub1 and Bub3 bind to all kinetochores in cells that have been arrested in mitosis by microtubule-polymerization inhibitors, and specifically localize to microtubule-free kinetochores during spindle assembly in normal cells. Furthermore, the Mps1, Mad1, Mad2, Mad3, Bub1 and Bub3 homologues have been shown to have a role in the spindle checkpoint[63,95–103]. In *Xenopus laevis,* Mps1 recruits CENPE and Mad1/Mad2 to kinetochores[102], and Mad1 recruits Mad2 to kinetochores[97]. Bub3 might recruit both Bub1 and Mad3 to kinetochores in humans[101]. However, although the fission yeast (*Schizosaccharomyces pombe*) homologue of Bub1 has been shown to localize to kinetochores[104], it has not been shown that any component of the budding yeast checkpoint is localized to kinetochores.

A physical link between spindle-checkpoint proteins and a kinetochore-bound motor protein has been found[63]. CENPE, a kinetochore kinesin motor, was found to interact with BUBR1 (a human homologue of Bub1), in a two-hybrid test and by co-immunoprecipitation. Using *Xenopus* egg extracts, CENPE was shown to be necessary for establishing and maintaining the spindle checkpoint *in vitro*[105].

The combination of the vertebrate and budding yeast results suggest a plausible pathway for the spindle checkpoint — microtubule-free kinetochores recruit the Bub and Mad checkpoint proteins which, in turn, inhibit the anaphase-promoting complex (APC), thereby preventing sister chromatids from separating (FIG. 4). We conclude that the signalling pathway is highly and functionally conserved between budding yeast and humans.

Conclusions and future prospects

We have described our present knowledge of how the kinetochore is conserved between budding yeast and humans by discussing individual kinetochore components. Although this field is at an early stage in fully understanding the molecular mechanisms of kinetochore function, we believe that the evidence indicates that the kinetochore and its regulating system are highly conserved between budding yeast and humans. Of course, there are probably human or mammalian-specific activities at the kinetochore — methylation, for example, or the relevance of kinetochore studies and the spindle checkpoint to cancer (BOX 4). However, we believe that research into the budding yeast will continue to reveal further conserved functions at the kinetochore.

We know very little about the regulation of kinetochore activity during cell-cycle progression. What, for example, controls the timing of assembly of the kinetochore proteins? We know that unattached or damaged kinetochores send a signal to the mitotic checkpoint to stop the cell cycle, but what is the molecular mechanism of the signalling from kinetochores to the checkpoint? As described above, the molecular linkage of individual proteins within the supramolecular complexes that define kinetochores are not yet known. In

ANEUPLOIDY
One or more chromosomes of a normal set of chromosomes are missing, or present in more than their usual number of copies.

addition, there is the question of how kinetochores link to microtubules. There might also be unknown activities at kinetochores.

Budding yeast is a good test-system for developing genome-wide technologies. Systematic protein–protein interaction analyses using the two-hybrid technique or mass spectrometry could provide insight into all these unanswered questions. Eventually, after determining all the players, it would be exciting and powerful if we could reconstitute the kinetochore dynamic activity *in vitro*. In this way, definitive experiments could be done to associate directly specific biochemical activities of individual proteins and subcomplexes (through direct biochemical analyses *in vitro*) with specific cellular functions (using mutational and phenotypic analyses *in vivo*). We suspect that such definitive experiments will show that the basic strategies and molecular components that define kinetochore activity and regulation have been conserved throughout eukaryotic evolution.

Links

DATABASE LINKS **Cbf1 | Cse4 | NDC10 | CEP3 | CTF13 | SKP1 | Sgt1 | CENPA | CENPB | SKP1 | SGT1 | Mif2 | Ctf19 | Mcm21 | Mtw1 | CEN3 | CEN15 | Slk19 | Plc1 | KAR3 | *CIN8* | phospholipase C | Bir1 | Ndc80 | Nuf2 | Spc24 | Spc25 | Spc19 | Spc34 | Dam1 | Stu2 | Bik1 | CENPC | CENPF | CENPE | MCAK | CLIP170 | SUMO-1 | Smt3 | survivin | Mad1 | Mad2 | Mad3 | Bub3 | Cdc20 | BUB1 | *MPS1* | BUBR1 | Smc1 | Smc3 | Scc1 | Rec8 | Esp1**

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