Replisome-cohesin interfacing: a molecular perspective

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Keywords

DNA replication, replisome structure, cohesin, replication forks, cohesin, sister chromatid cohesion, chromosome dynamics

Summary

Cohesion is established in S-phase through the action of key replisome factors as replication forks engage cohesin molecules. By holding sister chromatids together, cohesion critically assists both an equal segregation of the duplicated genetic material and an efficient repair of DNA breaks. Nonetheless, the molecular events leading the entrapment of nascent chromatids by cohesin during replication are only beginning to be understood. We describe here the essential structural features of the cohesin complex in connection to its ability to associate DNA molecules and review the current knowledge on the architectural-functional organization of the eukaryotic replisome, significantly advanced by recent biochemical and structural studies. In light of this novel insight, we discuss the mechanisms proposed to assist interfacing of replisomes with chromatin-bound cohesin and elaborate on models for nascent chromatids entrapment by cohesin in the environment of the replication fork.

1. Introduction

In eukaryotes, the structural maintenance of chromosomes (SMC) cohesin complex stably associates different DNA segments to influence key chromosomal transactions. Cohesin mediates the establishment of sister chromatid cohesion (SCC), required for a correct segregation of the genetic material to daughter cells and for an efficient repair of DNA breaks, the organization of chromosomal domains and the interaction of regulatory elements modulating gene expression.^[1–3] Chromosomal DNA replication is carried out by the replisome, a large molecular machine that unwinds chromatin, synthesises DNA complementary to parental strands and assembles nascent duplexes into chromatin, while dealing with obstacles potentially threatening its processivity and stability^[4–6]. Establishment of sister chromatid cohesion is intimately intertwined with chromosome duplication and is mediated by factors progressing with replication machineries^[7–9]. On the other hand, recent studies have linked cohesin function to the structural organization and stability of replication forks, particularly important in the presence of DNA damage or replicative stress^[10,11]. Hence, interfacing between cohesin and replication forks has emerged as a central event potentially impacting on chromosome metabolism, transmission and integrity. Nonetheless, there is little insight on the mechanisms orchestrating the molecular interaction between replisomes and cohesin rings. Recent studies have advanced our understanding on the structural arrangement of eukaryotic replisome components central to replication fork function, as well as on the molecular mechanisms that govern DNA entrapment and locking by cohesin. Here we review this novel evidence, focusing on budding yeast factors and mechanisms, with the aim of providing a molecular perspective on

how replication machineries deal with cohesin to achieve a stable association of nascent sister chromatids.

2. Cohesin conforms a ring-shaped structure that dynamically associates to DNA during the chromosome duplication cycle

The cohesin core structure is formed by two SMC proteins (Smc1 and Smc3) and an alpha-kleisin (Scc1) subunit, to which the accessory factors Scc3, Pds5 and Wpl1 associate (**Table 1**). SMC subunits are characterised by long antiparallel coiled-coils, that form upon folding at a hinge domain, and a globular nucleotide-binding domain arranged through the interaction of C- and N- terminal portions of the protein ^[12] (**Figure 1**). These globular domains mediate ATP hydrolysis, which is important for the entrapment of DNA molecules and determines cohesin association to chromatin ^[13,14]. Smc1 and Smc3 bind together through their hinge domains to form V-shaped dimers, which are bridged by Scc1 association to the globular ATPase heads to give rise to a closed ring-like structure ^[15]. The accessory subunits Scc3, Pds5 and Wpl1 contribute to the stabilization of cohesin structure and modulate the dynamic interaction of cohesin with DNA ^[16–18]. These factors associate to the cohesin core through Scc1 and are thought to influence the opening and closing of a DNA exit gate, formed at the interface between the distal portion of the Smc3 coiled-coil domain and the N-terminal portion of Scc1 ^[19,20]. Pds5 and Wpl1 have been proposed to dislodge this interface ^[12], thus promoting the exit of DNA molecules from ring and in turn determining a highly dynamic association of cohesin with chromatin ^[21,22]. Scc3 is a large protein, mostly containing HEAT-repeats arranged in a horseshoe shape, that interacts with Scc1/RAD21 through a concave inner surface ^[23,24]. Scc3 is important for cohesin structural stability and function, though its precise molecular roles and its overall position relative to the ring lumen are still obscure. Pds5, as Scc3, is significantly larger than Scc1 and is thought to associate to the N-terminal portion Scc1 close to the Smc3-Scc1 interface

serving as DNA exit gate^[12]. Of note, different cohesin-associated proteins may compete for binding similar regions in Scc1. Scc2 associates to Scc1 through an N-terminal region overlapping the Pds5-binding region, which suggests that Pds5 may contribute to cohesin release from Scc2-Scc4^[25]. This observation raises the notion whether the functions of associated factors in stimulating cohesin loading or release from DNA may involve competition for access to key cohesin interfaces.

Cohesin is loaded onto chromatin by the Scc2/Scc4 complex prior to DNA replication, in G1 phase in yeast and during telophase in mammalian cells. The Smc3-Smc1 hinge and Smc3-Scc1 interfaces have been proposed to be important to mediate DNA entry into the complex^[19], in a process that requires ATP hydrolysis^[13,14]. Cohesin association to chromatin is labile prior to replication due to the action of Wpl1 and Pds5 (**Figure 2**). This dynamic association changes during S-phase, as sister chromatids emerge and are stably trapped by cohesin. Establishment of sister chromatin cohesion is thought to be achieved by the locking of replicated duplexes inside cohesin, which is controlled through two key lysine residues in Smc3, K112 and K113. These residues are acetylated by the Eco1 acetyltransferase that associates with replication forks^[8,9], rendering the ring refractory to Pds5/Wpl1 action likely by interference with the opening of the Smc3-Scc1 exit gate located nearby^[12]. Eco1 associates to replisome factors in both yeast and human cells^[8,25] and, in addition, several fork proteins (such as Ctf4, Mrc1, Ctf18, Elg1 or Chl1, see below) contribute to sister chromatid entrapment and locking. Ablation of these factors results in cohesion defects, though to a lesser extent than mutations in cohesin genes^[26–29].

Acetylated cohesin remains bound to DNA until mitosis, when sister chromatids are segregated^[30]. In anaphase, Scc1 is cleaved by the Esp1/separase protease following the degradation of its inhibitor Pds1/securin upon activation of the Anaphase Promoting Complex (APC)/Cdc20 (**Figure 2**). α -kleisin

proteolysis promotes the release of cohesin rings from DNA and triggers the segregation of sister chromatids to daughter cells^[31]. Deacetylation of K112 and K113 in Smc3 by Hos1 has also been proposed to be important for the opening of the cohesin ring and its release from sister chromatids^[32]. While most of cohesin is removed by action of separase in yeast cells, other mechanisms operate in higher eukaryotes. In mammals, cohesin is removed from chromosome arms in prophase, in a manner dependent on the Plk1 (Polo-like kinase 1) and Wapl function^[33,34], while dissolution of centromeric cohesin also involves RAD21/Scc1 cleavage in anaphase^[35]. Hence, modulating the dynamic behaviour of cohesin interaction with chromatin, determined by the opening and locking of DNA gates in the ring structure, appears critical to both couple the synthesis and entrapment of daughter DNA strands and to maintain a close association of sister chromatids until their partition in mitosis.

3. Architecture and function of the eukaryotic replisome

3.1 Leading and lagging strand polymerases associate to a helicase replisome core

The eukaryotic replisome exerts three main functions during chromosomal replication^[6,36]: (i) parental DNA unwinding, (ii) priming and (iii) leading and lagging nascent strands synthesis. Chromatin unwinding is carried out by the Cdc45-MCM-GINS (CMG) helicase. The DNA polymerase α /Primase complex synthesizes primers, while DNA polymerases ϵ and δ perform most of DNA synthesis. The leading strand is synthesized continuously, primarily by Pol ϵ , while the lagging strand is synthesized discontinuously by Pol δ ; leading to the formation of short segments called Okazaki fragments. Additional factors assist these key functions, contribute to the functional coordination between replisome components and support a stable fork progression along chromosomal DNA (**Table 2**). The CMG helicase constitutes the structural core of the replisome to which other key

factors associate in order to build up a replication fork (**Figure 3**). CMG is conformed by the Mcm2-7 heterohexameric complex, which adopts a toroid structure encircling single stranded DNA, the GINS (standing for Go-Ichi-Ni-San, Japanese for “5-1-2-3” in reference to its Sld5, Psf1, Psf2 and Psf3 subunits) complex, and Cdc45^[37]. The MCM complex is considered the motor of CMG, though full helicase activity is only achieved upon association of GINS and Cdc45^[38,39], the latter sharing homology with RecJ nuclease but apparently devoid of a nuclease activity^[40]. The CMG complex has a global size of 785 KDa and single particle reconstructions by 3D-cryo-electron microscopy (EM) suggest an approximate width of 20 nm^[41,42]. Current models assume that the strand serving as leading template enters a central channel in Mcm2-7 thus melting the parental DNA duplex to generate two downstream single stranded filaments^[43,44]. Replication Protein A complex (RPA), constituted by three (Rfa1, Rfa2 and Rfa3) subunits, binds and stabilizes ssDNA to facilitate DNA synthesis and perform key functions in response to replication stress^[45–47]. DNA polymerase ϵ (Pol ϵ), composed of four polypeptides (Pol2, Dpb2, Dpb3 and Dpb4), tightly associates to the CMG helicase through the GINS and Cdc45 components^[48]. The largest subunit Pol2 bears both DNA polymerase and 3'-5' exonuclease activities and is arranged in two large domains that likely raised via duplication of a single ancestral protein^[49]. The N-terminal half of Pol2 harbours its catalytic activities, while the C-terminal portion, which contains inactive exonuclease and polymerase domains, is essential for viability in yeast cells^[50]. The smaller Dpb2-4 subunits interact through the C-terminal portion of Pol2 and lack catalytic activity. Dpb2 contributes to the stability and fidelity of the holozyme, while Dpb3 and Dpb4 are thought to assist Pol ϵ 's association to DNA and processivity^[51,52]. *In vitro*, Pol ϵ processivity is further enhanced by the replication clamp PCNA (Proliferating Cell Nuclear Antigen), a homotrimeric complex constituted by Pol30 polypeptides that also mediates key events for the tolerance of DNA damage during replication^[53,54].

While DNA unwinding and leading strand synthesis seems primarily coupled by physical association of CMG and Pol ϵ , recent evidence suggests that coordination with lagging strand synthesis takes place through the action of the Ctf4 homotrimeric adaptor complex^[55,56]. Ctf4 can bind different proteins harbouring CIP (Ctf4-interacting-peptide) boxes. Ctf4-associating proteins include the Psf2 subunit of the CMG helicase and the Pol1 subunit of DNA polymerase α /Primase. Through these interactions, Ctf4 may couple up to two Pol α complexes to CMG. CIP boxes also mediate interaction of Ctf4 with other proteins like the Dna2 nuclease, the Chl1 helicase (required for efficient establishment of sister chromatid cohesion) or Top2 (DNA topoisomerase-II factor 2), suggesting that Ctf4 behaves as a hub allowing the association of different factors to the replisome^[29,57]. Pol α /Primase is a highly conserved complex that initiates both leading and lagging strand synthesis, the latter by periodically priming Okazaki fragments. It is composed of four subunits Pol1, Pol12, Pri1 and Pri2, where Pol1 and Pri1 bear DNA polymerase and RNA primase activities, respectively. The large polymerase subunit, Pol1 is characterized by the presence of a C-terminal domain (CTD) connected to the catalytic core by a flexible linker, to which other subunits associate^[58,59]. During lagging strand synthesis, this linker enables large-scale rearrangements that mediate switching from generating short RNA primers to synthesizing DNA^[36]. Primers synthesized by Pol α are handed over to Pol δ , which extends DNA synthesis until the adjacent Okazaki fragment is reached. The Pol δ holozyne is constituted of 3 subunits, Pol3, Pol31 and Pol32, where Pol3 bears the polymerase and exonuclease catalytic activities. Pol δ is intrinsically less processive than Pol ϵ , but its highly stimulated in vitro by the replication clamp PCNA^[60]. Loading of PCNA by the replication factor C, an ATPase composed of the Rfc5, Rfc1, Rfc2, Rfc3 and Rfc4 subunits, is thought to stimulate a switch from Pol α - to Pol δ -mediated extension during lagging strand synthesis^[61,62]. Alternative RFC complexes can be formed through substitution of the Rfc1 subunit by the related Ctf18 or Elg1 proteins^[63,64], which have been proposed to carry out clamp loading at the leading strand and PCNA

unloading at lagging strands, respectively ^[63,65,66]. When Pol δ reaches the 5' end of the ensuing Okazaki fragment, it triggers its maturation that involves a switch in Pol δ to strand displacement synthesis mode and the action of the Fen1 nuclease, the Dna2 nuclease/helicase and Cdc9/DNA ligase I ^[36,67,68].

3.2 Additional factors contribute to replisome organization and promote its progression through chromatin.

In addition to Ctf4, the Mrc1, Tof1 and Csm3 proteins are thought to contribute to the architectural organization of the forks. These proteins are necessary for optimal replication rates *in vitro* and *in vivo* ^[69–72], though little information on their structure, arrangement within the replisome or molecular functions is available to date. The three proteins have been proposed to act within a Replisome Protection Complex ^[16,73–75], but they also exert distinct functions. Mrc1 is required to couple DNA unwinding and synthesis in conditions of stress ^[76,77], and has been proposed to link the CMG helicase with synthesis by DNA polymerase ϵ ^[78,79]. This function might be related to its function as an adaptor of DNA replication checkpoint signalling, which is thought to respond to ssDNA generated by helicase-polymerase uncoupling ^[4,80]. Tof1 and Csm3 contribute to maintain the stability of paused replication forks ^[75,81] and counteract the transfer of torsional stress generated during replication behind fork branching points ^[82], suggesting that they may promote a replisome architecture not permissive to the rotation of nascent strands around the fork axis. Enigmatic is also the function of the Mcm10 protein which has been involved in both replisome assembly and progression ^[83–85]. Mcm10 associates to Mcm2-7 and Cdc45 ^[86,87] and has been proposed to stimulate CMG function ^[88,89] and stabilize Pol α ^[90].

Several additional factors associate to replication forks to support replisome progression through chromatin and remove obstacles to DNA unwinding. These include the FACT complex, composed of

Spt16 and Pob3, transiently disrupting parental nucleosomes in front of replication forks ^[91,92], which are then promptly incorporated to nascent DNA by different chromatin assembly factors along with newly synthesized histones ^[93]. FACT biochemically associates to replisome components ^[94,95] and is essential for chromatin replication *in vitro* ^[92]. DNA topoisomerases I and II (Top1 and Top2) also act at replication forks to remove torsional stress generated by DNA unwinding that, if unresolved, would block helicase action ^[96]. Top1 shows a weak biochemical interaction with CMG ^[94], while the mechanism of Top2 recruitment to forks remains to be clarified. In addition, several DNA helicases assist replisome progression by removing obstacles to CMG function, some of which have been described to travel with replication forks, such as the Rrm3 Pif1-family helicase that removes bulky protein-DNA complexes ^[97], or the Sen1 RNA/DNA and DNA helicase which counteracts RNA-DNA hybrid structures accumulation ^[98].

In a nutshell, the replisome can be seen as a large robust helicase core to which Pol ϵ directly associates to carry out processive extension of the leading strand. A more dynamic physical continuity between CMG and the lagging strand synthesis machineries might instead be achieved through Ctf4, which would facilitate the periodic recruitment of primase, while Pol δ , primarily in charge of lagging strand extension, may not be directly linked to the nucleosome core. Such a dynamic arrangement would facilitate coupling between leading and lagging strand synthesis, possibly subject to modulation by factors as Mrc1, Tof1/Csm3 in specific conditions. Other essential replication factors would be dynamically recruited to unwind DNA in the environment of the replisome core to stabilize ssDNA, modulate sliding clamp dynamics, or mediate the maturation of Okazaki fragments. In addition, factors dedicated to sustain fork progression and negotiate the changes associated to the unwinding of chromatin might preferentially interact with the CMG helicase. This setup suggests a scenario in which the replisome would have a certain degree of

structural plasticity, which may allow transiently adjusting its architecture in response to replicative stress, such as in presence of roadblocks to DNA unwinding or in conditions impairing DNA synthesis.

4. Molecular interfacing between cohesin and replication machineries

Replisomes meet cohesin complexes previously loaded on chromatin as they progress throughout the genome in S-phase. Cohesin has been reported to enrich at sites of DNA synthesis ^[11,99] and cohesin and its regulators productively interact with replication machineries (these interactions were recently revised^[100]). Even if cohesion establishment is tightly intertwined with replication progression, the molecular mechanisms through which cohesin interfaces with replisome components are not yet understood. Several models for cohesin-fork interfacing have been considered (**Figure 4**). A simple possibility is that cohesin is stochastically removed from unreplicated chromatin by the action of Wpl1 and Pds5 and unrelated cohesin molecules are independently loaded on replicated DNA duplexes (**Figure 4a**). This option, in which cohesin and replisome factors may not happen to interact, fails to explain how cohesion establishment is achieved, as cohesin ring locking would still need to be coordinated with the fork-associated Eco1 locking function. In addition, recent studies suggest that cohesin molecules already bound to unreplicated chromatin are utilized to establish cohesion upon fork passage ^[10,101]. A simple solution to couple sister chromatid entrapment and cohesin locking is provided by models in which the replisome “replicates through” cohesin molecules encircling parental DNA (**Figure 4b**). In these scenarios, sister chromatids are directly synthesised into the ring structure that would readily lock them by action of Eco1 moving with the passing fork.

These models raise the key question of whether a functional replisome could traverse the pore of the cohesin complex while carrying out replication. The lumen of purified core cohesin complexes has been estimated to be around 30-40 and 20 nm in height and width, respectively, based on cryo-

EM and crystallographic analysis ^[12,102–104] (**Figure 1**). However, a recent single molecule analysis indicates that cohesin cannot overcome obstacles bigger in than 11 nm when sliding along DNA ^[105]. This is consistent with the observation that, *in vivo*, cohesin appears to be pushed by mRNA transcription ^[106], driven by RNA polymerase II which is approximately 12 nm in width and height ^[107]. Structural reconstructions indicate that the CMG replisome core has an approximate width of 20 nm ^[108] and association of the Pol ϵ holozyeme, Ctf4 trimers and the Pol α /primase complex would further increase the width of the essential replication machinery by about 10-15 nm ^[109]. In addition, a significant number of factors assisting DNA synthesis, replisome architectural organization and progression are known to strongly associate to the replisome core ^[94] (**Figure 3**), likely resulting in a much bulkier protein-DNA ensemble (over 2.5 MDa), to which numerous accessory factors are still recruited (**Table 2**). Hence, it seems unlikely that an ongoing replisome may topologically accommodate inside a cohesin ring, unless extensive remodelling of one or the other complex took place.

Recent evidence in yeast cells has suggested that the p97/Cdc48 ubiquitin selective segregase remodels cohesin complexes to promote their removal from unreplicated chromatin upon engagement by replication forks ^[10]. Mutants impairing the function of Cdc48 or the Rsp5^{Bul2} ubiquitin ligase show increased binding of cohesin close to replication forks but reduced association to nascent DNA, suggesting that cohesin ubiquitylation and remodelling by Cdc48 are required for its mobilization and translocation to nascent duplexes behind forks (**Figure 4c**). Consistent with this notion, Cdc48 and Rsp5^{Bul2} mutants exhibit mild sister chromatid cohesion defects, equivalent to those observed in Wpl1 ablated cells, and decreased Smc3 acetylation (our unpublished observations). Genetic evidence suggests that the role of Cdc48 in mobilizing cohesin is linked to Wpl1 function and may therefore be related to the opening of the DNA exit gate in the interface

between Smc3 and Scc1. A transient displacement from chromatin would determine a necessity for Scc2/Scc4 function to load cohesin back on replicated DNA duplexes in the wake of the replication fork. Concerning this notion, NIPBL/MAU2 (Scc2/Scc4) has been reported to interact with the MCM complex in human cells ^[110]. Of note, *in vitro* studies using purified fission yeast factors have demonstrated that Mis4/Ssl3 (Scc2/Scc4)-mediated capture of a second DNA molecule by cohesin that already embraces a dsDNA duplex is more efficient when ssDNA is provided as a substrate ^[111]. This observation has important implications for the loading of cohesin around nascent sister chromatids, as mobilized rings may be first loaded on double stranded DNA readily synthesized by Pol ϵ at the leading strand and, in a second step, capture single stranded DNA segments transiently present at the lagging strand (**Figure 4c**). This scenario would imply that lagging strand synthesis by Pol δ could occur across the cohesin ring, a notion reminiscent of “replisome through the ring” models. It also provides a stand point to interpret the observed contribution to cohesion establishment of factors thought to mediate a dynamic replisome architecture, the processivity of leading strand synthesis, or lagging strand maturation ^[26,28,29]. Impaired function of Ctf4, Mrc1, Tof1 or Csm3 is likely to alter the coordination between leading and lagging strand synthesis and might hence interfere with an orderly capture of double-stranded and single-stranded portions of nascent DNA molecules by cohesin. Similarly, in the absence of Ctf18 or Elg1 the dynamic persistence and extension of ssDNA segments, respectively, might be altered at nascent duplexes, which may interfere with their efficient capture within the window of opportunity provided by the presence of Eco1 in the close environment. The role of Chl1, exerted through association with Ctf4 but independent of its helicase activity ^[29], would remain intriguing in this scenario.

5. Concluding remarks

Recent structural, biochemical and molecular genetic evidence has provided seminal insight into how interfacing between chromatin-bound cohesin and replication forks might be achieved in order to establish sister chromatid cohesion. In the future, it will be interesting to gather structural evidence on factors as Mrc1, Tof1 and Csm3 to better understand their role within the replisome and integrate their functions into our knowledge of how a dynamic cohesin-replisome interfacing is achieved. It would also be insightful to analyse the ability of cohesin to entrap chromatinized DNA molecules *in vitro*, and to address the contribution to cohesin function of factors controlling nucleosome dynamics during replication, as chromatin is promptly assembled at forks and these factors can be expected to strongly influence nascent DNA entrapment by cohesin. All known cohesin roles are dictated by its ability to interact with and associate different DNA segments. Hence, the mechanisms mediating cohesin-replisome interfacing may have a relevant impact on cohesin-mediated functions other than sister chromatid cohesion, such as chromosome loop extrusion, the stabilization of the interaction between distant transcriptional regulatory elements or the protection of stalled replication forks. Some of these functions are thought to underlie the physiopathology of cohesin-related diseases ^[112,113] and are likely to also be affected in conditions in which the reshaping of cohesin interaction with chromatin upon replisome engagement is impaired.

Acknowledgements

We apologize for the relevant studies that could not be discussed in this review due to space limitations. We are thankful to current and past members of our laboratory for insightful discussions. This work was supported by the Ministry of Economy, Industry and Competitiveness -

MINECO (BFU2014-52529-R and BFU2017-87013-R to R.B) and the Spanish *Formación del Personal Investigador* (FPI) program (to S.V-H.).

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Figure legends.

Figure 1. The eukaryotic cohesin complex. In scale schematic model of the arrangement of budding yeast cohesin subunits and associated factors. Main structural domains of SMC proteins are indicated. A cartoon of a nucleosomal DNA fibre is depicted for size reference.

Figure 2. Cohesin function during the chromosome replication cycle. Schematic representation of the dynamic association of cohesin complexes to chromosomal DNA along the cell cycle. Cohesin is loaded on DNA by the Scc2/Scc4 complex prior to S-phase. Following replication, cohesin embraces sister chromatids which are lock by Eco1-mediated acetylation of Smc3, close to its interface with Scc1. Cohesin holds chromatids together until mitosis, in which Scc1 cleavage by separase and Smc3 deacetylation by Hos1 trigger the release of sister chromatids and their segregation to daughter cells. Blue circles represent acetylation of 113 and 114 lysines in Smc3.

Figure 3. The eukaryotic replication fork. Schematic model (approximately in scale based on crystallographic and electron microscope structural studies) of proteins conforming the replisome and associating to replication forks in budding yeast. Black and blue lines represent parental and

nascent DNA strands, respectively. RNA primers are depicted in green. Grey circles represent nucleosomes.

Figure 4. Interfacing between chromatin-bound cohesin and replication forks. Possible models proposed for the fate of chromatin-bound cohesin upon engagement by replication forks and sister chromatid cohesion establishment. Speculative interfacing scenarios based on random mobilization/loading (a), “replication through” the cohesin ring (b) and cohesin mobilization and translocation to nascent chromatids (c) are represented. ssDNA is represented in grey. See text for details.

	Subunits	Size (KDa)	Human ortholog
SMC	Smc1	141	SMC1
	Smc3	141	SMC3
α-Kleisin	Scc1	63	RAD21
Accessory	Scc3	133	SA1/SA2
	Pds5	147	PDS5
	Wpl1	75	WAPL
Loader	Scc2	171	NIPBL
	Scc4	72	MAU2

Table 1. Cohesin subunits. Names of budding yeast proteins, molecular weights and human orthologs are listed.

Complex	Subunits	Size (KDa)	Human ortholog
CMG		786	
Mcm2-7		605.6	MCM2-7
	Mcm2	98,7	MCM2
	Mcm3	107,5	MCM3
	Mcm4	105	MCM4
	Mcm5	86,4	MCM5
	Mcm6	113	MCM6
	Mcm7	95	MCM7
	Cdc45	74	CDC45
GIN5		105	GIN5
	Sld5	34	SLD5
	Psf2	25	PSF2
	Psf1	24	PSF1
	Psf3	22	PSF3
Polymerase α/primase		355	
	Pol1	166.8	POLA1
	Pol12	78	POLA2
	Pri1	47.7	PRIM1
	Pri2	62.3	PRIM1
DNA polymerase δ		220.6	
	Pol3	125	POLD1
	Pol31	55.3	POLD2
	Pol32	40.3	POLD3
			POLD4
DNA polymerase ϵ		378	
	Pol2	255.6	POLE
	Dpb2	78.3	POLE2
	Dpb3	22.6	POL3
	Dpb4	22	POL4

Factor / Complex	Subunits	Size (KDa)	Human ortholog
PCNA	Pol30	90 (3 x 30)	PCNA
RFC		249	
	Rfc1	95	RFC1
	Rfc2	40	RFC2
	Rfc3	38.2	RFC3
	Rfc4	36	RFC4
	Rfc5	40	RFC5
Ctf4	Ctf4	3x 104.4	AND-1
RPA		114	
	Rfa1	70.3	RPA70
	Rfa2	30	RPA32
	Rfa3	13.8	RPA14
Replication Pausing Complex	Mrc1	124	CLASPIN
	Tof1	141	TIMELESS
	Csm3	36.3	TIPIN
FACT		181.6	
	Spt16	118.6	SPT16
	Pob3	63	SSRP1
Alternative RFCs			
	Ctf18	84,4	CHTF18
	Elg1	91.2	ATAD5
DNA topoisomerase I	Top1	90	TOP1
Mcm10	Mcm10	66	MCM10

Table 2. Replication fork proteins. Names of budding yeast factors, molecular weights and human orthologs are listed.

Figure 1

Figure 2

Figure 3

Figure 4