Basic mechanism of eukaryotic chromosome segregation

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We now have firm evidence that the basic mechanism of chromosome segregation is similar among diverse eukaryotes as the same genes are employed. Even in prokaryotes, the very basic feature of chromosome segregation has similarities to that of eukaryotes. Many aspects of chromosome segregation are closely related to a cell cycle control that includes stage-specific protein modification and proteolysis. Destruction of mitotic cyclin and securin leads to mitotic exit and separase activation, respectively. Key players in chromosome segregation are SMC-containing cohesin and condensin, DNA topoisomerase II, APC/C ubiquitin ligase, securin–separase complex, aurora passengers, and kinetochore microtubule destabilizers or regulators. In addition, the formation of mitotic kinetochore and spindle apparatus is absolutely essential. The roles of principal players in basic chromosome segregation are discussed: most players have interphase as well as mitotic functions. A view on how the centromere/kinetochore is formed is described.

Keywords: sister chromatid separation; cohesion; separase; centromere; kinetochore; spindle

1. INTRODUCTION: A HISTORICAL PERSPECTIVE

This article is based on the author's concluding remarks at the discussion meeting on chromosome segregation, which was held at The Royal Society, 27–28 September 2004. Only a limited number of references could be included, and the author apologizes for not citing many important ones. Studies on eukaryotic chromosome segregation have been successful owing to proper selection of model organisms. Two eukaryotic microbes, the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, have proven to be excellent choices. These organisms, containing approximately 5000 genes, are evolutionarily quite separate, so that what was found to be true in both organisms has been generally also true in vertebrates. Many genes that are identified to be required for proper chromosome segregation in both *S. cerevisiae* and *S. pombe* are also present in the human genome. Studies on the chromosome behaviour of fly (e.g. Axton *et al.* 1990; Moore *et al.* 1998; Jager *et al.* 2001) and human mutants (e.g. Ohtsubo *et al.* 1989; Jallepalli *et al.* 2001) are consistent with the notions developed in yeast studies. We now have firm evidence that the basic mechanism of chromosome segregation is the same in eukaryotic cells. Even in prokaryotes, as discussed by Errington and Löwe (Errington *et al.* 2005; Leonard *et al.* 2005), the very basic feature of the chromosome segregation mechanism, such as the requirement of structural maintenance of chromosome (SMC) protein family (Hirano 2005; Huang *et al.* 2005), is similar to that of eukaryotes.

In budding yeast, a collection of *cdc* (cell division cycle) mutants pioneered by Hartwell and his associates (Pringle & Hartwell 1981) included those defective in chromosome segregation. Those *cdc* mutations displayed cell cycle defects and also aberrant chromosome segregation. Additional mutations that revealed chromosome instability (some called chromosome transmission fidelity (ctf) mutants; Spencer *et al.* 1990) were isolated (Hieter *et al.* 1985; Koshland *et al.* 1985). To analyse the mis-segregation behaviour in these mutants, the use of artificial circular minichromosome constructed by Clarke and Carbon (1980) was immensely helpful and also successful to identify the functional centromeric DNA sequence. The size of budding yeast centromere is exceptionally small, of the order of 100 base pairs containing three functional domains, but a large number of proteins are known to interact with the centromere for correct segregation (e.g. Westermann *et al.* 2003; De Wulf *et al.* 2003). Linear minichromosomes made and characterized by Murray and Szostak (1983) were attached to the telomeric sequences at their ends and opened a way to clone large pieces of human genome DNAs.

Three other approaches to identify budding yeast mutants distinctly defective in chromosome segregation are notable. One class showed an increased frequency of minichromosome nondisjunction (Larionov *et al.* 1985; Kouprina *et al.* 1988) that led to identification of *smc1* mutants (Strunnikov *et al.* 1993). Another is the isolation of spindle checkpoint mutants (Mad, Bub; Hoyt *et al.* 1991; Li & Murray 1991). These mutants are impaired in restraining mitotic progression in the absence of spindle function, suggesting the existence of a monitoring system for spindle function. Localization of the spindle checkpoint proteins at mitotic kinetochores (Chen *et al.* 1996) and the inactivation of APC/C complex by Mad2

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One contribution of 17 to a Discussion Meeting Issue 'Chromosome segregation'.

Figure 1. Fission yeast proteins required for chromosome segregation. See text for explanation. Most of them are conserved in higher eukaryotes. Gene products are grouped by different roles in chromosome segregation. Ub, ubiquitin; MT, microtubule; M-cyclin, mitotic cyclin.

(Kim *et al.* 1998; Hwang *et al.* 1998) explained, at least partly, the role of spindle checkpoint proteins in mitotic progression. But further experiments, such as discussed by Musacchio (DeAntoni *et al.* 2005), are necessary to fully understand how checkpoint proteins can arrest the mitotic progression. The other mutants are cohesion-defective *scc1* and *mcd1* mutants (Michaelis *et al.* 1997; Guacci *et al.* 1997; Ciosk *et al.* 1998). For *scc* mutants, a piece of the genomic DNA was fluorescently marked (Straight *et al.* 1997) so that premature sister chromatid separation could be recognized. These cohesion mutants have provided the insight into the cohesion mechanism: how sister chromatid DNAs are duplicated and held together until separation. In this issue, Nasmyth, Hirano and Koshland (Nasmyth 2005; Hirano 2005; Huang *et al.* 2005) discuss the current knowledge and concepts on how these important SMC proteins work.

In the fission yeast *S. pombe*, detailed mitotic nuclear and chromosome structures observed by DAPI (a fluorescent probe for DNA; Yanagida *et al.* 1983, 1985) staining were used as criteria for isolating temperature-sensitive mitotic mutants (Toda *et al.* 1981; Yanagida 1995, 1998; Hayashi *et al.* 2004). A large number of mutants were isolated and their gene products were identified. Such an approach succeeded

Figure 2. Three principal chromosome segregation defects in fission yeast mutants at the restrictive temperature. Arrest: β -tubulin *nda3* mutant; Cut phenotype: *top2* mutant; unequal segregation: *mis6* mutant. DNA is stained by DAPI.

to cover most of the essential genes now known to be required for chromosome segregation conserved in higher eukaryotes (figure 1). *Schizosaccharomyces pombe* mutants can be classified into three phenotypes: (i) the

Figure 3. Two-domain centromere structures in fission yeast. (a) Fission yeast has three chromosomes, and their centromeres (variable in length from 35 to 110 kb) are designated cen1, cen2 and cen3 (Takahashi *et al.* 1992). The central domain (cnt, imr) has approximately constant (approximately 15 kb) length (red line), while the outer region (blue) line is variable in length. The central domains have specialized chromatin, revealing the smeared micrococcal digestion patterns. The central sequences, if inserted into plasmid pYC310 or pYC306, no longer show the smeared patterns. The minichromosome Ch16 can be visualized in the mitotically arrested cells of *nda3* mutant. The central *cnt* and *imr* domain is associated with CENP-A, Mis6, Mis12 and Bub1, etc., while the outer *otr* domain contains HP-1/Swi6 and cohesin (see text). (b) The centromeric organization of fission yeast resembles that of human. The fission yeast central domain associates with CENP-A and Mis12 and can form the base for the protruded sister kinetochores. The outer repeats correspond to the heterochromatin that is enriched in cohesin Rad21. G. Goshima prepared this figure for his doctoral thesis.

arrest with condensed chromosomes (Hiraoka *et al.* 1984), (ii) the *cut* phenotype (cell untimely torn; Hirano *et al.* 1986), and (iii) the unequal chromosome mis-segregation (Takahashi *et al.* 1994; Hayashi *et al.* 2004). Examples of *arrest*, *cut* and *mis* mutant cells are shown in figure 2. Certain *cut* mutants are defective in

proteins directly implicated in chromosome segregation, such as those of *top2* defective in Top2/DNA topoisomerase II (Uemura *et al.* 1987), *cut1*/separase (Uzawa *et al.* 1990; Kumada *et al.* 1998), *cut2*/securin (Funabiki, Yamano *et al.* 1996; Funabiki, Kumada & Yanagida 1996), *cut3* and *cut14*/condensin SMC4 and

SMC2 subunits, respectively (Saka *et al.* 1994). Other *cut* mutants are implicated in spindle formation, anaphase promoting ubiquitin-dependent proteolysis and mitotic regulations, such as APC/cyclosome (*cut4*, *cut9*; Yamashita *et al.* 1996), spindle motor (*cut7*; Hagan & Yanagida 1990), 26S proteasome recruitment factor (*cut8*; Tatebe & Yanagida 2000), spindle pole body separation (*Cut12*; Bridge *et al.* 1998) and Bir1/survivin (*cut17*; Morishita *et al.* 2001).

The arrest phenotypes are often caused by the activation of spindle checkpoint in mutant cells, as the combination with the deletion of Mad2 abolished the arrest phenotypes. Spindle, kinetochore–microtubule linking, sister chromatid cohesion and protein dephosphorylation are defective in these arrest mutants (Hiraoka *et al.* 1984; Ohkura *et al.* 1989; Nakaseko *et al.* 2001; Toyoda *et al.* 2002). The *mis* mutants were initially isolated by the phenotype of minichromosome instability (Takahashi *et al.* 1994). Later, cytological screening was employed to isolate mutants that displayed the large and small daughter nuclei, such as *mis6* and *mis12* (Hayashi *et al.* 2004). These mutants are all defective in centromere/kinetochore functions: Mis6, Mis12, Mis13–Mis18 and Cnp1 proteins are all located at the central *cnt* and *imr* centromere regions (Takahashi *et al.* 1992, 2000; Saitoh *et al.* 1997; Goshima *et al.* 1999; Hayashi *et al.* 2004; Obuse *et al.* 2004).

Gene nomenclature in different organisms is a serious problem in communicating results, particularly outside of the field. Fortunately, many proteins required for chromosome segregation often form the complexes with other segregation proteins, such as APC/C, condensin and cohesin so that differences in individual subunit names may not be hugely problematic if appropriate names are given for all the supra-molecular complexes that will be identified in the future. Common names may be created in the future for the family of subunits of the complexes. Separase and securin are one example of an effort to unify the nomenclature. Securin/Cut2 in fission yeast, securin/Pds1 in budding yeast and securin/PTTG in humans shared little sequence similarity. But these proteins are bound to separase whose sequences are evolutionarily conserved (Funabiki, Kumada & Yanagida 1996; Yanagida 2000).

Mei-S332 and Sgo is another example (Orr-Weaver *et al.* 2005; Watanabe & Kitajima 2005). The long-standing fascinating *Drosophila* protein MEI-S332 that protects centromere cohesion during meiosis I is now understood in the broad context of all the eukaryotes through the discovery of Sgo proteins. One should be aware, however, that these named proteins might not be always perfectly appropriate. They might have additional, unexpected functions, and the names might not embrace the whole functions. Condensin has the interphase role such as DNA repair (Aono *et al.* 2002; Chen *et al.* 2004) and other functions (see Huang *et al.* 2005) besides mitotic chromosome condensation and segregation. A study on genetic interactions by the gene network method (Yuasa *et al.* 2004) showed that the fission yeast separase/*cut1*⁺ gene interacts with

approximately 15 other genes, some of which may be unrelated to chromosome segregation.

The centromere/kinetochore is fundamental for accurate chromosome segregation in eukaryotic cells. The fission yeast centromere defined by the stability test of minichromosomes constructed (Niwa *et al.* 1986) is very large in comparison with that of budding yeast, ranging from 35 to 110 kb (Nakaseko *et al.* 1986; Takahashi *et al.* 1992), as illustrated in figure 3a. It has two functionally distinct domains (essential central and outer heterochromatic repeats). Studies using the linear and circular minichromosomes show that the central domain and a small portion of heterochromatic outer repetitive regions are necessary for correct segregation (Niwa *et al.* 1989). While the central regions are bound to CENP-A, Mis6, Mis12 and Mad2 (Hayashi *et al.* 2004; see also Takahashi *et al.* 2005), the outer heterochromatic regions contain HP-1-like Swi6 and small inhibitory siRNA transcribed in the outer region (Hall *et al.* 2002; Volpe *et al.* 2002; see also Pidoux & Allshire 2005). Cohesin and aurora kinase B-like Ark1 kinase subunits are also present in the heterochromatic region of fission yeast centromeres (Tomonaga *et al.* 2000; Morishita *et al.* 2001). The centromeric heterochromatin sequences also exist in the mating type and telomeric regions (Grewal & Klar 1997; Pidoux & Allshire 2005). The centromeric protein–DNA architecture in fission yeast resembles, in many ways, that of human kinetochore (figure 3b), and will continue to serve as an excellent model.

2. SUPRA-MOLECULES REQUIRED FOR CHROMOSOME SEGREGATION

In figure 4, principal proteins (and complexes) required for chromosome segregation are arranged along the cell cycle stages where they are functionally implicated. The reason why centromere formation is placed at the earliest stage of the cell cycle (G1/S or before) is that fission yeast centromere/kinetochore protein mutants exhibit the requirement of the passage of G1/S, or previous mitosis at 36 °C, to produce the defective phenotypes (Saitoh *et al.* 1997; Goshima *et al.* 1999; Hayashi *et al.* 2004). When the centromere is established once at G1/S, or in the previous late mitosis at 26 °C, mitotic kinetochore functions seem to be normal in subsequent mitosis in cells transferred to 36 °C. Cohesin that consists of two SMC and two non-SMC subunits is loaded onto chromatin during the S phase, and this step requires Scc2/Mis4/Adherin (Ciosk *et al.* 2000; Tomonaga *et al.* 2000). Cohesin is proposed to form a large ring embracing sister chromatids duplicated during the S phase (Haering *et al.* 2002; Nasmyth 2005). The model is attractive as it explains the dual roles of cohesin in the S and M phases, and predicts the positive role for the removal of the cohesin from chromosomes by separase upon the onset of anaphase. Biochemical evidence for the big ring model is still scarce (Hirano 2005), and other models are proposed (Huang *et al.* 2005; Nasmyth 2005). In retrospect, the glue hypothesis (Holloway *et al.* 1993) predicted a protein like securin to hold sister chromatids, but

the actual 'glue' was found to be cohesin, which was the target of separase protease tightly bound to securin.

Condensin is required for both mitotic condensation and segregation of chromosomes such as DNA topoisomerase II in fission yeast (Top2; Uemura *et al.* 1987). Direct evidence that condensin was required for mitotic chromosome condensation was obtained by FISH (fluorescence *in situ* hybridization), which was introduced in fission yeast (Uzawa & Yanagida 1992). A cocktail of cosmid probes could reveal the rod-like arm in interphase, which became the compacted ball in metaphase, but the non-condensed arm-like FISH images remained in condensin mutant cells during metaphase (Saka *et al.* 1994). The mitotic role of condensin is obvious by its activation in frog extracts (Hirano 2005) and also by its mobilization to chromatin through Cdc2 phosphorylation in fission yeast (Sutani *et al.* 1999). The direct interaction between condensin (or cohesin) and Top2 has not been reported. Condensin contains two SMC and three non-SMC subunits. Single molecule analysis of condensin reveals an intriguing difference between ATP-dependent and ATP-independent activities with DNA (Hirano 2005). Top2 may be implicated in the compaction of interphase chromatin as well as mitotic chromosome: the nuclear chromatin in interphase is collapsed in the double mutant *top2* and *top1* (DNA topoisomerase I; Uemura & Yanagida 1984).

Aurora B, survivin/Bir1, INCENP and Borealin/Dasra, called 'passenger proteins', are essential for mitotic progression and control the interaction between spindle microtubules and chromosomes (Gassmann *et al.* 2004; Sampath *et al.* 2004). Aurora kinase is abundant in the pericentric regions where cohesin and Swi6/HP1 are also enriched. APC/C containing approximately 15 subunits is the E3 ubiquitin ligase that polyubiquitinates mitotic cyclin and securin for destruction in the destruction-box dependent manner. APC/C is the target of the spindle checkpoint protein Mad2 that inhibits APC/cyclosome through the tight association with the APC/cyclosome activator. Polyubiquitinated cyclin and securin are rapidly destroyed by 26S proteasome. The destruction of securin leads to the activation of separase.

How are kinetochore microtubules shortened in anaphase? Two articles in this issue (Mitchison 2005; Salmon *et al.* 2005) discuss dynamism of kinetochore-microtubule interactions and spindle microtubule flux. The poleward flux requires bipolar organization and Eg5 (BimC-like kinesin). As flux is important for transporting kinetochore-associated ends towards the pole, Eg5 may generate the poleward force on metaphase chromosomes. Another article (Tanaka 2005) discusses how bioriented sister kinetochores are established before anaphase in budding yeast. Mal-oriented and Miss-attached kinetochores are the major source of aneuploidy. It seems that correction mechanisms exist during metaphase and even in anaphase. Proteins like Ipl1/Aurora B, cohesin and Ndc80/Nuf2, a kinetochore protein, may be implicated in the correction mechanisms. A centromere protein, Mis6/CENP-I, seems to be also required

for establishing correct biorientation of sister centromeres in metaphase cells and this ability is originated during the passage of G1/S (Saitoh *et al.* 1997).

Kinesin-like MCAK and KCM1 (a Kin-I) is a microtubule depolymerization factor, and thought to be important for anaphase kinetochore microtubule shortening in higher organisms (Moore & Wordeman 2004; Noetzel *et al.* 2005). However, MCAK is not needed for the flux (see Mitchison 2005). A group of kinesins (budding yeast Kip3, *S. pombe* Klp5/6, fly Klp67A and human KIF18) may also be implicated in anaphase microtubules shortening (e.g. West *et al.* 2001; Savoian *et al.* 2004). The other microtubule-interacting proteins, frog XMAP215, human Tog, fission yeast Dis1 and Alp14/Mtc1 and budding yeast Stu2, are also implicated in the shortening of kinetochore microtubules in anaphase. Their functions may be overlapped (Cassimeris & Morabito 2004; Gard *et al.* 2004; Holmfeldt *et al.* 2004). Their roles in anaphase remain to be understood, however. The fission yeast Dis1 is a kinetochore protein in metaphase (Nakaseko *et al.* 2001).

It is evident that there are many steps to prepare chromosomes suitable for the proper occurrence of sister chromatid separation in anaphase. The term 'chromosome cooking' for these steps that culminate at the moment of anaphase (comparable to eating) is a good one, particularly because the period of anaphase is so short in contrast to hours of careful preparation. Another revealing analogy may be that mitotic events are like the festival for cells as it happens once in the cell cycle. Conspicuous cellular objects such as condensed chromosomes and the spindle appear briefly to commence the steps toward the fete of division. Note, like actual festival participants, mitotic molecular participants also have their own tasks in non-festive interphase and will activate their mitotic roles upon the entry into mitosis by incentive modifications.

3. BASIC SEGREGATION MECHANISM VERSUS QUALITY CONTROL

The physical principle for accurate DNA replication is based on the double helical DNA structure with complementary base pairing. DNA polymerases are instrumental for replication, and many other proteins, such as repair enzymes, ensure the high fidelity of replication. No single physical principle is known, however, to keep the error of chromosome segregation to a minimum. One view is that incremental contributions by many gene functions are summed up to the high fidelity observed in chromosome segregation (a loss or non-disjunction of chromosomes per 10^4 divisions; see also Salmon *et al.* (2005) for discussion on the frequency of missegregation in higher organisms). In yeast, approximately 500 genes (approximately 10% of the whole genome) may directly or indirectly affect the fidelity of chromosome segregation. Another view is that the basic segregation mechanism may be simple, made up of a relatively small number of genes, but there are a large number of genes implicated in the improvement of segregation fidelity. Such quality control genes for segregation may

Figure 4. Chromosome dynamics in cell cycle that leads to sister chromatid separation in anaphase. Interphase centromere is altered, upon the entry of mitosis into the kinetochore structure, which can associate with kinetochore microtubules. Sister chromatid cohesion is made during the S phase and maintained in the G2/M until anaphase. Two protein complexes, cohesin and condensin, which both contain structural maintenance of chromosome (SMC) and non-SMC subunits, are required for sister chromatid cohesion and mitotic chromosome condensation. Resulting condensed chromosomes interact with the spindle in prophase and prometaphase under the control of Aurora-passenger proteins and Bub, Mad spindle checkpoint proteins. The metaphase chromosomes are thus bi-oriented and their sister kinetochores are end-on connected with kinetochore microtubules. Chromosomes are congressed in the middle of the spindle prior to segregation. The onset of anaphase A is thought to be triggered by activation of APC/C, resulting in ubiquitin-mediated destruction of mitotic cyclin and securin by 26S proteasome. Exit of mitosis is accomplished by mitotic cyclin destruction, while sister chromatid separation requires the destruction of securin, followed by the activation of separase that can cleave the Scc1/Rad21 subunit of cohesin. Classes of proteins MCAK/KinI and Tog/Dis1/Stu2 are implicated in destabilization of kinetochore microtubules (e.g. microtubule shortening) in anaphase. The kinetochore microtubules are shortened in anaphase A and the pole-to-pole microtubules are fully elongated in anaphase B. The late anaphase and telophase events include the disassembly of the spindle, chromosome de-condensation, nuclear envelope reformation and the coordination with cytokinesis.

be implicated in cell cycle and checkpoint control, ubiquitination, phosphorylation, proteolysis, centromere/kinetochore and spindle function, as depicted in figure 5. These genes may be essential or dispensable for cell viability. Dispensable gene functions may serve to improve the quality control in segregation, and could become the cause of diseases. Even certain missense mutations in the essential genes such as Scc2/Mis4/Nipped-B/NIPBL/Adherin facilitating cohesion cause the genetically inherited disease Cornelia de Lange syndrome, which displays growth and learning deficiencies (Dorsett 2004).

The basic segregation mechanism may be low in fidelity so that many additional quality control mechanisms are developed. Without such fidelity control, aneuploidy and chromosome loss may frequently take place. Although the spindle (including poles) and centromere/kinetochore structures consist of a large number of proteins even in fungi, future work will clarify the minimal essential components for the spindle and kinetochores. In the author's opinion, other basic players for eukaryotic chromosome segregation are cohesin, condensin, Top2, aurora passenger complex, spindle motor, kinetochore microtubule

Figure 5. The basic chromosome segregation mechanism is ensured by quality control mechanisms.

destabilizers, APC/cyclosome and securin-separase complexes (figure 6).

Bacterial systems offer us a view that evolutionarily conserved proteins are required for chromosome segregation in bacterial cells. Centromere-like DNA sequences have been found in bacterial systems

Figure 6. Principal players of chromosome segregation.

(see Errington *et al.* 2005; Leonard *et al.* 2005). Proteins called SMC and kleisins are found in bacterial cells as well as eukaryotic cells, and are essential for chromosome segregation, as discussed many times in this issue. Actin-like protein forms the skeletal structure and appears to be implicated directly or indirectly in the partition of bacterial chromosome DNAs. Although bacterial cells do not have the mitotic spindle, they contain a tubulin-like protein, FtsZ, which forms the medial ring structure and promotes cell division. It is clear that the SMC–kleisin complex, tubulin and actin are ancient proteins and play the major roles in chromosome segregation and cell division in all organisms on Earth.

4. HOW MUCH FORCE IS NEEDED FOR PULLING EUKARYOTIC CHROMOSOMES?

Force is needed to pull chromosomes towards the opposite spindle poles during segregation. It is unclear how much force is exactly operated for pulling a chromosome, but it is probably in the range of 50 pN per kinetochore microtubule from the legendary experiments using grasshopper spermatocytes (Nicklas 1983; also see Rieder *et al.* 1986). Motors and/or kinetochore microtubule depolymerizing factors may generate the spindle force. The average mass of single *S. pombe* chromosomes is roughly 30 fg, equivalent to that of 50 T_{even} bacteriophage or 10 000 ribosome particles. The mass of an average human chromosome is 1 pg, approximately 30 times more than that of fission yeast chromosome. The 2–3 and 20–30 kinetochore microtubules, respectively, are reported to be associated with each sister kinetochore of *S. pombe* and mammalian chromosomes. The mass of chromosome per single kinetochore microtubule may be around 15–50 fg, and not greatly different between human and *S. pombe*.

What are the central issues for generating the force to bring separated sister chromatids towards the poles in anaphase? Three articles (Mitchison 2005; Noetzel *et al.* 2005; Salmon *et al.* 2005) discuss them. The pulling and/or pushing forces (alternatively called polar wind or polar ejection) of the spindle are generated in prometaphase to metaphase by dynamic properties of microtubules and/or motors. In anaphase A, kinetochore microtubules move towards the poles. Microtubule dynamics occur in spontaneous and also regulated fashion. For this question, one should be

Figure 7. A hypothesis to explain how condensin and cohesin affect the organization of centromere/kinetochore (see text for explanation).

aware that budding yeast anaphase is exceptional (Winey *et al.* 1995; Goshima & Yanagida 2000): anaphase A (the shortening between kinetochores and poles) takes place after anaphase B (elongation of the distance between poles).

Our principal concern is placed on the regulated behaviour of spindle and kinetochore microtubules. Do these properties abruptly change in anaphase? Perhaps they do, but our knowledge is still meagre. Microtubule- and kinetochore-interacting proteins that critically regulate the dynamic properties of kinetochore and spindle microtubules for shortening in the metaphase–anaphase progression have not been established. *In vitro* and *in vivo* studies have suggested that MCAK and KCM1 (a Kin-I) and XMAP215 are implicated as an accelerator or inhibitor, however (Kinoshita *et al.* 2002; Ohi *et al.* 2003). One should carefully investigate certain classes of kinesin motor that interact with kinetochore microtubules. In anaphase A, only kinetochore microtubules are shortened, while the pole to pole microtubules interdigitated at the middle of the spindle begin to elongate in anaphase B. In addition to these two classes of microtubules, the aster microtubules radiating from the spindle poles and their plus ends associating with the cellular cortex determine the positions of the spindle apparatus within the cell and may play an important role in chromosome segregation. Nicklas (1983) showed that the spindle apparatus and kinetochore microtubules are powerful enough to generate the 10^4 times force required for free movements of the chromosome. The force for the anaphase spindle in the absence of the opposing force generated by sister chromatid cohesion is thus not necessarily large.

The rate of chromosome movement during anaphase A in *S. pombe* and HeLa is approximately $1\text{--}3\ \mu\text{m min}^{-1}$ ($16\text{--}50\ \text{nm s}^{-1}$). In *S. pombe* the path of chromosome movement is approximately $1\ \mu\text{m}$ so that anaphase A is completed within 1 min. In HeLa, anaphase chromosomes move approximately $5\ \mu\text{m}$ within 2 min. Thus the duration of anaphase A is rather brief. Anaphase A movement of chromosomes in living yeast and HeLa cells is smooth and steady. Note, however, that kinetochore microtubule shortening seems to be slower than the maximum shortening

speed of cytoplasmic or isolated microtubules; owing to dynamic instability (Mitchison & Kirschner 1984) it is 10 times faster ($30 \mu\text{m min}^{-1}$). Salmon *et al.* (2005) discusses correction mechanisms for the merotelic kinetochore attachment in anaphase. The merotelic attachment can support biorientation and alignment near the metaphase plate so that it is not detected by spindle checkpoint. Because of its mal-attachment, lagging chromosomes are produced by the merotelic attachment. What are responsible for such correction mechanisms? Salmon *et al.* (2005) point out that the kinetocore protein complex containing Ndc80/Nuf2 may be implicated in the correction, but other kinetochore proteins may also be involved as the lagging chromosomes are also seen in HeLa cells after RNAi of CENP-A, hMis12 and other kinetochore proteins (e.g. Goshima *et al.* 2003; Obuse *et al.* 2004).

5. THE REMOVAL OF COHESIN IN MITOSIS AND INTERPHASE

The cohesin complex is loaded during the S phase and maintained in interphase cells. Upon the entry into mitosis, there are two ways to remove cohesin from the chromosome, one through phosphorylation by polo-like kinase and other through proteolytic cleavage by activated separase (Kimura *et al.* 1998; Uhlmann *et al.* 2000). In vertebrates, the bulk of cohesin is removed from chromosomes already in prophase and prometaphase by a mechanism that does not involve cohesin cleavage. Phosphorylation by polo-like kinase reduced the ability of cohesin to associate with chromosome (Sumara *et al.* 2002). While the bulk amount of Scc1 cohesin is cleaved during mitosis in budding yeast, only the residual level of Rad21/Scc1 is cleaved in fission yeast and human cells (Tomonaga *et al.* 2000; Gimenez-Abian *et al.* 2004). In human cells, the removal of residual cohesin in arms is sensitive to protease inhibitor.

A model proposed (Nasmyth *et al.* 2001; see also Nasmyth 2005) is that separase is a protease that triggers anaphase by abolishing the opposing force through the cleavage of cohesin that embraces sister chromatids by a large loop. Although cohesin is abundant in the pericentric region of budding yeast, the cleavage of cohesin may occur in the arms as the centromeres of budding yeast are known to be pre-separated in metaphase. Another possible explanation for the cohesin cleavage is that the residual cohesin in the metaphase chromosome of fission yeast or human cells may have to be removed by separase after metaphase, as even a minute amount of intact cohesin will become strongly inhibitory in anaphase. In this model, the opposing force in metaphase may be contributed by proteins other than those such as Top2, Sgo and unidentified ones. Alternatively, cohesin and these other proteins may contribute to the opposing force in metaphase. Variability in the degree of cohesin cleavage in different organisms may be explained by different contributions of cohesin to the opposing force.

The local removal of cohesin appears to occur in interphase upon DNA damage (Nagao *et al.* 2004).

A temperature-sensitive securin mutant, *cut2-EA2*, is sensitive to UV, X-ray and gamma-ray irradiation at 26 °C. The mutant protein fails to associate with separase so that separase becomes unstable and inactive. The expression of uncleavable cohesin subunit Rad21 in the damaged cells causes the failure to properly repair the damaged DNA. Radiation sensitivity of securin and separase mutants is hence probably due to the failure to cleave cohesin upon irradiation. In other words, proper repair of damaged DNA requires the cleavage of Rad21 by separase protease. Indeed, the cleaved fragment is not made in damaged *cut2-EA2* mutant cells. Consistent with a notion that cohesin cleavage is required for the repair, Rad21 is hyper-phosphorylated by Rad3 (ATR like) and Cds1 (Chk2) kinase and hyper-phosphorylated Rad21 may be cleaved by separase. The mechanism of securin destruction upon the damage is unknown, but a subpopulation of APC/C or other ubiquitin ligase might be a down stream target of Rad3. A mutation of Slp1, a fission yeast homologue of Cdc20, is radiation-sensitive (Matsumoto 1997). Separase thus executes its interphase role presumably by removing local cohesion by the cleavage of Rad21 for repair. Local sister chromatid separation is considered to be a correction mechanism in interphase for the damaged lesions of chromosomes, probably through recombination repair (Nagao *et al.* 2004).

6. CONSERVED KINETOCHORE SUBSTRUCTURES MADE ON DIVERSE CENTROMERE DNA SEQUENCES

Two articles describe different aspects of centromere structure and function (Pidoux & Allshire 2005; Takahashi *et al.* 2005). The centromere is linked to heterochromatin where gene expression is silenced owing to histone modifications. The outer centromeric repeats of fission yeast centromeres are underacetylated on histones H3 and H4, and methylated on lysine 9 of H3. The repressive gene expression is dependent on the RNA interference machinery (Hall *et al.* 2002; Volpe *et al.* 2002; Pidoux & Allshire 2005). For correct chromosome segregation in vertebrate cells, as well as in fission yeast, the RNAi system is necessary (Fukagawa *et al.* 2004). For loading of CENP-A, a centromere-specific histone H3, various proteins are needed (Takahashi *et al.* 2000; Pidoux *et al.* 2003; Chen *et al.* 2004; Hayashi *et al.* 2004). Mis16/RbAp46/48 and Mis18 are the upstream factors required for CENP-A loading and evolutionarily conserved proteins (Hayashi *et al.* 2004). Histone deacetylation in the central centromere regions requires the interaction with the complex between Mis16 and Mis18 in fission yeast. Mis16 is highly similar to human RbAp46/48, which is bound to retinoblastoma protein (RB). It is a nuclear protein enriched in the central centromeres, whereas Mis18 is conserved in vertebrates is a central centromere protein, presumably having the specificity directed towards centromeric chromatin. Ams2 is unique among those, as it is a transcription factor and is required for the periodic transcription of histones (Takahashi *et al.* 2005). Loading of CENP-A at the

boundary of G1/S and G2/M appears to occur in the deletion mutant of Ams2.

Centromere/kinetochore-interacting proteins are important as their defects often lead to incorrect chromosome segregation for various reasons. Centromere DNAs and a number of interacting proteins together make the specific centromere structure that becomes the basis of the kinetochore during mitosis so that kinetochore microtubules can interact with it. If the interactions of kinetochores with spindle microtubules are abnormal, chromosome segregation does not proceed normally, with the degree of abnormality depending on the type of deficiencies in the interactions. Spindle checkpoint proteins are associated briefly with kinetochores mainly in prophase and prometaphase. Recent studies have shown that many centromere-interacting proteins are evolutionarily conserved, whereas the centromeric DNA sequences are not. An example of conserved centromere proteins is CENP-A, which is present from fungi to vertebrates. How CENP-A is recruited to the centromere is a central question to understanding the centromere identity. Another question is how bioriented sister kinetochores are formed in metaphase. This is discussed by many authors in this issue, and in detail by Tanaka (2005) and Salmon *et al.* (2005). Many factors are clearly involved in establishing biorientation. In this regard, it should be realized that a number of centromere/kinetochore proteins so far identified are unclear in terms of their molecular functions. The whole consequences in the assembly of centromere/kinetochore that finally culminate in the bipolar attachments of kinetochore–microtubules are only poorly understood.

Centromere/kinetochore proteins can be classified into several groups, belonging to different substructures and executing their functions at different cell cycle stages. In fission yeast, proteins belonging to the CENP-A group are independent of the Mis12 group in their centromere localization (Hayashi *et al.* 2004; Obuse *et al.* 2004). In human cells, essentially the same relationships are obtained. These proteins probably form different substructures in the interphase centromeres and mitotic kinetochores. Consistent with the concept of kinetochore substructures, Mis12/Mtw1/hMis12 is now shown to be an evolutionarily conserved centromere substructure (De Wulf *et al.* 2003; Cheeseman *et al.* 2004; Obuse *et al.* 2004). Nine polypeptides are bound to human hMis12. Interestingly, human hMis12 complex contains additionally HP1- α and HP1- γ , a heterochromatin protein-1 similar to fission yeast Swi6, containing chromodomain that interacts with methylated histones. The unexpected connection of the hMis12 complex with HP-1 may reflect the existence for the centromere substructure bound to centromeric heterochromatin; hMis12 is present in the interphase centromeres. RNAi of HP1 therefore abolished kinetochore localization of hMis12 and DC8 (Obuse *et al.* 2004).

Certain proteins other than spindle checkpoint proteins are temporally bound to kinetochores. Condensin is highly enriched in the central centromeric region during mitosis in fission yeast (Sutani *et al.* 1999;

Aono *et al.* 2002). Cdc2 phosphorylation of Cut3/SMC4, a condensin subunit, promotes nuclear mobilization of condensin from cytoplasm upon the onset of prophase. To explain such centromeric association, the protruded loop-like formation may be proposed for the central regions (figure 7). Condensin is thought to interact with different regions of the same chromatid (see Hirano 2005). Such a model predicts the bioriented protrusion of the sister kinetochore at the central centromere during mitosis. If the model is correct, a vital step for the kinetochore formation in mitosis requires condensin. In condensin mutants, both condensation and segregation are defective while the checkpoint is not activated so that lethal mitotic progression takes place, resulting in the cut phenotype. A possible hypothesis for the failure to activate the spindle checkpoint in condensin mutants is that proper kinetochore structure essential for the association of spindle checkpoint protein is not made in condensin mutant cells during mitosis. In fission yeast, cohesin is enriched in the outer region (otr) of centromeres (Tomonaga *et al.* 2000; Watanabe & Kitajima 2005). Distinct localization of condensin and cohesin depicted in figure 7 explains the roles for intra-chromatid and inter-chromatid interaction, respectively, in the centromere. Condensin may act for pre-separation of sister kinetochore DNAs in metaphase, while cohesin holds peri-centromeric regions until the progression of metaphase to anaphase.

Mitotic condensation occurs with the compaction along the same chromatid. The condensin complex is known to have the interphase role in damaged DNA repair and intra-S phase checkpoint (Aono *et al.* 2002). Budding yeast condensin also has the interphase role (Huang *et al.* 2005). Condensin mutant *cnd2-1* was defective in DNA repair at 26 °C. In *cnd2-1* hypersensitive to hydroxyurea (HU) as well as UV irradiation, the activation of Cds1 (Chk2) kinase in the presence of hydroxyurea (HU) is impaired in condensin mutant cells. The condensin complex might be required for maintaining stalled replication fork. Consistently, the recent result in the author's laboratory (Y. Akai, unpublished results) suggests that condensin is required for maintaining the bubble structure in the presence of HU. Condensin might stabilize the loop-like structure formed at the bubble under the arrest of replication. Although the level of condensin in the interphase nucleus seems to be low, the actual function might be closely similar to that of mitotic condensin–chromosome condensation at the molecular level.

7. FUTURE PROSPECTS

Proper transmission of chromosomes is fundamental in life inheritance and the basis for reproduction of organisms. The failure of correct segregation causes various deficiencies. The relationship between chromosome instability and human cancer is especially important as genetic instability is a principal feature in cancer cells (Michor 2005). A main question is whether chromosomal instability is a cause and hence a driving force of tumorigenesis. Full identification of

human CIN (chromosome instability) genes is thus necessary. Integration of experimental and theoretical approaches is also needed for understanding the evolution of cancer. Finding that *Scc2/Mis4/Nipped-B/NIPBL/Adherin* is the gene for Cornelia de Lange syndrome is somewhat surprising, as the gene needed for facilitating cohesion causes a variety of symptoms in this human disease. But in future, many similar cases in which genes implicated in chromosome segregation cause diseases with complex properties in growth (also mental) retardation and abnormalities may be discovered. Understanding the mechanism to control ploidy will be an important subject for improving various useful plants, animals and microbes. Acquiring various mutations that alter ploidy and chromosome stability is of use to produce polyploidy or aneuploidy organisms. Principal properties of cultivated plants can be altered without DNA transformation.

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GLOSSARY

- Cdc: cell division cycle
 CIN: chromosome instability
 Ctf: chromosome transmission fidelity
 FISH: fluorescence in situ hybridization
 HU: hydroxyurea
 otr: outer region
 RB: retinoblastoma protein
 SMC: structural maintenance of chromosome