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Roles of SUMO in Replication Initiation, Progression, and Termination

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Abstract

Accurate genome duplication during cell division is essential for life. This process is accomplished by the close collaboration between replication factors and many additional proteins that provide assistant roles. Replication factors establish the replication machineries capable of copying billions of nucleotides, while regulatory proteins help to achieve accuracy and efficiency of replication. Among regulatory proteins, protein modification enzymes can bestow fast and reversible changes to many targets, leading to coordinated effects on replication. Recent studies have begun to elucidate how one type of protein modification, sumoylation, can modify replication proteins and regulate genome duplication through multiple mechanisms. This chapter summarizes these new findings, and how they can integrate with the known regulatory circuitries of replication. As this area of research is still at its infancy, many outstanding questions remain to be explored, and we discuss these issues in light of the new advances.

Keywords

DNA replication initiation; Replication progression; Posttranslational modifications; Sumoylation; Ubiquitination; Phosphorylation

1 Overview of Eukaryotic DNA Replication

1.1 Replication Initiation

DNA replication occurs in three stages, namely, initiation, progression, and termination. Each of these stages entails multi-step DNA transactions carried out by dozens of proteins. Most of the replication steps and proteins are highly conserved from simple model organisms, such as yeasts, to humans. Replication initiation begins with the licensing of genomic sites called origins. Origin licensing takes place in late M to G1 phase when origins become bound by the MCM complex, a ring-shaped complex composed of MCM2–7 subunits (Fig. 1). This process is achieved by interaction between MCM and MCM-loading factors. In budding yeast, wherein origin licensing is best understood, the MCM-loading

factors include the origin recognition complex (ORC), Cdc6, and the MCM binding partner Cdt1 (Fig. 1) [reviewed in (Diffley et al. 1994; Kelly and Brown 2000; Bell and Dutta 2002; Scalfani and Holzen 2007; Remus and Diffley 2009; Li and Araki 2013; Bell and Labib 2016)]. ORCs demarcate origins by interacting with specific DNA sequences and chromatin components [reviewed in (Hoggard and Fox 2016; Gutiérrez and MacAlpine 2016)]. Cdc6 recruits the MCM-Cdt1 complex to ORC through interactions with both factors (Santocanale and Diffley 1996; Donovan et al. 1997; Speck et al. 2005; Randell et al. 2006; Sun et al. 2013). Subsequently, ATP hydrolysis by ORC, Cdc6, and MCM enables a pair of MCM rings to enclose DNA at origins (Fig. 1) (Bowers et al. 2004; Randell et al. 2006; Remus et al. 2009; Fernández-Cid et al. 2013; Frigola et al. 2013; Coster et al. 2014). This multistep process produces an MCM double hexamer with its central channel enclosing DNA [reviewed in (Diffley et al. 1994; Kelly and Brown 2000; Bell and Dutta 2002; Scalfani and Holzen 2007; Remus and Diffley 2009; Wei and Zhao 2016b; Li and Araki 2013; Bell and Labib 2016)].

Once loaded, the MCM double hexamer must be kept inactive until the onset of S phase when origin firing takes place. One critical event during origin firing is the conversion of MCM into the replicative helicase, composed of MCM and its cofactors Cdc45 and the four-subunit GINS complex. This conversion requires two protein kinases, DNA polymerase ϵ , and several scaffolding proteins, including Sld2, Sld3–Sld7, and Dpb11 in budding yeast and their homologs in other organisms (Gambus et al. 2006; Moyer et al. 2006; Pacek et al. 2006; Ilves et al. 2010; Muramatsu et al. 2010; Kang et al. 2012). The first kinase, the Dbf4-dependent kinase (DDK), phosphorylates MCM subunits (Fig. 1). Mcm4 phosphorylation is particularly important as it is recognized by Sld3 in partnership with Sld7 and Cdc45 (Fig. 1) (Sheu and Stillman 2006; Sheu and Stillman 2010; Deegan et al. 2016). Subsequent to this step, the second kinase, cyclin-dependent kinase (CDK), phosphorylates Sld2 and Sld3, enabling their interaction with Dpb11 in cooperation with Pol ϵ and GINS (Fig. 1) (Tanaka et al. 2007; Zegerman and Diffley 2007; Muramatsu et al. 2010). As a result of this cascade of protein interactions, Cdc45 and GINS are delivered to MCM, resulting in the formation of the replicative helicase CMG (Cdc45-MCM-GINS) (Fig. 1) (Gambus et al. 2006; Moyer et al. 2006; Pacek et al. 2006; Ilves et al. 2010; Kang et al. 2012).

Subsequent to CMG formation, DNA polymerase α , the chromatin remodeling complex FACT, and several scaffolding proteins are recruited to CMG and Pol ϵ to form the replisome (Fig. 1) (Gambus et al. 2006, 2009; Morohashi et al. 2009). In the meantime, the origin firing scaffolds, such as Sld2, Sld3, and Dpb11, leave the CMG and are recycled to additional origins that fire later in S phase (Fig. 1) (Mantiero et al. 2011). The temporal order of the origin firing program is determined by multiple factors, such as the affinity between ORCs and origin sequences and local chromatin environment [reviewed in (Masai et al. 2010; Fragkos et al. 2015)]. At both early- and late-fired origins, the formation of a pair of replisomes establishes divergent replication forks that travel in opposite directions.

1.2 Replication Progression

As DNA synthesis begins, the DNA primase-Pol α complex generates primer sequences (Fig. 1). These primers can be extended by Pol ϵ for continuous leading strand synthesis and

by Pol δ to produce many Okazaki fragments during discontinuous lagging strand synthesis (Fig. 1) [reviewed in (Kelly and Brown 2000; Bell and Dutta 2002; Sclafani and Holzen 2007; Bell and Labib 2016)]. The maturation of Okazaki fragments requires several additional conserved enzymes. In budding yeast, these include the flap endonuclease Rad27, the DNA helicase-nuclease Dna2, the exonuclease Exo1, the DNA helicase Pif1, and the ligase Cdc9 [reviewed in (Waga and Stillman 1998; Bell and Labib 2016)]. Collaboration among these factors enables ligation of Okazaki fragments.

During replication progression, a major challenge is coping with many types of template blockages. These can include (i) the topological stress generated by DNA unwinding, (ii) tightly bound nonhistone proteins, (iii) difficult-to-replicate genomic loci, (iv) collision with transcription machinery, and (v) DNA lesions generated from both intrinsic and extrinsic sources. Topological stress is largely relieved by replisome-associated topoisomerases. Several scaffold proteins within the replisome play pivotal roles in dealing with other replication impediments. Depending on the types of blocks, different proteins and strategies are used, and in many cases, additional DNA metabolism proteins are recruited to overcome template blocks. For example, tightly bound nonhistone proteins can be removed by DNA helicases, such as Rrm3 in budding yeast, allowing the resumption of DNA synthesis (Ivessa et al. 2000; Calzada et al. 2005; Azvolinsky et al. 2006). In the case of template damage, such as UV-induced thymidine dimers, translesion polymerases can mediate synthesis bypass of these sites [reviewed in (Waters et al. 2009)].

Besides template blockage, other issues that must be managed during replication progression include the removal of template nucleosomes ahead of replication forks and reestablishment or recycling of nucleosomes with correct positioning behind replication forks. In addition, replication progression is coupled with the establishment of sister chromatid cohesion and inheritable epigenetic markers. These topics have been recently summarized, and we refer the readers to several reviews for details [reviewed in (Jeppsson et al. 2014; Almouzni and Cedar 2016; Bell and Labib 2016)].

1.3 Replication Termination

As two opposing replication forks from adjacent origins converge, replication terminates. In general, replication termination sites are determined by the meeting point of two forks, but in some instances, termination occurs at replication pausing sites where one fork has more retention time (Labib and Hodgson 2007; Fachinetti et al. 2010). Three major events are required for replication termination, including the completion of local DNA synthesis, decatenation of the two daughter strands by topoisomerases, and disassembly of replisome. Compared with replication initiation and progression, replication termination is less well understood. More recently, new findings have implicated MCM ubiquitination in the disassembly of replisomes during termination (Maric et al. 2014; Priego Moreno et al. 2014; Dewar et al. 2015).

2 The SUMO Modification Cycle

Protein modifications underpin many regulatory mechanisms during the three stages of replication. Phosphorylation and ubiquitination have been found to be critical in all stages of

replication [reviewed in (Wei and Zhao 2016b; Kelly and Brown 2000; Diffley 2004; Vodermaier 2004; Blow and Dutta 2005; Arias and Walter 2007; Sclafani and Holzen 2007; Moreno and Gambus 2015; Sivakumar and Gorbsky 2015; Garcia-Rodriguez et al. 2016)]. More recently, sumoylation has also been found to influence replication and is important for genome integrity. In this chapter, we summarize the findings that begin to unravel the mechanisms of SUMO-based replication regulation after a brief introduction of protein sumoylation.

2.1 Principles of the Sumoylation Process

SUMO (small ubiquitin-like modifier) is a highly conserved member of the ubiquitin family of protein modifiers. With approximately 100 amino acids, SUMO assumes an ubiquitin fold but with a distinct surface charge distribution. The SUMO-specific E1 (or activating enzyme), E2 (or conjugating enzyme), and E3 (or ligase) enzymes conjugate SUMO to the ϵ -amino group lysine residue of a substrate (Johnson 2004) (Fig. 2). Most organisms contain one SUMO E1 and E2 but multiple SUMO E3s. SUMO E2 can directly bind to the so-called sumoylation consensus or reverse consensus sequences, [Ψ KX(D/E)] or [(D/E)XK Ψ] (Ψ , a hydrophobic residue; X, any residue). With the help of E3s, SUMO is then transferred from E2 to the lysine within such sequences (Gareau and Lima 2010; Lamoliatte et al. 2014). However, proteomic studies found that many of these sites are not sumoylated, suggesting that additional factors must also influence the sumoylation process (Gareau and Lima 2010; Lamoliatte et al. 2014). For example, it has been noted that sumoylation sites are often in loop regions (Gareau and Lima 2010), likely because of the ability of such regions to adopt local conformational changes that favor productive contact between the E2~SUMO thioester bond and the acceptor lysine. This principle could also explain findings that while sumoylation is conserved among many protein homologs, the sites of modification often vary (Golebiowski et al. 2009; Dou et al. 2010; Elrouby and Coupland 2010; Cremona et al. 2012; Psakhye and Jentsch 2012; Hendriks et al. 2014; Ma et al. 2014; Tammsalu et al. 2014).

SUMO E3s play important roles partly by bridging E2 and substrates. For instance, budding yeast has the homologous Siz1 and Siz2 SUMO E3s and the Mms21 E3, all of which possess an SP-RING domain that can associate with Ubc9 (Johnson and Gupta 2001; Takahashi et al. 2001; Zhao and Blobel 2005; Gareau and Lima 2010). In addition, each E3 can associate with specific substrates. For example, Siz2 interacts with the ssDNA-binding protein RPA, promoting RPA sumoylation and Siz2 localization to DNA breaks (Chung and Zhao 2015). Mms21 is a part of the Smc5/Smc6 complex that localizes to several genomic loci to influence sumoylation events in these places (Murray and Carr 2008; De Piccoli et al. 2009; Hang et al. 2015; Bonner et al. 2016; Bermudez-Lopez et al. 2016). These interactions and the ability of E3s to enable productive alignment between E2 and substrates for SUMO transfer make SUMO E3s indispensable for sumoylation in vivo [reviewed in (Gareau and Lima 2010)]. Based on studies from several organisms, SUMO E3s often exhibit substrate redundancy, likely reflecting their similar SUMO transfer mechanisms (Hang et al. 2014; Sarangi et al. 2014).

Mammalian cells have larger numbers of SUMO E3s than yeast. At least ten E3s have been described in human cells thus far (Gareau and Lima 2010; Cappadocia et al. 2015; Eisenhardt et al. 2015). These can be divided into several groups, including (1) PIAS proteins that are homologs of the yeast Siz1 and Siz2, (2) the Mms21 homolog NSMCE2, (3) the ZNF451 type of SUMO E3s that utilize tandem SUMO-interacting motifs (SIMs) to enable sumoylation (Cappadocia et al. 2015; Eisenhardt et al. 2015), and (4) more specialized SUMO E3s that target specific processes, such as the nuclear pore protein Ran binding protein 2 (RanBP2), the polycomb group protein Pc2, and the promyelocytic leukemia (PML) protein. The increased numbers of SUMO E3s in human cells are associated with the presence of multiple SUMO isoforms (Gareau and Lima 2010; Liang et al. 2016). SUMO2 and SUMO3 have 97% sequence identity and can form SUMO chains, while the more divergent SUMO1 is less frequently found in SUMO chains [reviewed in (Gareau and Lima 2010)]. The acquirement of different SUMO isoforms and the many types of SUMO chains that they can form, in conjunction with the multiple types of SUMO E3s in human cells, can meet the needs of more complex genomes and increased demands for regulation.

2.2 Principles of the Desumoylation Process

Sumoylation can be reversed by multiple SUMO-specific cysteine proteases, known as desumoylation enzymes (Mukhopadhyay and Dasso 2007; Hickey et al. 2012) (Fig. 2). The substrate selectivity of desumoylation enzymes is partly achieved by their distinct localizations. Using budding yeast as an example, one of its desumoylation enzymes, Ulp1, primarily associates with nuclear pore complexes, whereas the other enzyme, Ulp2, can be seen concentrated in the nucleolus (Li and Hochstrasser 1999, 2000; Panse et al. 2003; Kroetz et al. 2009; Srikumar et al. 2013). Consistent with these localization patterns, Ulp1 and Ulp2 have different substrates (Makhnevych et al. 2009; de Albuquerque et al. 2016; Wei and Zhao 2016a). In addition, Ulp1 enables SUMO maturation by removing the tail of the precursor SUMO molecule (Li and Hochstrasser 1999), while Ulp2 has a major role in removing SUMO chains (Li and Hochstrasser 2000). Both Ulp1 and Ulp2 are required for cell fitness and resistance to a broad range of genotoxins (Li and Hochstrasser 1999, 2000; Schwartz et al. 2007). As Ulp2 mutant defects are suppressed to a large degree by mutating the lysine residues on SUMO, which prevents SUMO chain formation, accumulation of SUMO chains is deleterious (Bylebyl et al. 2003). Human cells contain at least six desumoylation enzymes, called sentrin-/SUMO-specific proteases (SENPs) (Hannoun et al. 2010; Hickey et al. 2012). SENP1, 2, 3, and 5 are more related to Ulp1, whereas SENP6 and 7 are similar to Ulp2 [reviewed in (Mukhopadhyay and Dasso 2007)]. As is the case for Ulp1 and Ulp2 in yeast, SENPs have distinct activities and cellular localization patterns, and their mutants cause a wide range of defects [reviewed in (Mukhopadhyay and Dasso 2007; Hickey et al. 2012)].

2.3 Biochemical Effects of Sumoylation

Protein sumoylation affects a myriad of biological processes, such as transcription, nuclear transport, DNA metabolism, and protein quality control [reviewed in (Sarangi and Zhao 2015)]. The conjugation and removal of SUMO from proteins can alter protein-protein interactions, partly because SUMO modules can interact with SIMs on other proteins or the

substrate itself. As such, SUMO-SIM interactions can promote the assembly of protein complexes and the formation of a membrane-free nuclear compartment [reviewed in (Shen et al. 2006)]. On the other hand, SUMO sometimes disrupts existing protein-protein interactions or protein aggregation, possibly due to steric hindrance posed by the SUMO moiety. Additionally, sumoylation can alter a protein's interaction with DNA or chromatin, its enzymatic activities, or its protein levels. Each of these effects has been observed in DNA metabolism processes, particularly in DNA repair. For example, sumoylation enhances inter-subunit association among the DNA helicase-topoisomerase Sgs1-Top3-Rmi1 complex and promotes its function in resolving Holliday junctions (Bermudez-Lopez et al. 2016; Bonner et al. 2016). On the other hand, sumoylation of the DNA nuclease cofactor Sae2 helps convert the protein from insoluble aggregates to a soluble form, which is required for DNA-end resection (Sarangi et al. 2015). In the case of the recombination mediator protein Rad52, sumoylation leads to association with the segregase Cdc48/p97 that removes proteins from DNA using its ATPase activity (Bergink et al. 2013). These examples illustrate some of the mechanisms by which sumoylation regulates genome maintenance. Recent studies have begun to reveal the roles of sumoylation during DNA replication, and the following sections summarize findings in this area.

3 SUMO-Based Regulation of Replication Initiation

Proteomic studies in multiple organisms have shown that protein factors that help replisomes cope with template obstacles are enriched among SUMO substrates (Golebiowski et al. 2009; Elrouby and Coupland 2010; Cremona et al. 2012; Hendriks et al. 2014; Ma et al. 2014; Tammsalu et al. 2014). Genetic and biochemical studies of individual SUMO substrates and SUMO enzymes have revealed some mechanisms of SUMO-based regulation of these proteins and how they affect DNA replication.

3.1 MCM Sumoylation Inhibits Replication Initiation in Budding Yeast

In budding yeast, all six subunits of MCM are sumoylated (Cremona et al. 2012; de Albuquerque et al. 2016; Wei and Zhao 2016a). MCM sumoylation has a distinct spatial and temporal pattern relative to the cycle of DNA replication (Wei and Zhao 2016a). Spatially, MCM is only sumoylated when loaded onto origins (Wei and Zhao 2016a). Temporally, Mcm2–6 sumoylation levels peak during G1 phase prior to DDK-mediated Mcm4 phosphorylation, then decline as cells enter S phase, and again increase during G2/M phase, coincident with the next MCM-loading cycle (Wei and Zhao 2016a). The opposing patterns of Mcm2–6 sumoylation and MCM phosphorylation during the cell cycle indicate a negative role of MCM sumoylation during replication initiation. Indeed, increased MCM sumoylation causes a reduction in the levels of Mcm4 phosphorylation, CMG, and origin firing (Wei and Zhao 2016a). These defects are partly because hyper-sumoylated MCM has increased association with the PP1 phosphatase, which reverses Mcm4 phosphorylation (Wei and Zhao 2016a; Davé et al. 2014; Hiraga et al. 2014; Mattarocci et al. 2014). MCM sumoylation levels subside at the start of S phase, partly through the action of Ulp2 (Wei and Zhao 2016a; de Albuquerque et al. 2016). These findings suggest that MCM sumoylation serves as a safeguard to prevent premature helicase function before S phase and that initiation of DNA synthesis requires removing this modification (Wei and Zhao 2016a). Future work is

needed to examine whether the observed effects are due to a particular MCM subunit or contributions from multiple subunits and whether sumoylation alters other MCM features in addition to PP1 regulation.

3.2 MCM Sumoylation in Higher Eukaryotes

MCM sumoylation has also been detected in higher eukaryotes (Golebiowski et al. 2009; Elrouby and Coupland 2010; Hendriks et al. 2014; Ma et al. 2014; Schimmel et al. 2014; Tammsalu et al. 2014). For example, human MCM2, 3, 4, and 7 proteins are sumoylated. Importantly, Mcm4 sumoylation levels exhibit a similar pattern to that of yeast Mcm2–6 during the cell cycle, peaking in G1, declining in S phase, and increasing again during the subsequent G1 phase (Schimmel et al. 2014). It is reasonable to envision that human MCM sumoylation also provides a regulatory mechanism to restrain origin firing. A negative role for sumoylation in replication initiation can also be inferred from findings in *Xenopus*, wherein increased origin firing occurs after reducing sumoylation, either by expression of a dominant-negative SUMO E2 or addition of SUMO-specific proteases (Bonne-Andrea et al. 2013). Given *Xenopus* MCM subunits are sumoylated (Ma et al. 2014), it is worthy of consideration whether this modification underlies the negative effect of sumoylation in replication initiation in this system. Considering that PP1- and DDK-mediated MCM regulation is conserved across species (Wotton and Shore 1997; Lee et al. 2003; Cho et al. 2006; Masai et al. 2006; Montagnoli et al. 2006; Tsuji et al. 2006; Cornacchia et al. 2012; Hayano et al. 2012; Yamazaki et al. 2012), the targeting of this pathway by MCM sumoylation to prevent premature initiation, as seen in yeast, may be conserved. Direct tests of these ideas will clarify the roles of MCM sumoylation in higher eukaryotes.

3.3 ORC2 Sumoylation Prevents Re-replication at Centromeric Regions

ORC, composed of ORC1–6 subunits, binds to replication origins and is critical for MCM loading during origin licensing (Diffley et al. 1994; Kelly and Brown 2000; Bell and Dutta 2002; Sclafani and Holzen 2007; Remus and Diffley 2009; Li and Araki 2013). In addition, ORC2 can dissociate from replication origins and localize to centromeric regions during G2/M phase (Craig et al. 2003; Prasanth et al. 2004; Lee et al. 2012), coincident with the appearance of SUMO2-modified ORC2 in human cells (Huang et al. 2016). Elimination of ORC2 sumoylation by mutating its two sumoylation sites leads to re-replication, polyploidy, and genome damage (Huang et al. 2016). Mechanistically, ORC2 sumoylation promotes the recruitment of KDM5A (Huang et al. 2016), a histone H3 lysine 4 (H3K4) demethylase (Defeo-Jones et al. 1991; Christensen et al. 2007; Klose et al. 2007). Loss of ORC2 sumoylation results in elevated levels of tri-methylated H3K4 (H3K4me3) in centromeric chromatin, reduced transcription of α -satellites at centromeres, and decondensation of pericentric heterochromatin, which correlates with re-replication of the pericentric region (Huang et al. 2016). It remains to be determined how the change in chromatin environment caused by the perturbation of the ORC2-KDM5A axis leads to re-replication, despite the presence of multiple mechanisms that prevent re-replication. In budding yeast, multiple ORC subunits are sumoylated (Cremona et al. 2012), but the functions of this modification have yet to be determined.

3.4 Other Potential SUMO Substrates Affecting Replication Initiation

Several other proteins involved in replication initiation are SUMO substrates, such as the ssDNA-binding protein RPA and CDK (Dou et al. 2010; Cremona et al. 2012; Bonne-Andrea et al. 2013). Sumoylation of both human and yeast RPA has been reported to promote homologous recombination during DNA repair, though whether it also has a role in replication initiation has not been tested (Dou et al. 2010; Psakhye and Jentsch 2012). Cyclin E has been shown to be a SUMO2/SUMO3 substrate in *Xenopus* (Bonne-Andrea et al. 2013). Its sumoylation is detectable during replication and is independent of its kinase activity (Bonne-Andrea et al. 2013). A direct effect of cyclin E sumoylation in replication initiation remains to be determined. Because multiple proteins involved in origin licensing and firing are SUMO substrates, we anticipate the presence of multiple mechanisms through which SUMO regulates timing and efficiency of origin firing and prevents harmful rereplication events.

4 SUMO-Based Regulation of Replication Progression

Genetic studies using SUMO E2 and E3 mutants have shown that reducing sumoylation retards replication progression, particularly under replicative stress (Cremona et al. 2012; Schimmel et al. 2014; Hang et al. 2015). For example, mutating SUMO E3s in budding yeast impairs replication when cells are treated with the DNA-alkylating agent methyl methanesulfonate (MMS) (Cremona et al. 2012). In human cell lines, reducing UBC9 function leads to a prolonged S phase (Schimmel et al. 2014). The effects of sumoylation in replication progression may be broad, as many proteins central for this process are subject to sumoylation. Aside from MCM, several other replisome components are sumoylated, including Pol ϵ , Pol δ , the processivity factor PCNA, topoisomerases, DNA primase, and the nucleosome remodeling factor FACT (Golebiowski et al. 2009; Elrouby and Coupland 2010; Cremona et al. 2012; Hendriks et al. 2014; Ma et al. 2014; Tammsalu et al. 2014). In addition, several proteins that collaborate with replisome for DNA synthesis are sumoylated (Golebiowski et al. 2009; Elrouby and Coupland 2010; Cremona et al. 2012; Hendriks et al. 2014; Ma et al. 2014; Tammsalu et al. 2014). Some examples include subunits of the SMC complexes (cohesin, condensin, and Smc5/Smc6), the SMC-like Mre11 complex, and the clamp loader RFC complex (Golebiowski et al. 2009; Elrouby and Coupland 2010; Cremona et al. 2012; Hendriks et al. 2014; Ma et al. 2014; Tammsalu et al. 2014). Among these proteins, the sumoylation of PCNA has been well examined and shown to recruit the anti-recombinase Srs2 to impaired replication forks in order to prevent toxic recombination events (Papouli et al. 2005; Armstrong et al. 2012). The molecular function of this modification, in conjunction with other types of PCNA modifications, has been extensively reviewed elsewhere [reviewed in (Mailand et al. 2013; Ulrich and Takahashi 2013)]. Below we focus on recent findings regarding additional effects SUMO has on replication progression, mostly derived from studying the combined effects of loss of sumoylation of many substrates, but with a few mechanistic studies as well.

4.1 SUMO-Based Regulation of Replisome Components

Following up earlier observations that Mms21 SUMO ligase mutations impair replication, our group showed that under MMS conditions, Mms21 and the associated Smc5/Smc6

complex promote sumoylation of Mcm6 and Pol2, the catalytic subunit of Pol ϵ (Hang et al. 2015). As physical interactions are detected between the Smc5/Smc6 complex and these substrates, the effects seen are likely to be direct (Hang et al. 2015). In addition, as Smc5/Smc6 deficiency impairs replication at regions far from fired origins, the Smc5/Smc6 complex may use sumoylation to facilitate later stages of replication. Future tests can help us understand whether MCM sumoylation has a distinct role during replication stress and whether Pol2 sumoylation influences DNA polymerization.

Several replisome members have been found to be sumoylated in human cells (Golebiowski et al. 2009; Hendriks et al. 2014; Schimmel et al. 2014; Tammsalu et al. 2014). Interestingly, under ATR inhibition conditions, sumoylation of replisome components can lead to fork collapse (Ragland et al. 2013). This largely depends on the SUMO-targeted ubiquitin ligase RNF4 and endonuclease scaffold protein SLX4 (Ragland et al. 2013). It is thought that under such conditions, RNF4 can target sumoylated replisomes for degradation, rendering replication forks accessible for SLX4-mediated cleavage (Ragland et al. 2013). In another study, sumoylation of two Fanconi anemia (FA) proteins, namely, FANCD2 and FANCI, was shown to trigger RNF4-mediated ubiquitination of these proteins and subsequent removal from DNA damage sites (Gibbs-Seymour et al. 2015). Lack of this regulation reduces the ability of cells to cope with replication stress, likely due to blockage of FANCD2 and FANCI from recycling among different damage sites (Gibbs-Seymour et al. 2015). Both of these studies highlight the complex interplay between sumoylation and ubiquitination for replication fork management.

The above notion is further extended by another study utilizing iPOND (isolation of proteins on nascent DNA) coupled with mass spectrometry-based protein analyses in human cells. It has been reported that SUMO is enriched on nascent chromatin, while ubiquitin molecules are enriched on mature chromatin (Lopez-Contreras et al. 2013). A follow-up study showed that the SUMO deubiquitinase, USP7, contributes to the establishment of this SUMO-high and ubiquitin-low nascent chromatin environment (Lecona et al. 2016). USP7 can remove ubiquitin molecules that are conjugated to SUMO2 *in vitro* and *in vivo* and is associated with nascent chromatin and MCM4 (Lecona et al. 2016). The authors suggest that through this mechanism, USP7 reduces ubiquitin levels and allows enrichment of SUMO2 on replisome components. This role likely contributes to USP7's function in maintaining normal rates of fork speed and origin firing (Lecona et al. 2016). These studies suggest that enrichment of SUMO and reduction of ubiquitin at or near replisomes can be advantageous for replication progression. Further investigation is needed to provide mechanistic insights into the relevant sumoylation events critical for DNA synthesis and the full spectrum of the effects of USP7 and RNF4 in keeping a balance between sumoylation and ubiquitination of replisome components under different conditions.

4.2 SUMO-Based Regulation of Lagging Strand Synthesis

Lagging strand Okazaki fragment processing involves sequential reactions of gap filling by polymerase δ , flap cleavage by flap endonuclease 1 (FEN1), and nick ligation by DNA ligase 1 (LIG1) (Waga and Stillman 1998). In human cells, modification of FEN1 by SUMO3 at lysine 168 occurs in S phase and peaks in G2/M phase (Guo et al. 2012). The

sumoylation of FEN1 is dependent on its phosphorylation, which occurs during G1 phase, peaking in late S phase. Sumoylation of FEN1 appears to occur after its phosphorylation, as the non-phosphorylatable FEN1 mutant (S187A) is not sumoylated (Guo et al. 2012). Furthermore, sumoylation of FEN1 triggers its ubiquitination and subsequent proteasome-mediated degradation, presumably by recruiting the SIM-containing ubiquitin E3 ligase PRP19 (Guo et al. 2012). The timely degradation of FEN1 via this cascade of modifications is critical for maintaining genome stability, as its deregulation leads to cell cycle delay and polyploidy (Guo et al. 2012). Other than FEN1, polymerase δ is also reported to be SUMO substrate in organisms from yeast to humans (Cremona et al. 2012; Hendriks et al. 2014; Tammsalu et al. 2014). It remains to be determined if polymerase δ sumoylation has a role in DNA lagging strand synthesis.

4.3 SUMO-Based Regulation of Sister Chromatid Cohesion

As replication progresses, the two sister chromatids stay close together partly through the function of cohesin [reviewed in (Blow and Tanaka 2005; Sherwood et al. 2010)]. The ring-shaped cohesin complex is loaded onto chromatin before S phase and encloses sister chromatids to keep them connected during S phase [reviewed in (Blow and Tanaka 2005; Sherwood et al. 2010)]. The resulting sister chromatid cohesion is important for supplying faithful templates for DNA repair and for ensuring each daughter cell receives one set of chromosomes during mitosis [reviewed in (Blow and Tanaka 2005; Sherwood et al. 2010)]. Four subunits of cohesin, including the rod-shaped Smc1 and Smc3 proteins and the associated Scc1 and Scc3 proteins, are sumoylated in yeast and human cells, partly in an Mms21-dependent manner (Denison et al. 2005; Potts et al. 2006; Almedawar et al. 2012; Golebiowski et al. 2009; Hendriks et al. 2014; Tammsalu et al. 2014). In budding yeast, sumoylation of Smc1 and Scc1 occurs after cohesin loading, and Scc1 sumoylation is independent of another important modification for cohesion establishment, namely, Smc3 acetylation (Almedawar et al. 2012). Decreasing cohesin sumoylation impairs cohesion, without affecting the integrity of the cohesin complex (Almedawar et al. 2012). Based on these observations, it was proposed that cohesin sumoylation is required for the establishment of cohesion.

Another study further highlights the importance of SUMO-based regulation of cohesion. When a cohesion establishment factor, Pds5, is mutated in budding yeast, Scc1 becomes hyper-sumoylated and is degraded (D'Ambrosio and Lavoie 2014). This correlates with precocious separation of sister chromatids (D'Ambrosio and Lavoie 2014). Such a defect can be suppressed through removal of the SUMO E3 ligase Siz2 or the SUMO-targeted ubiquitin ligase Slx5/Slx8, supporting the hypothesis that toxic sumoylation events underlie *pds5-1* defects (D'Ambrosio and Lavoie 2014). The authors suggest that Pds5 plays a role in preventing hyper-sumoylation of cohesin and maintaining cohesin levels until mitosis. The full picture of how SUMO regulates cohesion is likely more complex. For example, Pds5 itself is sumoylated by Siz2 from S to G2/M phase, though the biochemical effect of this modification is not known (Stead et al. 2003). The timing of sumoylation and ubiquitination of cohesin is critical, and it will be of interest to determine how their temporal order is established, perhaps through the regulation of sumoylation and desumoylation enzymes or through other cohesion regulators.

4.4 SUMO-Based Regulation of Topoisomerase Function

Topoisomerases are essential for releasing topological stress and promoting replication fork progression (Champoux 2001; Wang 2002; Vos et al. 2011). They also contribute to the removal of transcription-generated DNA-RNA hybrids, known as R-loops, which pose a barrier for replication (Gan et al. 2011; Aguilera and Garcia-Muse 2012). A recent study showed that sumoylation of human TOP1 provides a means to reduce R-loop-mediated replication fork stalling via two distinct mechanisms (Li et al. 2015). Upon sumoylation by PIAS1, TOP1 showed improved interactions with the active form of RNA polymerase II (RNAPII α), leading to recruitment of splicing factors to avoid R-loop formation (Li et al. 2015). In addition, sumoylation of TOP1 reduces its enzymatic activity, potentially leading to reduced TOP1-induced DNA nicking at transcriptionally active regions (Li et al. 2015). Both effects of TOP1 sumoylation could contribute to lessening barriers for replication forks. It remains to be tested if these effects of TOP1 sumoylation are conserved in other organisms.

5 Potential SUMO Substrates Affecting Replication Termination

Although a full understanding of replication termination is still elusive, several new discoveries have shed light into this process. In budding yeast, *C. elegans*, and *Xenopus*, ubiquitination of Mcm7 has been shown to be a key event for disassembly of the replisome. In budding yeast, the Dia2 ubiquitin ligase that is part of the replisome can ubiquitinate Mcm7 when replication forks converge. This modification is then recognized by the segregase Cdc48/p97, leading to removal of MCM from chromatin and replisome disassembly (Maric et al. 2014; Priego Moreno et al. 2014). In *C. elegans* and *Xenopus*, replisome-associated E3 ligase *CUL-2^{LRR-1}* and the segregase remove CMG during termination (Sonneville et al. 2017; Dewar et al. 2017). Interestingly, in budding yeast, Mcm7 sumoylation appears to be regulated distinctly from that of Mcm2–6, with its levels only declining when the bulk of DNA replication has been completed (Wei and Zhao 2016a). It will be interesting to investigate whether Mcm7 sumoylation could trigger its ubiquitination or contribute to replication termination.

During replication termination, decatenation of sister chromatids requires Top2. Top2 sumoylation has been found in human, mouse, *Xenopus*, and yeast [reviewed in (Lee and Bachant 2009)]. Sumoylation of Top2 in vertebrates promotes the recruitment of Top2 or the chromosomal passenger complex to kinetochores during mitosis to facilitate chromosome segregation [reviewed in (Lee and Bachant 2009)]. Whether Top2 sumoylation plays a role in decatenation during replication termination is not known. With more molecular details of replication termination becoming available in the future, the examination of SUMO substrates involved in replication termination will reveal more details of this process.

6 Concluding Remarks

Each stage of DNA replication is intricately regulated to ensure precise genome duplication. Posttranslational modifications provide a dynamic regulatory means at multiple stages of the replication process. Phosphorylation- and ubiquitination-based modes of regulation are essential for replication, and the role of sumoylation in replication is emerging from several

recent studies. During the replication initiation stage, sumoylation of MCM (yeast) and ORC2 (human) can influence origin firing. In addition, sumoylation promotes replication progression through multiple mechanisms, such as lagging strand synthesis, reducing R-loops, replication fork metabolism, and sister chromatid cohesion. However, only a small number of SUMO substrates have been studied thus far as summarized in Table 1, and our understanding of how sumoylation regulates replication is still at an early stage. With more advanced methods to map sumoylation sites and tools to alter the sumoylation status of substrates, detailed molecular mechanisms of how sumoylation regulates each substrate will be elucidated. Future work will also help to establish a clear picture of how sumoylation is coordinated with other types of protein modifications during DNA replication. In addition, examination of how sumoylation and desumoylation enzymes are themselves regulated can also reveal how SUMO modification cycles facilitate DNA replication. As SUMO enzyme deficiencies, such as SUMO E1 and E2 depletion or SUMO E3 mutations, have been implicated in cancer and inherited human syndromes (Eifler and Vertegaal 2015; He et al. 2015; Yu et al. 2015), understanding their roles in genome duplication will provide new avenues for disease detection and treatment strategies.

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Fig. 1.

Summary of main steps of DNA replication initiation in budding yeast. The origin licensing step during late M to G1 phases entails free MCM being loaded onto replication origins as a double hexamer. This process requires several MCM-loading factors, including Cdc6, Cdt1, and the ORC complex. During the origin firing step in S phase, DDK and CDK kinases activate the loaded MCM. DDK-mediated phosphorylation of loaded MCM recruits Sld3 and its binding partners Cdc45 and Sld7. CDK-mediated phosphorylation of Sld2 and Sld3 promotes the recruitment of Pol ϵ , Dpb11, and the GINS complex. Cdc45, MCM, and GINS form the active replicative helicase CMG. Subsequent recruitment of additional protein factors results in the formation of the replisome. SUMO can counteract DDK-mediated MCM phosphorylation. A fraction of loaded Mcm2–7 subunits are sumoylated to prevent premature replication initiation. This is partly achieved as SUMO aids the recruitment of the Rif1-PP1 phosphatase complex that can antagonize DDK action in G1. As S phase starts,

rising DDK levels are associated with increased MCM phosphorylation and decreased MCM sumoylation levels. Such a switch of MCM modification states is also seen in other organisms. Note that recently findings suggest that MCM also switches from enclosing dsDNA to enclosing mostly leading strand ssDNA template when DNA synthesis begins and that the N-terminal tier of the MCM ring faces the moving replication fork

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Fig. 2.

The SUMO conjugation cycle and SUMO's effects on substrate proteins. SUMO E1, E2, and E3 enzymes can conjugate a SUMO molecule to a lysine residue of a substrate. Sumoylation can also occur on multiple lysines of a substrate or in the form of SUMO chain (not shown). Sumoylation can have several biological effects on the substrates, and three frequently observed molecular consequences are indicated. Sumoylation can be reversed by SUMO isopeptidases

Table 1

Summary of sumoylated substrates involved in DNA replication

Replication	Substrate (function)	Molecular effect(s) of sumoylation
Initiation	<i>sc</i> MCM (replicative helicase)	Inhibit replication initiation via phosphatase recruitment
	<i>hs</i> ORC2 (replication origin recognition)	Prevent centromeric region re-replication by recruitment of the histone demethylase KDM5A
Progression	<i>hs</i> FANCD2 and <i>hs</i> FANCI (Fanconi anemia proteins)	Promote replication stress survival by triggering self-ubiquitination and removal from DNA damage sites
	<i>hs</i> FEN1 (flap endonuclease)	Promote genome stability, prerequisite for its ubiquitination and degradation
	<i>sc</i> Cohesin subunits (chromatid cohesion)	Promote sister chromatid cohesion
	<i>hs</i> TOP1 (topoisomerase)	Prevent R-loop formation by promoting interaction with splicing factors; reduce DNA nicking

Note that only the major function is indicated for each substrate

sc *Saccharomyces cerevisiae*, *hs* *Homo sapiens*