Chapter 10
The Structure and Function of Replication Protein A in DNA Replication

Aishwarya Prakash and Gloria E.O. Borgstahl

Abstract In all organisms from bacteria and archaea to eukarya, single-stranded DNA binding proteins play an essential role in most, if not all, nuclear metabolism involving single-stranded DNA (ssDNA). Replication protein A (RPA), the major eukaryotic ssDNA binding protein, has two important roles in DNA metabolism: (1) in binding ssDNA to protect it and to keep it unfolded, and (2) in coordinating the assembly and disassembly of numerous proteins and protein complexes during processes such as DNA replication. Since its discovery as a vital player in the process of replication, RPA's roles in recombination and DNA repair quickly became evident. This chapter summarizes the current understanding of RPA's roles in replication by reviewing the available structural data, DNA-binding properties, interactions with various replication proteins, and interactions with DNA repair proteins when DNA replication is stalled.

Keywords Replication protein A • DNA replication • Single-stranded DNA binding protein • OB-fold • Protein-protein interaction • G-quadruplex

A. Prakash
Department of Microbiology and Molecular Genetics, The Markey Center for Molecular Genetics, University of Vermont, Given Medical Building, 89 Beaumont Avenue, Burlington, VT 05405, USA

G.E.O. Borgstahl (✉)
The Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 987696 Nebraska Medical Center, Omaha, NE 68198-7696, USA
e-mail: gborgstahl@unmc.edu

S. MacNeill (ed.), *The Eukaryotic Replisome: A Guide to Protein Structure and Function*, Subcellular Biochemistry 62, DOI 10.1007/978-94-007-4572-8_10,

10.1 Introduction

DNA replication is a cleverly orchestrated, fundamental process occurring within cells that allows organisms to duplicate the vast amounts of genetic information carried within DNA. This process occurs during the S-phase of the cell cycle and must be completed for healthy cells to divide. Replication of eukaryotic chromosomes is initiated at replication origins. These origins, ~30–100 kb apart and scattered along each chromosome, serve to recruit several proteins that constitute the replisome. The replisome, an enormous multiprotein-DNA complex, comprises proteins that unwind the DNA-double helix, stabilize ssDNA regions generated during the initial steps, and copy the DNA with accuracy and speed.

Briefly, replication begins at the origins upon binding of the origin recognition complex and proceeds bidirectionally in a semi-discontinuous manner (Campbell 1986; Wold 2010). The double-stranded DNA (dsDNA) is melted and unwound by a DNA helicase after which the ssDNA regions produced are coated rapidly by RPA (Oakley and Patrick 2010; Wold 1997). RPA is in abundance in cells and its binding protects ssDNA. It is thought to unfold DNA secondary structures, and keep them from reforming, before the DNA is replicated. During the initiation of replication, RPA functions to recruit the DNA polymerase α-primase complex (Pol α-primase) to the replication origins (see Chap. 9). Pol α-primase lays down an RNA-DNA primer to initiate leading and lagging strand synthesis, after which the leading strand is extended continuously. The clamp loader, replication factor C (RFC), assembles the sliding clamp, proliferating cell nuclear antigen (PCNA), at the end of the primer which then displaces Pol α-primase (see Chaps. 14 and 15). During the elongation phase RPA is believed to play a role in stimulating DNA polymerase δ (Pol δ) and DNA polymerase ε (Pol ε) which carry out highly processive DNA synthesis (see Chaps. 12 and 13). The lagging strand is constructed in a similar fashion to the leading strand but in the opposite 3′–5′ direction and as a series of short Okazaki fragments, each of which is synthesized 5′–3′. When Pol δ approaches the RNA primer of the downstream Okazaki fragment, ribonuclease (RNase) H1 removes all but the last RNA nucleotide of the DNA primer. RPA is involved in the recruitment of the Dna2 endonuclease, which cleaves the RPA bound primers and RPA is therefore thought to play a role in Okazaki fragment processing (Bae et al. 2001, 2003; MacNeill 2001). Following this, the flap endonuclease 1 (FEN1) exonuclease complex (Chap. 16) removes the last RNA nucleotide and the gap is filled in by Pol δ. DNA ligase joins the Okazaki fragment to the growing strand (Wold 2010; Kunkel and Burgers 2008) (Chap. 17). DNA replication is regarded as a tightly regulated process that involves the coordinated action of numerous factors that function to copy the DNA efficiently with minimal error, in order to maintain genomic stability.

In the 1980s, when the molecular biology of DNA replication was still in its infancy, scientists relied on *in vitro* reconstitution analyses to study this process. Due to its simplistic genome organization, Simian Virus 40 (SV40) virus replication was used as a model system. T-antigen, a virally encoded protein, plays a central role in

the binding and unwinding of the viral DNA during the initial stages of replication. This protein in addition to six others was necessary for proper replication (Weinberg et al. 1990). The T-antigen requires ATP and another cellular protein to successfully perform its unwinding functions. This cellular protein was determined to be RPA (Wold and Kelly 1988). RPA has been studied extensively since its discovery and is thought to be the primary eukaryotic ssDNA binding protein that is involved in several facets of DNA metabolism including replication, recombination, and repair (Binz et al. 2004; Bochkarev and Bochkareva 2004; Broderick et al. 2010; Fanning et al. 2006; Iftode et al. 1999; MacNeill 2001; Mer et al. 2000a; Oakley and Patrick 2010; Sakaguchi et al. 2009; Turchi et al. 1999; Wold 1997; Zou et al. 2006).

RPA is an abundant protein in cells: in humans it is the most abundant ssDNA binding protein with 5×10^4 to 2.4×10^5 molecules of RPA per cell (Kenny et al. 1990; Seroussi and Lavi 1993). RPA is essential for cell survival and there is a constant level of RPA protein during the cell cycle (Din et al. 1990). Down regulation of RPA with small-interfering RNA (siRNA) results in prolonged S-phase during the cell cycle, accumulation of DNA strand breaks, G2/M arrest, and cell death (Haring et al. 2008). Since the primary function of RPA is to bind any naked ssDNA generated during cellular processes, it is not surprising that cells cannot survive without it. RPA accumulates at sites of replication, called replication foci, in the nucleus just prior to the initiation of replication and remains localized during the DNA synthesis phase with 10–50 RPA molecules per replicating strand in the replication fork (Seroussi and Lavi 1993).

RPA is heterotrimeric in nature: in humans the three subunits are named RPA1, RPA2 and RPA3 in decreasing order of size where RPA1 is 70 kDa, RPA2 is 32 kDa and RPA3 is 14 kDa (Fig. 10.1a). Each of these subunits have folded domains called "oligonucleotide binding folds" (OB-folds) (Fig. 10.1b, c). RPA1 contains four OB-folds (F, A, B, and C – see Fig. 10.1) that are separated by intrinsically disordered linkers. OB-folds A, B, and C bind DNA, whereas OB-fold F is a protein interaction domain. RPA2 has a disordered N-terminus, OB-fold D at its center and a winged-helix-loop-helix (wHLH) protein interaction domain at its C-terminus connected by a disordered linker. RPA3 is composed of OB-fold E. The OB-folds are conserved in structure, with more structural homology than sequence homology (Figs. 10.1c and 10.2). Each of these domains is involved in specialized functions that involve ssDNA binding, recognition of damaged DNA and noncanonical DNA, protein-protein interactions, inter-subunit interactions, and post-translational modifications such as phosphorylation (Iftode et al. 1999; Oakley and Patrick 2010; Wold 1997).

10.2 Evolution of RPA

Single-strand DNA binding (SSB) proteins are essential in mediating several aspects of DNA metabolism. These proteins have been identified in organisms from prokaryotes to eukaryotes, and in archaea (Chedin et al. 1998). The bacterial SSB is

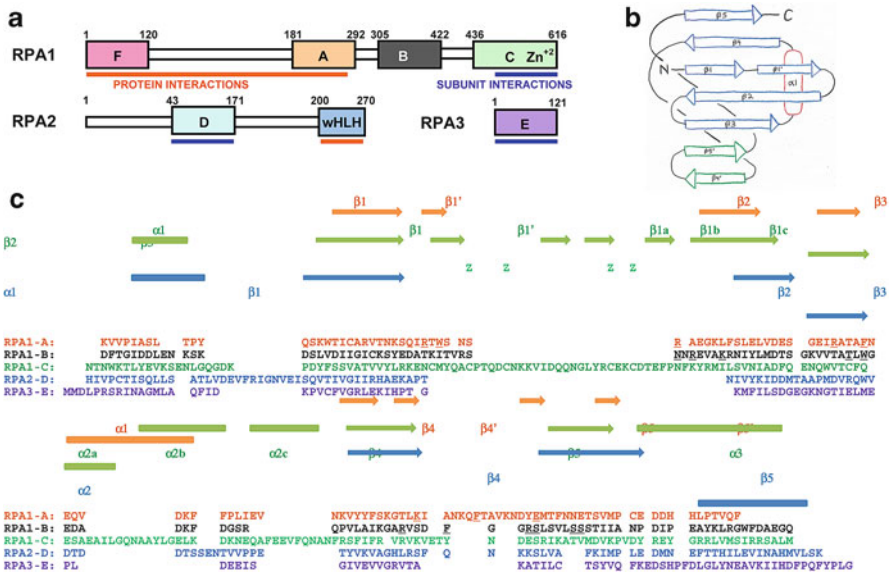

Fig. 10.1 The domain structure of RPA. (**a**) Schematic drawing of the three subunits (RPA1 = 70 kDa, RPA2 = 32 kDa, and RPA3 = 14 kDa), the folded domains (*thick colored rectangles*) and flexible linkers defined by limited proteolysis and NMR studies (Brosey et al. 2009; Gomes et al. 1996; Gomes and Wold 1995, 1996) (*thin white rectangles*). The OB-folds are labeled *A–F*, individually colored, and this color code is used in all figures in this chapter. The zinc finger on RPA1 is indicated (Zn^{+2}). The winged-helix-loop-helix domain is labeled wHLH. (**b**) General topology of the OB-folds (Bochkarev et al. 1997). The β-strands are indicated by *arrows* and the α-helix by an oval. The *blue* β-strands correspond to those that comprise the OB-fold. The L12 loop lies between β1′ and β2 and the L45 loop lies between β4′ and β5′. (**c**) Sequence and secondary structure alignment of domains *A–E* based on structure (Bochkarev et al. 1997, 1999; Bochkareva et al. 2002). *Orange* secondary structure elements represent domains *A* and *B*, *green* elements represent domain *C*, and *blue* elements represent domains *D* and *E*. Lower case *z* indicates the Cys residues in domain *C* that bind zinc. Residues in RPA1-A and RPA1-B that bind ssDNA are *underlined*

expressed from one gene and functions as a homotetramer. In contrast, eukaryotic RPA is expressed from three separate genes forming three subunits and functions as a heterotrimer. Despite these differences, the two proteins are structurally similar suggesting they originated from a common ancestor before evolving into the proteins they are today. In the archaeal homologs features like the zinc finger motif (present at the C-terminal domain of RPA1) developed, which is not present in the bacterial homologs, representing a link between prokaryotic (archaeal) and eukaryotic proteins.

In addition to the three canonical subunits of RPA, a homolog of human RPA2 (with 47% amino acid identity and 63% similarity) called RPA4 was discovered (Kemp et al. 2010). RPA4 readily forms an alternative heterotrimeric complex with RPA1 and RPA3, called aRPA and is expressed in all human tissues, albeit at different levels. This alternate form of RPA failed to support replication in the *in vitro*

SV40 replication system indicating that the roles for this protein are significantly different than canonical RPA (Haring et al. 2010). RPA4 has been implicated in the initial steps of nucleotide excision repair (NER) and also during the Rad51 dependent strand exchange step of homologous recombination (HR), indicating a role for this protein in DNA repair (Mason et al. 2010).

10.3 RPA Structure

RPA is a dynamic complex in solution and not surprisingly, the quest for a full-length crystal structure of RPA has resembled that for the Holy Grail. However, over the past few decades, several groups have reported either NMR or crystal structures of various domains and truncated subunits of RPA (Fig. 10.2). These structures have enabled researchers to piece together some of the structural basis for the numerous essential interactions of RPA with DNA and various interacting proteins.

The structures of RPA2-wHLH and RPA1-F protein interaction domains have been determined. An NMR structure of the C-terminal region of RPA2 comprising residues 172–270, revealed a wHLH domain formed by a right-handed three-helix bundle and three short anti-parallel β-strands (Fig. 10.2a) (Mer et al. 2000b). This wHLH domain is an important protein-protein interaction domain in DNA repair, mediating interactions with, for example, XPA, UNG2 and RAD52 (Jackson et al. 2002; Mer et al. 2000b; Stigger et al. 1998). The N-terminal RPA1-F domain, encompassing residues 8–108 was also studied with NMR; it forms a five-stranded β-barrel which is capped on both ends by a short helix (Jacobs et al. 1999) (Fig. 10.2b). This region was shown to associate with various proteins including p53, VP16, Gal4 and XPG (Bochkareva et al. 2005; He et al. 1993, 1995) and is very important in DNA replication. Residues 109–168 form an unstructured flexible linker to RPA1-A. The β-barrel contains two loops on one side that form a basic cleft containing one lysine and five arginine residues which extend from one end of the β-barrel. This basic cleft was proposed to form a binding surface for the acidic motifs of transcriptional activators, repair proteins and replication proteins.

X-ray crystallography was used to study the structure of the primary DNA binding domains of RPA1: A (residues 180–290) and B (residues 300–420). RPA1-AB are arranged in a tandem orientation and connected with an extended, flexible interdomain linker (Fig. 10.2c). The crystal structure revealed that each domain contains an OB-fold structure with an N-terminal extension with RPA1-B also having a C-terminal helix (Bochkareva et al. 2001). Without ssDNA bound, the flexible linker between RPA1-A and RPA1-B can adopt multiple conformations. RPA1-AB was co-crystallized with a poly dC_8 oligonucleotide (Fig. 10.2d) (Bochkarev et al. 1997; Pfuetzner et al. 1997). This structure clearly showed that both OB-folds contain ssDNA binding sites. Upon binding ssDNA the OB-folds reorient, the interdomain linker is stabilized and the binding surfaces coalign to tightly bind the oligonucleotide (Fig. 10.3a, b). The oligonucleotide crosses on the β-strands on both OB-folds and between the loops L12 and L45 (Fig. 10.2d) and these loops

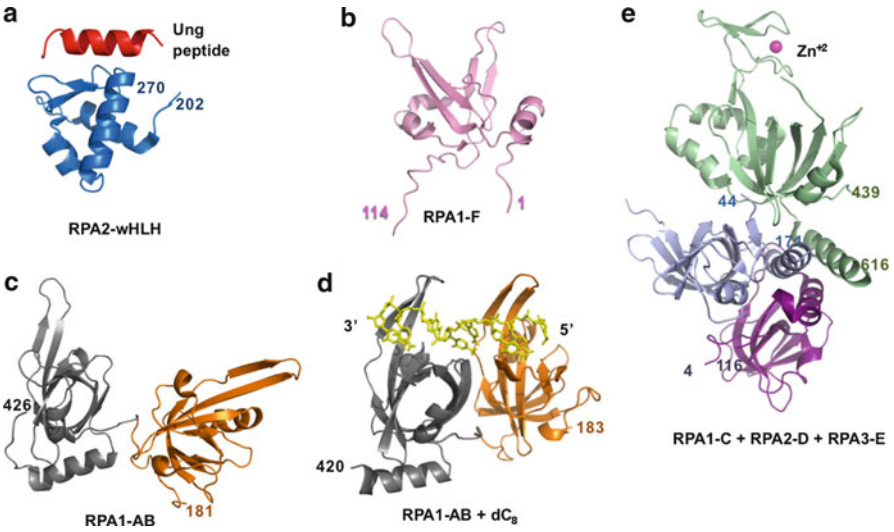

Fig. 10.2 X-ray and NMR structures of RPA domains. (**a**) Domain RPA2-wHLH with Ung peptide bound (Mer et al. 2000b); (**b**) Domain RPA1-F; (**c**, **d**) Major ssDNA binding domains RPA1-AB with and without ssDNA bound (Bochkarev et al. 1997; Bochkareva et al. 2001); (**e**) Trimerization core containing domains RPA1-C, RPA2-D and RPA3-E (Bochkareva et al. 2002). Domains colored as in Fig. 10.1. PDB IDs used were 1LIO, 1JMC, 1FGU, 1EWI and 1DPU, respectively

significantly change their conformation when DNA is bound (Fig. 10.3a, b). The cytosine bases are tucked into the binding cleft and the phosphodiester backbone interacts with basic patches on the surface (Fig. 10.3b). The loops move to a closed conformation by folding around the oligonucleotide and securely hold it in the depths of the charge and shape compatible binding cleft. Each OB-fold makes contacts with three nucleotides and there are two nucleotides between the OB-folds (Fig. 10.2d). RPA1-A makes more extensive contacts with the ssDNA than RPA1-B. Thus we have a structural description for how RPA's primary DNA binding domains bind pyrimidine-rich ssDNA with high affinity.

The crystal structure of the RPA2/3 core was solved (Bochkarev et al. 1999). This construct included only the central region of RPA2 (residues 43–171) and full-length RPA3 which were resistant to limited proteolytic digestion (Fig. 10.4a). This structure revealed that both RPA2 core and RPA3 contain canonical OB-fold structures with an N-terminal extension and a C-terminal helix. The heterodimer interface is mediated by the C-terminal helices on both subunits through a helix-helix interaction, while a higher order (dimer of dimers) interaction is mediated by a four-helix bundle (Fig. 10.4a). This helix bundle was proposed to play a role in trimerization of the full-length protein.

Full-length RPA2/3 was solved in several crystal forms (Deng et al. 2007). In these crystals the N-terminus (residues 1–42) and C-terminal wHLH domain (residues 175–270) were disordered with very weak electron density. The ordered OB-fold

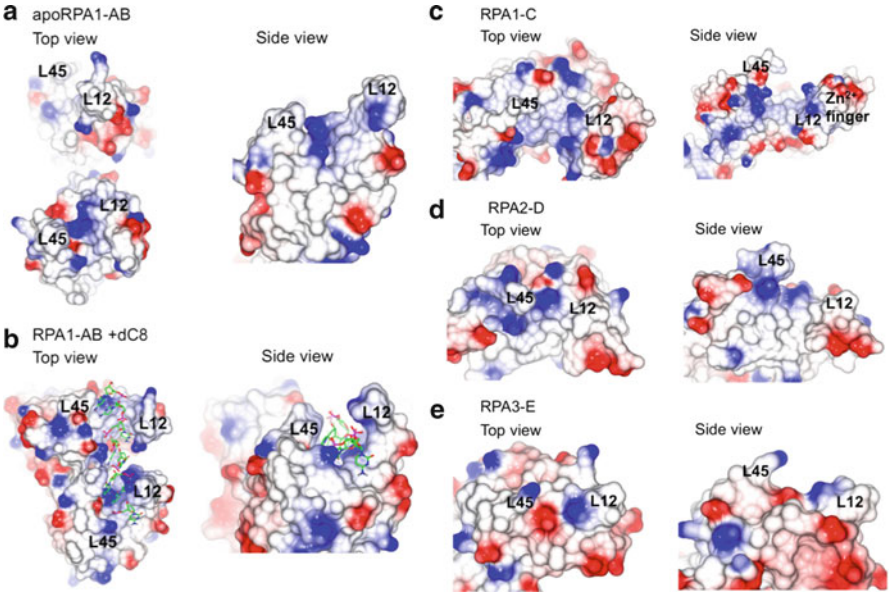

Fig. 10.3 Electrostatic surfaces of RPA's DNA binding domains. (**a**, **b**) Major ssDNA binding domains RPA1-AB with and without ssDNA bound. In the side view, the A domain is in the foreground. In the top view, the A domain is at the bottom; (**c**) RPA1-C domain; (**d**) RPA2-D domain; (**e**) RPA3-E. Surface figures were created using ccp4mg with −0.5 V (*red*) to +0.5 V (*blue*) (Potterton et al. 2004)

regions were very similar to the previously solved RPA2/3 core crystal structure. However, the higher order quaternary structures formed between the heterodimers were significantly different. The four-helix bundle previously thought to be important for forming the heterotrimer only occurred when the deletion construct was crystallized. The full-length RPA2/3 crystals contained different dimer-dimer interfaces (Fig. 10.3b, c). These differences in quaternary structure may reflect the actual structural repertoire of the RPA heterotrimer and the relative locations of the RPA2/3 α2 helices may represent alternate locations for the RPA1 α3 helix when the trimer forms.

The crystal structure of the RPA trimerization core included the C-terminus of RPA1 (residues 436–616), the core of RPA2 (residues 45–171) and RPA3 (Fig. 10.2e) (Bochkareva et al. 2002). All three domains comprise an OB-fold structure flanked by a C-terminal α-helix and are structurally similar (Fig. 10.1c). The hydrophobic interactions present between these helices form a three-helix bundle and mediate the trimerization of the domains (Fig. 10.4d). Also, a six-helix bundle forms between trimers in the crystal lattice (Fig. 10.4e). The individual RPA2 and RPA3 core regions in this structure are identical to those found in the dimer core structure. The binding surface of RPA2 and RPA3 is much shallower than seen with RPA1-AB (Fig. 10.3d, e), which corresponds with their weaker binding to ssDNA. Also, the floor of the binding cleft on RPA2-D is positively charged, and for RPA3-E the floor

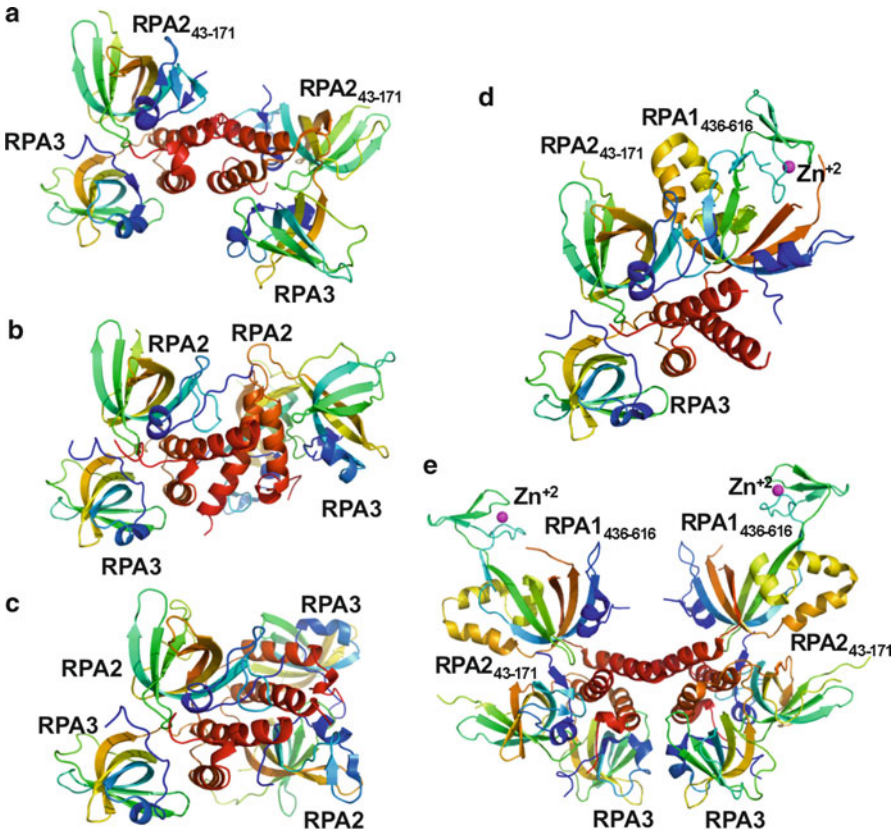

Fig. 10.4 Four-helix bundle quaternary structures formed by the RPA2/3 heterodimer and by the RPA trimer core. (**a**) Dimer core (Bochkarev et al. 1999); (**b**) Orthorhombic; and (**c**) Hexagonal crystal forms of full length dimer (Deng et al. 2007); (**d**) Three helical bundle formed by one trimer core; (**e**) Six helical bundle formed in the crystal lattice of the trimer core (Bochkareva et al. 2002). Domains colored by sequence number with N-terminal residues shown in *blue* and C-terminal residues *red*. For parts **a**, **b** and **c**, PDB IDs 1QUQ, 2PI2 and 2PQA were used, respectively. For parts **d** and **e**, PDB ID 1LIO was used

is negatively-charged with positively-charged loops. These observations imply that these OB-folds bind ssDNA differently than RPA1-AB.

RPA1-C contains a zinc-finger motif that sets it apart from the other OB-folds (Bochkareva et al. 2000). This zinc finger is present between strands ß1 and ß2, and the zinc ion present in the structure is coordinated by four cysteine residues (Cys 481, 486, 500 and 503; Fig. 10.1c). This is similar to transcription factors involved in DNA binding that have also been shown to possess zinc finger domains (Krishna et al. 2003). The binding cleft is deep and the surface charges for RPA1-C are very similar to RPA1-AB (Fig. 10.3c), implying that it will bind ssDNA in a similar conformation. Unfortunately, at this time we have no structural data on how RPA1-C, RPA2-D and RPA3-E interact with ssDNA.

Small angle x-ray scattering (SAXS) has been used to study the structural dynamics of the N-terminal half of RPA1 (including domains RPA1-F, -A and -B) when bound to ssDNA (Pretto et al. 2010). Consistent with previous reports, SAXS data indicate that binding of ssDNA to RPA1-FAB reduces the interdomain flexibility between RPA1-A and -B but has no effect on RPA1-F which is available for protein interactions. These data support a model where RPA1-F remains structurally independent of RPA1-AB when RPA is bound to ssDNA, thereby allowing RPA to form critical protein-protein interactions.

Structural studies on various domain constructs of RPA have provided a wealth of structural knowledge that has helped considerably in understanding the complex functions of RPA. Unfortunately, we still do not have a complete structural model for RPA. Methods have been developed using mass spectrometry to follow the reactivity of amino acids to proteolytic and chemical modification to test theoretical models of RPA built using the available domain structures (Nuss et al. 2006). These reactivities have been employed to construct and test a complete model for the structure of RPA (Nuss et al. 2009). This RPA structural model contains stable domains and highly flexible non-domain regions. The overall structure is discoidal, and its surface is predominantly negatively charged with neutral and positive patches coinciding with ssDNA or protein binding sites. This leaves one face of the structure largely negative for interaction with basic protein molecules. The DNA binding OB-folds (A, B, C, and D) are exposed to solvent and, with the exception of OB-fold D, they are on the periphery of the complex. This structure is consistent with ssDNA binding simultaneously to domains A–D. Most of the protein binding sites on RPA are also exposed and accessible to protein ligands. Four relatively long (>20 amino acids) regions of the RPA primary structure are coiled or intrinsically disorganized as judged by primary structure analysis. This model is helpful in understanding RPA function but is still limited in the understanding of full-length RPA because of the flexible nature of the protein.

Full-length heterotrimeric RPA was analyzed using NMR and gave rich insight into the folding and structural dynamics of this multidomain, flexible protein (Brosey et al. 2009). The NMR spectra on the RPA trimer contained over 350 of the 550 expected signals domains F, A, B, wHLH and the N-terminus of RPA2. The signals were nearly identical in position on the spectra as those from the isolated domains. This indicates these domains are structurally independent from each other in the absence of DNA. Signals from RPA-CDE core were absent in the spectra from the full-length protein, indicating it had a slow rate of tumbling due to the drag caused by the attachment of the five other domains. Experiments conducted in the presence of DNA confirmed that the basic RPA1-F domain and the acidic RPA2-wHLH domain played no role in binding to ssDNA and remained available for binding to other protein factors. Upon binding to DNA, a structural rearrangement and alignment of RPA1-AB with RPA-CDE was observed. Changes were also seen in the NMR signals of the N-terminal region of RPA2 reflecting remodeling of this region. This last observation may explain how the N-terminus of RPA2 with ssDNA bound is more accessible to kinase activity during DNA repair processes than the free form (Fotedar and Roberts 1992). We look forward to more NMR experiments on intact RPA and the full-length crystal structure in the future.

10.4 Interactions of RPA with Single-Stranded DNA

The DNA binding properties of RPA have been extensively studied and reveal several important features. Importantly, its ssDNA binding function protects DNA from nucleases and aids in unfolding any secondary structures that DNA forms which may disrupt DNA processing. RPA binds ssDNA with a much greater affinity when compared with dsDNA or RNA, and binds ssDNA with low cooperativity and a 5′→3′ molecular polarity. In fact, RPA binds ssDNA over 1,000-fold better than dsDNA with an association constant in the range of 10^9–10^{11} M^{-1} (Kim et al. 1992, 1994). The binding of RPA also appears to be sequence dependent, as it prefers to bind polypyrimidine sequences over polypurine sequences. One can summarize the order of RPA binding to nucleic acids in order of decreasing affinity as follows: polypyrimidine > mixed ssDNA > polypurine ssDNA >> damaged dsDNA > dsDNA ≡ RNA. RPA-ssDNA binding depends on two important factors, the length of the ssDNA sequence and salt conditions used in the assay. Shorter ssDNA sequences have lower binding constants for RPA with association constants ranging from 10^7 to 10^9 M^{-1}. The binding of RPA to pyrimidine-rich sequences is so tight that salt concentrations >1.5 M are necessary to weaken its interaction with the ssDNA for comparative studies (Kim et al. 1992; Wold 1997).

Several lines of evidence indicate that the binding of RPA to ssDNA causes a significant change in its conformation. Limited proteolysis experiments revealed that without ssDNA present, RPA1 and RPA2 are degraded within minutes (Gomes et al. 1996). RPA3 was resistant to proteolysis in these experiments. When a polypyrimidine oligonucleotide (dT_{30}) was present, RPA1 and RPA2 became more resistant to degradation and the domain structure of RPA, used in the many structural studies already discussed, was revealed. Additionally, electron microscopy (EM) images of the RPA-ssDNA complex indicate that the complex can adopt three different molecular shapes: globular, elongated, or contracted depending on the salt concentrations present in the reactions (Treuner et al. 1996). Using scanning-transmission electron microscopy (STEM), RPA was shown to adopt different conformations upon DNA binding (Blackwell et al. 1996). These complexes were observed as either an 8 nt mode which is more compact and globular or a 30 nt elongated binding mode. These early observations were then incorporated into the models for ssDNA binding described next.

The versatility of RPA's numerous possible interactions with ssDNA comes from the multiple DNA binding domains of RPA (Fanning et al. 2006; Sakaguchi et al. 2009). RPA1-A and-B are known as the primary DNA binding domains. These domains bind DNA with 10–50-fold lower affinity when compared to full-length RPA depending on the length and nature of the DNA sequence. RPA1-C and RPA2-D have some, albeit weak, DNA binding activity on the order of 10^{-5}–10^{-6} M^{-1}. Based on the numerous ssDNA interactions performed with RPA, a sequential model was proposed for DNA binding by RPA (Bastin-Shanower and Brill 2001; Bochkarev and Bochkareva 2004; Fanning et al. 2006). In these models, RPA1-A recognizes ssDNA first and this is followed by the binding of RPA1-B. Together RPA1-A and -B bind a footprint of 8 nt. This is followed by the binding of RPA1-C

which is then involved in binding a 12–23 nt segment of DNA. The binding of RPA2-D covers a length of 25–30 nt which is the most characterized, well-known footprint of RPA. This sequential model, updated with the recent data about RPA1-F and RPA2-wHLH being protein binding domains, is summarized in Fig. 10.5.

In the absence of the primary DNA binding domains, a construct containing only the trimer core RPA-CDE was capable of recognizing a primer-template junction (Dickson et al. 2009; Kolpashchikov et al. 2000a, b; Pestryakov et al. 2003, 2004, 2007; Pestryakov and Lavrik 2008; Weisshart et al. 2004). RPA3-E plays a vital role in the recognition of this primer-template junction since the same core with RPA3 deleted could not properly recognize the 3′ end of the primer-template junction. There is evidence for RPA3-E interacting with ssDNA molecules bound to trimeric RPA (Pestryakov et al. 2007); the polarity of this interaction is on the 3′ side of the oligonucleotide (Salas et al. 2009). Despite RPA's traditional preference for pyrimidine-rich sequences, more light has been shed on the interaction of RPA with biologically-relevant mixed ssDNA sequences (Deng et al. 2009) and the binding preferences of individual domains (Prakash et al. 2011b). Additionally, RPA is now known to bind non-canonical ssDNA sequences capable of forming complex secondary structures (Fan et al. 2009; Salas et al. 2006; Wu et al. 2008). These secondary structures pose a difficult challenge for DNA replication and the involvement of RPA in conquering them appears to be important, as described below.

10.5 DNA Structure and Requirement for RPA

The versatile nature of DNA and its ability to form stable secondary structures has intrigued scientists for a long time (see Mirkin 2008, for review). Some of these structures include DNA hairpins, cruciforms, triple-helical DNA, *i*-motif and G-quadruplex structures (Fig. 10.6). The formation and stabilization of these secondary structures *in vivo* has sparked the interest of researchers all over the world for decades because of their potential role in stalling replication thereby leading to disease progression (Voineagu et al. 2009; Wells 2007). The section below discusses some of these DNA arrangements in a disease-relevant context emphasizing the requirement for proteins like RPA to help unfold these structures and/or to signal a stress-response.

DNA hairpins are formed when ssDNA bends back onto itself forming duplex DNA and terminating in a loop (Voineagu et al. 2008). These are commonly formed by inverted repeat sequences. The stability of these hairpins is dependent upon the GC content of the sequence. The most common sequences that have the capability to form hairpins are trinucleotide repeats (TNRs), where the trinucleotide (for example, CNG or GAA, where N is any nucleotide) sequence is repeated multiple times (Lahue and Slater 2003). There are now over 20 known neurological disorders that involve TNRs, including Huntington's disease, Fragile X syndrome, and myotonic dystrophy (Cummings and Zoghbi 2000; Mirkin 2006, 2007). TNRs can occur in non-coding sequences as well as within coding sequences. NMR structural studies of these repeat

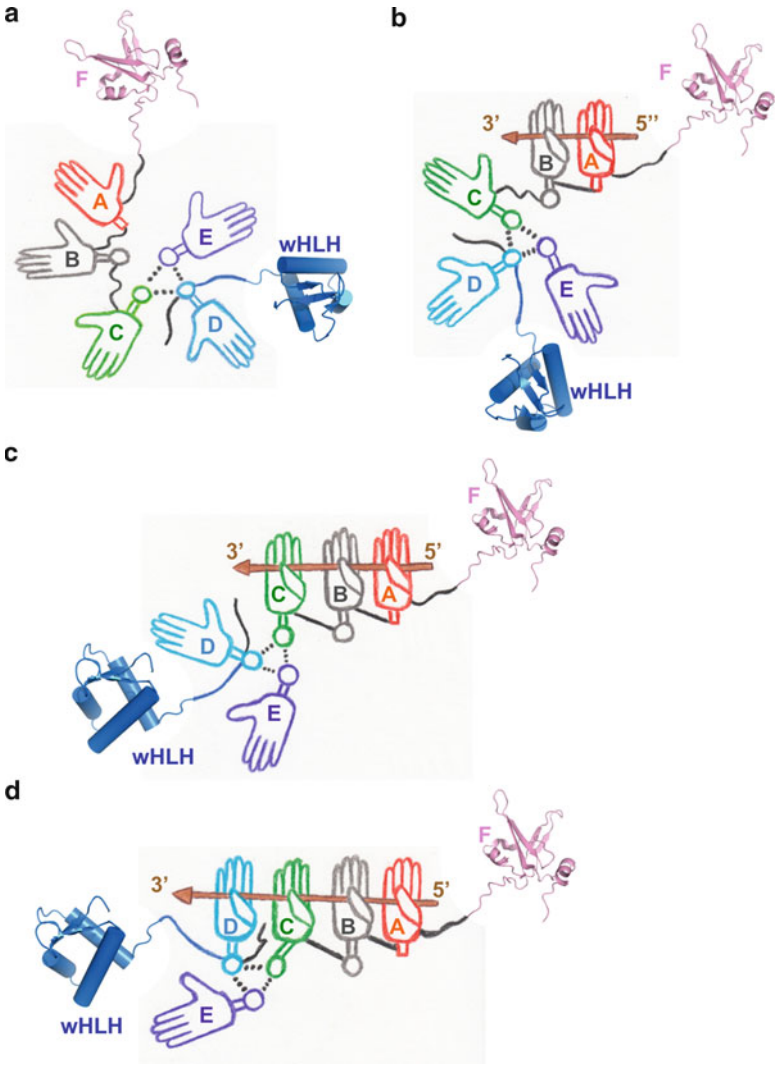

Fig. 10.5 Sequential binding model for RPA. (**a**) Unbound RPA in globular conformation. (**b**) Binding of 8–10 nt by RPA1-A and RPA1-B; (**c**) Binding of 13–15 nt by RPA1-A, RPA1-B and RPA1-C; (**d**) The 30 nt binding mode with all four DNA binding OB-fold domains. Domains RPA1-F and RPA2-wHLH are involved in protein-protein interactions. This model was created combining information from what is known about the flexible regions of RPA, the order of DNA binding, which domains primarily bind ssDNA (*A–D*) and which are involved in protein-protein interactions (*F* and *wHLH*) and speculation that various helical bundles might form the heterotrimer quaternary interface (Figs. 10.3 and 10.4) (Bastin-Shanower and Brill 2001; Bochkarev and Bochkareva 2004; Bochkareva et al. 2002; Brosey et al. 2009; Deng et al. 2007; Fanning et al. 2006; Gomes et al. 1996)

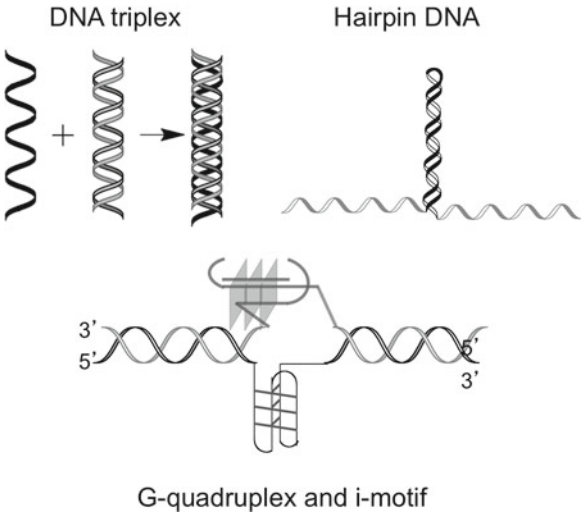

Fig. 10.6 RPA must bind ssDNA secondary structures and keep them from forming during DNA replication. Schematic representation of the structures of a DNA triplex (*upper left*), a DNA hairpin (*upper right*) and a G-quadruplex opposite an *i*-motif structure (*lower part*)

sequences reveal and confirm the formation of hairpin and mismatched DNA duplex structures (Mariappan et al. 1998). In such instances when the replicative polymerase encounters a stable secondary structure that was not unwound by a helicase or DNA binding protein, it skips over the region resulting in a loss of genetic information, genome instability and disease progression.

Another non-canonical DNA secondary structure that appears to have an effect on process like replication and transcription in the formation of triple helical DNA, often called triplex DNA (Bissler 2007). These structures are formed when a third strand of DNA binds to the major groove of a double-stranded DNA, using Hoogsteen base-pairing. Triplexes can form intermolecularly where the third strand originates from a second DNA molecule or from a triplex forming oligonucleotide (TFO), whereas in the case of intramolecular triplexes, also commonly referred to as H-DNA, the third triplex forming strand originates from a region within the same DNA molecule. TFOs are being exploited as therapeutic agents to target specific genes because of their ability to bind duplex DNA with high-affinity (Jain et al. 2008). Through the years, it has been noted that H-DNA structures can be formed by triple repeat structures. In the case of Friedreich's ataxia, an expansion of the intronic sequence d(GAA)n forms a triplex structure that halts DNA polymerization *in vitro* (Mirkin 1999). Thus, triplex DNA structures also pose a challenge for DNA replication.

The knowledge that G-rich regions in DNA form non-B DNA secondary structures like G-quadruplexes (often called tetraplexes or G4 DNA) has been known for a long time; contrary to their being a nuisance, these sequences have potentially important roles in regulating cellular metabolism (Dai et al. 2010; Gellert et al. 1962; Huppert 2008). *In vitro*, G-rich sequences can form a variety of G-quadruplex structures. Four planar guanine residues interact via Hoogsteen hydrogen bonds to form a G-quartet (Huppert and Balasubramanian 2007; Patel et al. 2007). A G-quadruplex results from the stacking of two or more G-quartets. The formation

of G-quadruplexes also depends on the presence of monovalent cations such as sodium or potassium ions. The precise ion preference depends on the sequence and nature of the G-quadruplexes (Marathias and Bolton 1999). G-quadruplexs form at the telomere and in the promoter regions of proto-oncogenes, such as c-MYC, VEGF, c-KIT and Bcl-2 (Eddy and Maizels 2006, 2009; Patel et al. 2007). An *i*-motif can form on the strand opposite the G-quadruplex (Dai et al. 2010). These locations indicate that the occurrence of G-quadruplexes and *i*-motifs might be regulatory and play a role in the formation and progression of many cancers.

10.6 RPA Binding to Non-canonical DNA Structures

As discussed previously, hairpin structures present the replication machinery with a challenge and a roadblock if not properly unwound or melted. *In vitro*, RPA was shown to bind preferentially to hairpin structures with a 3′ protruding end. However, in this study, RPA did not significantly melt or unfold the hairpin structures (de Laat et al. 1998). However, as will be discussed in later sections, RPA also serves to recruit other DNA binding proteins such as helicases that enable successful unwinding of DNA. Thus RPA binding could be a crucial initial first step in binding the ssDNA regions generated by hairpin structures in DNA and further aiding in the unfolding of these structures through the recruitment of other proteins. In contrast to *E. coli* and T4 ssDNA binding proteins, RPA was shown to melt a DNA triplex containing a pyrimidine third strand annealed to duplex DNA (Wu et al. 2008). In the same study, cellular analyses using HeLa cells indicated that depletion of RPA caused an increase in triplex DNA content. This emphasizes a physiological role for RPA in binding and unfolding such secondary structures.

Compared with the above-mentioned secondary structures, a significantly greater number of studies were performed with RPA binding to G-quadruplex DNA. Some of these studies are summarized here. Native gel electrophoresis, cross-linking, and fluorescence resonance energy transfer experiments indicate that RPA can bind and unfold a 21-mer telomeric G-quadruplex sequence (Salas et al. 2006). Most recently, studies employing CD (circular dichroism) indicate that RPA can bind and melt intramolecular G-quadruplex structures (Fan et al. 2009). In fact, it was demonstrated that RPA could bind a purine-rich, G-quadruplex forming sequence with a similar affinity as the complementary pyrimidine-rich sequence. Interestingly, the above studies showing RPA unfolding G-quadruplexes were all done in the presence of Na^+ ions. It was subsequently shown that K^+ (and a porphyrin drug) can stabilize G-quadruplex forming sequence from RPA unfolding (Prakash et al. 2011a). G-quadruplex forming sequences can induce instability during leading-strand replication when cells are treated with a G-quadruplex stabilizing drug or in the absence of the G-quadruplex unwinding Pif1 helicase (Lopes et al. 2011). It is possible that RPA may have a role in these types of errors in DNA replication.

RPA helps prevent the accumulation of telomeric DNA in cells employing alternative lengthening of telomeres, supports telomerase activity in yeast, restores

human telomerase activity *in vitro*, and causes telomere shortening in human cancer cells (Grudic et al. 2007; Kobayashi et al. 2010). The human Dna2 protein possesses both helicase and nuclease activities during lagging strand DNA replication and it specifically binds to telomeric regions that have the propensity to form G-quadruplexes (Masuda-Sasa et al. 2008). Although the helicase activity of Dna2 is effective in unwinding G-quadruplex DNA, this secondary structure causes attenuation of nuclease activity. The presence of RPA bound to the G-quadruplex DNA restores the nuclease activity of Dna2, thus emphasizing the requirement for RPA during telomere biogenesis.

The diverse nature of RPA binding to ssDNA has been explored by several groups. However, so far the data are limited since most studies on RPA, and its domains, have been performed using primarily poly-pyrimidine ssDNA sequences. The specific ssDNA sequences preferred by the DNA binding OB-fold domains of RPA were studied using SELEX (Systematic Evolution of Ligands by EXponential enrichment) methods (Prakash et al. 2011b). Not surprisingly, SELEX with full-length RPA revealed no specific sequence preference. The most interesting SELEX result was obtained with RPA-CDE which selected a 20-mer G-rich sequence that formed an intramolecular G-quadruplex. Fluorescence polarization (FP) binding studies to verify and understand the SELEX results were conducted where the selected G-quadruplex, a TC-rich complement of the G-quadruplex, a polyA and a polyG sequence were tested using five different RPA constructs: (i) full length RPA, (ii) RPA1-AB, (iii) RPA-CDE-core, (iv) RPA-DE, and (v) RPA1-C. These extensive FP binding studies indicate that domains RPA1-A, -B and -C of contribute to the "universal binder" functions of RPA. The similarities of their binding surfaces support this observation (Fig. 10.3). Binding affinity, with the RPA-C construct indicated that this construct binds to TC-rich and G-rich sequences alike with a binding constant ~3 μM. Most importantly RPA2-D and RPA3-E appear to contribute to a more specialized function for binding preferentially to G-rich sequences. CD studies showed that full length RPA and RPA-CDE core bind and unfold the G-quadruplex. RPA-DE on the other hand stabilized the G-quadruplex secondary structure. Note RPA2-D is unique in that is features positive charge on the floor of the binding cleft and a model for how RPA2-D could bind a folded G-quadruplex was built (see Fig. 8(e) in Prakash et al. 2011b). Taken together, it is likely that RPA-DE can recognize the G-quadruplex fold and in the context of the RPA heterotrimer, the G-quadruplex becomes unfolded. Also RPA-DE might recognize DNA secondary structures, such as G-quadruplexes or DNA hairpins and then recruit DNA helicases, like Dna2, to help unwind and unfold these structures for proper DNA replication.

10.7 RPA Binding to Damaged DNA

DNA is constantly being subjected to assault by either exogenous or endogenous factors that cause damage. Some exogenous agents include ultra-violet (UV) light, ionizing radiation (IR), toxic chemicals, and chemotherapeutic drugs. Endogenous

agents include reactive oxygen species (ROS), free radicals, secondary structures formed within DNA, and others. When the replicative machinery encounters lesions in the DNA caused by one or more of these factors, stalling occurs, causing replication arrest which further leads to a cascade of events to take place that signal the damage is present so that it is either repaired or bypassed (Hyrien 2000). The binding of RPA to damaged DNA has also been studied extensively. The first such report indicated RPAs interaction with DNA damage adducts and crosslinks, mediated by cisplatin (Clugston et al. 1992). In another study involving cisplatin induced DNA damage, RPA was seen to bind the damaged duplex DNA with a 10–50-fold increase in affinity over undamaged duplex DNA (Patrick and Turchi 1998). Conflicting reports exist in the literature as to whether RPA prefers to bind to the damaged DNA strand or the undamaged strand *in vitro* (Hermanson-Miller and Turchi 2002; Schweizer et al. 1999). Further, the binding of RPA has been studied with UV-induced damaged DNA where RPA bound preferentially to the 6-4- photoproduct thus formed. Therefore, not only does RPA have functions in binding and coating ssDNA regions formed during replication, but also binds to sites of DNA damage that can occur as part of the process. The binding and interactions of proteins involved during replication, either upon replication stalling or during normal replication, to RPA will be discussed in the next section.

10.8 Role in Recruiting Proteins to the Replication Fork

While RPA is binding ssDNA, it also helps coordinates DNA replication by binding to other replicative proteins at the appropriate place and time. RPA's primary replicative protein interaction domain appears to be the N-terminal RPA1-F domain (Figs. 10.1a and 10.5). Large T-antigen, some of the helicases, replication factor C (RFC), Dna2 and Pol α-primase all interact with RPA1-F (Fanning et al. 2006). The C-terminal RPA2-wHLH domain has been shown to also be important in binding T-antigen and proteins involved in processing stalled replication forks. RPA binds ssDNA at the replication fork immediately after the initiation of replication and then these interactions between RPA and other proteins are essential for forming an active DNA replication fork indicating that RPA is a proteinaceous glue of sorts.

Human RPA was originally recognized as a component necessary for SV40 DNA replication *in vitro* (Fairman and Stillman 1988). The interaction between RPA and the SV40 large T-antigen was shown to be essential for primosome assembly (Melendy and Stillman 1993). More specifically, interaction with the SV40 large T-antigen is mediated by both the RPA2-wHLH and the RPA1-F domains (Han et al. 1999; Taneja et al. 2007) and both of these domains were absolutely required for successful DNA replication. Large T-antigen residues 164–249, located within the DNA binding domain, are responsible for mediating this interaction with RPA (Weisshart et al. 1998). Large T-antigen actively loads RPA onto nascent ssDNA after initiation. NMR analyses indicated that the T-antigen, RPA1-F and a short 8-mer oligonucleotide can form a stable ternary complex (Jiang et al. 2006). This complex was disrupted by increasing the length of the DNA bound to RPA,

thereby indicating a conformational change within the protein that is required for loading onto the DNA (Arunkumar et al. 2005). Thus, T-antigen protein interactions with RPA, plus RPA's conformational change upon binding ssDNA (Fig. 10.5) load RPA on to ssDNA during initiation.

In addition, during the initial stages of replication RPA also forms a stable complex with DNA Pol α-primase and as well as with T-antigen (Dornreiter et al. 1992). The primase domain of Pol α-primase and RPA1-F domain mediate this interaction (Braun et al. 1997). RPA was shown to stimulate Pol α-primase activity and reduce misincorporation by this polymerase, thereby increasing its processivity.

During the process of replication, Pol α-primase is replaced by a switching mechanism where RFC, the eukaryotic clamp loader (see Chap. 14), binds to the 3′ end of the nascent DNA and loads PCNA and Pol δ (Waga and Stillman 1994). This switch occurs in the presence of RPA where RPA1-F binds Rfc4, one of the five subunits of RFC (Kim and Brill 2001). Thus, RPA participates in loading PCNA through an RFC protein-protein complex.

Furthermore, during the elongation stage of DNA replication RPA stimulates the action of Pol δ and Pol ε, an activity that could be the result of RPA's interaction with PCNA. Pol δ is one of the replicative polymerases which functions mainly in lagging strand synthesis (McElhinny et al. 2008) (see Chap. 12). This polymerase competes with RFC for RPA, resulting in displacement of RFC from the 3′ end (Yuzhakov et al. 1999). During the processing of Okazaki fragments, the Dna2 helicase/endonuclease aids in removing the RNA primers of these fragments. RPA plays a role in the stimulation of Dna2 endonuclease activity mediated by direct protein-protein interactions at the N-terminal domains of Dna2 and RPA1-F (Bae et al. 2001, 2003).

Another group of proteins that interact with RPA during the replication process are the RecQ family of helicases. The Werner syndrome protein (WRN), a member of this class of helicases that localizes to sites of stalled replication, directly interacts with RPA and the Mre11 complex upon replication arrest (Constantinou et al. 2000). In contrast to human RPA, *E. coli* SSB and bacteriophage T4 gene 32 protein (gp32) failed to stimulate WRN helicase unwinding of long DNA duplexes, indicating a specific interaction between WRN and RPA (Brosh et al. 1999). The interaction of WRN and RPA is substantially increased at stalled replication forks (Machwe et al. 2011). Similarly, a Bloom syndrome helicase (BLM) interacts with RPA using its N-terminal acidic domain. The basic N-terminal RPA1-F domain interacts with both the WRN and BLM helicases (Doherty et al. 2005). This interaction stimulates the helicases' ability to unwind long DNA substrates. These results suggest that the critical interactions between RPA and WRN or BLM helicases play an important role in the mechanism of RPA stimulated DNA unwinding during replication.

As indicated by the above examples, protein-protein interactions mediated by RPA are essential for successful replication. However, when the replication fork stalls, a DNA damage response (DDR) ensues involving the recruitment of repair proteins, several of which require an initial interaction with RPA. RPA is involved in cell cycle checkpoint signaling in addition to the DDR. Signaling from a stalled replication fork involves proteins that are sensors, mediators, transducers or effectors. Sensor proteins like ATM/ATR, the 9-1-1 complex and the MRN complex sense the

damage and through mediator proteins such as 53BP1, TopBP1, claspin, etc., mediate and recruit proteins that aid in restoring the replication fork (Sogo et al. 2002; Zou and Elledge 2003). RPA is required for the recruitment of the ATR kinase to sites of DNA damage and for ATR-mediated CHK1 phosphorylation and activation *in vivo*. The N-terminal region of RPA1 also stimulates the binding of ATR interacting protein (ATRIP) to ssDNA *in vitro* enabling the ATR-ATRIP complex to associate with DNA. The N-terminal region of RPA1 binds ATRIP, Rad9 and Mre11. Binding of RPA to Rad9 promotes ATR signaling (Xu et al. 2008). The Rad9 protein is part of the 9-1-1 (Rad9-Rad1-Hus1) clamp protein complex that plays a key role in cellular response to DNA damage (Kemp and Sancar 2009). The pro-apoptotic BH3-interaction death domain agonist (BID) associates with RPA1-F and stimulates the recruitment/stabilization of ATR-ATRIP to the DNA damage sensor complex (Liu et al. 2011). The Rad17 protein aids in loading the clamp complex onto the DNA via an RPA-mediated interaction. Further phosphorylation of the Rad17 protein activates the downstream cell cycle check-point to mediate DNA repair or alternatively leads to apoptosis (Gottifredi and Prives 2005). It has also been indicated that RPA-coated ssDNA recruits the protein Cut5 which facilitates the binding of the sensor protein ATR, Pol α-primase and Rad1 to damaged DNA (Parrilla-Castellar and Karnitz 2003). Another protein SMARCAL1 localizes to stalled replication forks via an interaction with the RPA2-wHLH domain. Silencing of SMARCAL1 causes an increase in RPA binding to chromatin (Bansbach et al. 2009). From all the above examples, it is evident that these proteins that are necessary for successful replication require an interaction with RPA.

It is noteworthy to mention here that RPA itself is phosphorylated in a cell cycle dependent manner and is hyperphosphorylated in response to DNA damage (Oakley et al. 2001; Oakley and Patrick 2010). Studies on RPA phosphorylation have been primarily focused on the N-terminal region of RPA32 because this domain is conserved in higher eukaryotes and up to ten phosphorylation sites have been noted on RPA32 (Ser4, Ser8, Ser11-13, Thr21, Ser23, Ser29, Ser33 and Thr98). The kinases that are known to phosphorylate RPA are ATM, ATR and DNA-protein kinase (DNA-PK). Although, the phosphorylation of RPA does not directly impact the process of DNA replication, some studies report an inhibitory effect (Vassin et al. 2004). It has been shown that RPA mediates recombination-based repair during replication stress (Sleeth et al. 2007). RPA's interaction with RAD52 in this repair pathway involves RPA1 and RPA2-wHLH domains (Jackson et al. 2002) and is activated by phosphorylation (Deng et al. 2009). So it can be surmised that the phosphorylation of RPA forms a link between signaling from a stalled replication fork to the initiation of DNA repair, mediated via extensive protein-protein interactions.

10.9 Concluding Remarks: Future Research on RPA

Despite the vast knowledge of RPA gained over the past three decades, RPA still poses an enigma to scientists interested in facets of DNA metabolism involving replication, recombination and repair. Although various aspects of RPA's binding to

DNA have been elucidated, mechanistically, the recruitment of RPA to ssDNA regions is still largely unknown. Thus, a key question that still remains is, how does RPA sense ssDNA regions? Does it remain loosely bound to DNA at all times in a "*cis*-fashion" or are there other signals that lead to a "*trans*" recruitment of RPA. The spatial-temporal regulation of RPA binding to DNA within a cell remains a mystery. Other related questions that influence our thinking about RPA include: What is the mechanism by which RPA is released from DNA so it can be handed-off to the next protein? Does phosphorylation of RPA play a role in facilitating the release of RPA from DNA by causing a conformational change in the protein? How does RPA recognize, bind and relax secondary structures formed in ssDNA regions? What is the global organization of RPA domains during all of its different functional states? How are these changes in architecture used to drive function? How do the various interaction domains serve as exchange points for different proteins and drive transitions in the DNA processing machinery?

Since the interactions between RPA and DNA are crucial in several different pathways, it is fathomable that disrupting this interaction could have disastrous deleterious effects on a cell. However, in the case of rapidly dividing cancer cells, targeting this interaction with small molecule inhibitors might enhance the efficacy of DNA damaging agents currently in use as chemotherapeutics. Recent studies by the Turchi lab have indicated that small molecule inhibitors *in vitro* can target the OB-folds of RPA. One such compound prevented cell cycle progression, induced cytotoxicity, and increased the efficacy of chemotherapeutic damaging agents (Anciano Granadillo et al. 2010; Shuck and Turchi 2010). In addition, through high-throughput screening, small molecule inhibitors of the N-terminal protein-protein interaction domain of RPA1 were discovered. Such novel compounds that disrupt RPA's interactions with other proteins also possess further therapeutic potential (Glanzer et al. 2011). The knowledge of the full-length structure of RPA would aid in a more complete understanding of the protein and perhaps assist in the design of more potent small molecule inhibitors. In addition to being a targeted by chemotherapeutic drugs, RPA has also been shown to be a prognostic indicator for patients with astrocytomas (Kanakis et al. 2011).

Until recently, RPA was thought to be the sole SSB involved in several processes involving DNA metabolism, however two novel proteins human SSB (hSSB1 and 2) were recently discovered to participate in DNA-damage signal transduction. These proteins are more closely related to the archael SSB in terms of domain structure. The relationship between RPA and the two hSSB proteins has not been completely teased out, although the roles for hSSB1 in DSB repair have been well documented (Richard et al. 2008).

For proper cellular function, it is apparent that the DNA within the cell has to be properly replicated and protected. Disturbing the peaceful equilibrium in the cell by DNA damaging agents can lead to replication stress, errors in replication, genomic instability, disease progression and/or cell-death. RPA is one of the key players in maintaining genomic integrity by its involvement in not only the complex replication process but also in the interrelated DNA-repair processes. Future experimental work on this complex protein is necessary and will help define how RPA performs its numerous roles in the cell.

Acknowledgements This work was supported by the American Cancer Society [RSG-02-162-01-GMC], NCI Eppley Cancer Center Support Grant [P30CA036727] and the Nebraska Department of Health and Human Services grants [2011-05 & 2012-04]. Aishwarya Prakash was supported by a University of Nebraska Medical Center graduate fellowship and Presidential graduate fellowship and would also like to thank Dr. Sylvie Doublié for her support [NIH/NCI P01CA098993].

References

Anciano Granadillo VJ, Earley JN, Shuck SC, Georgiadis MM, Fitch RW, Turchi JJ (2010) Targeting the OB-folds of replication protein A with small molecules. J Nucleic Acids 2010:304035

Arunkumar AI, Klimovich V, Jiang X, Ott RD, Mizoue L, Fanning E, Chazin WJ (2005) Insights into hRPA32 C-terminal domain-mediated assembly of the simian virus 40 replisome. Nat Struct Mol Biol 12:332–339

Bae SH, Bae KH, Kim JA, Seo YS (2001) RPA governs endonuclease switching during processing of Okazaki fragments in eukaryotes. Nature 412:456–461

Bae KH, Kim HS, Bae SH, Kang HY, Brill S, Seo YS (2003) Bimodal interaction between replication-protein A and Dna2 is critical for Dna2 function both *in vivo* and *in vitro*. Nucleic Acids Res 31:3006–3015

Bansbach CE, Betous R, Lovejoy CA, Glick GG, Cortez D (2009) The annealing helicase SMARCAL1 maintains genome integrity at stalled replication forks. Genes Dev 23:2405–2414

Bastin-Shanower SA, Brill SJ (2001) Functional analysis of the four DNA binding domains of replication protein A. The role of RPA2 in ssDNA binding. J Biol Chem 276:36446–36453

Binz SK, Sheehan AM, Wold MS (2004) Replication protein A phosphorylation and the cellular response to DNA damage. DNA Repair (Amst) 3:1015–1024

Bissler JJ (2007) Triplex DNA and human disease. Front Biosci 12:4536–4546

Blackwell LJ, Borowiec JA, Mastrangelo IA (1996) Single-stranded-DNA binding alters human replication protein A structure and facilitates interaction with DNA-dependent protein kinase. Mol Cell Biol 16:4798–4807

Bochkarev A, Bochkareva E (2004) From RPA to BRCA2: lessons from single-stranded DNA binding by the OB-fold. Curr Opin Struct Biol 14:36–42

Bochkarev A, Pfuetzner RA, Edwards AM, Frappier L (1997) Structure of the single-stranded-DNA-binding domain of replication protein A bound to DNA. Nature 385:176–181

Bochkarev A, Bochkareva E, Frappier L, Edwards AM (1999) The crystal structure of the complex of replication protein A subunits RPA32 and RPA14 reveals a mechanism for single-stranded DNA binding. EMBO J 18:4498–4504

Bochkareva E, Korolev S, Bochkarev A (2000) The role for zinc in replication protein A. J Biol Chem 275:27332–27338

Bochkareva E, Belegu V, Korolev S, Bochkarev A (2001) Structure of the major single-stranded DNA-binding domain of replication protein A suggests a dynamic mechanism for DNA binding. EMBO J 20:612–618

Bochkareva E, Korolev S, Lees-Miller SP, Bochkarev A (2002) Structure of the RPA trimerization core and its role in the multistep DNA-binding mechanism of RPA. EMBO J 21:1855–1863

Bochkareva E, Kaustov L, Ayed A, Yi GS, Lu Y, Pineda-Lucena A, Liao JC, Okorokov AL, Milner J, Arrowsmith CH, Bochkarev A (2005) Single-stranded DNA mimicry in the p53 transactivation domain interaction with replication protein A. Proc Natl Acad Sci USA 102:15412–15417

Braun KA, Lao Y, He Z, Ingles CJ, Wold MS (1997) Role of protein-protein interactions in the function of replication protein A (RPA): RPA modulates the activity of DNA polymerase α by multiple mechanisms. Biochemistry 36:8443–8454

Broderick S, Rehmet K, Concannon C, Nasheuer HP (2010) Eukaryotic single-stranded DNA binding proteins: central factors in genome stability. Subcell Biochem 50:143–163

Brosey CA, Chagot ME, Ehrhardt M, Pretto DI, Weiner BE, Chazin WJ (2009) NMR analysis of the architecture and functional remodeling of a modular multidomain protein, RPA. J Am Chem Soc 131:6346–6347

Brosh RM Jr, Orren DK, Nehlin JO, Ravn PH, Kenny MK, Machwe A, Bohr VA (1999) Functional and physical interaction between WRN helicase and human replication protein A. J Biol Chem 274:18341–18350

Campbell JL (1986) Eukaryotic DNA replication. Annu Rev Biochem 55:733–771

Chedin F, Seitz EM, Kowalczykowski SC (1998) Novel homologs of replication protein A in archaea: implications for the evolution of ssDNA-binding proteins. Trends Biochem Sci 23:273–277

Clugston CK, McLaughlin K, Kenny MK, Brown R (1992) Binding of human single-stranded DNA binding protein to DNA damaged by the anticancer drug *cis*-diamminedichloroplatinum (II). Cancer Res 52:6375–6379

Constantinou A, Tarsounas M, Karow JK, Brosh RM, Bohr VA, Hickson ID, West SC (2000) Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. EMBO Rep 1:80–84

Cummings CJ, Zoghbi HY (2000) Fourteen and counting: unraveling trinucleotide repeat diseases. Hum Mol Genet 9:909–916

Dai J, Hatzakis E, Hurley LH, Yang D (2010) i-motif structures formed in the human c-MYC promoter are highly dynamic–insights into sequence redundancy and i-motif stability. PLoS One 5:e11647

de Laat WL, Appeldoorn E, Sugasawa K, Weterings E, Jaspers NG, Hoeijmakers JH (1998) DNA-binding polarity of human replication protein A positions nucleases in nucleotide excision repair. Genes Dev 12:2598–2609

Deng X, Habel JE, Kabaleeswaran V, Snell EH, Wold MS, Borgstahl GE (2007) Structure of the full-length human RPA14/32 complex gives insights into the mechanism of DNA binding and complex formation. J Mol Biol 374:865–876

Deng X, Prakash A, Dhar K, Baia GS, Kolar C, Oakley GG, Borgstahl GE (2009) Human replication protein A-Rad52-single-stranded DNA complex: stoichiometry and evidence for strand transfer regulation by phosphorylation. Biochemistry 48:6633–6643

Dickson AM, Krasikova Y, Pestryakov P, Lavrik O, Wold MS (2009) Essential functions of the 32 kDa subunit of yeast replication protein A. Nucleic Acids Res 37:2313–2326

Din S, Brill SJ, Fairman MP, Stillman B (1990) Cell-cycle-regulated phosphorylation of DNA replication factor A from human and yeast cells. Genes Dev 4:968–977

Doherty KM, Sommers JA, Gray MD, Lee JW, von Kobbe C, Thoma NH, Kureekattil RP, Kenny MK, Brosh RM Jr (2005) Physical and functional mapping of the replication protein a interaction domain of the Werner and Bloom syndrome helicases. J Biol Chem 280:29494–29505

Dornreiter I, Erdile LF, Gilbert IU, von Winkler D, Kelly TJ, Fanning E (1992) Interaction of DNA polymerase α-primase with cellular replication protein A and SV40 T antigen. EMBO J 11:769–776

Eddy J, Maizels N (2006) Gene function correlates with potential for G4 DNA formation in the human genome. Nucleic Acids Res 34:3887–3896

Eddy J, Maizels N (2009) Selection for the G4 DNA motif at the 5′ end of human genes. Mol Carcinog 48:319–325

Fairman MP, Stillman B (1988) Cellular factors required for multiple stages of SV40 DNA replication *in vitro*. EMBO J 7:1211–1218

Fan JH, Bochkareva E, Bochkarev A, Gray DM (2009) Circular dichroism spectra and electrophoretic mobility shift assays show that human replication protein A binds and melts intramolecular G-quadruplex structures. Biochemistry 48:1099–1111

Fanning E, Klimovich V, Nager AR (2006) A dynamic model for replication protein A (RPA) function in DNA processing pathways. Nucleic Acids Res 34:4126–4137

Fotedar R, Roberts JM (1992) Cell cycle regulated phosphorylation of RPA-32 occurs within the replication initiation complex. EMBO J 11:2177–2187
Gellert M, Lipsett MN, Davies DR (1962) Helix formation by guanylic acid. Proc Natl Acad Sci USA 48:2013–2018
Glanzer JG, Liu S, Oakley GG (2011) Small molecule inhibitor of the RPA70 N-terminal protein interaction domain discovered using *in silico* and *in vitro* methods. Bioorg Med Chem 19:2589–2595
Gomes XV, Wold MS (1995) Structural analysis of human replication protein A. Mapping functional domains of the 70-kDa subunit. J Biol Chem 270:4534–4543
Gomes XV, Wold MS (1996) Functional domains of the 70-kilodalton subunit of human replication protein A. Biochemistry 35:10558–10568
Gomes XV, Henricksen LA, Wold MS (1996) Proteolytic mapping of human replication protein A: evidence for multiple structural domains and a conformational change upon interaction with single-stranded DNA. Biochemistry 35:5586–5595
Gottifredi V, Prives C (2005) The S phase checkpoint: when the crowd meets at the fork. Semin Cell Dev Biol 16:355–368
Grudic A, Jul-Larsen A, Haring SJ, Wold MS, Lonning PE, Bjerkvig R, Boe SO (2007) Replication protein A prevents accumulation of single-stranded telomeric DNA in cells that use alternative lengthening of telomeres. Nucleic Acids Res 35:7267–7278
Han Y, Loo YM, Militello KT, Melendy T (1999) Interactions of the papovavirus DNA replication initiator proteins, bovine papillomavirus type 1 E1 and simian virus 40 large T antigen, with human replication protein A. J Virol 73:4899–4907
Haring SJ, Mason AC, Binz SK, Wold MS (2008) Cellular functions of human RPA1. Multiple roles of domains in replication, repair, and checkpoints. J Biol Chem 283:19095–19111
Haring SJ, Humphreys TD, Wold MS (2010) A naturally occurring human RPA subunit homolog does not support DNA replication or cell-cycle progression. Nucleic Acids Res 38:846–858
He Z, Brinton BT, Greenblatt J, Hassell JA, Ingles CJ (1993) The transactivator proteins VP16 and GAL4 bind replication factor A. Cell 73:1223–1232
He Z, Henricksen LA, Wold MS, Ingles CJ (1995) RPA involvement in the damage-recognition and incision steps of nucleotide excision repair. Nature 374:566–569
Hermanson-Miller IL, Turchi JJ (2002) Strand-specific binding of RPA and XPA to damaged duplex DNA. Biochemistry 41:2402–2408
Huppert JL (2008) Four-stranded nucleic acids: structure, function and targeting of G-quadruplexes. Chem Soc Rev 37:1375–1384
Huppert JL, Balasubramanian S (2007) G-quadruplexes in promoters throughout the human genome. Nucleic Acids Res 35:406–413
Hyrien O (2000) Mechanisms and consequences of replication fork arrest. Biochimie 82:5–17
Iftode C, Daniely Y, Borowiec JA (1999) Replication protein A (RPA): the eukaryotic SSB. Crit Rev Biochem Mol Biol 34:141–180
Jackson D, Dhar K, Wahl JK, Wold MS, Borgstahl GE (2002) Analysis of the human replication protein A:Rad52 complex: evidence for crosstalk between RPA32, RPA70, Rad52 and DNA. J Mol Biol 321:133–148
Jacobs DM, Lipton AS, Isern NG, Daughdrill GW, Lowry DF, Gomes X, Wold MS (1999) Human replication protein A: global fold of the N-terminal RPA-70 domain reveals a basic cleft and flexible C-terminal linker. J Biomol NMR 14:321–331
Jain A, Wang G, Vasquez KM (2008) DNA triple helices: biological consequences and therapeutic potential. Biochimie 90:1117–1130
Jiang X, Klimovich V, Arunkumar AI, Hysinger EB, Wang Y, Ott RD, Guler GD, Weiner B, Chazin WJ, Fanning E (2006) Structural mechanism of RPA loading on DNA during activation of a simple pre-replication complex. EMBO J 25:5516–5526
Kanakis D, Levidou G, Gakiopoulou H, Eftichiadis C, Thymara I, Fragkou P, Trigka EA, Boviatsis E, Patsouris E, Korkolopoulou P (2011) Replication protein A: a reliable biologic marker of prognostic and therapeutic value in human astrocytic tumors. Hum Pathol 10:1545–1553
Kemp M, Sancar A (2009) DNA distress: just ring 9-1-1. Curr Biol 19:R733–R734

Kemp MG, Mason AC, Carreira A, Reardon JT, Haring SJ, Borgstahl GE, Kowalczykowski SC, Sancar A, Wold MS (2010) An alternative form of replication protein a expressed in normal human tissues supports DNA repair. J Biol Chem 285:4788–4797

Kenny MK, Schlegel U, Furneaux H, Hurwitz J (1990) The role of human single-stranded DNA binding protein and its individual subunits in simian virus 40 DNA replication. J Biol Chem 265:7693–7700

Kim HS, Brill SJ (2001) Rfc4 interacts with Rpa1 and is required for both DNA replication and DNA damage checkpoints in *Saccharomyces cerevisiae*. Mol Cell Biol 21:3725–3737

Kim C, Snyder RO, Wold MS (1992) Binding properties of replication protein A from human and yeast cells. Mol Cell Biol 12:3050–3059

Kim C, Paulus BF, Wold MS (1994) Interactions of human replication protein A with oligonucleotides. Biochemistry 33:14197–14206

Kobayashi Y, Sato K, Kibe T, Seimiya H, Nakamura A, Yukawa M, Tsuchiya E, Ueno M (2010) Expression of mutant RPA in human cancer cells causes telomere shortening. Biosci Biotechnol Biochem 74:382–385

Kolpashchikov DM, Ivanova TM, Boghachev VS, Nasheuer HP, Weisshart K, Favre A, Pestryakov PE, Lavrik OI (2000a) Synthesis of base-substituted dUTP analogues carrying a photoreactive group and their application to study human replication protein A. Bioconjug Chem 11:445–451

Kolpashchikov DM, Pestryakov PE, Wlassoff WA, Khodyreva SN, Lavrik OI (2000b) Study of interaction of human replication factor A with DNA using new photoreactive analogs of dTTP. Biochemistry (Mosc) 65:160–163

Krishna SS, Majumdar I, Grishin NV (2003) Structural classification of zinc fingers: survey and summary. Nucleic Acids Res 31:532–550

Kunkel TA, Burgers PM (2008) Dividing the workload at a eukaryotic replication fork. Trends Cell Biol 18:521–527

Lahue RS, Slater DL (2003) DNA repair and trinucleotide repeat instability. Front Biosci 8:s653–s665

Liu Y, Vaithiyalingam S, Shi Q, Chazin WJ, Zinkel SS (2011) BID binds to replication protein A and stimulates ATR function following replicative stress. Mol Cell Biol 31:4298–4309

Lopes J, Piazza A, Bermejo R, Kriegsman B, Colosio A, Teulade-Fichou MP, Foiani M, Nicolas A (2011) G-quadruplex-induced instability during leading-strand replication. EMBO J 30:4033–4046

Machwe A, Lozada E, Wold MS, Li GM, Orren DK (2011) Molecular cooperation between the Werner syndrome protein and replication protein A in relation to replication fork blockage. J Biol Chem 286:3497–3508

MacNeill SA (2001) DNA replication: partners in the Okazaki two-step. Curr Biol 11:R842–R844

Marathias VM, Bolton PH (1999) Determinants of DNA quadruplex structural type: sequence and potassium binding. Biochemistry 38:4355–4364

Mariappan SV, Silks LA 3rd, Chen X, Springer PA, Wu R, Moyzis RK, Bradbury EM, Garcia AE, Gupta G (1998) Solution structures of the Huntington's disease DNA triplets, (CAG)n. J Biomol Struct Dyn 15:723–744

Mason AC, Roy R, Simmons DT, Wold MS (2010) Functions of alternative replication protein A in initiation and elongation. Biochemistry 49:5919–5928

Masuda-Sasa T, Polaczek P, Peng XP, Chen L, Campbell JL (2008) Processing of G4 DNA by Dna2 helicase/nuclease and replication protein A (RPA) provides insights into the mechanism of Dna2/RPA substrate recognition. J Biol Chem 283:24359–24373

McElhinny AS, Li JL, Wu L (2008) Mastermind-like transcriptional co-activators: emerging roles in regulating cross talk among multiple signaling pathways. Oncogene 27:5138–5147

Melendy T, Stillman B (1993) An interaction between replication protein A and SV40 T antigen appears essential for primosome assembly during SV40 DNA replication. J Biol Chem 268:3389–3395

Mer G, Bochkarev A, Chazin WJ, Edwards AM (2000a) Three-dimensional structure and function of replication protein A. Cold Spring Harb Symp Quant Biol 65:193–200

Mer G, Bochkarev A, Gupta R, Bochkareva E, Frappier L, Ingles CJ, Edwards AM, Chazin WJ (2000b) Structural basis for the recognition of DNA repair proteins UNG2, XPA, and RAD52 by replication factor RPA. Cell 103:449–456

Mirkin S (1999) Structure and biology of H DNA. In: Malvy C, Harel-Bellan A (eds) Triple helix forming oligonucleotides. Kluwer Academic, Norwell, pp 193–222

Mirkin SM (2006) DNA structures, repeat expansions and human hereditary disorders. Curr Opin Struct Biol 16:351–358

Mirkin SM (2007) Expandable DNA repeats and human disease. Nature 447:932–940

Mirkin SM (2008) Discovery of alternative DNA structures: a heroic decade (1979–1989). Front Biosci 13:1064–1071

Nuss JE, Sweeney DJ, Alter GM (2006) Reactivity-based analysis of domain structures in native replication protein A. Biochemistry 45:9804–9818

Nuss JE, Sweeney DJ, Alter GM (2009) Prediction of and experimental support for the three-dimensional structure of replication protein A. Biochemistry 48:7892–7905

Oakley GG, Patrick SM (2010) Replication protein A: directing traffic at the intersection of replication and repair. Front Biosci 15:883–900

Oakley GG, Loberg LI, Yao J, Risinger MA, Yunker RL, Zernik-Kobak M, Khanna KK, Lavin MF, Carty MP, Dixon K (2001) UV-induced hyperphosphorylation of replication protein a depends on DNA replication and expression of ATM protein. Mol Biol Cell 12:1199–1213

Parrilla-Castellar ER, Karnitz LM (2003) Cut5 is required for the binding of ATR and DNA polymerase α to genotoxin-damaged chromatin. J Biol Chem 278:45507–45511

Patel DJ, Phan AT, Kuryavyi V (2007) Human telomere, oncogenic promoter and 5′-UTR G-quadruplexes: diverse higher order DNA and RNA targets for cancer therapeutics. Nucleic Acids Res 35:7429–7455

Patrick SM, Turchi JJ (1998) Human replication protein A preferentially binds cisplatin-damaged duplex DNA *in vitro*. Biochemistry 37:8808–8815

Pestryakov PE, Lavrik OI (2008) Mechanisms of single-stranded DNA-binding protein functioning in cellular DNA metabolism. Biochemistry (Mosc) 73:1388–1404

Pestryakov PE, Weisshart K, Schlott B, Khodyreva SN, Kremmer E, Grosse F, Lavrik OI, Nasheuer HP (2003) Human replication protein A. The C-terminal RPA70 and the central RPA32 domains are involved in the interactions with the 3′-end of a primer-template DNA. J Biol Chem 278:17515–17524

Pestryakov PE, Khlimankov DY, Bochkareva E, Bochkarev A, Lavrik OI (2004) Human replication protein A (RPA) binds a primer-template junction in the absence of its major ssDNA-binding domains. Nucleic Acids Res 32:1894–1903

Pestryakov PE, Krasikova YS, Petruseva IO, Khodyreva SN, Lavrik OI (2007) The role of p14 subunit of replication protein A in binding to single-stranded DNA. Dokl Biochem Biophys 412:4–7

Pfuetzner RA, Bochkarev A, Frappier L, Edwards AM (1997) Replication protein A. Characterization and crystallization of the DNA binding domain. J Biol Chem 272:430–434

Potterton L, McNicholas S, Krissinel E, Gruber J, Cowtan K, Emsley P, Murshudov GN, Cohen S, Perrakis A, Noble M (2004) Developments in the CCP4 molecular-graphics project. Acta Crystallogr D Biol Crystallogr 60:2288–2294

Prakash A, Kieken F, Marky LA, Borgstahl GE (2011a) Stabilization of a G-quadruplex from unfolding by replication protein A using potassium and the porphyrin TMPyP4. J Nucleic Acids 2011:529828

Prakash A, Natarajan A, Marky LA, Ouellette MM, Borgstahl GE (2011b) Identification of the DNA-binding domains of human replication protein A that recognize G-quadruplex DNA. J Nucleic Acids 2011:896947

Pretto DI, Tsutakawa S, Brosey CA, Castillo A, Chagot ME, Smith JA, Tainer JA, Chazin WJ (2010) Structural dynamics and single-stranded DNA binding activity of the three N-terminal domains of the large subunit of replication protein A from small angle X-ray scattering. Biochemistry 49:2880–2889

Richard DJ, Bolderson E, Cubeddu L, Wadsworth RI, Savage K, Sharma GG, Nicolette ML, Tsvetanov S, McIlwraith MJ, Pandita RK, Takeda S, Hay RT, Gautier J, West SC, Paull TT,

Pandita TK, White MF, Khanna KK (2008) Single-stranded DNA-binding protein hSSB1 is critical for genomic stability. Nature 453:677–681

Sakaguchi K, Ishibashi T, Uchiyama Y, Iwabata K (2009) The multi-replication protein A (RPA) system-a new perspective. FEBS J 276:943–963

Salas TR, Petruseva I, Lavrik O, Bourdoncle A, Mergny JL, Favre A, Saintome C (2006) Human replication protein A unfolds telomeric G-quadruplexes. Nucleic Acids Res 34:4857–4865

Salas TR, Petruseva I, Lavrik O, Saintome C (2009) Evidence for direct contact between the RPA3 subunit of the human replication protein A and single-stranded DNA. Nucleic Acids Res 37:38–46

Schweizer U, Hey T, Lipps G, Krauss G (1999) Photocrosslinking locates a binding site for the large subunit of human replication protein A to the damaged strand of cisplatin-modified DNA. Nucleic Acids Res 27:3183–3189

Seroussi E, Lavi S (1993) Replication protein A is the major single-stranded DNA binding protein detected in mammalian cell extracts by gel retardation assays and UV cross-linking of long and short single-stranded DNA molecules. J Biol Chem 268:7147–7154

Shuck SC, Turchi JJ (2010) Targeted inhibition of replication protein A reveals cytotoxic activity, synergy with chemotherapeutic DNA-damaging agents, and insight into cellular function. Cancer Res 70:3189–3198

Sleeth KM, Sorensen CS, Issaeva N, Dziegielewski J, Bartek J, Helleday T (2007) RPA mediates recombination repair during replication stress and is displaced from DNA by checkpoint signalling in human cells. J Mol Biol 373:38–47

Sogo JM, Lopes M, Foiani M (2002) Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. Science 297:599–602

Stigger E, Drissi R, Lee SH (1998) Functional analysis of human replication protein A in nucleotide excision repair. J Biol Chem 273:9337–9343

Taneja P, Boche I, Hartmann H, Nasheuer HP, Grosse F, Fanning E, Weisshart K (2007) Different activities of the largest subunit of replication protein A cooperate during SV40 DNA replication. FEBS Lett 581:3973–3978

Treuner K, Ramsperger U, Knippers R (1996) Replication protein A induces the unwinding of long double-stranded DNA regions. J Mol Biol 259:104–112

Turchi JJ, Henkels KM, Hermanson IL, Patrick SM (1999) Interactions of mammalian proteins with cisplatin-damaged DNA. J Inorg Biochem 77:83–87

Vassin VM, Wold MS, Borowiec JA (2004) Replication protein A (RPA) phosphorylation prevents RPA association with replication centers. Mol Cell Biol 24:1930–1943

Voineagu I, Narayanan V, Lobachev KS, Mirkin SM (2008) Replication stalling at unstable inverted repeats: interplay between DNA hairpins and fork stabilizing proteins. Proc Natl Acad Sci USA 105:9936–9941

Voineagu I, Freudenreich CH, Mirkin SM (2009) Checkpoint responses to unusual structures formed by DNA repeats. Mol Carcinog 48:309–318

Waga S, Stillman B (1994) Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication *in vitro*. Nature 369:207–212

Weinberg DH, Collins KL, Simancek P, Russo A, Wold MS, Virshup DM, Kelly TJ (1990) Reconstitution of simian virus 40 DNA replication with purified proteins. Proc Natl Acad Sci USA 87:8692–8696

Weisshart K, Taneja P, Fanning E (1998) The replication protein A binding site in simian virus 40 (SV40) T antigen and its role in the initial steps of SV40 DNA replication. J Virol 72:9771–9781

Weisshart K, Pestryakov P, Smith RW, Hartmann H, Kremmer E, Lavrik O, Nasheuer HP (2004) Coordinated regulation of replication protein A activities by its subunits p14 and p32. J Biol Chem 279:35368–35376

Wells RD (2007) Non-B DNA conformations, mutagenesis and disease. Trends Biochem Sci 32:271–278

Wold MS (1997) Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. Annu Rev Biochem 66:61–92

Wold MS (2010) Eukaryotic replication protein A. Encyclopedia of life science. Wiley, Chichester

Wold MS, Kelly T (1988) Purification and characterization of replication protein A, a cellular protein required for *in vitro* replication of simian virus 40 DNA. Proc Natl Acad Sci USA 85:2523–2527

Wu Y, Rawtani N, Thazhathveetil AK, Kenny MK, Seidman MM, Brosh RM Jr (2008) Human replication protein A melts a DNA triple helix structure in a potent and specific manner. Biochemistry 47:5068–5077

Xu X, Vaithiyalingam S, Glick GG, Mordes DA, Chazin WJ, Cortez D (2008) The basic cleft of RPA70N binds multiple checkpoint proteins, including RAD9, to regulate ATR signaling. Mol Cell Biol 28:7345–7353

Yuzhakov A, Kelman Z, Hurwitz J, O'Donnell M (1999) Multiple competition reactions for RPA order the assembly of the DNA polymerase δ holoenzyme. EMBO J 18:6189–6199

Zou L, Elledge SJ (2003) Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300:1542–1548

Zou Y, Liu Y, Wu X, Shell SM (2006) Functions of human replication protein A (RPA): from DNA replication to DNA damage and stress responses. J Cell Physiol 208:267–273