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Review

Review

Replication protein A and more: single-stranded DNA-binding proteins in eukaryotic cells

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Abstract

Single-stranded DNA-binding proteins (SSBs) play essential roles in DNA replication, recombinational repair, and maintenance of genome stability. In human, the major SSB, replication protein A (RPA), is a stable heterotrimer composed of subunits of RPA1, RPA2, and RPA3, each of which is conserved not only in mammals but also in all other eukaryotic species. In addition to RPA, other SSBs have also been identified in the human genome, including sensor of single-stranded DNA complexes 1 and 2 (SOSS1/2). In this review, we summarize our current understanding of how these SSBs contribute to the maintenance of genome stability.

Key words: genome instability, single-stranded DNA-binding proteins, replication protein A, sensor of single-stranded DNA complex

Introduction

Although genetic information is carried by double-stranded DNA (dsDNA), the practical use of that information requires the unwinding of duplex DNA [1,2]. As a result, single-stranded DNA (ssDNA) is probably one of the most abundant and important DNA intermediate structures in cells [1]. However, ssDNA is less stable and very easily forms spontaneous duplex DNA or is attacked by chemical and nucleolytic reagents. To deal with these risks, cells have evolved a group of protective ssDNA-binding proteins (SSBs) that bind ssDNA with high affinity and specificity [1,2].

SSBs are found in all bacterial, archaeal, and eukaryotic cells. Although they have a wide range of sequences and differ markedly in their subunit composition and oligomerization states, they all bind to ssDNA with high affinity and in a sequence-independent manner [1,2]. In this way, SSBs form nucleoprotein complexes with ssDNA, which serve as substrates for DNA replication, recombination, and repair processes. The only conserved motif that all SSBs have is the DNA-binding oligonucleotide/oligosaccharide binding (OB) fold, which is responsible for both ssDNA binding and oligomerization [3,4]. The primary sequence of OB folds is not well conserved, and they can vary in length from 70 to 150 amino acids, but each consists of at least a five-stranded β-sheet arranged as a β-barrel capped by an α-helix [3,5].

Replication Protein A: The Major SSB in Eukaryotic Cells

Replication protein A (RPA) is a nuclear SSB complex found in all eukaryotes and is required for many aspects of DNA metabolism [1,2,6,7]. It is produced by three separate genes encoding three subunits and functions as a heterotrimer. The three subunits of RPA are RPA1 (70 kDa), RPA2 (32 kDa), and RPA3 (14 kDa) [8–10]. RPA1 has four domains and is responsible for most of the complex's DNA-binding activity, including one N-terminal protein–protein interaction domain (amino acids 1–110, DBD-F) and three ssDNA-binding domains (DBD), arranged in tandem [DBD-A (amino acids 181–290), DBD-B (amino acids 301–422), and DBD-C (amino acids 436–616)] [11–14] (**Fig. 1**). RPA2 is the medium-sized subunit and has three domains, with the central domain called DBD-D (**Fig. 1**).

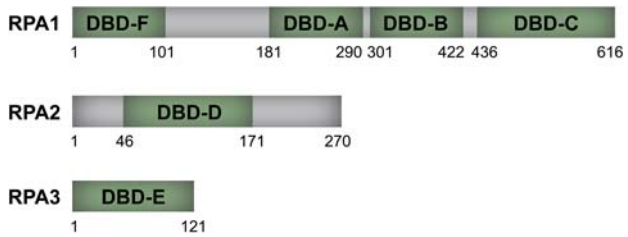

Figure 1. Schematic representation of human RPA

As the smallest subunit, RPA3 has only one domain (DBD-E) (**Fig. 1**). RPA was first defined and purified from human HeLa cell extracts in a study of DNA replication using a simian virus 40 (SV40) model system, as an indispensable component of SV40 DNA replication [15–17]. In a highly original study, RPA was found to successfully perform unwinding functions with T antigen, a virally encoded protein [18]. Since then, RPA has been studied extensively and is thought to be a crucial eukaryotic SSB involved in DNA replication, DNA recombination, and DNA repair. Homologs of the human RPA protein complex have been identified from almost every examined eukaryotic organism, and significant homology among species has been revealed for RPA proteins, especially RPA1, at the amino acid level [19–25].

Interactions of RPA with DNA

Over the past few decades, the DNA-binding properties of RPA have been investigated extensively, and several important features have been revealed. First, RPA binds to ssDNA with much higher affinity than to dsDNA or RNA [15,16,26]. Specifically, it was reported that RPA binds to ssDNA over 1000-folds more effectively than to dsDNA [27]. Second, the binding of RPA also exhibits sequence dependence, since it occurs preferentially at polypyrimidine sequences, rather than at polypurine ones [28,29]. Third, the length of the ssDNA sequence is also important for RPA binding [27]. Shorter ssDNA sequences have much lower binding affinity for RPA than the longer ones [27].

As mentioned above, the RPA complex contains six OB folds, four of which belong to the largest subunit, RPA1 [15,26,30]. Initial studies of RPA in human cells indicated that only the RPA1 subunit has significant ssDNA-binding activity, and that the ability of RPA1 to bind to ssDNA is similar to that for the whole heterotrimer [30,31]. Following the identification of residues ~180–420 as the DBD, further analysis in scRPA revealed that this central region of RPA1 contains two subdomains, DBD-A and DBD-B [11]. Moreover, studies of the crystal structure confirmed that the central DBD of hsRPA1 (residues 181–422) is composed of two subdomains with similar structures: DBD-A (residues 198–291) and DBD-B (residues 305–402), connected by a 15-amino acid linker [6,11,12,32–35].

However, the idea that RPA1 is the only DNA-binding subunit was soon challenged. First, a new RPA 8–10-nucleotide (nt) complex was identified, in addition to the known RPA 30-nt complex, which suggested the possibility that RPA could bind to ssDNA via multiple sites [28,36]. Researchers subsequently found that RPA2 could also bind to ssDNA. In contrast, human RPA2–RPA3 complex purified from *Escherichia coli* did not show significant ssDNA-binding activity and did not support SV40 DNA replication *in vitro* [37]. Despite such contradictory results, further research has suggested that the RPA2 DBD only becomes accessible after RPA1–ssDNA binding has occurred [38].

It was further revealed that the C-terminal region (residues 416–621) of RPA1 also contains a DBD (DBD-C), which is the fourth DBD that has been defined in the RPA complex [14]. It is now believed that such binding occurs sequentially, with the DBD-A and DBD-B motifs in RPA1 binding weakly to an 8–10 nt segment, followed by conformational changes that allow DBD-C and DBD-D to interact with longer ssDNA substrates (~30 nt) [39].

RPA and DNA Replication

As a main ssDNA-binding protein in eukaryotic cells, RPA is an essential factor in cellular DNA metabolism and plays key roles in multiple physiological processes, including DNA replication, cell division, and DNA repair [6].

Polyomavirus DNA replication systems such as SV40, BKV, and JCV are perfect model systems to study eukaryotic DNA replication. Among them, SV40 DNA replication is now best understood [17,40]. In this system, the viral genome is replicated via the collaboration of large T-antigen helicase and multiple proteins supplied by the host cell [41]. Researchers found that RPA, together with T antigen, assists in the origin unwinding [42]. Biochemical and genetic evidence has revealed that RPA interacts with T antigen and DNA polymerase α complex directly [43–49]. Taken together, these results suggest that the role of the RPA complex in SV40 DNA replication initiation does not depend solely on the protein–protein interaction of RPA with other proteins and its ssDNA-binding activity [48]. Besides T antigen and DNA polymerase α, other proteins involved in DNA replication, such as bovine papillomavirus E2, Epstein–Barr virus, and PCNA, have also been reported to interact physically with RPA [50–52]. Researchers even found some transactivator proteins, such as VP16 and GAL4, that bind to the RPA complex through direct interaction with RPA1, either to stabilize ssDNA at the replication origin or to recruit DNA polymerase α to the replication initiation complex [53].

hPrimpol1 is a recently identified DNA primase–polymerase that is involved in the response to DNA replication stress [54–62]. It possesses both primase and DNA polymerase activities *in vitro* and is recruited to sites of DNA damage and stalled replication forks through its direct interaction with RPA1 [57–62]. Evidence has indicated that RPA1 binding is required for the cellular function of hPrimol1 in response to DNA replication stress [57–62].

RPA and DNA Repair

Besides DNA replication, RPA is critical in many DNA repair processes, including nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), double-strand break (DSB) repair, and even telomere maintenance [2,6].

RPA and NER

NER is a DNA repair mechanism that repairs a broad spectrum of DNA damage, including cyclobutane pyrimidine dimers resulting from ultraviolet (UV) irradiation, as well as other types of lesions [63,64]. It was reported that RPA interacts with xeroderma pigmentosum group A (XPA), an early response factor in the NER pathway, and then stimulates its ability to bind to the damaged DNA site [65–68]. XPA is one of the pivotal factors in NER, since XPA deficiency results in relatively high sensitivity of cells in

response to UV light [69]. However, XPA itself has no enzyme activity and must function through a complex that includes multiple proteins (RPA, ERCC1, DDB2, and TFIIH) and damaged DNA [66,70,71]. It has been reported that RPA1 binds to the N-terminal region of XPA, and RPA2 binds to its central region [68]. The interaction between XPA and RPA is the first critical step in the entire NER pathway. Once the XPA–RPA complex is formed, the ERCC1–XPF and XPG endonucleases are recruited to the damaged site for excision [65,66,72]. It has also been reported that RPA interacts with XPG directly [73]. Although the direct interaction between RPA and ERCC1–XPF has not been detected, a ternary interaction among XPA, RPA, and ERCC1–XPF has been observed [72]. In addition, RPA is also involved in the gap-filling stage of NER, along with PCNA, RFC, and DNA polymerase δ or ε [73].

RPA and BER

Besides the NER pathway, RPA also participates in BER, although the function of RPA in BER remains somewhat unclear, and the results obtained thus far are confusing. One important protein named uracil-DNA glycosylase (UNG) plays critical roles in BER and has been reported to physically interact with RPA [74]. However, RPA has shown only very weak inhibitory effects on UNG activity [74]. In addition, researchers have observed that RPA markedly stimulates long-patch BER by facilitating gap filling along with DNA polymerase ε [75].

RPA and MMR

The MMR system recognizes and repairs base–base mismatches, insertion–deletion loops, and heterologies generated during DNA replication and recombination [76,77]. Defects in MMR lead to genomic instability and a strong predisposition to cancer [77]. RPA has been shown to play important roles in each step of MMR, including stimulating EXO-catalyzed excision, protecting the ssDNA gap generated during excision, facilitating the termination of MMR excision, and repairing/synthesizing DNA [78–83].

RPA and DSB repair

DSBs are highly cytotoxic lesions induced by ionizing radiation and many other exogenous and endogenous DNA-damaging agents [84,85]. Since DSBs involve both DNA strands in the double helix, they are extremely hazardous to cells. A failure to appropriately repair DSBs leads to severe genomic instability [86,87]. Two major signaling pathways, non-homologous end joining (NHEJ) and homologous recombination (HR), are recruited by cells to repair DSBs [88–90]. While HR relies on the homologous duplex to serve as a template for repair, NHEJ ligates the DSB ends directly without requiring a homologous DNA template [89,91–94].

The function of RPA in HR has been well studied. HR requires the generation of ssDNA intermediates which are necessary for homology searching and pairing [95,96]. This explains why RPA proteins are required in this process. After a DSB has arisen, the MRN complex (comprising MRE11, RAD50, and NBS1/Xrs2) and CtIP initiate a process called DNA end resection. This 5′-end resection generates 3′ single-strand overhang which is then rapidly recognized and subsequently coated by RPA to remove the secondary structure and protect the ssDNA tail [88].

However, once DNA end resection has occurred and ssDNA intermediates appeared, a somewhat dangerous alternative pathway called microhomology-mediated end joining (MMEJ) may occur instead of HR. MMEJ creates deletions, translocations, and even chromosome rearrangements in DNA; thus, it is one of the most dangerous pathways to repair DSB in DNA [97,98]. It has been demonstrated that the presence of sufficient amounts of RPA proteins to bind to naked ssDNA is the most important factor for preventing the occurrence of MMEJ [99,100].

In addition to ssDNA, RPA can also bind to HR proteins. The recombinase RAD51 binds to ssDNA after RPA loading, mediating the process of pairing and strand exchange. Studies have revealed that RPA can bind to RAD51 directly and stimulate its strand transfer activity [101,102]. Another important factor involved in HR is RAD52. While RAD51 binds to ssDNA to promote pairing and strand exchange processes, RAD52 facilitates strand annealing in HR [103]. Studies have revealed that RPA can also bind to RAD52, and the interaction between them is essential for HR [104].

Recently, we found that a human protein associated with cancer, SLFN11, interacts with RPA1 and is recruited to sites of DNA damage in an RPA1-dependent manner [105]. By destabilizing the RPA–ssDNA complex, SLFN11 inhibits checkpoint maintenance and HR repair, and then sensitizes certain cancer cell lines to DNA-damaging agents [105].

Other SSBs in Eukaryotic Cells

Although RPA is suggested to be the most abundant and thoroughly studied SSB in eukaryotic cells, other SSBs have also been identified in the human genome, including hSSB1 and hSSB2 (SOSSB1/2), both of which contain a single OB domain [106]. We and others found that SSB1 and SSB2 interact with INTS3 (SOSS-A) and C9orf80 (SOSS-C) to form the SOSS DNA complex and revealed the structural basis by which the SOSS1 complex is assembled and recognizes ssDNA [107–111]. It was demonstrated that the SOSS1/2 complexes are involved in the checkpoint response and DNA repair regulation in response to DSBs [107,109,110,112].

Other than the nuclear SSBs, mitochondrial SSBs (mtSSBs) have also been reported, which are conserved from yeast to human. mtSSBs bind to ssDNA and stabilize the single-stranded regions of mtDNA within its displacement loops [113].

Summary and Perspective

Mounting evidence indicates that RPA interacts with a large and growing number of proteins or protein complexes to regulate DNA metabolism. Future studies are needed to understand the molecular mechanisms that allow RPA to participate in specific DNA metabolic pathways.

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