Front. Biol. 2014, 9(6): 472–480
DOI 10.1007/s11515-014-1334-y

REVIEW

Comparative analysis of chromosome segregation in human, yeasts and trypanosome

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Abstract **Chromosome segregation is a tightly regulated process through which duplicated genetic materials are equally partitioned into daughter cells. During the past decades, tremendous efforts have been made to understand the molecular mechanism of chromosome segregation using animals and yeasts as model systems. Recently, new insights into chromosome segregation have gradually emerged using trypanosome, an early branching parasitic protozoan, as a model organism. To uncover the unique aspects of chromosome segregation in trypanosome, which potentially could serve as new drug targets for anti-trypanosome chemotherapy, it is necessary to perform a comparative analysis of the chromosome segregation machinery between trypanosome and its human host. Here, we briefly review the current knowledge about chromosome segregation in human and *Trypanosoma brucei*, with a focus on the regulation of cohesin and securin degradation triggered by the activation of the anaphase promoting complex/cyclosome (APC/C). We also include yeasts in our comparative analysis since some of the original discoveries were made using budding and fission yeasts as the model organisms and, therefore, these could provide hints about the evolution of the machinery. We highlight both common and unique features in these model systems and also provide perspectives for future research in trypanosome.**

Keywords cohesin, separase, securin, anaphase promoting complex, spindle assembly checkpoint, *Trypanosoma brucei*

Introduction

To maintain genome stability during cell division, duplicated chromosomes must be faithfully segregated into daughter cells. Segregation of sister chromatids or anaphase onset is triggered by the activation of the APC/C through the spindle assembly checkpoint (SAC). SAC monitors the accuracy of kinetochore-microtubule attachment and delays anaphase onset by inhibiting APC/C activity. Once activated, APC/C degrades securin, an inhibitor of the protease separase, allowing cleavage of cohesin by separase and subsequent sister chromatid segregation. This pathway appears to be well conserved throughout eukaryotes, but distinctions do exist in different organisms, especially between the early diverged protozoa and humans. In this review, we first briefly summarize our current understanding of the mechanisms of chromosome segregation mediated by the SAC and APC/C pathways. We then provide a detailed comparison of the features and functions of individual regulatory proteins involved in chromosome segregation in human, yeasts, and trypanosome, a unicellular microbial eukaryote and a pathogen causing human sleeping sickness. By highlighting the distinctions in chromosome segregation between trypanosome and its human host, we hope to provide the rationale and potential targets for anti-trypanosome drug development.

Received July 2, 2014; accepted September 19, 2014
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Regulation of sister chromatid segregation in eukaryotes: a brief summary

Entry into mitosis in eukaryotes is known to depend on the activity of the mitotic cyclin-dependent kinase Cdk1, which is bound and activated by a mitotic cyclin. Abundance of the mitotic cyclin Cyclin B is tightly regulated by the equilibrium of protein synthesis and degradation during the cell cycle. During mitosis, a surveillance mechanism called spindle assembly checkpoint ensures that all kinetochores on the chromosomes are correctly attached to spindle microtubules before sister chromatids are segregated in anaphase. SAC

inhibits the activity of APC/C, which involves the mitotic checkpoint complex (MCC) consisting of Bub3, BubR1, Cdc20, and Mad2 in near equal stoichiometry (Sudakin et al., 2001). However, precisely how MCC components are activated by unattached kinetochores is still poorly understood. Nonetheless, it is well established that the activated Mad2, in cooperation with other components of the MCC, binds to Cdc20, thereby preventing APC/C activation. When there is no kinetochore-microtubule attachment error and SAC is satisfied, APC/C is activated, which ubiquitinates securin for degradation by the 26S proteasome. A protease called separase is inactivated by securin binding and Cdk1/cyclin B-mediated inhibitory phosphorylation. As a consequence of securin degradation, separase is released and becomes active, which subsequently cleaves the cohesin protein, releasing the sister chromatids for separation. Moreover, the activated APC/C also ubiquitinates cyclin B for degradation by the 26S proteasome, which is required for mitotic exit (Fig. 1).

Like in humans, sister chromatid cohesion in yeasts is also established during DNA replication, which depends on a multi-protein complex called cohesin that consists of Scc1, Scc3, Smc1, and Smc3. During the metaphase-to-anaphase transition in budding yeast, Scc1 is destroyed by Esp1, a homolog of human separase. Esp1 is tightly bound by the anaphase inhibitor Pds1, which is destroyed by APC/C and proteasome before anaphase onset (de Gramont and Cohen-Fix, 2005). Cleavage of Scc1 likely initiates the release of the Cdc14 phosphatase from the nucleolus (Shou et al., 1999), which dephosphorylates Cdk1 targets and is required for mitotic exit. It has been shown that Esp1 activates Cdc14, which in turn acts on Pds1. Cdc14 can remove an inhibitory phosphorylation on Pds1, thereby facilitating Pds1 degradation to release Esp1. In this system, a positive feedback loop is adopted to coordinate sister chromatid separation (Holt et al., 2008).

As an early branching protozoan, trypanosome has emerged as a model organism for understanding many fundamental cellular processes due to its genetic tractability. Trypanosome possesses many unique features of chromosome segregation (Li, 2012; Akiyoshi and Gull, 2013); however, our knowledge about the regulation of chromosome segregation in trypanosome is still very limited. Although homologs of separase and cohesin have been identified, the

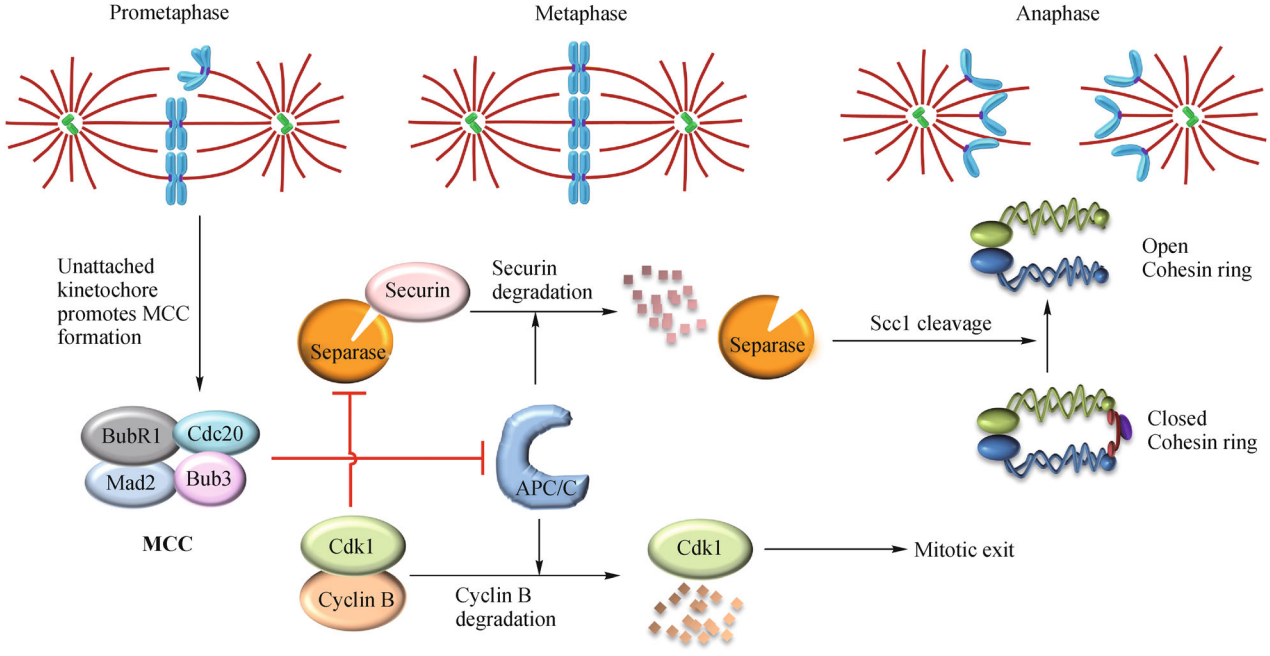

Figure 1 Regulation of chromosome segregation in eukaryotes. During prometaphase, unattached kinetochores trigger the formation of the mitotic checkpoint complex (MCC), which is composed of Mad2, Bub3, BubR1, and Cdc20. MCC inhibits the activity of the APC/C. Once all kinetochore-microtubule attachment errors are corrected and all chromosomes are aligned at the metaphase plate (metaphase). MCC is no longer formed, thus producing free Cdc20 to activate the APC/C. Activated APC/C ubiquitinates securin, leading to securin degradation by the 26S proteasome. Degradation of securin releases separase, and the latter further cleaves the Scc1 subunit of the cohesin ring complex and produces an open cohesin ring, allowing the bound sister chromatids to separate (anaphase). Cdk1/cyclin B complex also exerts an inhibitory effect on separase, preventing premature chromosome segregation. When cyclin B is ubiquitinated by the activated APC/C and degraded by the 26S proteasome, Cdk1 is inactivated and, consequently, the inhibitory phosphorylation on separase is removed. Degradation of cyclin B also allows mitotic exit. Adapted and modified from (Lara-Gonzalez et al., 2012).

securin homolog in trypanosome is still missing. Genetic evidence suggests the presence of securin in trypanosome (Kumar and Wang, 2006), but no effort has been undertaken to identify this important protein.

Essential factors involved in regulating sister chromatid segregation

Anaphase promoting complex/cyclosome

Human APC/C

The APC/C is a large multi-subunit complex and is conserved in eukaryotes (for a detailed review, see (Buschhorn and Peters, 2006)). The human APC/C is composed of 12 core subunits (Table 1). APC1, the largest APC/C subunit, is homologous to the RPN1 and RPN2 subunits of the 19S proteasome regulatory complex and likely acts as a scaffold protein in the APC/C complex. APC2 and APC11 contain cullin domain and RING-H2 finger domain, respectively, which mediate the interaction between APC2 and APC11. These two domains are also found in the SCF ubiquitin ligase complexes. The RING-H2 domain in APC11 interacts with E2 enzymes such as UbcH5 and UbcH10 and, therefore, may function to recruit E2 enzyme to the APC/C. Five subunits of APC/C, APC3/CDC27, APC5, APC6/CDC16, APC7, and APC8/CDC23, contain a 34-residue tetratricopeptide (TPR) motif that is known to mediate protein–protein interactions in multi-protein complexes. APC10 contains a Doc domain and likely functions in substrate recognition (da Fonseca et al., 2011). Little is known about the function of the remaining subunits, but presumably they may play roles in maintaining APC/C structure. The fully assembled APC/C is made of two large domains, the so-called "platform" and the "arc lamp". When the APC/C is bound by its co-activator Cdh1 or Cdc20, there is a change in the relative positions of the two domains, which may activate APC/C.

Table 1 Subunit composition of APC/C in different organisms

H. sapiens	*S. cerevisiae*	*S. pombe*	*T. brucei*
APC1	Apc1	Cut4	APC1
APC2	Apc2	Apc2	APC2
APC3	Cdc27	Nuc2	CDC27
APC4	Apc4	Lid1	APC4
APC5	Apc5	Apc5	–
APC6	Cdc16	Cut9	CDC16
APC7	–	–	–
APC8	Cdc23	Cut23	CDC23
–	Apc9	–	–
APC10	Doc1	Doc1	DOC1
APC11	Apc11	Apc11	APC11
CDC26	Cdc26	Hcn1	–
APC13	Swm1	Apc13	–
–	–	Apc14	–
–	Mnd2	Apc15	–
–	–	–	AP2
–	–	–	AP3

APC/C subunits from human (*Homo sapiens*), budding yeast (*Saccharomyces cerevisiae*), fission yeast (*Schizosaccharomyces pombe*), and trypanosome (*Trypanosoma brucei*) were listed. Proteins listed in the same row are the homologs in these organisms. "–" denotes that homolog is not found in this organism.

Yeast APC/C

The first APC/C subunit genes were originally identified by genetic screening in budding yeast (Irniger et al., 1995) and by biochemical approach in clam and *Xenopus* egg extracts (King et al., 1995; Sudakin et al., 1995). The budding yeast APC/C is composed of 13 different proteins, 11 of which are homologous to human APC/C subunits. However, the APC/C in budding yeast lacks the APC7 homolog, but contains two new proteins, APC9 and Mnd2 (Table 1). The APC/C complex in fission yeast is also composed of 13 different proteins, 12 of which are homologous to their counterparts in the budding yeast, with the only exception of APC9, which is replaced by APC14 (Table 1). There appears to be only a slight difference in the subunit composition of the APC/C from yeasts and human, suggesting that the complex is well conserved during evolution.

Trypanosome APC/C

Seven APC/C subunit homologs, APC1, APC2, APC3/CDC27, APC6/CDC16, APC8/CDC23, APC10/DOC1, and APC11, were initially identified in the *T. brucei* genome (Kumar and Wang, 2006). RNAi-mediated silencing of individual APC/C subunits showed that only APC1 and APC3/CDC27 are essential for cell viability (Kumar and Wang, 2006), suggesting that either RNAi is insufficient to knock down the remaining five subunits or these five subunits are dispensable in trypanosome. A subsequent study was carried out to affinity purify the APC/C complex from different cell cycle stages of *T. brucei*, which led to the purification of a 10-subunit APC/C complex. The purified APC/C complex contains the seven previously characterized subunits, an APC4 homolog, and two novel proteins (AP2 and AP3) that do not have close homologs in other systems (Table 1). Intriguingly, neither the Cdc20 homolog nor any SAC protein was co-purified with the APC/C at any stages of the cell cycle, suggesting an unusual regulatory mechanism of spindle assembly checkpoint control mediated by the APC/C in *T. brucei* (Bessat et al., 2013).

Spindle assembly checkpoint proteins

Human SAC proteins

The discovery of SAC can be dated back to 1930s. When vertebrate cells were treated with a spindle-depolymerizing

drug, they were arrested in mitosis (Brues and Cohen, 1936). Although SAC proteins were originally discovered in budding yeast and are conserved among eukaryotes, MCC, an APC/C inhibitor consisting of Mad2, BubR1, Bub3, and Cdc20, was first purified from HeLa cells (Sudakin et al., 2001). Nevertheless, human SAC proteins share high sequence homology to their yeast counterparts and carry out the same function in the SAC-mediated surveillance pathway (Table 2).

Table 2 Spindle assembly checkpoint proteins in different organisms

H. sapiens	*S. cerevisiae*	*S. pombe*	*T. brucei*
Mad1	Mad1	Mad1	–
Mad2	Mad2	Mad2	Mad2
BubR1	Mad3	Mad3	–
Bub1	Bub1	Bub1	–
Bub3	Bub3	Bub3	–
Mps1	Mps1	Mps1	–

See Table 1 legend for the names of the organisms. Proteins listed in the same row are the homologs in these organisms. "–" denotes that homolog is not found in *T. brucei*.

Yeast SAC proteins

The SAC genes were originally discovered by genetic screens in budding yeast, which identified Mad1, Mad2, Mad3, Bub1, and Bub3 (Hoyt et al., 1991; Li and Murray, 1991). Subsequent studies identified Mps1, a protein kinase, as another component of the SAC in budding yeast, which is well conserved in eukaryotes (Weiss and Winey, 1996) (Table 2). Mps1 activity promotes recruitment of Mad1-Mad2 to kinetochores. Mps1 kinase and Aurora B kinase are kinetochore components upstream of the SAC proteins. In fission yeast and most other organisms, Aurora B kinase promotes Mps1 targeting to kinetochores, which help recruit other SAC components to kinetochores (Heinrich et al., 2012). However, in budding yeast, Aurora B kinase (Ipl1) and Mps1 are recruited to kinetochores independently (Maure et al., 2007). Bub1 is another protein kinase in the SAC, but its kinase activity is not required for SAC function. Bub1 is the first SAC protein recruited to kinetochores (Li and Murray, 1991) and is required to recruit other SAC proteins, including Mad1, Mad2, Mad3, and Bub3, to kinetochores (Vigneron et al., 2004). Unlike its human homolog BubR1 that contains a C-terminal kinase domain, Mad3 lacks the C-terminal kinase domain. Mad3 might function as a pseudosubstrate of the APC/C and, thus, inhibits the APC/C.

Trypanosome SAC proteins

A functional SAC in trypanosome has not been identified so far. Strikingly, the trypanosome genome only encodes a single spindle checkpoint protein, Mad2 (Table 2), which, surprisingly, is localized to the flagellar basal body but not the nucleus or the kinetochore region (Akiyoshi and Gull, 2013). This unusual feature of Mad2 argues against its role in regulating kinetochore-microtubule attachment and sister chromatid segregation. Although trypanosome Mad2 homolog is highly conserved, it appears to lack the CDC20 binding motif (Akiyoshi and Gull, 2013). Moreover, Cdc20 and Mad2 were not co-purified with the APC/C at any cell cycle stages in trypanosome (Bessat et al., 2013), raising the questions of whether Cdc20 and Mad2 form an MCC complex and whether APC/C is regulated by MCC in trypanosome. Moreover, there is no strong evidence to support the presence of spindle assembly checkpoint in trypanosome. For example, disruption of spindle assembly by a microtubule poison causes mitotic defects but does not arrest cytokinesis (Ploubidou et al., 1999).

Cohesin

Human cohesin

The human cohesin complex consists of four core subunits, Smc1, Smc3, Scc1 (known as Rad21), and Scc3 (known as SA1 and SA2) (Michaelis et al., 1997) (Table 3). Smc1 and Smc3 form a large ring structure that is linked at one end by their respective globular hinge domains and at the other end by Scc1 and Scc3 (Fig. 2). Engagement and disengagement of the cohesin ring is regulated by ATP binding and hydrolysis, respectively. Upon anaphase onset, cleavage of Scc1 by separase opens up the cohesin ring and releases the bound sister chromatids for segregation. During meiosis, this process is extended in preparation for the first reduction division cycle, and in most organisms Rad21 is replaced by a meiosis-specific paralog, Rec8. Recently, another meiosis-specific cohesin subunit, Rad21L, was identified (Gutiérrez-Caballero et al., 2011; Ishiguro et al., 2011; Lee and Hirano, 2011). Moreover, vertebrates appear to express additional meiosis-specific variants of Smc1 (Smc1β) and Scc3 (SA3) (Uhlmann, 2011).

In human cells, it is generally accepted that cohesin is removed from chromosomes in two steps. The first step is the separase-independent dissociation of cohesin from chromosome arms driven by Polo-like kinae 1 (Plk1), which

Table 3 Cohesin complex in different organisms

	H. sapiens	*S. cerevisiae*	*S. pombe*	*T. brucei*
Smc1	Smc1	Smc1	Psm1	TbSMC1
Smc3	Smc3	Smc3	Psm3	TbSMC3
Scc1	Rad21	Mcd1/Pds3	Rad21	TbSCC1
Scc3	SA1, SA2	Scc3	Psc3	TbSCC3

See Table 1 legend for the names of the organisms. Proteins listed in the same row are the homologs in these organisms.

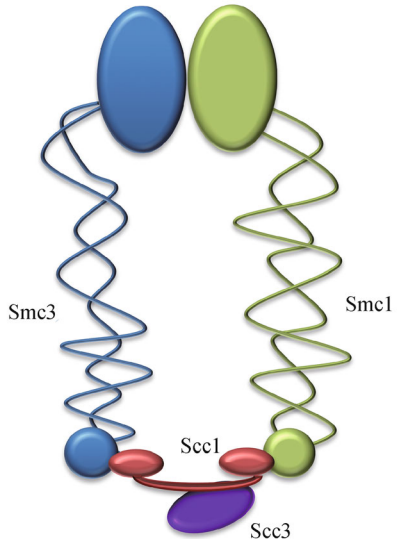

Figure 2 Molecular architecture of the cohesin complex. Smc1 (green) and Smc3 (blue) are each composed of a long coiled-coil arm flanked by an ATP binding head domain and a hinge domain. The N- and C-terminal domains of the kleisin subunit Scc1 (red) bind to the head domains of Smc3 and Smc1, respectively, leading to the formation of a tripartite ring. The fourth subunit of the cohesin complex, Scc3 (purple), binds to Scc1.

phosphorylates Scc1/Rad21, thus allowing the cohesin complex to dissolve (Hauf et al., 2005). However, cohesin in the centromeric region is protected by Shugoshin (Sgo1 and Sgo2) by retaining PP2A at the centromere to antagonize Plk1-mediated phosphorylation of cohesin (Kateneva and Higgins, 2009). The second step is the separase-dependent cleavage of Scc1/Rad21, which leads to removal of the cohesin complex from the centromeric region.

Yeast cohesin

Cleavage of Scc1 by separase (Esp1 in yeast) as the trigger for anaphase onset was first discovered in the budding yeast *Saccharomyces cerevisiae* (Uhlmann et al., 1999; Uhlmann et al., 2000) and subsequently in metazoa (Jäger et al., 2001; Wirth et al., 2006). In yeasts, the cohesin complex is also composed of four core subunits, Smc1, Smc3, Scc1, and Scc3 (Table 3), and is constitutively loaded onto the chromatin, albeit with low binding affinity, from the G1 phase and throughout the cell cycle. Loading of cohesin onto chromosomes is necessary, but not sufficient, to ensure that sister chromatids are ultimately connected to each other. Establishment of cohesin is also crucial for sister chromatid cohesion (Lyons and Morgan, 2011). Many accessory factors are involved in cohesin loading and establishment. In *S. cerevisiae*, loading of cohesin is facilitated by another protein complex composed of Scc2 and Scc4 (Ciosk et al., 2000). These two proteins are conserved from yeast to human. An acetyltransferase called Eco1 (known in mammalian cells as Esco1 and Esco2), which acetylates Smc3, is crucial for cohesin establishment (Ivanov et al., 2002). Smc3 acetylation is reversed at anaphase by the Hos1 deacetylatase as a consequence of Scc1 cleavage (Beckouët et al., 2010; Xiong et al., 2010). Several other proteins, including Ctf18, Csm3, Tof1, Mrc1, Ctf4, and Chl4, are also involved in cohesin establishment, but their roles are still unclear (Fernius et al., 2013).

Trypanosome cohesin

The genome of *Trypanosoma brucei* is composed of 11 pairs of mega-base chromosomes with the size ranging from 1 to 6 Mb, several intermediate chromosomes with the size ranging from 200 to 900 kb, and ~100 mini-chromosomes with the size ranging from 50 to 150 kb (Ersfeld et al., 1999). The mega-base chromosomes are believed to attach to spindle microtubules via kinetochores, and their segregation follows the same mechanism as in yeasts and animals (Ersfeld and Gull, 1997). However, due to the lack of kinetochore structure on mini-chromosomes, segregation of mini-chromosomes does not follow the same mechanism as that for the mega-base chromosomes. Instead, it is likely to be mediated by the association of mini-chromosomes with the central mitotic spindle (Ersfeld and Gull, 1997).

Orthologs of cohesin components are present in trypanosome (Table 3), which are closely related to their human and yeast counterparts. Although the molecular organization of trypanosome cohesin complex is not defined, presumably the four subunits could form a similar ring structure as in human and yeast. Despite the lack of structural information of the trypanosome cohesin complex, functional studies suggest that the cohesin proteins are essential for chromosome segregation in trypanosome. TbSCC1 has a dynamic pattern during trypanosome cell cycle. It is expressed at late G1 phase prior to DNA synthesis, remains in the nucleus throughout S- and G2-phases, and disappears at anaphase (Gluenz et al., 2008). RNAi knockdown of TbSCC1 or expression of a separase-non-cleavable TbSCC1 blocks nuclear division (Gluenz et al., 2008). Moreover, RNAi-mediated silencing of TbSMC3 also interferes with the separation of mega-base chromosomes, but it has no detectable effect on the segregation of mini-chromosomes (Bessat and Ersfeld, 2009).

Separase

Human separase

Separase, a CD clan protease, appears to be well conserved throughout evolution (Uhlmann et al., 2000). Human separase is detected on centrosomes only before anaphase and then abruptly disappears upon anaphase onset (Chestukhin et al., 2003). Unlike in budding yeast where Pds1 (securin) is the only known inhibitor of separase, human separase is inhibited by securin binding and Cdk1 phosphorylation (Stemmann et al., 2001). With the inhibitory phosphorylation exerted by Cdk1, separase likely becomes

more accessible to cyclin B1, which induces a conformational change. Intriguingly, Cdk1 itself is inactivated when bound to separase (Gorr et al., 2005). Therefore, in the separase-Cdk1 complex, both the protease and the kinase are held inactive.

Separase itself is also subjected to proteolytic auto-cleavage at three cluster sites, all of which are well conserved in vertebrates (Zou et al., 2002). However, the cleaved products of separase remain bound to each other and are still catalytically active. It is speculated that auto-cleavage of separase may prepare itself for destruction, thus allowing establishment of sister chromatid cohesion in the next cell cycle (Waizenegger et al., 2002). Separase may have other substrates in addition to securin. For example, kendrin (also named pericentrin) was found to be a substrate of separase in mammalian cells (Matsuo et al., 2012).

Human separase might be an oncogene because over-expression of separase induces premature separation of sister chromatids, lagging chromosomes, and anaphase bridges. Additionally, overexpression of separase in mammary epithelial cells is sufficient to induce aneuploidy and tumorigenesis in a p53 mutant background (Pati, 2008; Zhang et al., 2008). RNAi-mediated knockdown of separase results in genomic instability (Waizenegger et al., 2002), and knockout of separase in mice causes embryonic lethality (Wirth et al., 2006). Loss of separase also blocks centriole disengagement during mitotic exit and delays the assembly of new centrioles during the following S-phase (Tsou et al., 2009).

Yeast separase

Separase was originally identified in yeast and was named Esp1 in budding yeast (McGrew et al., 1992) and Cut1 (cells ultimately torn) in fission yeast (Uzawa et al., 1990). The separase proteins from different organisms, with the exception of *Drosophila* separase, share a similar structure with a variable N-terminal super-helical motif comprising more than two-dozen ARM or HEAT repeats and two C-terminal caspase domains. However, only the second caspase domain appears to be active (Viadiu et al., 2005).

Studies in budding yeast showed that Pds1 (securin) can bind to both the evolutionary conserved C terminus of Esp1 and the diverged N-terminal region (Hornig et al., 2002). Pds1 binding not only prevents access of substrate to Esp1, but also prevents interaction between the N-terminal domain and the C-terminal domain of Esp1, which may contribute to Esp1 function (Hornig et al., 2002). Unlike in humans, however, no evidence was presented to show that Esp1 and Cut1 undergo auto-cleavage. In addition to Pds1, Esp1 may cleave other substrate proteins during anaphase. For example, Slk19, a protein that associates with spindle and kinetochores, is also a target of Esp1 (Sullivan et al., 2001). However, it remains unclear how many other proteins are substrates of Esp1.

Trypanosome separase

The trypanosome homolog of separase (TbSep) has been identified, which shares similar structure as its yeast and human orthologs (Bessat and Ersfeld, 2009). TbSep has a calculated molecular mass of only 126 kDa, which is considerably smaller than the yeast and mammalian separase (~210 kDa) (Viadiu et al., 2005). TbSep is expressed throughout the cell cycle and is excluded from the nucleus until metaphase, which potentially prevents premature cleavage of cohesion (Bessat and Ersfeld, 2009). TbSep depletion by RNAi causes mis-segregation of both mega-base chromosomes and mini-chromosomes (Bessat and Ersfeld, 2009). The defect in mini-chromosome segregation is surprising because it is known that mini-chromosome segregation does not follow the spindle-kinetochore mechanism utilized by the mega-base chromosomes (Ersfeld and Gull, 1997). Since mini-chromosomes associate with the spindle (Ersfeld and Gull, 1997), the defective segregation of mini-chromosomes in TbSep RNAi cells likely is attributed to the spindle assembly defects (Bessat and Ersfeld, 2009).

Securin

Human securin

The human securin protein is encoded by *PTTG1* (pituitary tumor-transforming gene-1), which was originally isolated from rat pituitary tumor cells (Pei and Melmed, 1997) and later identified as a vertebrate homolog of securin (Zou et al., 1999). PTTG1 functions as an anaphase inhibitor by inhibiting separase activity and preventing premature chromosome separation. PTTG1 is extremely hydrophilic and contains a basic amino acid-rich N-terminal region (Zhang et al., 1999). Its C terminus contains two proline-rich motifs that form a predicted Src-homology 3 (SH3)-interacting domain (Kakar and Jennes, 1999). PTTG1 may function as an oncogene through SH3-mediated signal transduction and activation of growth factor(s). PTTG1 contains in the N terminus a KEN-box and a destruction box (D-box), which are recognized by APC/C and are responsible for PTTG1 degradation (Vlotides et al., 2007). Securin may play functions in addition to inhibiting separase activity. For example, securin binding may ensure separase to adopt a proper fold that is required for its proteolytic activity (Jallepalli et al., 2001).

Yeast securin

Pds1 in budding yeast (Cohen-Fix et al., 1996) and Cut2 (cells untimely torn) in fission yeast (Funabiki et al., 1996) function as the securin to prevent premature chromosome segregation. In *S. pombe*, deletion of Cut2 is lethal and produces similar defects as deletion of Cut1 (separase), i.e., a complete blockage of chromosome segregation (Funabiki

et al., 1996). In *S. cerevisiae,* Pds1 mutant displays delayed anaphase entry and is synthetic lethal with Esp1 mutant (Ciosk et al., 1998). This suggests that Pds1 and Esp1 may act synergistically rather than antagonistically in regulating anaphase onset in budding yeast. Two studies demonstrated that the securin homolog in fission and budding yeasts not only inhibits separase but also regulates the localization of separase to spindles (Kumada et al., 1998; Jensen et al., 2001). Another study showed that Pds1 plays dual roles in regulating Esp1 in budding yeast: promoting nuclear accumulation of Esp1 and ensuring full proteolytic activity of Esp1 at anaphase (Hornig et al., 2002). A positive roles of securin on separase was also observed in human (Jallepalli et al., 2001), suggesting that the dual function of securin in regulating separase likely is conserved across evolution.

Trypanosome securin

The securin homolog in trypanosome has not been identified, but it is generally accepted that securins from different organisms are very divergent in sequence (Jäger et al., 2001). Therefore, search for securin homologs by homology-based sequence analysis is very challenging and often fails to identify securin homologs, especially in the early-diverged organisms. Indeed, genetic data suggest that a securin-like protein is functioning in the cell cycle control because RNAi-mediated ablation of the 26S proteasome or the APC/C both inhibits chromosome segregation (Li and Wang, 2002; Kumar and Wang, 2006).

Concluding remarks and perspectives

The basic chromosome segregation machinery appears to be well conserved across evolution, but distinctions in the regulatory pathways have been identified among different organisms, particularly between human and trypanosome. Nevertheless, among the chromosome segregation regulators, such as separase, securin, and the components of SAC, APC/C, and cohesin, only securin has a very divergent sequence and is not conserved. This raises an interesting question of why such an essential regulator was not well preserved during evolution. Although the securin homolog in trypanosome remains to be identified, genetic evidence suggests its presence and function in regulating sister chromatid segregation. Therefore, the regulatory pathway from APC/C activation to separase activation and finally to cohesin cleavage is an ancient mechanism that is conserved from early diverged protozoa to humans and likely was present in the last eukaryotic common ancestor (LECA). Surprisingly, the SAC-mediated surveillance mechanism is missing in trypanosome, with its single component Mad2 localizing to the flagellar basal body region but not nucleus and kinetochores. This unusual discovery raises the question of how trypanosome cells monitor and correct kinetochore-microtubule attachment errors to ensure faithful chromosome segregation. It is also unclear how the APC/C is activated in response to kinetochore-microtubule attachment errors. Further, it raises another intriguing question of when and how the SAC mechanism was evolved since the LECA.

The regulation of sister chromatid segregation in trypanosome is still poorly understood despite the efforts made by several groups during the last decade. Unfortunately, only a few regulators have been identified. Many other essential regulators are likely present in trypanosome, but they may be very divergent in sequence and, therefore, are difficult to be identified by homology-based search. New approaches, such as genetic screening and proteomics, appear to be necessary for identifying those trypanosome-specific regulators, which might serve as potential drug targets for anti-trypanosome chemotherapy.

Acknowledgements

We apologize to our colleagues whose work could not be cited due to space constraints. Work in the Li laboratory is supported by NIH grants R01AI101437 and R21AI093897.

Compliance with ethics guidelines

Xianxian Han and Ziyin Li declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional reiew board or ethics committee.

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