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REVIEWS

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Replication Protein A as a Major Eukaryotic Single-Stranded DNA-Binding Protein and Its Role in DNA Repair

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Abstract—Replication protein A (RPA) is a key regulator of eukaryotic DNA metabolism. RPA is a highly conserved heterotrimeric protein and contains multiple oligonucleotide/oligosaccharide-binding folds. The major RPA function is binding to single-stranded DNA (ssDNA) intermediates forming in DNA replication, repair, and recombination. Although binding ssDNA with high affinity, RPA can rapidly diffuse along ssDNA and destabilizes the DNA secondary structure. A highly dynamic RPA binding to ssDNA allows other proteins to access ssDNA and to displace RPA from the RPA–ssDNA complex. As has been shown recently, RPA in complex with ssDNA is posttranslationally modified in response to DNA damage. These modifications modulate the RPA interactions with its protein partners and control the DNA damage signaling pathways. The review considers up-to-date data on the RPA function as an active coordinator of ssDNA intermediate processing within DNA metabolic pathways, DNA repair in particular.

Keywords: replication protein A, DNA repair, DNA replication

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INTRODUCTION

Replication protein A (RPA) is a key single-stranded DNA (ssDNA)-binding (SSB) protein in eukaryotic cells [1–7]. The understanding of how RPA forms contacts with ssDNA was substantially improved in the past years [8–10]. RPA binding to ssDNA is known to be a dynamic process [11, 12] and to determine the role of DNA containing single-stranded regions in a particular biological process [13]. SSB activity of RPA does not directly correlates with its ability to form contacts with other proteins involved in DNA metabolism [14, 15]. Lower-level RPA expression increases the mutation rate and total genome instability and, in the limiting case, causes cell death [16], the effects supporting the key role RPA plays in maintaining genome stability.

RPA AS A COORDINATOR OF DNA PROCESSING

Transmission of genetic information and maintenance of its stability are extremely complex multistep processes. It is inconceivable that one multifunctional protein alone would perform a variety of catalytic reactions involved. In fact, multiprotein ensembles are responsible for DNA replication, repair, recombination, and transcription, certain enzymes and protein factors playing a role in several, rather than one, of the processes [17, 18] and thereby acting as links between them. RPA is one of these proteins, being involved in the basic processes of DNA replication, repair, and recombination [1, 19–21]. In addition, RPA plays a role in coordinating the cell response to DNA damage and subsequent activation of a cell cycle checkpoint mechanism [22–24]. A main feature is that single-stranded regions form as DNA intermediates in all RPA-involving processes, varying in structure, length, and protein partner set. RPA binds to ssDNA and thereby prevents the formation of duplex structures, which hinder enzymatic processes. RPA occurs in large amounts in the cell, and its high affinity for ssDNA ($K_d < 10^{-9}$ M) makes it possible to think that any single-stranded region appearing in the cell is immediately bound with RPA [25, 26]. The mechanism whereby the RPA • DNA complex coordinates the DNA processing is still not fully understood.

STRUCTURAL ORGANIZATION OF RPA

Structurally, human RPA (hsRPA) is a stable heterotrimer composed of subunits of 70, 32, and 14 kDa

Abbreviations: dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; ATR, ataxia telangiectasia and Rad3-related protein; NER, nucleotide excision repair; OB domain, oligosaccharide/oligonucleotide-binding domain; polprim, DNA polymerase α–primase complex; RPA, replication protein A; hsRPA, human (*Homo sapiens*) RPA; umRPA, *Ustilago maydis* RPA; SSB, ssDNA binding.

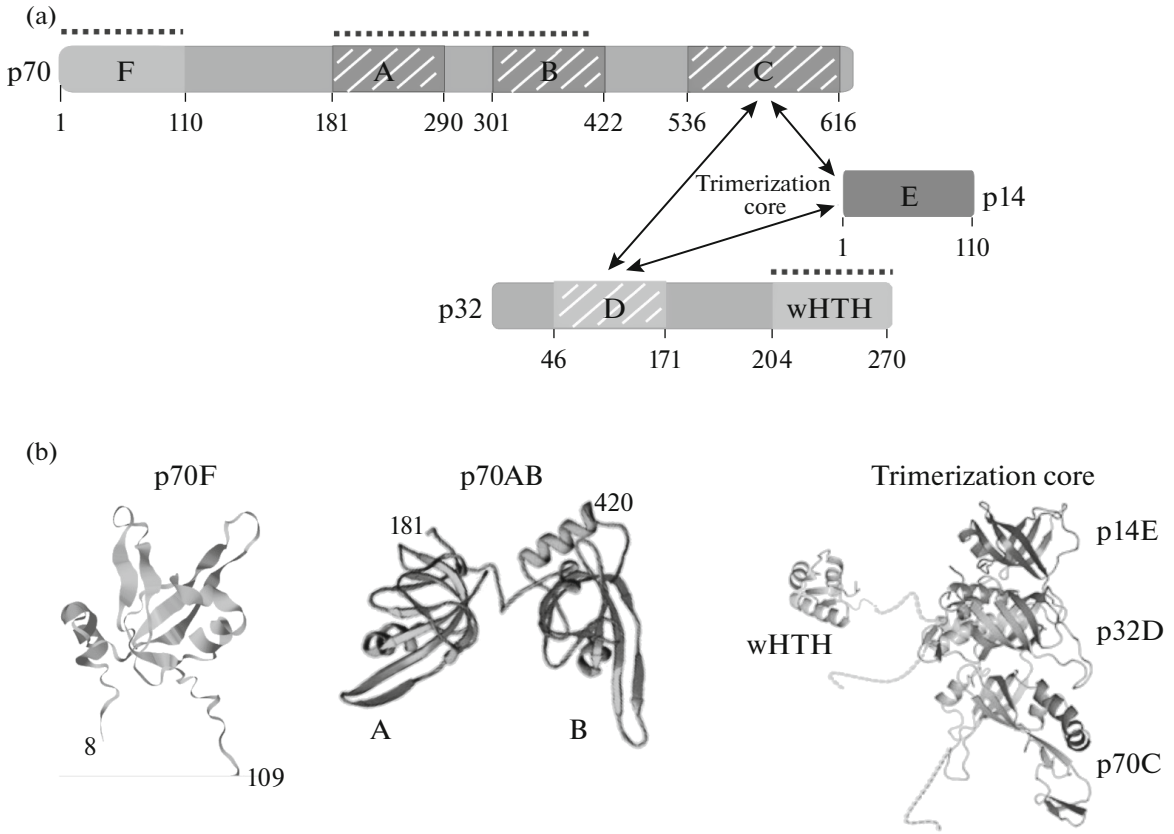

Fig. 1. RPA structure. (a) Schematic RPA structure. Arrows show the inter-subunit interactions producing the trimerization core. The DNA-binding domains A–C and D are crosshatched. Domains responsible for protein–protein interactions are indicated with a dotted line. (b) Structures of the OB domains p70F (PDB 1EWI) and p70AB (PDB 1JMC) and the trimerization core ([30], PDB 1L1O) formed by the OB domains C, D, and E and the wHTH domain.

(RPA1, RPA2, and RPA3, respectively) [1, 19, 27]. The RPA subunits cannot separately be expressed in a soluble form. Each of the subunits harbors one or several oligosaccharide/oligonucleotide-binding (OB) domains [28]. The OB domains can be involved in binding nucleic acids and oligosaccharide fragments and possess additional peptide-binding activity according to the Structural Classification of Proteins (SCOP) database [29].

Seven domains are recognized in the RPA structure (Fig. 1a), and six of them are OB domains, including four involved in high-affinity DNA binding [1, 27]. The hsRPA OB domains differ in both structure and function [27].

The large RPA subunit p70 has four OB domains [1]. The N-terminal domain p70F is separated from the other part of the subunit by a relatively extended linker (Fig. 1b). Although p70F does interact with DNA, a regulation via protein–protein interactions is thought to be its major function [14, 23, 31]. Two DNA-binding domains, p70A and p70B, occur in tandem in the central region of the subunit and each possess DNA-binding activity [32]. The dissociation constants (K_d) of DNA complexes with one of the domains alone fall within a micromolar range; i.e., individual domains have only low affinity for ssDNA [33]. A short linker between the two domains allows them to function as one tandem, p70AB, whose affinity for DNA is substantially higher than that of individual domains, the K_d of the p70AB • ssDNA complex falling in the nanomolar range [33]. Another DNA-binding domain (p70C) is in the C-terminal region of p70 and plays a role in the formation of the RPA heterotrimer in addition to performing its other functions [30]. A zinc finger, or zinc ribbon, is recognized in the p70C structure, being conserved among all RPA homologs and occurring in certain homidimeric SSB proteins of Euryarchaeota [34, 35].

The p32 central RPA subunit also harbors a DNA-binding OB domain (p32D) [36]. One domain, p32CTD, is the only RPA domain that does not belong to the OB domain family, but is classed with wHTH (helix–turn–helix) domains. The domain is involved exclusively in protein–protein interactions in hsRPA [27, 37], while related domains of the same family are found in certain transcription factors and *Escherichia coli* helicase RecQ and interact with double-stranded DNA (dsDNA) [37, 38]. The unstruc-

tured N-terminal region of the p32 subunit provides a target for phosphorylation by kinases responsible for the cell cycle and involved in the cell response to DNA damage [6].

The third RPA subunit p14 is a single domain (p14E) based on the OB domain [39]. The domain possesses weak DNA-binding activity [40] and is most likely involved in the formation of a RPA heterotrimer [39].

The three RPA subunits form a stable complex, one domain contributing to its maintenance from each of the subunits: p70C, p32D, and p14E. Their interaction region is known as a trimerization core [30]. Flexible linkers connect all other RPA regions to the trimerization core, providing RPA with high conformational mobility and the capability of using a broad range of DNA sequences as ligands [41].

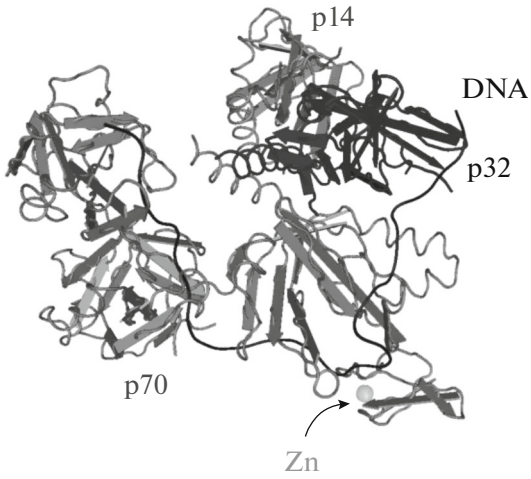

Fig. 2. RPA binding with ssDNA. Structure of umRPA (PDB 4GOP) in complex with ssDNA [42].

RPA INTERACTION WITH SINGLE-STRANDED DNA

Eukaryotic RPA homologs bind ssDNA with high affinity to produce complexes varying in architecture and binding site length. The conformational flexibility of RPA in complex with ssDNA explains why X-ray quality crystals could not be obtained for a long time. Three-dimensional structures were first solved for all DNA-binding domains of hsRPA [30, 42] and a complex of the p70AB tandem with an 8-mer ssDNA fragment [32]. In 2012, crystals were obtained and the structure was solved for a maize smut *Ustilago maydis* heterotrimeric RPA ortholog (umRPA) in complex with a 32-mer single-stranded oligonucleotide [43]. According to this structure and available biochemical data, four umRPA DNA-binding domains (A–D) form a stable complex with 30 ssDNA nucleotide residues (RPA30 complex) (Fig. 2).

A mechanism of RPA • DNA complex formation was proposed before the spatial structure had been solved for the RPA heterotrimer [30]. Several complexes of different types were thought to form upon RPA binding to DNA, one successively evolving into another. An unstable RPA10-like complex is the first to form via interactions between the p70A and p70B DNA-binding domains with 8–10 nucleotides of a DNA strand (Fig. 3a). The possibility of its formation is supported by covalent crosslinking data [44] and an X-ray analysis of the p70AB DNA-binding tandem in complex with octadeoxycytidine [32, 45]. A spatial model of the p70AB • ssDNA model suggests that each of the OB domains is in direct contact with three ssDNA nucleotides and that two other nucleotides are between the protein domains. Thus, eight nucleotide residues are shielded in total by the tandem of the p70A and p70B domains, determining the RPA–ssDNA binding site length in RPA10-like complexes. The K_d of the p70AB • ssDNA complex is ~10^{-7} M, being ~1.7×10^{-6} M for the p70A • ssDNA complex and ~16×10^{-6} M for the p70B • ssDNA complex. High affinity of the p70AB tandem for ssDNA is due to a short linker connecting the p70A and p70B domains (Fig. 3a) [33]. It is possible to conclude that the p70A domain, which possesses higher affinity, is the first to bind to ssDNA, thus increasing the local concentration of the other DNA-binding domains in the ssDNA vicinity and allowing the domains to be consecutively incorporated in the complex with DNA. The binding mechanism is supported by the finding that the p32 RPA subunit interacts with ssDNA only after p70 is bound [46].

When the ssDNA fragment bound with the A and B RPA subunits exceeds 8–10 nucleotides, the protein conformation may change so that two other DNA binding domains, p70C and p32D, come into contact with ssDNA [47]. A thermodynamics analysis of RPA–DNA binding showed that protein complexes with 18–20 and 28–30 nucleotides are possible, corresponding to DNA interactions with three (A, B, and C) and four (A, B, C, and D) DNA-binding domains (Fig. 3b) [8, 12, 26, 42, 48]. The greater the length of the bound ssDNA region, the more stable is the RPA • DNA complex.

Data from X-ray studies of umRPA (Fig. 2) shed further light on how RPA unfolds on DNA, that is, the RPA10 complex transforms into RPA30 [43]. The protein–DNA contacts formed in RPA30 by the p70A and p70B domains were found to be nonequivalent, in contrast to contacts in the RPA10 complex [32]. The contacts are nonequivalent because the BC linker (nine amino acid residues between the p70B and p70C domains) enters the DNA-binding channel of the p70B domain in the RPA30 complex, thereby changing the DNA-contacting region conformation that has formed in RPA10. The p70C domain is positioned in a manner optimal for its binding to DNA in the (p70AB–BC linker) • DNA complex. Thus, the BC linker plays an important role of a conformational

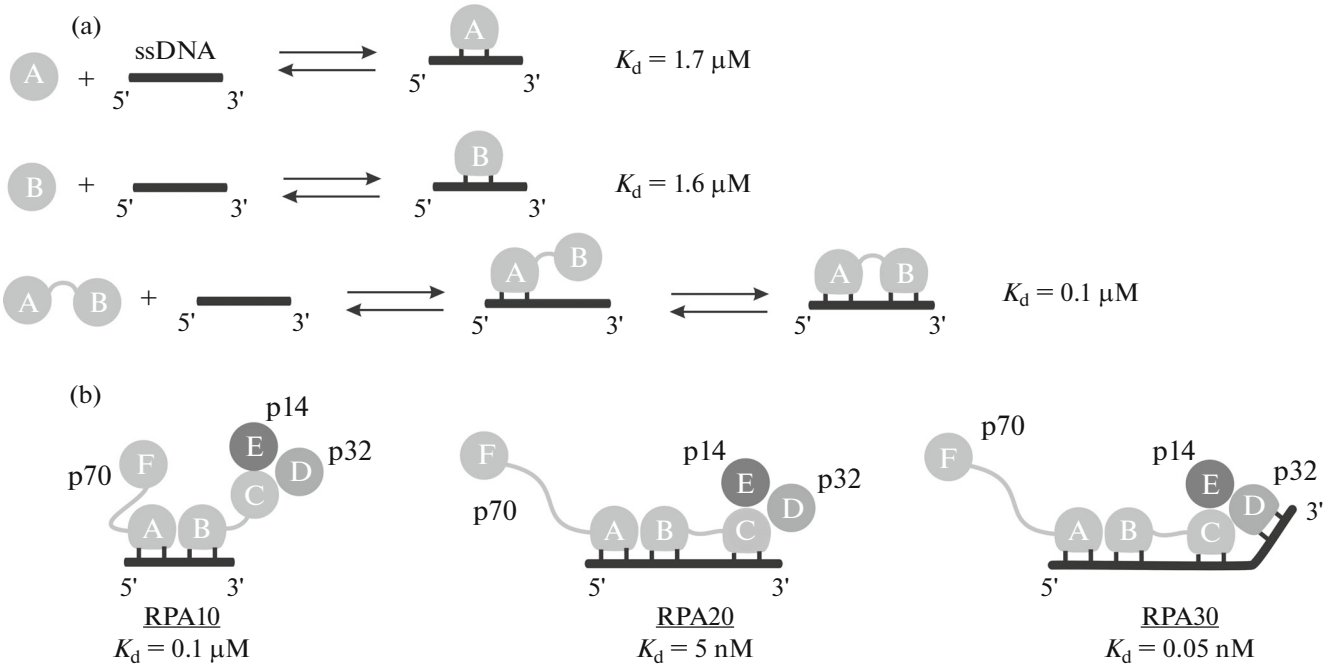

Fig. 3. RPA affinity for ssDNA. (a) Affinity for ssDNA of the AB tandem is more than 100 times higher than that of the individual A or B domain. The dissociation constants of the ssDNA complexes with RPA domains were measured using the oligonucleotide d(CTTCA) for the A and B domains and d(CTTCACTTCA) for the AB tandem [33]. (b) Strong 5'→3' polarity of RPA–ssDNA binding and the effect the number of DNA-bound RPA domains exerts on affinity and complex architecture [8, 11].

inducer upon the transition from RPA10 to RPA30. A 12-nt ssDNA region is involved in the (p70AB–BC linker) • DNA transition complex [43, 47]. The binding site length increases to 20 nt upon a subsequent interaction of the p70C domain with DNA, and other 5 nt are additionally involved as the region between p70C and p32D and the p32D domain are included in the complex. It should be noted that conformational mobility in hsRPA was already reported for the p70 subunit on evidence of limited proteolytic cleavage [49, 50].

Before X-ray data became available for the umRPA spatial structure, biochemical studies were performed to understand how the number of hsRPA subunits bound to ssDNA affects the architecture of the RPA–DNA complex. Affinity modification and proteolytic cleavage experiments showed that RPA10 and RPA30 differ in both types and number of DNA–protein contacts [49, 50] and, consequently, in stability [27]. RPA10-like complexes proved to be the least stable, while RPA30-like complexes showed the greatest stability. By affinity modification data, the RPA subunits have a certain orientation in complex with ssDNA. The p70 subunit more efficiently interacts with the 5' ssDNA region; and p32, with the 3' ssDNA region. A polar RPA arrangement on ssDNA in RPA30-like complexes arises because the p32D domain of the p32 subunit binds closer to the 3' ssDNA end, while the p70A, p70B, and p70C domains of the p70 subunit bind predominantly closer to the 5' end [21, 47, 48, 51–53]. According to electron microscopy data, DNA is not wrapped around the protein molecule in RPA30-like complexes, in contrast to what was observed for the prokaryotic SSB protein in complexes formed in the SSB65-like mode [54–56]. DNA is U-shaped following the arrangement of DNA-binding channels in the protein domains according to structural data on the upRPA–DNA complex [43] (Fig. 2).

A current paradigm implies that DNA-binding domains of RPA consecutively bind to ssDNA, i.e., they are included in the RPA–DNA complex in a stepwise manner, and the complex grows in stability as more DNA-binding domains are involved in its formation. The model provides a simple explanation for the findings that RPA affinity for DNA decreases with decreasing DNA length [25] and that the type of the RPA • DNA complex depends not only on the length of the accessible ssDNA platform, but also on the RPA–DNA concentration ratio [48]. Several requirements should be met to allow a RPA30-like binding mode, wherein all DNA-binding domains of the protein interact with ssDNA. First, the length of a continuous ssDNA fragment should correspond to the total size of the consecutive binding sites of all SSB domains of the protein. Second, the conditions of RPA interactions with ssDNA should be such that only one RPA molecule land on the continuous ssDNA region. Given that the binding of the RPA domains is low cooperative in the RPA30-like mode [57], the conditions are as necessary when the protein

concentration is lower than the ssDNA concentration. When the RPA concentration exceeds the DNA concentration, it is more likely that several RPA molecules are consecutively loaded on ssDNA. The low-affinity DNA-binding domains p70C and p32D of one RPA molecule are competitively displaced by the high-affinity domains p70A and p70B of another RPA molecule in this case [48]. The possibility of ssDNA complexes that do not fit in the model was demonstrated for hsRPA by photoaffinity modification [53]. An architecture wherein the p14 small subunit is involved in heterotrimer contacts with ssDNA is possible for a hsRPA • DNA complex [53]. The binding site length is no more than 10 nucleotides in the putative complex, and the p70 and p14 subunits interact with DNA. The p14 hsRPA gene is absolutely essential for cell viability [58, 59], the fact being associated with an important role p14-containing complexes play in the dynamic interaction of hsRPA with DNA during replication and repair.

DYNAMICS OF RPA INTERACTIONS WITH ssDNA

The main RPA function in all processes of DNA metabolism is to protect ssDNA from damage and nuclease cleavage so that intact ssDNA be presented to protein factors and enzymes involved in restoring the duplex structure. It is important for its adequate functioning that RPA has high affinity for ssDNA and, on the other hand, rapidly dissociates from ssDNA to provide binding sites for specific repair, replication, or transcription proteins. As was recently shown, a dynamic model better describes all steps of the RPA–DNA interaction than the model of consecutive binding and dissociation of individual SSB domains in a protein–DNA complex.

For instance, a study of the interactions between individual hsRPA components with ssDNA showed that RPA rapidly moves along ssDNA tracts via diffusion without dissociation [12]. The diffusion rate of hsRPA is one order of magnitude higher than the rates of bacterial SSB proteins, suggesting different mechanisms of the process. Rapid diffusion of hsRPA along a ssDNA tract destabilizes the duplex structures encountered.

The binding of yeast RPA with ssDNA was studied using fluorescence visualization of individual molecules [11]. Once formed, a RPA • DNA complex was found to be stable and to rare dissociate in the absence of the free protein in solution. When free RPA is present, bound and free components are rapidly exchanged. This is explained by the fact that, in spite of the general stability of the RPA • DNA complex ($K_d = 5 \times 10^{-11}$ M [11]), individual SSB domains of RPA dissociate rather easily, and the resulting free ssDNA platforms are subsequently occupied by other SSB domains. Thus, dissociation of the RPA • DNA complex proceeds through several transition states, wherein the number of RPA domains bound to ssDNA decreases, and the ssDNA platforms freed in the process provide access to DNA for other proteins.

Thus, a dynamic model of RPA binding is as follows. RPA forms a stable complex with ssDNA tracts. Yet the dissociation potential of individual SSB domains of RPA is higher than that of the total complex and, together with their conformational mobility, leads to rapid conformational changes in the protein domains and the RPA movement along ssDNA (Fig. 4). Owing to this locally "breathing" structure (continuous individual domain microdissociation events), RPA is readily adjusted to various ssDNA structural variants, rapidly diffuses along the ssDNA strand, destabilizes unwanted secondary structure elements (e.g., hairpins), or is displaced from ssDNA by other DNA-binding proteins [8]. The mechanism allows SSB proteins to bind to ssDNA by replacing RPA domains connected through flexible linkers without requiring extended ssDNA regions to be exposed.

INTERACTION WITH PARTIAL DNA DUPLEXES

The above variants of interactions with RPA pertain to ssDNA. However, DNA occurs in a more intricate form when involved in key cell processes such as replication, repair, or recombination. Partial DNA duplexes with single-stranded overhangs better imitate the functionally important DNA forms in the processes as compared with ssDNA [48, 51]. DNA polymerases, which play a central role in replication, fill in the gaps in double-stranded regions and synthesize new DNA strands, utilizing the 3' terminal nucleotide as a primer. It is therefore of particular interest to study the RPA interaction with the 3' primer end in the region of ssDNA–dsDNA (ss/dsDNA) junction. A model that describes the RPA arrangement in the vicinity of a junction between dsDNA and the 5'-protruding template ssDNA (Fig. 5) is based on photoaffinity modification and partial proteolytic cleavage data [48, 50, 59, 60]. An architectural feature common for RPA complexes with partial DNA duplexes and with ssDNA is that the p70A and p70B DNA-binding domains of the large subunit make a major contribution to the interaction with the ssDNA region, providing RPA with affinity for DNA as is necessary for their binding [51, 58, 61]. The p70C and p32D domains can also occur in contact with ssDNA in RPA complexes with extended ssDNAs, but they occur in the region of the ss/dsDNA junction in complexes with partial DNA duplexes.

Evidence for the interaction model were obtained using partial DNA duplexes with a template overhang varying in length and carrying a photoreactive group in the region of the ss/dsDNA junction [48, 52, 60]. The position of the p32 subunit on the ssDNA tract determines a major difference between the binding modes

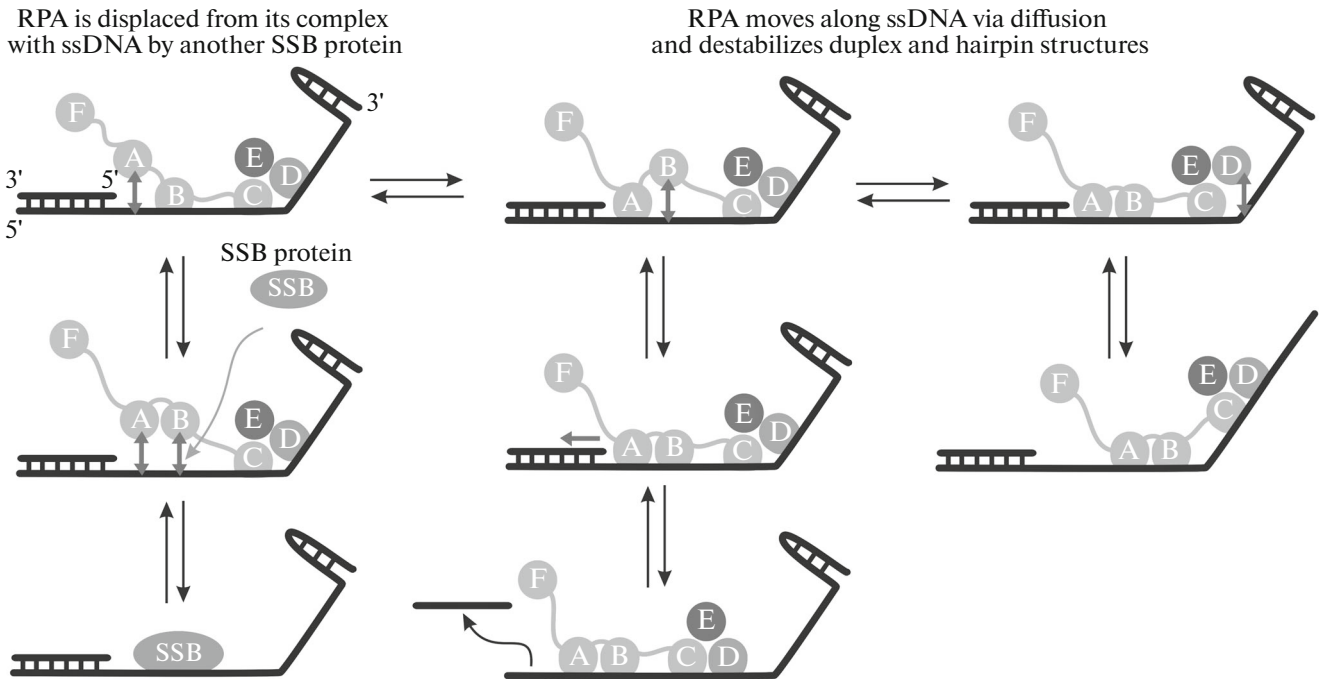

Fig. 4. Dynamic model of RPA binding to ssDNA. The idea of the figure is taken from [8].

in question. The p32 subunit directly interacts with a single-stranded region in RPA complexes with ssDNA and occurs in the vicinity of the ss/dsDNA junction in complexes with partial DNA duplexes [48, 52, 53], p70C occupying a site in the single-stranded region and p32D forming a direct contact with the 3' end of the primer [50, 61]. Polarity of RPA–DNA interactions is common for all binding modes in question. Photoaffinity modification with DNA duplexes carrying a photoreactive group at the ss/dsDNA junction showed that, upon RPA binding to partial DNA duplexes, the 5' end of the downstream oligonucleotide interacts with the p70 subunit, while the 3' end of the upstream oligonucleotide interacts with p32 when RPA is in deficiency relative to DNA and p70 when RPA is in excess relative to DNA [48, 60]. Thus, the orientation of these subunits is the same in RPA complexes with ssDNA and with partial DNA duplexes containing extended gaps. A detailed study of the binding mechanism showed that the p70AB main DNA-binding domain tandem is not essential for a specific and oriented interaction of RPA with partial DNA duplexes [61]. At the same time, the p14 small subunit is absolutely essential, and RPA loses its capability of a polar arrangement on DNA in the absence of p14 [62].

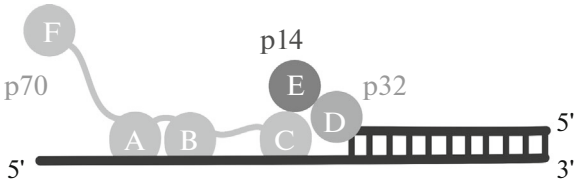

Fig. 5. Model of the oriented interaction of hsPRA with DNA at the junction of dsDNA with 5'-protruding template ssDNA. The hsRPA domains A, B, and C interact with the template DNA strand.

RPA IS A KEY REPLICATION PROTEIN

It is known that RPA is absolutely required for all steps of DNA replication [1]. DNA polymerase α–primase (pol-prim), which plays a key role in replication initiation by synthesizing and elongating the RPA primer in early replication, acts as an RPA partner in a replication model system [1]. Mutant RPA forms devoid of the p32 or p14 subunit were found to be incapable of ensuring the RNA primer synthesis and elongation, which are catalyzed by pol-prim [62]. Thus, a productive ternary complex pol-prim • substrate DNA • RPA cannot form in the absence of the small RPA subunits. The oriented RPA interaction with substrate DNA plays an important role in the formation of the ternary complex along with protein–protein interactions between RPA and pol-prim. A complex formed in the absence of the small RPA subunits is incapable of ensuring a polar arrangement of RPA subunits on DNA and sustaining the synthesis and elongation of an RNA primer [62].

The mechanism of RNA–DNA primer elongation was studied in replicating SV40 chromosomes, and different RPA binding modes were observed at differ-

ent steps of the process [63, 64]. Early primer synthesis products mostly contact the p32 subunit, indicating that a RPA30-like complex results from RPA–DNA binding. The binding mode changes to RPA10 at later synthesis steps, only the p70 subunit being available for contacts with the 3' end of the growing strand [63, 64]. The findings perfectly agree with the results of RPA photoaffinity modification with model DNA duplexes [48, 60]. As one binding mode changes to another, the effect RPA exerts on the relevant DNA polymerase may change because a new pattern of protein–protein contacts arises. For instance, RPA was found to exert no effect on DNA synthesis catalyzed on a damaged template by DNA polymerase λ when the template region is 36 nt, while RPA-dependent stimulation of DNA synthesis was observed with substrate DNA containing a 16-nt template overhang [65]. The binding mode corresponds to RPA30 in the former case and mostly to RPA10 in the latter case according to the model assumed for RPA interactions with partial DNA duplexes. Thus, experimental findings indicate that the capability of forming complexes with a proper architecture is important for RPA to perform its function in DNA replication.

It was observed that RPA improves the fidelity of DNA synthesis catalyzed by DNA polymerase α [66]. The fidelity of DNA synthesis by DNA polymerase λ is also increased by RPA [67]. More recent studies showed that the effect is especially great when a damaged template is used in synthesis. DNA polymerase λ is capable of synthesizing DNA on a template containing 8-oxoguanine or 1,2-dihydro-2-oxoadenine lesions, but the fidelity of this synthesis is low; i.e., the probability of adding a proper nucleotide (dCTP and or dTTP, respectively) is comparable with that of adding a mismatch (dATP or dGTP, respectively) [68, 69]. RPA substantially reduces the erroneous dATP incorporation rate on 8-oxoguanine-containing templates. The proliferating cell nuclear antigen (PCNA) added to the mixture together with RPA facilitates dCTP incorporation, increasing the probability of correct nucleotide addition by DNA polymerase λ by a factor of 1200 [68]. When the template contains the other lesion (1,2-dihydro-2-oxoadenine), PCNA and RPA acting together increase the probability of correct dTTP incorporation by a factor of 166 [69]. Thus, RPA and PCNA are important components of a functional replication complex and affect not only major replicative DNA polymerases (α and δ), but also specialized polymerases that synthesize DNA in a replication fork blocked by a lesion in the template strand.

ROLE OF RPA IN DNA REPAIR AND OTHER PROCESSES MAINTAINING THE GENOME STABILITY

As a key component of DNA metabolism, RPA is involved in recruiting many proteins on substrate DNA and is responsible for assembly of DNA–protein complexes with a proper architecture. Many partner proteins presumably interact with RPA, while direct proten–protein interactions were demonstrated using NMR for Rad51, XPA, UNG2, Rad52, SV40 T-antigen helicase, DNA primase, ATRIP, MRE11, Rad9, BID, TIPIN, and HDHB [23, 24, 37, 70–75]. With this multiplicity of partner proteins, overlapping RPA sites should be involved in interactions with them.

It is clear now that the dynamic model of RPA–ssDNA interactions makes it possible to explain why RPA activity depends on the functions performed. A mutation analysis of the DNA-binding domains showed that high affinity for ssDNA is insufficient for the adequate RPA function. Certain RPA mutants with affinity for ssDNA two orders of magnitude lower than that of the wild-type protein are completely functional [14]. RPA forms with mutations affecting the conserved regions of p70A and p70B sustain replication, but not repair [15]. The following explanation is possible for these findings in the context of the dynamic binding model. Changes in the binding of individual domains, that is, microscopic binding constants, affect the dynamics of the RPA • DNA complex, while the macroscopic binding constant may still not be affected. Therefore, different RPA–DNA contacts sustain the RPA functions in replication and repair.

A functional analysis of the RPA interaction with ssDNA showed that RPA binding to single-stranded regions during double-strand break repair facilitates the most error-free pathway of restoring the DNA structure. In other words, RPA stimulates error-free homologous recombination and inhibits erroneous microhomology-mediated end joining [13]. To exert its inhibitory effect, RPA binds to ssDNA and prevents the annealing of microhomology regions; i.e., dynamic RPA binding destabilizes incorrect duplexes.

Nucleotide excision repair (NER) was assumed to involve RPA because RPA was found to have higher affinity for damaged DNA duplexes compared with intact ones [76] and to form a stable RPA • XPA complex [77]. More recently, RPA was alternatively ascribed with a major role in recognizing DNA lesions [78] or thought to play no role in the process [79]. Although the role RPA plays in recognizing DNA lesions during NER is unclear, there is evidence that RPA is absolutely essential for both excision of a damaged DNA site and DNA resynthesis [80]. RPA interacts with many factors involved in the pre-incision complex. Namely, RPA binds with XPA and XPG [76], increases the binding efficiency and activity of XPF-ERCC1 [81], stimulates the XPC-HR23B binding to DNA [82], and interacts with centrin 2 in the course of DNA binding [83]. Moreover, a complex RPA • XPA • TFIIH • XPC • DNA was demonstrated by the electrophoretic mobility shift assay [84]. A regulatory role in triggering the NER function was recently observed for RPA [85], and the role RPA plays as a NER-limiting factor was verified [86].

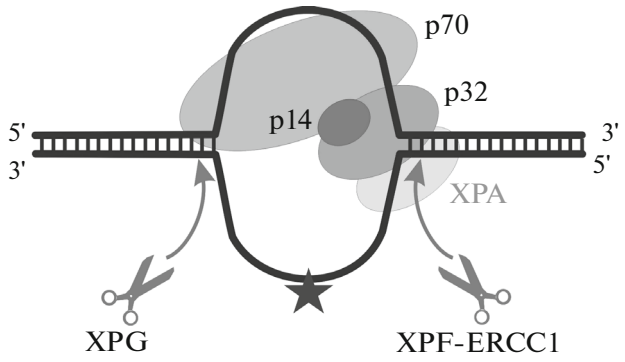

Fig. 6. Localization of RPA and XPA on DNA in the pre-incision NER complex [87]. Arrows indicate the putative contact sites for NER-specific endonucleases XPF-ERCC1 and XPG; a star shows a bulky lesion in the DNA structure.

Photoaffinity modification experiments showed that RPA as a component of the NER complex interacts predominantly with the intact DNA strand during the step that precedes excision of a damaged site [87]. RPA is thought to protect the intact DNA strand from a nuclease attack (Fig. 6). RPA acts together with XPA in NER. In fact, affinity for damaged DNA of the XPA • RPA complex is more than one order of magnitude higher than that of RPA [88]. RPA stabilizes XPA–DNA binding [89]. XPA interacts with two RPA subunits, p70 and p32 [90]. An overlap between the DNA-binding domain of XPA and the XPA region responsible for contacting p70 suggests a cooperative interaction between the two proteins in the course of their binding to DNA [91].

Relatively recent studies revealed RPA in telomeric chromosome ends, the association level being maximal in the S phase [92–94]. RPA renders the telomeric ends accessible for Est1p, an important component of the telomerase complex, and thereby plays a substantial role in telomere processing [95, 96]. It seems that RPA stabilizes DNA in the single-stranded form, which interacts with components of the telomerase complex. Another possible mechanism of the role RPA plays in activating telomerase is related to the findings that RPA is capable of destabilizing guanosine-rich G-quadruplexes [92, 97–100] and that RPA promotes the binding of shelterin complex proteins to telomeric DNA and facilitates the telomerase function [101].

It is well known that RPA interacts with many partner proteins in replication, repair, recombination, and checkpoint control. RPA regions other than DNA-binding domains are usually involved in these protein–protein interactions, and all of them are necessary for the RPA functions in the above processes. RPA–protein contacts may change the conformation of individual domains and thereby modulate the DNA-binding activity of RPA. Interactions with the p70F and p32CTD domains are presumably involved in regulating activation of checkpoint control and replication proteins, respectively [19, 23, 24, 102, 103]. The association of RPA with the checkpoint control system was observed in the yeast *Saccharomyces cerevisiae*; i.e., a deletion of the Ddc1 checkpoint protein leads to proteolytic cleavage of the p70 RPA subunit [104].

ALTERNATIVE RPA FORM AND ITS ROLE IN DNA REPAIR

Apart from the three canonical RPA subunits (p70, p32, and p14), one more subunit, RPA4, is found in human cells and possess 63% homology with p32 [105]. The RPA4 subunit was first identified in a screening for proteins interacting with the p70 subunit [106]. Genes homologous to the RPA4 gene were found in primates and horses. RPA4 can replace p32 in the RPA heterotrimer to produce the so-called alternative RPA form, which is identical in biochemical properties to the canonical form [107]. The alternative RPA form does not sustain SV40 replication in vitro [107], and p32-mutant HeLa cells expressing the RPA4 gene fail to proceed through the cell cycle in the S phase [105, 107]. Compared with the canonical RPA, the alternative form has higher affinity for damaged DNA, but is less efficient in sustaining NER because of weaker contacts with XPA and lack of a stimulating effect on endonuclease activity of XPF-ERCC1 [108]. In addition, the alternative form interacts with Rad51 and Rad52, which are involved in homologous recombination, and stimulates Rad51-dependent DNA strand exchange [108]. The alternative form can functionally substitute for the canonical RPA in the mechanism activating checkpoint control proteins [109]. The alternative RPA form is currently thought to play a role in DNA repair, but its functional significance and the distribution of functions between the canonical and alternative RPA forms are still poorly understood.

RPA INTRACELLULAR LEVEL AND GENOME STABILITY

Loss of any of the RPA subunits is lethal for the cell [14, 58], and nonlethal RPA mutations are capable of causing DNA repair defects and destabilizing the genome [110–112]. The total RPA pool is necessary for DNA-related processes, and a decrease in the RPA level has adverse consequences for the cell [14, 105]. Microdeletions and microduplications in the gene for the RPA1 subunit already cause defects in the cell cycle control mechanism, while the total protein level decreases insignificantly in the cell [113, 114].

Many single-stranded regions arise in DNA when replication forks are stalled (replication stress). Binding to these regions, RPA helps to stabilize the replication forks. The resulting RPA • DNA complexes provide a signal for ATR (ataxia telangiectasia and Rad3-related protein, a serine/threonine protein kinase)-dependent system activating the checkpoint control,

and replication is thereby stopped and the cell cycle arrested [115]. On the other hand, RPA helps the cell to continue replication in the absence of replication stress [116–118]. A study of the role RPA plays in maintaining genome stability showed that single-stranded regions arising in DNA in severe replication stress may be so numerous that the total RPA pool is not enough to bind all of them; i.e., the RPA pool is exhausted [16]. Double-strand breaks rapidly arise in the ssDNA regions that are not covered with RPA in these conditions, triggering cell death. An increase in RPA expression improves the cell resistance to replication stress. Moreover, ATR-dependent checkpoint activation plays an important role in preventing RPA pool exhaustion, i.e., ssDNA regions covered with RPA activate ATR, and, in turn, ATR inhibits generation of new single-stranded regions (activation of new replication sites).

A study of the mechanism regulating the consecutive NER steps [85] showed that inhibition of gap-filling synthesis and ligation as last steps of NER causes RPA to remain on the single-stranded region in the gap, while other protein factors involved in the pre-incision complex freely dissociate after the incision and may initiate assembly of new NER complexes on other damaged sites. Assembly of new pre-incision complexes cannot be completed without RPA, and RPA is not involved in other NER steps until gap filling and ligation are complete. In other words, the RPA pool may be exhausted when repair initiation sites are too many. According to a model assumed, RPA regulates the process so that new repair events do not start as long as NER initiated earlier at other sites is incomplete.

POSTTRANSLATIONAL MODIFICATIONS MODULATING RPA ACTIVITY

In response to DNA damage, RPA is hyperphosphorylated in the cell [20]. Phosphorylation modulates the protein–protein and protein–DNA interactions [20, 119, 120], but it is unclear how this modification regulates the cell response to DNA damage. The N-terminal region of the p32 subunit provides a major phosphorylation target, and minor modification sites occur in other subunits as well [119, 121]. RPA phosphorylation plays an important role in the cell response to DNA damage and replication stress in the S phase [115, 118, 122] and to genotoxic stress in mitosis [118, 123]. RPA phosphorylation is thought to regulate homologous recombination in replication-blocking conditions [118, 124, 125]. Hyperphosphorylation of p32 was recently found to facilitate a cell transition to apoptosis in replication stress with dysfunction of the ATR-CHK1 signaling pathway [126].

SUMOylation of RPA at lysine residues of the p70C domain also plays an important role in the cell response to DNA damage [127]. Cells carrying RPA with mutations of the SUMOylation sites display higher sensitivity to DNA damage [127]. SUMOylated RPA is necessary for Rad51 binding to DNA damage sites [127]. In baker's yeast, RPA was shown to interact with Siz2 ligase in response to induction of DNA breaks and to trigger SUMOylation of recombination factors, thus improving the cell resistance to lesions [128]. It remains unclear how SUMOylation affects RPA activity.

All RPA subunits are ubiquitinated in response to DNA damage [129, 130]. An interaction of RPA • ssDNA with the PRP19 E3 ubiquitin ligase complex, which is involved in ubiquitinating proteins in response to DNA damage, was recently revealed by proteome screening [129]. The p32 subunit is modified with ubiquitin residues in response to DNA damage. RPA ubiquitination by the PRP19 complex was shown to facilitate recruitment to damaged sites and activation of the ATR–ATRIP kinase complex [9]. PRP19 was found to promote homologous recombination [131]. Thus, the RPA • ssDNA complex simultaneously performs two functions, providing a platform for PRP19 recruitment and acting as a substrate for E3 ligase. It should be noted that RPA • ssDNA is capable of recruiting other ubiquitin ligases to the sites of DNA lesions as well [24, 132–134]. For instance, ubiquitination of the SUMOylated p70 subunit by the RNF4 ubiquitin ligase complex plays an important role in regulating RPA activity in double-strand break repair [135].

Poly(ADP-ribosyl)ation by poly(ADP-ribose) polymerase 1 is another important posttranslational modification that was recently found to affect RPA [136]. Both p70 and p32 subunits are modified to affect RPA interaction with ssDNA.

Thus, the RPA • ssDNA complex is intensely phosphorylated, SUMOylated, ubiquitinated, and poly(ADP-ribosyl)ated in response to DNA damage. These posttranslational modifications of RPA regulate activation of the ATR-dependent checkpoint mechanism and facilitate DNA repair. In other words, posttranslational modifications provide for a fine regulation of RPA activity to ensure the proper RPA function and transmission of cell signals.

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