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Replication Protein A (RPA): The Eukaryotic SSB

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ABSTRACT: Replication protein A (RPA) is a heterotrimeric single-stranded DNA-binding protein that is highly conserved in eukaryotes. RPA plays essential roles in many aspects of nucleic acid metabolism, including DNA replication, nucleotide excision repair, and homologous recombination. In this review, we provide a comprehensive overview of RPA structure and function and highlight the more recent developments in these areas. The last few years have seen major advances in our understanding of the mechanism of RPA binding to DNA, including the structural characterization of the primary DNA-binding domains (DBD) and the identification of two secondary DBDs. Moreover, evidence indicates that RPA utilizes a multistep pathway to bind single-stranded DNA involving a particular molecular polarity of RPA, a mechanism that is apparently used to facilitate origin denaturation. In addition to its mechanistic roles, RPA interacts with many key factors in nucleic acid metabolism, and we discuss the critical nature of many of these interactions to DNA metabolism. RPA is a phosphorylation target for DNA-dependent protein kinase (DNA-PK) and likely the ataxia telangiectasia-mutated gene (ATM) protein kinase, and recent observations are described that suggest that RPA phosphorylation plays a significant modulatory role in the cellular response to DNA damage.

KEY WORDS: replication protein A, eukaryotes, nucleic acid metabolism.

I. INTRODUCTION

Single-stranded DNA-binding proteins (SSBs) play multiple and essential roles in almost every aspect of DNA metabolism, including replication, repair, and recombination. In eukaryotic cells, the role of the SSB is played by replication protein A (RPA), a heterotrimeric protein complex. At the most basic level, RPA stabilizes DNA in the single-stranded conformation, a form more active in many aspects of nucleic acid enzymology. As the characterization of RPA structure and function proceeds, however, it is becoming more apparent that RPA plays a dynamic modulatory role in each of these processes through specific physical interac-

tions with other protein molecules, and with the DNA. As an excellent comprehensive review of RPA was published recently,[1] this review concentrates on the more recent advances in our understanding of RPA structure and function.

RPA was first identified and purified from human (HeLa) cell extracts as an indispensable component of simian virus 40 (SV40) DNA replication.[2–4] Analysis of hsRPA* indicates that it is a heterotrimer composed of 70 kDa (RPA1), 29 kDa (RPA2), and 14 kDa (RPA3) subunits.[3,4] Subsequent to its identification in human cells, heterotrimeric homologues of hsRPA have been isolated or RPA-encoding genes have been identified from virtually every eukaryotic organism examined, including *Saccharomyces cerevisiae*,[5,6] *Schizosaccharomyces pombe*,[7] *Xenopus laevis*,[8] *Drosophila melanogaster*,[9,10] as well as in plants such as deepwater rice.[11] RPA is apparently the most abundant SSB in eukaryotic cells.[12] Systems that overexpress hsRPA in bacteria or in insect cells infected with recombinant baculovirus have been developed.[13,14]

Comparison of the amino acid sequence of various RPA subunits has revealed significant homology between species at the amino acid level, with the genes for RPA1 showing the highest homology (Table 1). For example, amino acid sequence comparison shows 44.5, 50.0, and 80.7% similarity for scRPA1, spRPA1, and xlRPA1 subunits, respectively, vs. hsRPA1 (Table 1A). The amino acid homology between the derived sequences of RPA2 and its RPA4 homologue[15] (see below), and RPA3 is less strong (Tables 1B and 1C). For example, the scRPA2 and scRPA3 subunits show a 37.8 and 35.2% similarity when compared with the hsRPA2 and hsRPA3 subunits, respectively. Even with this evolutionary divergence, RPA activities are conserved to a certain extent as SV40 DNA replication *in vitro* can be efficiently supported when hsRPA is replaced by bovine (Bos taurus) RPA[16] and dmRPA.[17] However, even though hsRPA and scRPA are somewhat homologous and can substitute for each other in certain reactions (e.g., Ref. 6), the yeast protein cannot replace hsRPA in SV40 DNA replication *in vitro*,[18] and the essential scRPA genes can not be replaced by the corresponding human coding sequences.[5,6]

II. RPA STRUCTURE

A. Primary DNA-Binding Domains

Initial studies of hsRPA indicated that only the RPA1 subunit had significant single-stranded DNA (ssDNA)-binding activity. For example, testing of the individual hsRPA subunits renatured after separation by SDS-PAGE showed that only the large subunit could bind ssDNA[19,20] and had an affinity for ssDNA similar to that found for the heterotrimer.[19] Similarly, UV-cross-linking of hsRPA bound to ssDNA found that only RPA1 was tightly associated with the DNA.[19] Supporting this interpretation, a single DNA-binding domain (DBD) was mapped to the center of the hsRPA1 subunit (residues ~180 to 420; Figure 1).[21–25] Moreover, analysis of the similar region in

* This review continues the nomenclature of Wold [1] and designates the source of RPA by the species from which it was derived (e.g., RPA from *Saccharomyces cerevisiae* is scRPA). In addition, to facilitate comparison with RPA isolated from different organisms, the large, middle, and small RPA subunits are referred to as RPA1, RPA2, and RPA3, respectively.

TABLE 1
Amino Acid Homology of the RPA Subunits from Various Species

TABLE 1A

Species	*H. sapiens* RPA1	*X. laevis* RPA1	*D. melanogaster* RPA1	*S. pombe* RPA1
S. cerevisiae RPA1	44.5 (32.5)	44.4 (33.1)	46.9 (34.5)	50.4 (39.2)
S. pombe RPA1	50.0 (38.7)	51.9 (41.4)	49.4 (36.9)	
D. melanogaster RPA1	54.6 (45.3)	54.3 (44.5)		
X. laevis RPA1	80.7 (74.1)			

TABLE 1B

Species	*H. sapiens* RPA2	*H. sapiens* RPA4	*M. musculus* RPA2	*S. pombe* RPA2
S. cerevisiae RPA2	37.8 (29.9)	35.3 (26.9)	37.4 (29.5)	41.7 (35.7)
S. pombe RPA2	43.5 (34.2)	38.7 (29.2)	44.2 (34.6)	
M. musculus RPA2	88.5 (87.4)	54.1 (45.6)		
H. sapiens RPA4	56.8 (47.5)			

TABLE 1C

Species	*H. sapiens* RPA3	*S. pombe* RPA3
S. cerevisiae RPA3	35.2 (27.6)	35.3 (27.4)
S. pombe RPA3	37.0 (26.0)	

Note: For each comparison, the first value indicates the percent similarity, while the second value (in parentheses) indicates the percent identity. Three comparisons were made: amino acid homologies between RPA1 subunits (panel A), between the RPA2/4 subunits (panel B), and between the RPA3 subunits (panel C). Sequence analysis was performed using the GAP program from the Wisconsin Package, version 10.0 (Genetics Computer Group, 1998). Note that GAP rather than BESTFIT was used, because BESTFIT was unable to recognize significant homology between the RPA3 subunits. For the RPA1, RPA2, RPA4 subunits, the GAP and BESTFIT programs yielded similar homology values. The following accession numbers were used: hsRPA1, M63488; hsRPA2, J05249; hsRPA3, L07493; hsRPA4, U24186; xlRPA1, Q01588, dmRPA1, Q24492; mmRPA2, D00812; spRPA1, U59385; spRPA2, U59386; spRPA3, U59387; scRPA1, P22336; scRPA2, P26754; scRPA3, P26755.

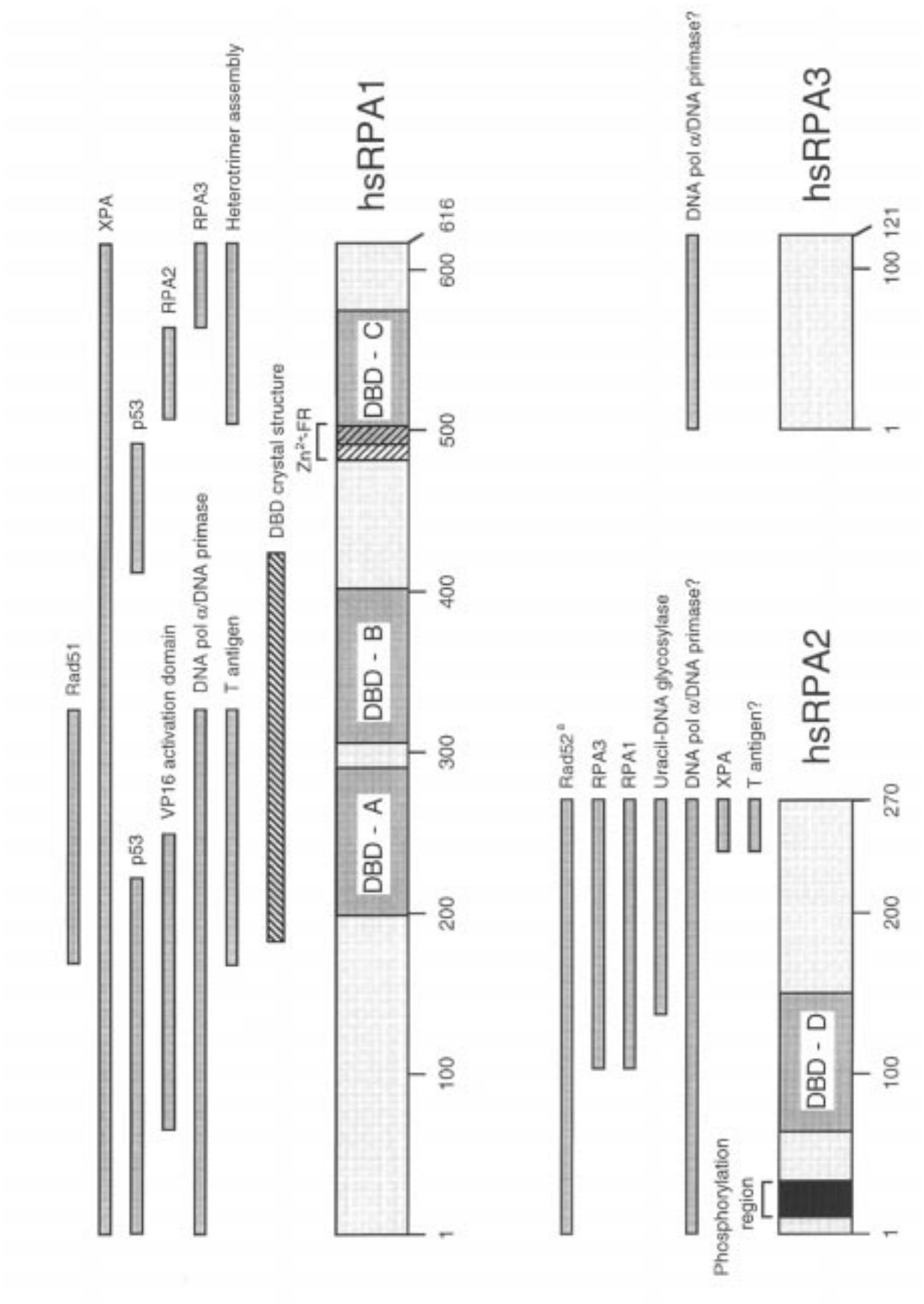

FIGURE 1. DNA-binding domains, protein-interaction sites, and other hsRPA subunit motifs. Each subunit is depicted with the amino acid position given below. The ssDNA-binding domains A, B, C, and D are shaded and indicated by DBD-A, etc., while the zinc-finger region of hsRPA1 is shown by a cross-hatched area (Zn^{2+}-FR). Note that there is an overlapping region for the zinc-binding domain and DBD-C indicated by double shading. Also depicted is the region of hsRPA2 phosphorylation by cdk-cyclin kinases and DNA-PK, and the hsRPA1 region for which the crystal structure was recently solved.[27] hsRPA regions to which protein-protein interactions have been mapped are shown by shaded lines above each subunit, with the interacting protein indicated to the right. The EBV EBNA-1 protein has been found to interact with the hsRPA1 subunit,[167] but this interaction has not been further delimited and hence is not included. [a]Note that the scRPA-Rad52p interaction, in contrast to the interaction between the human proteins, appears to be primarily mediated through the scRPA1 subunit.[207]

scRPA1 suggested that the central region contained two interchangeable subdomains, A and B.[26]

The crystal structure of the central DBD of hsRPA1 (residues 181 to 422) complexed to a single molecule of octadeoxycytosine was obtained recently at 2.2 Å resolution.[27] This domain is composed of two adjacent subdomains of similar structure (~100 amino acids each; designated domains A [residues 198 to 291] and B [residues 305 to 402]) connected by a 15 amino acid linker and forming a channel with a width of ~17 Å. Three nucleotides are contacted by each subdomain, and two additional nucleotides bridge the contacted residues. Each subdomain resembles a protein structure (the OB fold) found previously in various oligonucleotide- and oligosaccharide-binding proteins, including SSBs encoded by the f1 phage (gene V) and bacteriophage Pf3, the yeast aspartyl-tRNA synthetase, and, to a lesser extent, the ssDNA binding-domain of the bacteriophage T4 SSB, gp32. In addition, the OB fold was observed in a recent structure of the *Escherichia coli* SSB (EcoSSB).[28]

Two types of RPA-DNA contacts were noted in the $hsRPA1_{181-422}$ structure: hydrogen bonds between side chains and the phosphate backbone and individual bases, and hydrophobic base-stacking interactions between four aromatic amino acid residues (F238, F269, W361, and F386) and individual bases.[27] A recent study indicates that while mutation of two of the aromatic residues (F238 and W361) caused the mutant heterotrimer to bind ssDNA extremely poorly, mutation of the other two aromatic amino acids (F269 and F386) had no effect on ssDNA binding.[29] In addition, mutation of F238 and W361 generated a protein with an altered proteolytic digestion pattern, indicating that these amino acids are critical for the maintenance of proper hsRPA conformation.[29] These results support the argument that the key interactions between hsRPA and ssDNA are ionic rather than hydrophobic.

B. Secondary DNA-Binding Domains

1. RPA2

That domains A and B comprise the sole ssDNA-binding motif in RPA was questioned as its DNA-binding properties underwent further scrutiny. Blackwell and Borowiec[30] found that in addition to the previously characterized 30 nt binding form[12,31] (termed the $hRPA_{30nt}$ complex by our laboratory), hsRPA was also able to bind ssDNA more unstably using an 8 nt binding mode (the $hRPA_{8nt}$ complex) that required the use of a crosslinking agent for detection (see also below). Thus, the possibility that RPA bound ssDNA using multiple binding sites was raised. In addition, the identification of the minimum essential domains for the scRPA2 and scRPA3 subunits indicated that each domain contained regions that bore weak similarity to the ssDNA binding domain of EcoSSB[26] and to each other. The conserved region in RPA2 contains three hydrophobic residues, and a double mutation of two of these residues (Y133A and F143A) in scRPA2 were found to be lethal to yeast viability.[26]

More direct evidence demonstrating that RPA2 binds ssDNA has surfaced recently. It has been found that the scRPA2 subunit, when present in the heterotrimer, could be weakly crosslinked to ssDNA in a ssDNA-length- and salt-dependent manner.[26] In addition, the hsRPA2 subunit was able to be crosslinked to synthetic primer-template DNA molecules *in vitro*.[32] Photocrosslinking studies performed using SV40 chromosomes

replicating in African green monkey CV-1 cells (*Cercopithecus aethiops*) showed that ceRPA2 was crosslinked to RNA-DNA primers that were 10 to 35 nt in length.[33] As lagging strand synthesis appears to involve synthesis of short RNA-DNA primers by DNA polymerase α prior to a switch to DNA polymerase δ,[34–36] these data suggest that RPA2-DNA contacts may play a significant role in the polymerase switching mechanism.

While these data reveal contacts between RPA2 and DNA, isolation of a bacterially expressed hsRPA2·hsRPA3 complex was unable to bind ssDNA and could not support SV40 DNA replication *in vitro* (Ref. 13, see also Ref. 26). These somewhat contradictory results were resolved with the finding that while the isolated hsRPA2·hsRPA3 did not show significant ssDNA-binding activity the dimeric complex containing an N- and C-terminal deletion of hsRPA2 (residues 43 to 171) did bind ssDNA.[37] The binding affinity of this complex was relatively weak (~$10 - 50 \times 10^{-6}$ *M*), compared with the primary DBD in the central region of RPA1 (~$50 - 100 \times 10^{-9}$ *M*).[37,39] These data could suggest that the RPA2 ssDNA-binding domain is normally cryptic, and only becomes accessible after RPA1 binding to ssDNA.[37]

2. C-Terminus of RPA1

Recent characterization of various fragments of the scRPA1 subunit demonstrated that the C-terminal region (residues 416 to 621) also contains a fourth ssDNA-binding domain that weakly binds ssDNA as detected by UV-crosslinking analysis.[38] Similarly, a mutated hsRPA heterotrimer lacking the N-terminal 382 residues of hsRPA1 (including the central DBDs A and B) could be weakly crosslinked to ssDNA through the truncated hsRPA1 subunit.[44] That the RPA1 C-terminus contains a DBD is also supported by the observation that a truncated hsRPA heterotrimer containing the hsRPA1 C-terminal complexed with the central domain of hsRPA2 (residues 43 to 171) and hsRPA3 has a three- to fivefold greater affinity for ssDNA than the truncated hsRPA2-hsRPA3 dimer.[37]

The different DBDs of RPA have been designated in the following manner: the central high-affinity RPA1 sites, domains A and B; the low-affinity site at the RPA1 C-terminal, domain C; and the central RPA2 site, domain D. Domains C and D have limited homology with domains A and B, most especially with respect to the presence and location of amino acid residues important for RPA function.[26,37,38] Domains C and D also appear to be constructed using an OB-fold motif.[37,38] The role of the secondary ssDNA-binding domains is still unclear as their contribution to the overall RPA-ssDNA binding affinity is relatively marginal (e.g., Refs. 21, 39, 44). However, as discussed in greater detail below, they may contribute to the molecular polarity of RPA binding to ssDNA and could facilitate the multistep binding pathway that RPA uses to bind DNA.

C. Other RPA Domains

1. RPA1

Apart from its ssDNA binding role, the C-terminus of hsRPA (residues ~503 to 616) is necessary for the correct assembly of the heterotrimer.[21–24] Deletion analysis suggests that the extreme C-terminal 50 amino acids are required for interaction between hsRPA1 and hsRPA3, while the adjacent 60 residues interact with hsRPA2.[23] The C-terminal region is highly hydrophobic and its deletion renders hsRPA1 soluble in the absence of

the other two subunits.[21,22] Assembly of the heterotrimer has been shown to occur in an ordered process, with the initial formation of a stable RPA2-RPA3 complex followed by the association of RPA1.[13,14]

The C-terminal portion of RPA1 also contains a highly conserved zinc-finger motif (residues 481 to 503 of hsRPA1).[5,8,40–42] Complete deletion has only modest effects on the affinity of hsRPA for ssDNA.[21–24,29,43,44] Testing the effect of zinc-finger mutations on RPA function has led to contradictory findings. Lee and colleagues have observed that deletion of the zinc finger motif or mutation of all four of the critical cysteine residues generated a protein that was unable to stimulate the activity of DNA polymerase α or δ, was inactive in excision nucleotide repair, yet functional in SV40 DNA replication.[23,43,45] In contrast, the laboratory of Dutta showed that a similarly mutated hsRPA stimulated each polymerase to levels observed with the wild-type hsRPA, could not support SV40 DNA replication or mismatch repair, yet could functionally replace the wild-type protein in excision nucleotide repair.[24,46] Similarly, a recent study by Wold and co-workers found that a recombinant hsRPA mutated in the zinc-binding motif was inactive in SV40 DNA replication.[29]

It is possible that the observed differences in the effect on zinc-finger mutation on RPA activities relate to the source of the protein. Lee and co-workers prepare hsRPA using a baculovirus overexpression system, while the laboratories of Dutta and Wold have each generated recombinant hsRPA in bacteria. Although the source of the protein did not affect hsRPA activity in other assay systems,[13,14,47] it is possible that mutations in the zinc-finger motif result in an RPA heterotrimer with a less-stable conformation that requires the participation of the eukaryotic chaperonin system to generate properly folded molecules (e.g., Ref. 48). In support of this hypothesis, hsRPA mutated in the zinc-finger has a somewhat different pattern of trypsin proteolysis, although only in the presence of DNA.[29] Therefore, it is possible that the zinc-finger motif might play a role in the correct folding of the RPA1 subunit, similar to the T4 gp32 motif (e.g., Ref. 49).

Because trypsin cleavage of hsRPA causes initial cleavage of hsRPA1 into ~18 kDa N-terminal and ~52 kDa C-terminal fragments, it appears that ~170 amino acids of the N-terminal are constructed as a discrete domain.[50] This region is apparently nonessential because its deletion does not affect either ssDNA-binding or the ability of RPA to function in SV40 replication *in vitro*.[22,23] Although various regions of hsRPA1 are responsible for binding other proteins, the N-terminal region has been implicated in many of the known interactions. For example, hsRPA, able to stimulate DNA synthesis by DNA polymerases α and δ,[23,40,51,52] can physically interact with DNA polymerase α using the N-terminal half of hsRPA1.[23,53] In addition, the N-terminal 327 amino acids of RPA1 contain overlapping regions that interact with the activation domain of VP16 and the SV40 large tumor antigen (T antigen).[53] hsRPA interacts with p53 (see below),[54–56] and this interaction maps to two hsRPA1 regions (residues 1 to 221 and 411 to 492).[24]

Outside of the primary DBD, the biochemical characterization of the *S. cerevisiae* RPA1 subunit has been less extensive, and most of our knowledge has been obtained from genetic studies. In apparent contrast to hsRPA1, both the extreme N- and C-termini of scRPA1 are critical for RPA function as their truncation causes either extremely poor growth or loss of viability.[26] Recently, three temperature-sensitive mutations within the yeast *RFA1* gene (encoding the large subunit) were found to confer mutator phenotypes, resulting in various types of mutations, including deletions, base substitutions, frameshifts, and nonreciprocal transloca-

tions.[57] A test of one of these *rfa1* alleles in *rad10*, *rad51*, or *rad52* strains showed it to be growth defective, leading to the suggestion that the mutant scRPA was causing an increase in double-strand breaks that were then repaired by mutagenic processes. Consistent with this view, a point mutation (G77D) in the N-terminal domain of the scRPA1 causes cells to be defective in DNA repair induced by X rays, double-strand breaks, and high UV doses.[58] Similarly, a D228Y mutation in scRPA1 appears to yield a protein defective in interactions with the replication, repair, and recombination machinery that deleteriously affects the ability of RPA to function in repair or recombination.[59,60]

2. RPA2

Similar to the RPA1 subunit, RPA2 appears to contain three distinct functional domains. In addition to the secondary ssDNA-binding domain located in the center of RPA2 (described above), both the N- and C-terminal regions have distinct functions. As discussed in greater detail below, RPA is phosphorylated in a cell-cycle-dependent fashion,[61,62] and the sites of phosphorylation are located in the N-terminal region. For scRPA2 and hsRPA2, the N-terminus is nonessential for growth or *in vitro* SV40 DNA replication, respectively.[26,63]

Deletion of the ~100 C-terminal residues from scRPA2 allowed yeast growth, although in a temperature-sensitive fashion.[26] However, conflicting results regarding the importance of the C-terminus of hsRPA2 have been reported. While Braun et al. found that deletion of up to 50 residues from the hsRPA2 C-terminus efficiently supported SV40 DNA replication *in vitro*,[53] Lee and Kim observed that hsRPA2 mutants lacking the C-terminal 33 residues were nonfunctional in SV40 DNA replication.[63] Again, it is possible that the source of the recombinant protein (*E. coli* vs. insect cells) is a contributing factor. The C-terminal two-thirds of hsRPA2 has been found to be required for interactions with both hsRPA1 and hsRPA3.[24]

The RPA2 subunit can interact with other protein factors, including the various kinases that are able to phosphorylate RPA2 (see below). Deletion of the hsRPA2 C-terminal 33 amino acids has been found to prevent hsRPA-T antigen complex formation, suggesting that the C-terminus physically interacts with the viral protein.[63] However, analysis of similar hsRPA2 mutants by the Wold laboratory found no effect on the ability of hsRPA to interact with Tantigen.[53] The latter group has suggested that the hsRPA2 mutation alters RPA conformation, and thus indirectly affects protein-protein interactions.[53] Mutation of RPA2 (and RPA3) also caused diminished association of RPA with DNA polymerase α, but this may likewise be a consequence of differences in RPA conformation.[53] The hsRPA2 C-terminal has been found to be required for interaction with the *Xeroderma pigmentosum* group A complementing protein (XPA)[29,45,64] and with the human uracil-DNA glycosylase,[65] implicating the RPA2 subunit in both nucleotide and base excision repair.

A homologue of RPA2 in human cells, hsRPA4, has been isolated using hsRPA1 as bait in the yeast two-hybrid system.[15] hsRPA4 shares moderate (47%) identity with hsRPA2 and is expressed primarily in placenta and is present in colon mucosa cells. Although hsRPA4 is capable of assembling a heterotrimeric complex together with RPA1 and RPA3 that is able to bind ssDNA, placental cells express little RPA1, suggesting that most hsRPA4 is not present in a heterotrimeric hsRPA1-3-4 structure. The role of RPA4 is open to speculation.

3. RPA3

At present, apart from bridging the RPA1 and RPA2 subunits and stabilizing their interaction, the role of RPA3 is not yet established.[13,14] It has been proposed that RPA3 can directly bind DNA,[26] although such binding has not yet been demonstrated.[32,33,37,39] Mutational analysis of scRPA3 indicates that loss of the N-terminal 70 residues from the 122 amino acid subunit can be accommodated even though viability is temperature sensitive.[26] Small deletions from the C-terminus of scRPA3 are inviable.[26]

Examination of the effects of various *rfa2* and *rfa3* (encoding the scRPA2 and scRPA3 subunits, respectively) mutations on chromosomal replication indicates differing roles for each subunit. A majority of temperature-sensitive mutations of *rfa2* show a 'fast-stop' DNA synthesis phenotype at the nonpermissive temperature, with RPA1 and RPA2 no longer associated at the elevated temperature.[66,67] Thus, the continued presence of the RPA2 subunit is required for replication fork progression. In contrast, all temperature-sensitive *rfa3* mutations were seen to have a 'slow-stop' replication phenotype at the nonpermissive temperature and had no apparent effect on RPA1-RPA2 association.[67] The *rfa3* cells were primarily arrested in the G1 phase and apparently were functional in both the initiation and elongation stages of chromosomal replication. The *rfa2* and *rfa3* alleles were seen to have various degrees of methyl methane sulfonate (MMS)- and ultraviolet (UV)-sensitivity and slight to significant increases in mutation frequency.

4. Archaeal Homologues of RPA

Interestingly, examination of the genomic sequence of various archaeons (*Methanococcus jannaschii*, *Methanobacter thermoautotrophicum*, and *Archaeoglobus fulgidas*) found candidate SSB genes that contained regions that bore significant homology to the ssDNA-binding domain A of hsRPA1 and are predicted to be structurally similar.[68,69] For each candidate gene, the homologous region was present in multiple copies with four and five repeats found for *M. jannaschii* and *M. thermoautotrophicum*, respectively, and two repeats were found in each of two putative SSB genes in *A. fulgidas*. When expressed in bacteria, the mjSSB showed high-affinity ssDNA-binding activity and a binding site size of ~20 nt.[68] As mentioned, the RPA1 and RPA2 subunits of yeast and humans show weak homology with EcoSSB,[26,40] also known to form using an OB-fold.[28] Overall, it appears that the eukaryotic RPA and archaeal SSBs evolved from a common precursor, perhaps present in an early prokaryotic ancestor.

5. Location of the RPA Genes

Mapping of the individual hsRPA genes indicate that the RPA1 subunit is located on chromosome 17 at 17p13.3, RPA2 on chromosome 1 (1p35), and RPA3 on chromosome 7 (7p22).[70–72] In yeast, the *RFA1*, *RFA2*, and *RFA3* genes are located on the right arm of chromosome I, the left arm of chromosome XIV, and the left arm of chromosome X, respectively.[5,73] Null mutations of the three yeast genes are nonviable and result in a terminal phenotype consistent with an essential role in chromosomal DNA replication.[5,73] As yet, no known genetic disorders map to an hsRPA gene, but it is clear that mutation of any of the three yeast genes can result in genetic instability (e.g., Refs. 57 and 67).

III. INTERACTION OF RPA WITH NUCLEIC ACIDS

A. Binding to ssDNA

The binding parameters of various RPA homologues (hsRPA, scRPA, dmRPA) have been characterized extensively and are reviewed in detail by Wold.[1] The strength of the interaction between RPA and its ssDNA substrate, as reflected by the apparent association constant (Ka), is in the range of 10^8 to 10^{11} M^{-1}, depending on the conditions used.[9,22,31,39,47,74–76] The Ka value is dependent on DNA length, increasing ~50-fold as the DNA length is increased from 10 to 20 nt, and ~6-fold as the length further increases to 60 nt.[75] Base composition of the ssDNA substrate significantly modulates the RPA binding affinity, with ~50-fold higher affinity noted for hsRPA and scRPA binding to single-stranded polypyrimidine sequences when compared with polypurine sequences.[31] Similarly, both human and yeast RPA have been shown to strongly prefer binding to the pyrimidine-rich strands of both the SV40 origin of replication and an *S. cerevisiae* chromosomal origin, respectively.[31,77] hsRPA has also been found to bind preferentially to short (8 nt) single-stranded pyrimidine sequences, relative to purine elements, located either in the center or at one end of 48-bp DNA molecules.[78]

The various RPA homologs were found to bind DNA with various degrees of cooperativity. dmRPA, hsRPA, and btRPA have been shown to bind ssDNA with low cooperativity with ω determined to be 10 to 20 for hsRPA and 10 to 300 for dmRPA.[9,75,76,79] Although scRPA was initially found to bind with high cooperativity ($w = 10^4 - 10^5$),[74] a recent study disputes these observations and finds the cooperativity of scRPA binding similar to hsRPA.[39] The conformation of hsRPA-DNA complexes is salt sensitive, with higher salt levels (i.e., 100 m*M* NaCl or >2 m*M* $MgCl_2$) inducing their significant compaction.[80]

B. Binding to Double-Stranded DNA (dsDNA)

The binding affinity of RPA for nonspecific dsDNA and RNA is at least three orders of magnitude weaker than that for ssDNA.[6,20,31,44,81] However, significant binding by hsRPA was observed to duplex DNA containing thermally unstable sequences.[80] In addition, high-affinity binding of RPA was detected with particular dsDNA sequences, which are apparently involved in the regulation of transcription in various eukaryotic species.[82–84] As no DNA consensus sequence was identified for these interactions, it is likely that RPA recognizes a specific DNA structure associated with the sequence rather than the sequence itself.

Interestingly, RPA has been shown to recognize specific types of damaged DNA. For example, hsRPA has four- to sixfold higher affinity for duplex DNA containing a single 1,2-d(GpG) cisplatin adduct than to an unmodified control.[85,86] These results are consistent with previous observations showing a 50-fold greater binding of hsRPA to dsDNA containing pyrimidine(6-4) primidone photoproducts induced by UV irradiation.[87] hsRPA has also been observed to bind with increased affinity to DNA damaged with *N*-acetoxy-2-acetylaminofluorene.[64] As discussed in greater detail below, binding to certain damaged substrates is synergistic with other repair factors (e.g., XPA).[64] Although the structure of the primary DNA-binding domain of hsRPA1 has been solved,[27] the molecular requirements of damaged DNA that are recognized by hsRPA are not yet understood. However, a recent study by Patrick and Turchi indicates

that hsRPA does not recognize the damaged base per se, but rather the structural distortion caused by the damage.[88]

C. Mechanism of RPA Binding

hsRPA has been found to bind ssDNA in multiple binding modes,[30,89,90] similar to that previously noted for EcoSSB (e.g., see Ref. 91). First identified was a stable binding mode that occludes 30 nt (the $hRPA_{30nt}$ complex).[12,30,31,75,76,89] hsRPA has also been found to associate less stably with ~8 nt of ssDNA to form the $hRPA_{8nt}$ complex, which can be detected in the presence of glutaraldehyde.[30,89,90] Interestingly, the primary DBDs A and B bind 8 nt of ssDNA,[27] perhaps indicating that hsRPA binding in the 8 nt mode involves ssDNA occupancy of both primary DBDs.

Visualization of both complexes by scanning transmission electron microscopy (STEM) revealed a topological reorientation of hsRPA after ssDNA binding, dependent on the ssDNA length (Figure 2). In the absence of ssDNA or using short ssDNA lengths that prevent $hRPA_{30nt}$ complex formation, hsRPA appears as globular monomer or as globular contracted oligomers coating the DNA, respectively. Using longer DNA lengths that permit formation of the $hRPA_{30nt}$ complex, hsRPA takes a more elongated shape along the DNA.[89] These conformational changes appear to affect both the hsRPA1 and hsRPA2 subunits, as DNA-

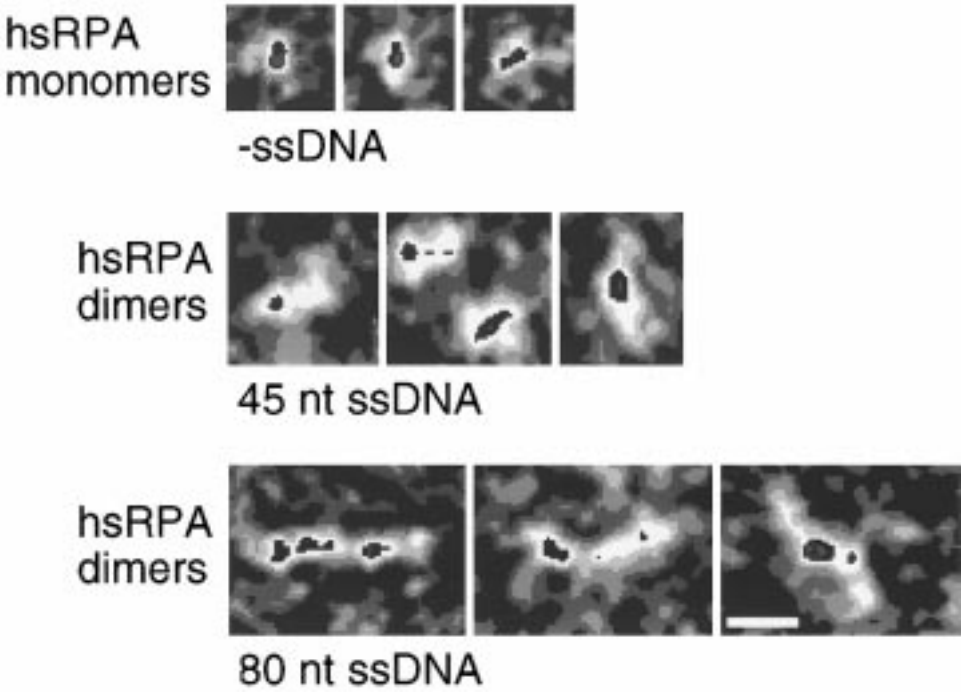

FIGURE 2. STEM visualization of conformational changes induced in hsRPA after binding to ssDNA of sufficient length. Images of representative hsRPA molecules or hsRPA-ssDNA complexes, in the absence of glutaraldehyde, are shown. Image shading cycles progressively through black, three gray levels of increasing lightness, and white with each shading change representing an increase in the mass per unit area. The hsRPA stoichiometries are indicated to the left of each panel. Note that the central image in the middle panel contains two hsRPA (dimer)-ssDNA complexes. Bar indicates 10 nm.

binding causes increased phosphorylation of hsRPA2 by activated DNA-dependent protein kinase (DNA-PK)[89] and alters the sensitivity of the two larger hsRPA subunits to protease cleavage.[50] Notably, the crystal structure of hsRPA1 domains A and B revealed that each domain contained a large loop (L_{45}) in the OB-fold that was postulated to occur in different orientations dependent on the presence of ssDNA,[27] and may be at least partially responsible for the observed hsRPA conformational changes.[50,89] In addition, recent characterization of the ability of hsRPA to be photocrosslinked to template-primer substrates indicates that while the formation of the $hRPA_{8nt}$ complex involves major contacts of the primer with RPA1, formation of the $hRPA_{30nt}$ complex utilizes additional primer contacts with RPA2.[90]

Characterization of the two hsRPA binding modes has led to the suggestion that hsRPA binding to ssDNA occurs through a multistep pathway.[89] RPA first binds 8 nt of DNA unstably, using the high-affinity ssDNA-binding domains A and B, and forming the $hRPA_{8nt}$ complex. RPA then aligns along the DNA, using additional contacts with one or both of the secondary DBDs (C and D), forming the stable $hRPA_{30nt}$ complex (Figure 3). [89]

In addition to the 8 and 30 nt complexes identified for hsRPA binding, use of electron microscopy and fluorescence quenching has indicated that scRPA can bind ssDNA in a 90 to 100 nt mode, forming nucleosome-like structures.[74] More recent studies indicate that scRPA uses a smaller binding site of 20 to 30 nt[92] or 45 nt.[39] In the latter study, scRPA was observed to bind ssDNA oligonucleotides with a 5-fold higher affinity compared with hsRPA. The differences in DNA-binding properties between hsRPA and scRPA are apparently due to distinct binding activities of the RPA2/RPA3 subcomplexes, in part because the yeast RPA2/RPA3 subcomplex bound ssDNA with 1000-fold stronger affinity than the human subcomplex.[39]

1. Molecular Polarity of RPA Binding

The polarity of hsRPA binding to ssDNA has been examined by de Laat and co-worker,[93] in conjunction with the role of RPA in the nucleotide excision repair (NER) reaction. In this study, hsRPA showed preferential binding to hairpin substrates containing 3′-protruding arms shorter than 19 nt. The results were interpreted to indicate that the strong ssDNA-binding domain of hsRPA lies on the 5′ portion of the ssDNA, while a weaker binding entity was located on the 3′ portion of the DNA (Figure 3). Equivalent binding to both the 3′- and 5′-protruding arms was seen when longer sequences were used. The polar binding appears to allow positioning of the XPG and ERCC1-XPF factors during NER to facilitate processing of repair intermediates.[93]

Using somewhat similar substrates, our laboratory has found that hsRPA binds preferentially to 8 nt ssDNA arms protruding in the 5′ direction, or to the 5′ arm of a fork structure.[78] Although the arm that hsRPA bound was the opposite of that found by de Laat et al.,[93] these data are also consistent with a strong ssDNA binding site located on the 5′ portion of the site, while a secondary site(s) is used to contact the 3′ portion of the ssDNA binding site. Moreover, using photocrosslinking methodology to study the interaction of hsRPA with primer-template substrates, Lavrik et al. found that a 19 nt ssDNA region allowed RPA2 to be crosslinked to the primer end 15-fold better than RPA1, while use of a 9 nt ssDNA region showed that only RPA1 was covalently bound to the primer.[90] The laboratory of Kaufmann, studying SV40 replication

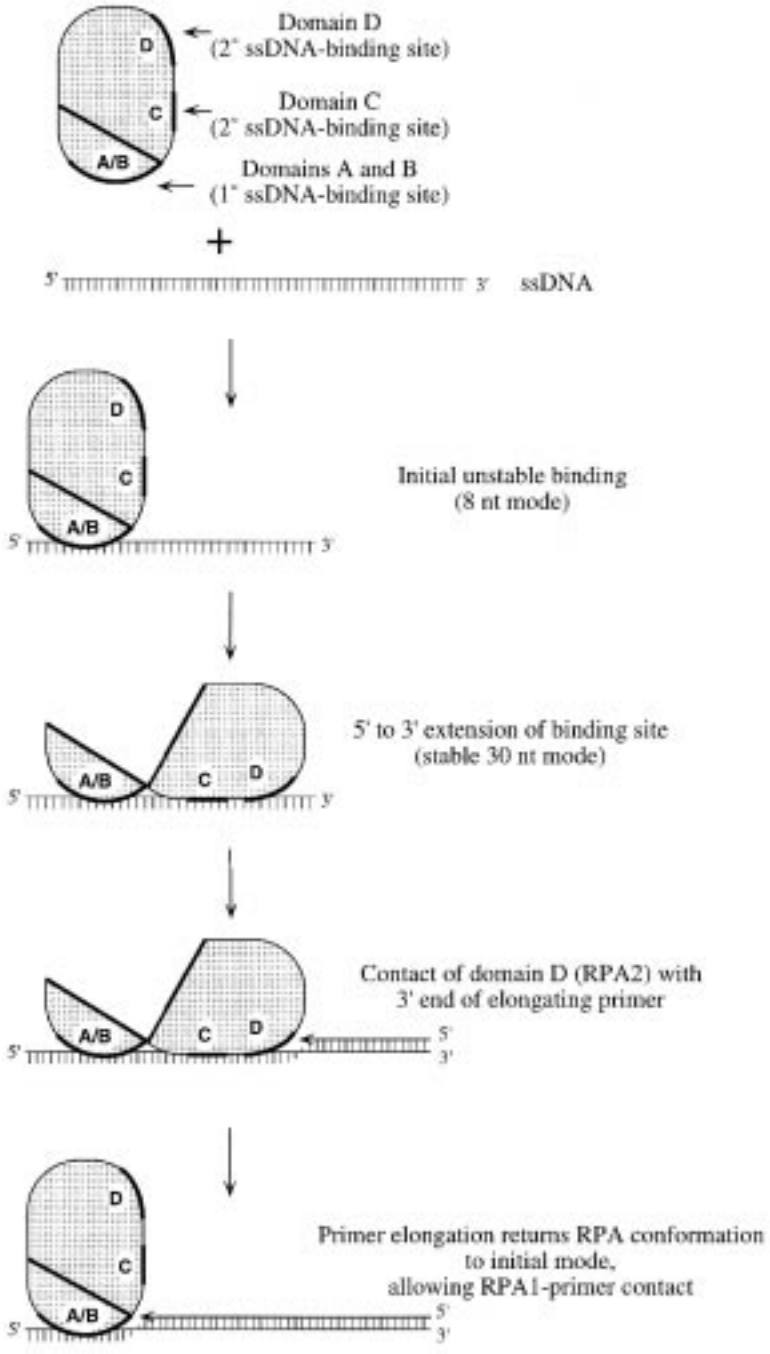

FIGURE 3. Model of RPA binding to ssDNA. In the first step, RPA recognizes the ssDNA unstably using the high-affinity ssDNA-binding domains A and B to contact ~8 nt. In a step involving a significant change to RPA conformation, RPA then occludes 30 nts using the primary and secondary ssDNA-binding domains of RPA1, and perhaps the D domain of RPA2. During the process of DNA synthesis (e.g., by DNA replication or repair), elongation of the nascent strand first contacts the RPA2 subunit, and then the RPA1 subunit, in the process returning RPA to the nonextended configuration. The unstably bound RPA thus can be easily released from the template by further elongation of the nascent strand.

in vivo, observed that RPA2 was crosslinked to the growing lagging strand earlier (at slightly shorter DNA lengths) than RPA1.[33,94] As the RNA-DNA primer was extended further, RPA2 also dissociated from the primer end earlier than RPA1. These data suggest that RPA2 is positioned at the 3′ end of the ssDNA binding site relative to RPA1, again consistent with a 5′ → 3′ molecular polarity of RPA binding (Figure 3).

Although no evidence of polarity for the binding of subsequent molecules of RPA has yet been observed (e.g., see Ref. 95), it is possible that the 5′ → 3′ polarity is also reflective of the mechanism of hsRPA loading onto the lagging DNA strand of a replication fork (which would expose the single- and lagging-strand template in the 5′ → 3′ direction). RPA polarity was also suggested to be involved in homologous recombination because the protein binds to the 3′-ssDNA tails created as a result of double-strand break (DSB) resection (see below).[93]

D. Unwinding of Duplex DNA by RPA

RPA can unwind dsDNA substrates with varying efficiencies. Short (24 nt) oligonucleotides hybridized to M13 DNA are completely denatured by btRPA.[95,96] hsRPA also was observed to denature very lengthy (>1 kb) duplex DNA regions, leading to the production of RPA-DNA filaments.[80] hsRPA has been shown to stimulate the unwinding of the *S. cerevisiae* ARS1 element when present on a negatively supercoiled plasmid.[97] The latter two examples of DNA unwinding by RPA appear to require thermally unstable DNA sequences (e.g., AT-rich regions).[80,97] These 'general' DNA unwinding activities of RPA are salt sensitive, being strongly inhibited by moderate salt levels.[80,96] This inhibition may be due in part to salt-induced compaction of RPA-DNA complexes seen by electron microscopy.[80] Note that the duplex DNA-unwinding activity of RPA does not require ATP hydrolysis. RPA-mediated DNA unwinding therefore is not due to any intrinsic DNA helicase activity but rather to a helix-destabilizing activity, similar to that identified for T4 gp32,[98] AdDBP,[99,100] and ICP8.[101,102]

In addition to completely duplex DNA substrates, 'pseudo-origin' substrates have also been employed that contain an 8-nt bubble flanked by 20-bp duplex flanks,[44,47,103] and thus resemble a DNA structure found within the ATP-dependent initiation complex formed between the SV40 T antigen and the viral origin.[104–106] hsRPA cooperatively binds the pseudo-origin substrate across both strands, causing localized regions of the flanking dsDNA to become reactive to a chemical probe specific for distorted DNA, and leading to the denaturation of a fraction of the bound substrate.[47,103] Although it has been suggested that dsDNA binding is a consequence of the ssDNA-binding activity of hsRPA,[44] Iftode and Borowiec have found that hsRPA binds to the pseudo-origin substrate with an intermediate affinity between ssDNA and dsDNA.[47] In addition, DNA unwinding can be distinguished from DNA binding both kinetically and by temperature.

Unwinding of the pseudo-origin structure has been proposed to occur by hsRPA first binding the 8-nt bubble using the $hRPA_{8nt}$ binding mode. Then, as a result of the conversion of the $hRPA_{8nt}$ to the $hRPA_{30nt}$ complex, the duplex DNA becomes denatured. Because the 8-nt bubble structure has been identified within the SV40 T antigen-origin complex,[105,106] the DNA unwinding activity of RPA rather than the T antigen DNA helicase activity apparently induces the complete denaturation of the viral origin. Loss of the duplex DNA recognition elements releases the T antigen DNA helicase activity to subsequently unwind the DNA bidirectionally outward from the ori-

gin.[107–115] We postulate that the DNA unwinding activity of RPA also serves a similar role during the initiation of chromosomal DNA replication and may be involved in the denaturation of the damaged DNA region during NER.

The large subunit of RPA has been suggested to be important for dsDNA unwinding.[44,95] While it is not clear whether the secondary ssDNA-binding domains of RPA play an important role in its helix-destabilization activity, it is notable that mutation of the hsRPA1 zinc-finger motif reduced its ability to unwind duplex DNA.[44] RPA2 may also play a role in modulating dsDNA denaturation, as phosphorylation of this subunit stimulates unwinding of short oligonucleotides.[95]

IV. RPA PHOSPHORYLATION

One of the most intriguing aspects of RPA that remains functionally undefined is its phosphorylation. Both human and yeast RPA are phosphorylated in a cell-cycle-dependent fashion, with phosphorylation of RPA2 seen at the G1/S transition and continuing through late mitosis.[61,62] In addition to its cell-cycle-dependent phosphorylation, both hsRPA2 and scRPA2 become hyperphosphorylated *in vivo* in response to DNA-damaging agents such as UV or ionizing radiation, treatment with the replication-inhibitor hydroxyurea, and during cellular apoptosis.[116–121] Phosphorylation of hsRPA2 *in vitro* occurs during SV40 DNA replication, with hsRPA binding to ssDNA stimulating its modification.[122]

Characterization of the hsRPA kinases has shown that certain cdk-cyclin complexes (cyclin A-cdk2 and cyclin B-cdc2) are able to phosphorylate hsRPA2 *in vitro,* while others (cyclin E-cdk2) do not.[62,63,122–126] This differential phosphorylation activity apparently results from the ability of hsRPA2 to physically interact with the individual cyclins.[125] A second kinase active in hsRPA phosphorylation is DNA-PK.[89,123,127–129] As its name suggests, DNA-PK requires ssDNA or duplex DNA ends to stimulate kinase activity,[127,130,131] and partially explains the enhanced RPA phosphorylation when DNA-bound. In addition, hsRPA binding to ssDNA causes changes to the conformation of RPA allowing more efficient phosphorylation by DNA-PK.[50,89] Interestingly, RPA–DNA-PK complexes are present in unstressed cells, but are disrupted by the topoisomerase I-inhibitor camptothecin that induces DNA damage during replication.[120] Thus, RPA–DNA-PK complexes may serve to target DNA-PK to sites of DNA damage.

While extracts from DNA-PK-deficient cell lines (e.g., mouse SCID cells) show an inability to phosphorylate hsRPA, irradiation of murine or human cell lines lacking DNA-PK activity still induces RPA2 hyperphosphorylation.[129] As an earlier report found that SCID cells were deficient in the hyperphosphorylation of murine RPA (mus musculus, mmRPA),[128] this point remains controversial. Overall, these data suggest that a kinase different from DNA-PK can also phosphorylate RPA in response to ionizing radiation.

Very recently, hsRPA has been shown to be a phosphorylation target for immunoprecipitates specific for the ataxia telangiectasia-mutated gene (ATM) protein kinase.[132] Lack of ATMp activity results in defective cellular check-point control and causes cells to be hypersensitive to ionizing radiation.[133] Notably, the ionizing-radiation-dependent phosphorylation of hsRPA is delayed in ataxia telangiectasia (AT) cells,[116,119] indicating that either an ATM-mediated pathway or ATMp kinase activity itself play an important role in hsRPA phosphorylation. Consistent with this notion, although *S. cerevisiae* apparently lacks

DNA-PK activity, yeast cells contain an ATMp homologue, Mec1p (product of the *MEC1* gene), which is responsible for scRPA phosphorylation after radiation treatment.[118] mmRPA and ATMp have been found to co-localize during meiotic prophase in nodules on synapsed chromosomes, suggesting a functional interaction during meiotic recombination.[134,135] Thus, it is possible that the kinase responsible for the early radiation-induced phosphorylation of mammalian RPA is actually ATMp, and the delayed RPA phosphorylation in AT cells results from DNA-PK activity.

hsRPA2 can be phosphorylated at numerous sites, as indicated by the presence of ~five different species when its modification is examined by SDS-PAGE analysis (e.g., Ref. 117). The extreme N-terminal of hsRPA2 contains both the cyclin-cdk and DNA-PK phosphorylation sites.[62,63,116,124,126,141] Two consensus cyclin-cdk sites (Ser-Pro or Thr-Pro)[136] are present at Ser-23 and Ser-29 of hsRPA2.[62] Mutation of both these residues to alanine was observed to greatly reduce hsRPA2 phosphorylation when the subunit was expressed in mouse cells,[62] in crude extracts of human cells,[63] and prevented the phosphorylation of recombinant hsRPA (expressed in bacteria) by cyclin B-cdc2.[124] Single mutations of the two Ser residues indicate that, while both sites are cdk targets,[124] Ser-29 is phosphorylated more efficiently.[124,126]

The primary DNA-PK phosphorylation sites on hsRPA2 have been localized to Thr-21 and Ser-33, with Thr-21 appearing to be the primary site.[126,137] In addition, DNA-PK likely phosphorylates another site at either Ser-11, Ser-12, or Ser-13, and perhaps either Ser-4 or Ser-8.[137] Mutation of the Ser-23 and Ser-29 cdk phosphorylation targets does not affect hsRPA2 phosphorylation by DNA-PK.[63,124] Importantly, phosphopeptide maps of hsRPA phosphorylated *in vivo* and *in vitro* with cyclin-cdk and DNA-PK are identical,[62,137] verifying that the sites determined from *in vitro* phosphorylation reactions are identical to those modified *in vivo*. Although it is probable that the sites of hsRPA2 phosphorylation by DNA-PK and ATM are identical (e.g., Ref. 132), this assumption should be verified.

Unfortunately, the functional significance of RPA phosphorylation remains unclear. Although SV40 DNA replication is stimulated by the addition of an S-phase cdk activity,[62,138] no significant differences were detected between the activities of wild-type phosphorylated or nonphosphorylated hsRPA in SV40 DNA replication or NER.[127,139,140] In addition, deletion of the N-terminal hsRPA2 residues 2 to 30 prevents hsRPA phosphorylation, yet has no significant effects on the ability of the mutant heterotrimer to support SV40 DNA replication.[63,141] Similarly, replacement of wild-type hsRPA by an hsRPA mutant containing alanine residues at hsRPA2 positions 23 and 29 had no effect on the ability of hsRPA to bind ssDNA or support SV40 DNA replication.[124] With respect to checkpoint control, expression of dominant-negative fragments of ATM (which prevent S-phase arrest after exposure to ionizing radiation) are not defective in RPA phosphorylation.[119] Moreover, AT cells transformed to express the ATM kinase domain are able to undergo cell-cycle arrest, yet show no significant hsRPA phosphorylation.[119]

On the other hand, various pieces of evidence suggest that RPA phosphorylation plays an important role in eukaryotic DNA metabolism. First, extracts from human cells exposed to ultraviolet radiation (causing hsRPA hyperphosphorylation) are deficient in supporting SV40 DNA replication *in vitro*, but can be restored to the activity of non-treated cell extracts by the addition of purified hsRPA.[117] Second, characterization of the general DNA unwinding activity of btRPA[96] indicates that phosphorylation of

btRPA2 by the cdc2 kinase stimulated 5- to 6-fold the displacement of a 24 nt oligonucleotide bound to M13 ssDNA.[95] Third, a test of a mutant recombinant hsRPA lacking the N-terminal 33 residues of hsRPA2 found that the heterotrimer was inactive in SV40 replication only in the presence of DNA-PK, perhaps suggesting that differential phosphorylation of particular replication components induces the inhibition of replication.[141] Finally, two recent studies indicate that RPA phosphorylation modulates the ability of RPA to interact with other proteins. The laboratory of Wold has found that RPA phosphorylation by DNA-PK or mutation of the hsRPA2 phosphorylation sites to aspartic acids reduced RPA-T antigen complex formation and prevented RPA from physically interacting with DNA polymerase α-DNA primase.[142] Moreover, the mutated RPA was unable to stimulate DNA polymerase activity. Similarly, Abramova et al. found that RPA phosphorylation prevented the association of RPA with p53.[143] As hsRPA interacts with each of these proteins through the RPA1 subunit (see above), these data suggest that RPA2 phosphorylation alters the conformation of the heterotrimer.[142]

In conclusion, current evidence suggests that RPA phosphorylation appears to act in cellular signaling pathway(s) involving DNA-PK, ATM, and related kinases. At sites of DNA damage and on DNA recombination intermediates found during meiosis and during DNA damage repair, hyperphosphorylated RPA may act to recruit additional cellular repair/recombination factors, and thereby facilitate these processes. In addition, RPA phosphorylation has the potential of modulating the enzymatic activities of the proteins with which it interacts. A future experimental direction is clearly the identification of cellular factors that interact specifically with hyperphosphorylated RPA, perhaps when bound to DNA substrates resembling DNA repair intermediates (e.g., formed following a double-strand DNA break). Elucidation of such factors has the potential of yielding new information not only on the role of RPA phosphorylation in cellular signaling relating to the metabolism of DNA, but also on the actual mechanism of these pathways.

V. CELLULAR LOCALIZATION OF RPA

Examination of the cell-cycle location of hsRPA showed that, during the G1 and G2 phases, each RPA subunit displays a diffuse nuclear localization when examined by immunofluorescence,[144,145] with RPA3 observed additionally to be localized to the nucleolus.[145,146] A significant fraction of total hsRPA appears to be associated with double-stranded chromatin during the G1-phase of the cell cycle, although it is unclear whether this interaction is mediated through other DNA-binding proteins.[147] After entering S-phase, the RPA distribution changes to show a punctate nuclear pattern,[144,145,148] consistent with previous observations that DNA replication occurs in so-called nuclear replication factories (e.g., Refs. 149, 150). Study of recruitment of RPA to *S. cerevisiae* replication origins indicates that, at the onset of replication initiation, RPA loading requires a functional MCM complex, and it is regulated by S phase cyclin-dependent kinases and the Dbf4/Cdc7 kinase complex.[151] In Chinese hamster ovary (CHO) cells, association of RPA with replication foci occurs concurrently with the beginning of nucleotide incorporation for both early- and late-replicating origins.[152]

Using *X. laevis* cell-free replication extracts in which replication was blocked by depletion of the membrane components,

xlRPA was seen to bind the decondensed chromatin at numerous sites that appear to represent prereplication centers.[8,153,154] After membrane addition, initiation of replication occurred at the RPA binding sites, indicating that aggregation of RPA around these foci takes place prior to the initiation of replication and could even occur before formation of the nucleus. Subsequent examination showed that the formation of these foci requires ATP hydrolysis and the presence of the focus forming activity-1 (FFA-1).[155,156] FFA-1 has DNA helicase activity and its cloning revealed it to be a homologue of the human WRN gene product whose mutation leads to the Werner syndrome.[156] Presently, it is unclear as to whether FFA-1 is an essential factor for chromosomal DNA replication in *Xenopus*, or rather if FFA-1 is involved in some aspect of DNA repair (see also below).

Examination of the cellular localization of hsRPA during metaphase indicated that, while RPA2 remained attached to chromosomes, RPA1 became associated with the spindle poles and RPA3 was cytoplasmic.[145] Although these data could suggest a cell-cycle-regulated assembly of the hsRPA heterotrimer,[144,145] a more recent reexamination of RPA localization in mitotic CHO or Hela cells found no evidence for dissociation of the heterotrimeric RPA, and no significant association of RPA subunits with metaphase chromosomes.[152]

VI. RPA-PROTEIN INTERACTIONS

RPA is an essential factor in cellular DNA metabolism, and as such the interactions between RPA and other proteins are important for almost every aspect of genome maintenance. Isolation and identification of proteins that physically interact with RPA (Table 2) have further elucidated the various functions of this protein and the reactions in which this protein participates.

A. RPA Interactions with Replication Factors

Study of the initiation and elongation phases of SV40 replication indicates that T antigen and RPA first catalyze the denaturation of the viral origin.[157] These two factors then recruit the DNA polymerase α-DNA primase complex to the unwound origin using specific protein-protein interactions between each protein.[158–162] The hsRPA-binding site maps to T antigen residues 164 to 249,[163] while hsRPA interacts with the DNA polymerase α complex via its primase subunits (see above for the reciprocal binding sites on hsRPA).[16,161] Genetic evidence also reveals an interaction between RPA and the DNA polymerase α complex in yeast.[164]

The physical interactions between each protein results in a variety of effects on the various activities these proteins mediate. For example, the presence of both hsRPA and the DNA-polymerase α complex inhibits the rate of T antigen-mediated unwinding of an SV40 origin-containing template, likely as a consequence of RNA primer synthesis by the polymerase complex.[165] While hsRPA stimulates polymerase activity and primer elongation, it inhibits primer synthesis on M13 ssDNA, and both of these effects are reversed to a degree by T antigen.[18,53,160,166] As T antigen and the DNA polymerase α complex have overlapping binding sites on hsRPA, it is possible that competition between T antigen and the polymerase for RPA is responsible for the observed effects.[53] Although an association with the RPA1 subunit is essential for the stimulatory effects on DNA polymerase α activity, ssDNA-binding by the RPA1 sub-

TABLE 2
Proteins Known or Postulated to Interact with RPA

Chromosomal or viral DNA replication proteins	Species[a]	Ref.
DNA polymerase α-DNA primase	*H. sapiens, B. taurus, S. cerevisiae*	16, 161, 164
SV40 large tumor antigen	*H. sapiens*	161
RTH1 nuclease	*S. cerevisiae*	169
Bovine Papillomavirus E2	*H. sapiens*	56
Epstein-Barr virus EBNA-1	*H. sapiens*	167
DNA repair proteins[b]		
XPA	*H. sapiens*	64, 182–185
XPG	*H. sapiens*	64
ERCC1-XPF	*H. sapiens*	186
Uracil-DNA glycosylase	*H. sapiens*	65
DNA recombination proteins		
Rad52p	*H. sapiens, S. cerevisiae*	58, 206, 207
Rad51p	*H. sapiens*	209
Proteins involved in cell-division or checkpoint control, or DNA damage response		
p53	*H. sapiens*	54–56
cdc2	*H. sapiens, S. cerevisiae*	62
DNA-PK	*H. sapiens*	123, 127, 128
ATMp (MEC1)	*H. sapiens, S. cerevisiae*	118, 132
Transcription factors		
Herpes simplex virus VP16 (activation domain)	*H. sapiens*	55, 56
S. cerevisiae GAL4 (activation domain)	*H. sapiens*	55
Other factors		
Murine FM3A DNA helicase	*H. sapiens*	231

Note: See text for details.

[a] Indicates the species from which RPA and the RPA-interacting factor (if appropriate) were isolated to demonstrate the physical interaction.
[b] Proteins that are involved in nucleotide or base excision repair.

unit alone is not sufficient for the observed stimulation.[53] Recently, two monoclonal antibodies have been identified that are specific for the RPA-binding domain of T antigen and prevent T antigen-hsRPA complex formation.[163] Although these antibodies have no significant effects on the ability of T antigen to bind or denature the SV40 origin, they were observed to inhibit both the synthesis of RNA primers and the elongation of these primers by the DNA polymerase α complex. Taken together, these results suggest that the stimulatory role of RPA on DNA polymerase α activity utilizes specific protein-protein contacts and requires the ssDNA-binding activity of RPA.

A direct interaction between hsRPA and EBNA1, the latent origin-binding protein of the Epstein-Barr virus, has been reported recently.[167] The interaction is mediated through the hsRPA1 subunit and occurs in solution as well as when EBNA1 is bound to the viral origin. As the hsRPA-EBNA1 interaction does not appear to induce structural alterations to the viral origin, hsRPA-EBNA1 complex formation may serve to recruit other host-cell replication proteins to viral initiation sites.

The *S. cerevisiae RTH1* gene product is a 5′ → 3′ exonuclease that also has flap endonuclease activities similar to the mammalian FEN-1.[168,169] Like its mammalian counterparts,[36,170–173] the RTH1 nuclease has been implicated in the processing of chromosomal replication intermediates and in DNA damage repair.[168,169,174,175] The addition of scRPA stimulates the endonucleolytic cleavage of flap substrates, while hsRPA does not, suggesting a species-specific physical interaction between scRPA and the RTH1 nuclease.[169]

hsRPA can physically interact with the bovine papillomavirus (BPV) E2, a transcriptional activator and a required factor for BPV replication *in vivo*.[56] hsRPA has also been reported to bind with significant affinity to a PCNA-affinity column.[176] Because purified hsRPA and human PCNA do not interact by various criteria,[176a] this interaction is likely mediated by other factors.

B. RPA Interactions with Repair Factors

1. Nucleotide Excision Repair

Aside from its absolute requirement in DNA replication, RPA is also a critical factor in NER that is used to repair cyclobutane pyrimidine dimers resulting from UV irradiation as well as other types of lesions.[64,164,177–181] Contribution of RPA to the NER process occurs stepwise. At an early stage, hsRPA interacts with the damage recognition factor XPA, stimulating its ability to bind the damaged site.[64,182–185] XPA-RPA complex formation utilizes interactions across the hsRPA1 and hsRPA2 subunits,[29,64,183–185] with XPA residues 153 to 176 determined to be important for hsRPA1 binding.[183] The interaction with RPA1 seems to be critical because deletion of this interacting domain on XPA results in the inhibition of XPA-mediated NER activity.[183] The dissociation constant of the hsRPA-XPA complex has been determined to be 1.9×10^{-8} *M* using a surface plasmon resonance biosensor.[185] The interaction of hsRPA with XPA leads to the inhibition of SV40 DNA replication *in vitro*.[184]

Once the XPA-RPA complex is formed, the XPG and ERCC1-XPF endonucleases are recruited for excision of the damaged site. XPG and RPA can physically interact, with this interaction mediated by a highly acidic region (residues 668 to 747) within XPG.[64] hsRPA but not scRPA can facilitate the binding of ERCC1-XPF to DNA substrates containing a 30-nt bubble, also suggesting that hsRPA and ERCC1-XPF can form a specific complex.[186] Although a ternary interaction between XPA, RPA, and ERCC1-XPF was observed, an RPA–ERCC1-XPF interaction was not seen using surface plasmon resonance.[185] As mentioned above, the molecular polarity of RPA appears to be a key determinant in the proper positioning of the XPG and ERCC1-XPF nucleases onto the damaged DNA molecule.[93] In addition to recruitment of the two nucleases, the presence of RPA modulates the ability of these nucleases to cleave the damaged strand.[186–188] RPA also participates in a later stage of NER, the gap-filling reaction, along with PCNA, RFC, and DNA polymerases δ or ε.[189]

RPA was initially reported to complement the NER defect observed in extracts of

xeroderma pigmentosum group E (XP-E) cells.[190] More recently, however, it has been found that RPA stimulates NER in extracts from wild-type and various mutant cell lines, so the observed effect does not appear to be specific for the XP-E cells.[191]

RPA has been implicated recently in transcription-coupled repair, in which lesions (generally pyrimidine dimers) present in transcribed regions undergo selective repair such that the transcribed strand is repaired at a higher rate than the nontranscribed strand.[192] Characterization of the yeast *rfa1-M4* strain (that contains a two amino acid insertion in the scRPA1 ssDNA-binding domain A between residues 211 and 212)[164] showed it to be completely defective in NER on the nontranscribed strand of the *MFA2* gene, yet was able to repair the transcribed strand, albeit at reduced levels relative to the wild-type strain.[193] Although it is possible that other factors can functionally replace RPA for transcription-coupled repair in the *rfa1-M4* strain, these data suggest that the primary ssDNA-binding domain of scRPA1 plays a less important role in repair of the transcribed strand compared with the nontranscribed strand.

2. Base Excision Repair

As mentioned above, a role for RPA in base excision repair has been implicated by virtue of its ability to physically interact with human uracil-DNA glycosylase (UNG).[65] The interacting domain on UNG has been localized to residues 28 to 79, a region that bears homology to the RPA-binding region of XPA. The significance of this interaction is unclear, because RPA has only weak inhibitory effects on UNG activity. Nagelhus et al. have suggested that RPA may function to localize UNG to replication foci, so that the template DNA is scanned for the presence of uracil prior to passage of the replication fork.[65]

Two other results implicate RPA in base excision repair. First, RPA has been shown to be a stimulatory factor in long patch DNA base excision repair by facilitating gap filling by DNA polymerase ε.[194] Second, mutation of the *S. cerevisiae RFA1* gene led to the identification of a variety of mutants that were MMS sensitive.[195] Although many of the recovered *rfa1* mutants were also defective in double-strand break repair (DBR), suggesting an impairment of homologous recombination (see below), others were not. As MMS-induced lesions are often repaired by base excision repair (see Ref. 194), these data provide suggestive genetic evidence for a role of RPA in this process.

Finally, an essential function for RPA in mismatch repair has been reported because the use of anti-RPA antibodies inhibits the repair reaction, and the inhibition can be overcome by addition of wild-type RPA but not by various RPA mutants.[46] It is not clear whether RPA is only required for the gap-filling stage of mismatch repair, or if it also acts during the preexcision phase.

C. RPA Interactions with Recombination Factors

Recently, one of the more significant advances that has been made toward our understanding of RPA function is in regard to DNA recombination. Although RPA has been known for a number of years to play a role in recombination from genetic studies in *S. cerevisiae*,[58,59,164] a number of laboratories have explored the role of RPA in homologous genetic recombination and in double-strand break repair. Both of these pathways are mediated by members of the highly conserved *RAD52* and *RAD51* families, the latter encoding a protein that is the eukaryotic homologue of the *E. coli* recA.[196–198]

The Rad51 protein has DNA-dependent ATPase activity and promotes homologous

pairing and strand exchange of complementary ssDNA molecules,[199–201] while Rad52p is a ssDNA-binding protein that facilitates strand annealing.[202] It is known that the two proteins can physically interact.[199,203–205] Studies of human and yeast RPA have shown that each can interact with the cognate Rad52,[58,206,207] with the human protein interaction occurring via the hsRPA2 subunit and residues 221 to 280 of hsRad52p.[206] hsRPA-Rad52p complex formation appears to be essential for recombination, because overexpression of a mutant Rad52p that is unable to interact with RPA2 failed to enhance homologous recombination in monkey cells.[206] In contrast, use of the yeast two-hybrid system indicates that scRad52p can interact with all three RPA subunits, with the strongest interaction observed with scRPA1.[207] Mutation of Rad52p residues 121 or 142 within the putative DNA-binding domain (generating a Rad52p defective in HO-mediated gene conversion[58]) abolished binding of Rad52p to RPA1 but had no effect on the interaction with RPA2.[207] It is unclear at this point whether the observed requirements for the Rad52p-RPA interaction actually differ between yeast and humans, or if the assay systems used to test Rad52p-RPA complex formation led to the divergent findings.

hsRPA has been found to stimulate the strand transfer activity of hsRad51p and these two proteins can directly interact.[208,209] The RPA-Rad51p interaction is mediated through residues 169 to 326 of the RPA1 subunit.[209] A significant interaction between the scRPA1 and the cognate Rad51p was not observed using the two-hybrid system.[207]

Study of the strand exchange reaction *in vitro* has indicated that scRPA could reduce the amount of secondary structure in the ssDNA substrate, yet lessened the amount of presynaptic Rad51p-ssDNA complex formation under limiting Rad51p concentrations by competing for available ssDNA.[92,210] Interestingly, Rad52p is able to overcome the inhibitory effect of RPA by recruiting additional Rad51p to the RPA-ssDNA complex.[210–212] The effect was not observed if EcoSSB or RecA was substituted for RPA or Rad51p, respectively, indicating that specific RPA-Rad52p-Rad51p interactions are required.[212] These data suggest a stepwise pathway in which a double-strand DNA break is first resected to generate a lengthy 3′ ssDNA tail (e.g., Ref. 213). RPA binds to the ssDNA, leading to a reduction in the amount of ssDNA secondary structure. Rad52p acts to displace RPA and facilitate Rad51p binding to the ssDNA, using specific interactions with both proteins and causing the formation of the presynaptic filament that can then begin the search for homologous sequences.

Even with these clear demonstrations of the role of RPA in presynaptic filament formation, the *in vivo* process is undoubtedly more complex. A recent study reports the extensive colocalization of scRPA and scRad52p in discrete foci during meiotic recombination, and a less strong co-localization with scRad51p.[214] Examination of genetic requirements for the appearance of the Rad51p foci demonstrated that the *RAD55* and *RAD57* genes were required, in addition to *RAD52*. In contrast, formation of the RPA-Rad52p foci did not require *RAD51*, *RAD55*, *RAD57*, or *DMC1* (a recA homologue with meiotic specificity). Colocalization of RPA and Rad51p in nuclear foci following γ-irradiation has also been observed in mammalian cells.[209] In addition, RPA and Rad51p have been found to colocalize on newly synapsed axes formed during murine meiotic prophase.[135] As mentioned above, RPA was seen to colocalize with ATMp at nodules within the synaptonemal complex, suggesting that RPA, Rad51p, and ATMp are members of a larger nucleoprotein complex.[134,135]

Genetic studies have found that an allele of the yeast *RFA1* gene (*rfa1-D228Y*) suppresses defects in direct-repeat recombination observed in *rad1 rad52* strains.[59,60] Both *rfa1-D228Y* mutant strains and wild-type strains overexpressing the *rfa1-D228Y* mutant protein were seen to show a hyper-recombinogenic phenotype.[59] Analysis of the processing intermediates caused by induction of the HO-endonuclease revealed that, in a *rad52* background, the *rfa1-D228Y* mutation decreases formation of large-ssDNA intermediates.[60] Therefore, these results lead to the suggestion that RPA containing the *rfa1-D228Y* mutation increases the processing rate of an intermediate in the double-strand break repair pathway that occurs subsequent to the generation of a 3′ ssDNA-tailed molecule (e.g., Refs. 213 and 215).

Mutagenesis of *RFA1* led to the isolation of a number of *rfa1* alleles that were either defective in DNA replication yet insensitive to UV-irradiation or MMS treatment, or UV or MMS sensitive but able to efficiently support chromosomal DNA replication.[195] These data demonstrate that, in addition to common determinants needed for both processes (e.g., ssDNA binding), specific features of the RPA1 subunit are selectively required for repair of DNA damage and for DNA replication.

D. RPA and Cell-Division Control

hsRPA has been shown to physically interact with the tumor suppressor p53,[54–56] a cellular protein critical for a variety of processes, including transcriptional activation, cell division, and apoptosis.[216] While the activation domain of p53 stimulates BPV-1 DNA replication *in vitro* (when bound adjacent to the origin in a GAL4 fusion),[56] formation of the RPA-p53 complex inhibits SV40 replication.[54] The p53-RPA interaction is mediated through the RPA1 subunit (see above),[24,54] and the binding constant of the RPA-p53 complex has been determined to be from 1 to 4×10^8 M^{-1}.[217] Two RPA-interacting domains on p53 have been detected that map to residues 40 to 60 and 289 to 356, although each domain was suggested to consist of smaller subdomains.[54,143,218] Interestingly, Dutta and colleagues found that a synthetic protein constructed from tandem repeats of a p53 peptide (residues 48 to 58) bound RPA efficiently.[218] The monomeric peptide contains an aromatic core surrounded by acidic amino acids, and it was suggested that other proteins used such a motif to bind RPA (e.g., XPA). Mutation of either of two pairs of aromatic amino acids in the N-terminal domain of p53 (D48H-D49H; W53S-F54S) rendered p53 unable to interact with RPA. These same mutations only modestly affected the ability of p53 to activate or repress transcription and had little effect on growth suppression, demonstrating that these p53 activities are not mediated through an interaction with RPA.[218]

The RPA-p53 interaction is greatly down-regulated after the damage-induced hyperphosphorylation of hsRPA2.[143] The diminishment of RPA-p53 complex formation depends on a viable NER process, because XP-A, XP-C, or XP-G cells did not show a disruption of p53 binding to RPA after UV irradiation.[143] The RPA-p53 complex, which is abolished by the presence of ssDNA, inhibits p53 binding to the WAF1 p53-recognition elements.[219] These data suggest that mobilization of RPA in DNA damage repair releases p53 to mediate the global damage response mechanism, including regulation of the cell cycle.[143,219]

Recently, it has been reported that p53 localizes to hsRPA-containing viral replication centers in the nuclei of cells infected with human cytomegalovirus.[220] In contrast, co-localization of RPA and p53 has not been observed during the early development of *X. laevis*.[221]

Two yeast studies have implicated RPA in modulating cellular checkpoint mechanisms. A UV- and MMS-sensitive strain with the *rfa1-M2* allele (containing a two amino acid insertion between scRPA1 residues 96 and 97)[164] still underwent an abortive S-phase after UV-irradiation in G1 phase, in contrast to the wild-type strain that became arrested at G1/S.[222] In that UV-irradiation of the *rfa1-M2* strain during the G2 phase results in a normal G2/M arrest, these data suggest that RPA is an important element of the G1/S and S-phase damage checkpoints.

More recently, examination of yeast DSB repair has also suggested a role for RPA in the cellular DNA damage-sensing and checkpoint control mechanisms. Induction of a single unrepairable DSB during G1 causes yeast cells to undergo a lengthy arrest at G2/M, subsequent to which the cells adapt to the break, undergo cellular division, and eventually die because of the loss of essential genetic information (e.g., Ref. 223). Mutant cells that have an increased rate of 5′ to 3′ degradation at the broken ends (e.g., as a result of a mutation of *hdf1* that encodes Ku70) and hence have longer 3′ ssDNA tails are unable to adapt and fail to escape from the arrest.[224] Notably, cells defective in DSB repair resulting from a K45E mutation in scRPA1 (the *rfa1-t11* allele)[195] can adapt to the DSB in *hdf1* cells and divide, while having no effect on adaptation in an otherwise wild-type strain.[224] While the *rfa1-t11* mutation does not apparently affect the rate of degradation of the DSB in a wild-type strain, the degradation pattern of a *rfa1-t11 hdf1* double mutant was similar to wild type. These results indicate that the mutant RPA modulates the adaptation response to DNA damage, allowing release from the G2/M arrest. It is unclear whether the altered adaptation seen in the *rfa1-t11* strain is a result of a different length of ssDNA generated after resection of the DSB in the *hdf1* strain, or if the altered response is caused by changes in RPA-mediated communication with the cell-cycle arrest machinery.

E. Role of RPA in Transcription Regulation

Physical interactions of RPA with several transcription factors have been described, although the functional significance of this interaction is still unclear. A possible role for transcription factors in eukaryotic replication has been suggested because the binding of such factors (e.g., CTF, c-Jun, and Oct-1) to origin-proximal sites can stimulate the initiation of replication.[225–227] RPA was shown to interact with VP16, a structural component of herpes simplex virus that contains a transcription- and replication-activation domain, and mutagenesis experiments demonstrate a correlation between the ability to interact with hsRPA and enhance polyomavirus DNA replication.[55,56] Interactions between hsRPA and the GAL4 activation domain were also found in these studies.

RPA has been reported to bind to dsDNA upstream repressing sequences (URS) sites in *S. cerevisiae*, including 'MAG-type' URS elements shared by at least 12 DNA repair and metabolism genes.[82,83,228] However, a recent study found that RPA manifests significantly higher binding-affinity to single-stranded than double-stranded URS1 sites, implying that RPA does not have a functional role in transcriptional repression through this site.[229] RPA has also been found to repress transcription from the promoter of the human metallothionein IIA gene.[84]

F. Other Interactions

RPA has been observed to associate with or stimulate the activity of a variety of ei-

ther functionally characterized or orphan helicases as described previously.[230] Most recently, Hughes and Baldacci described the isolation of a novel helicase from the mouse mammary carcinoma cell line FM3A using an hsRPA-affinity chromatography technique.[231] Intriguingly, xlRPA has been observed to co-localize in nuclear foci with FFA-1, a factor that recently has been found to have DNA helicase and exonuclease activity and bears significant homology to the human WRN protein. Notably, mutation of the human WRN gene leads to the Werner syndrome, a rare progeria epitomized by a significantly shortened life span resulting from changes to the organism that resemble premature aging.[232] The functional significance of this observation is presently unclear, but may indicate that WRN acts during replication to properly process DNA replication intermediates, perhaps by reducing the level of intrachromosomal recombination (e.g., see Refs. 233 and 234). Further biochemical characterization of a possible FFA-1– or WRN–RPA interaction may shed light on the genetic derangement seen in cells from Werner syndrome patients, particularly deletions and rearrangements of large DNA segments.

VII. CONCLUDING REMARKS

As can be gleaned from the many different reactions in which RPA acts directly and indirectly, RPA is involved in central aspects of DNA metabolism. The mechanistic aspects of RPA function in these pathways is becoming more apparent as seen from recent structural and mutational characterization of the RPA subunits, and from biochemical and genetic study of these processes. In contrast, the degree to which RPA exerts regulatory control over these processes is still only poorly understood, and this likely will continue to be a profitable avenue of investigation. It is our opinion that novel roles of RPA in the coordination of major aspects of cellular growth remain to be discovered. We anticipate that the next few years will continue to yield important information on RPA, consistent with it being a central player in nucleic acid metabolism.

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REFERENCES

1. **Wold, M. S.** 1997. Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. *Ann. Rev. Biochem.* **66:** 61–92.
2. **Wobbe, C. R., L. Weissbach, J. A. Borowiec, F. B. Dean, Y. Murakami, P. Bullock, and J. Hurwitz.** 1987. Replication of simian virus 40 origin-containing DNA *in vitro* with purified proteins. *Proc. Natl. Acad. Sci. USA* **84:** 1834–1838.
3. **Fairman, M. P. and B. Stillman.** 1988. Cellular factors required for multiple stages of SV40 DNA replication *in vitro*. *EMBO J.* **7:** 1211–1218.

4. **Wold, M. S. and T. J. Kelly.** 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.

5. **Heyer, W.-D., M. R. S. Rao, L. F. Erdile, T. J. Kelly, and R. D. Kolodner.** 1990. An essential *Saccharomyces cerevisiae* single-stranded DNA binding protein is homologous to the large subunit of human RP-A. *EMBO J.* **9:** 2321–2329.

6. **Brill, S. J. and B. Stillman.** 1989. Yeast replication factor-A functions in the unwinding of the SV40 origin of replication. *Nature* **342:** 92–95.

7. **Parker, A. E., R. K. Clyne, A. M. Carr, and T. J. Kelly.** 1997. The Schizo-saccharomyces pombe rad11+ gene encodes the large subunit of replication protein A. *Mol. Cell. Biol.* **17:** 2381–90.

8. **Adachi, Y. and U. K. Laemmli.** 1992. Identification of nuclear pre-replication centers poised for DNA synthesis in Xenopus egg extracts: immunolocalization study of replication protein A. *J. Cell. Biol.* **119:** 1–15.

9. **Mitsis, P. G., S. C. Kowalczykowski, and I. R. Lehman.** 1993. A single-stranded DNA binding protein from *Drosophila melanogaster*: characterization of the heterotrimeric protein and its interaction with single-stranded DNA. *Biochemistry* **32:** 5257–5266.

10. **Marton, R. F., P. Thommes, and S. Cotterill.** 1994. Purification and characterisation of dRP-A: a single-stranded DNA binding protein from *Drosophila melanogaster*. *FEBS Lett.* **342:** 139–44.

11. **van der Knaap, E., S. Jagoueix, and H. Kende.** 1997. Expression of an ortholog of replication protein A1 (RPA1) is induced by gibberellin in deepwater rice. *Proc. Natl. Acad. Sci. USA* **94:** 9979–83.

12. **Seroussi, E. and S. Lavi.** 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:** 7147–54.

13. **Henricksen, L. A., C. B. Umbricht, and M. S. Wold.** 1994. Recombinant replication protein A: expression, complex formation, and functional characterization. *J. Biol. Chem.* **269:** 11121–11132.

14. **Stigger, E., F. B. Dean, J. Hurwitz, and S.-H. Lee.** 1994. Reconstitution of functional human single-stranded DNA-binding protein from individual subunits expressed by recombinant baculoviruses. *Proc. Natl. Acad. Sci. USA* **91:** 579–83.

15. **Keshav, K. F., C. Chen, and A. Dutta.** 1995. Rpa4, a homolog of the 34–kilodalton subunit of the replication protein A complex. *Mol. Cell. Biol.* **15:** 3119–3128.

16. **Nasheuer, H. P., D. von Winkler, C. Schneider, I. Dornreiter, I. Gilbert, and E. Fanning.** 1992. Purification and functional characterization of bovine RP-A in an in vitro SV40 DNA replication system. *Chromosoma* **102:** S52–9.

17. **Kamakaka, R. T., P. D. Kaufman, B. Stillman, P. G. Mitsis, and J. T. Kadonaga.** 1994. Simian virus 40 origin- and T-antigen-dependent DNA replication with *Drosophila* factors in vitro. *Mol. Cell. Biol.* **14:** 5114–22.

18. **Melendy, T. and B. Stillman.** 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.

19. **Kenny, M. K., U. Schlegel, H. Furneaux, and J. Hurwitz.** 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.

20. **Wold, M. S., D. H. Weinberg, D. M. Virshup, J. J. Li, and T. J. Kelly.** 1989. Identification of cellular proteins required for simian virus 40 DNA replication. *J. Biol. Chem.* **264:** 2801–2809.

21. **Gomes, X. V. and M. S. Wold.** 1995. Structural analysis of human replication protein A. Mapping functional domains of the 70–kDa subunit. *J. Biol. Chem.* **270:** 4534–4543.

22. **Gomes, X. V. and M. S. Wold.** 1996. Functional domains of the 70–kilodalton subunit of human replication protein A. *Biochemistry* **35:** 10558–10568.

23. **Kim, D.-K., E. Stigger, and S.-H. Lee.** 1996. Role of the 70–kDa subunit of human replication protein A. *J. Biol. Chem.* **271:** 15124–15129.

24. **Lin, Y.-L., C. Chen, K. F. Keshav, E. Winchester, and A. Dutta.** 1996. Dissection of functional domains of the human DNA replication protein complex replication protein A. *J. Biol. Chem.* **271:** 17190–17198.

25. **Pfuetzner, R. A., A. Bochkarev, L. Frappier, and A. M. Edwards.** 1997. Replication protein A. Characterization and crystallization of the DNA binding domain. *J. Biol. Chem.* **272:** 430–434.

26. **Philipova, D., J. R. Mullen, H. S. Maniar, J. Lu, C. Gu, and S. J. Brill.** 1996. A hierarchy of SSB protomers in replication protein A. *Genes Dev.* **10:** 2222–2233.

27. **Bochkarev, A., R. A. Pfuetzner, A. M. Edwards, and L. Frappier.** 1997. Structure of the single-stranded-DNA-binding domain of replication protein A bound to DNA. *Nature* **385:** 176–181.

28. **Raghunathan, S., C. S. Ricard, T. M. Lohman, and G. Waksman.** 1997. Crystal structure of the homo-tetrameric DNA binding domain of Escherichia coli single-stranded DNA-binding protein determined by multiwavelength x-ray diffraction on the selenomethionyl protein at 2.9–Å resolution. *Proc. Natl. Acad. Sci. USA* **94:** 6652–7.

29. **Walther, A. P., X. V. Gomes, Y. Lao, C. G. Lee, and M. S. Wold.** 1999. Replication protein A interactions with DNA: 1. Functions of the DNA-binding and zinc-finger domains of the 70–kDa subunit. *Biochemistry* **38:** 3963–3973.

30. **Blackwell, L. J. and J. A. Borowiec.** 1994. Human replication protein A binds single-stranded DNA in two distinct complexes. *Mol. Cell. Biol.* **14:** 3993–4001.

31. **Kim, C., R. O. Snyder, and M. S. Wold.** 1992. Binding properties of replication protein A from human and yeast cells. *Mol. Cell. Biol.* **12:** 3050–3059.

32. **Lavrik, O. I., H.-P. Nasheuer, K. Weisshart, M. S. Wold, R. Prasad, W. A. Beard, S. H. Wilson, and A. Favre.** 1998. Subunits of human replication protein A are crosslinked by photoreactive primers synthesized by DNA polymerases. *Nucl. Acids Res.* **26:** 602–607.

33. **Mass, G., T. Nethanel, and G. Kaufmann.** 1998. The middle subunit of replication protein A contacts growing RNA-DNA primers in replicating simian virus 40 chromosomes. *Mol. Cell. Biol.* **18:** 6399–407.

34. **Nethanel, T., S. Reisfeld, G. Dinter-Gottlieb, and G. Kaufmann.** 1988. An Okazaki piece of simian virus 40 may be synthesized by ligation of shorter precursor chains. *J. Virol.* **62:** 2867–73.

35. **Nethanel, T. and G. Kaufmann.** 1990. Two DNA polymerases may be required for synthesis of the lagging DNA strand of simian virus 40. *J. Virol.* **64:** 5912–5918.

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

37. **Bochkareva, E., L. Frappier, A. M. Edwards, and A. Bochkarev.** 1998. The RPA32 subunit of human replication pro-

tein A contains a single-stranded DNA-binding domain. *J. Biol. Chem.* **273:** 3932–3936.

38. **Brill, S. J. and S. Bastin-Shanower.** 1998. Identification and characterization of the fourth single-stranded-DNA binding domain of replication protein A. *Mol. Cell. Biol.* **18:** 7225–34.

39. **Sibenaller, Z. A., B. R. Sorensen, and M. S. Wold.** 1998. The 32– and 14–kilodalton subunits of replication protein A are responsible for species-specific interactions with single-stranded DNA. *Biochemistry* **37:** 12496–506.

40. **Erdile, L. F., W.-D. Heyer, R. Kolodner, and T. J. Kelly.** 1991. Characterization of a cDNA encoding the 70–kDa single-stranded DNA-binding subunit of human replication protein A and the role of the protein in DNA replication. *J. Biol. Chem.* **266:** 12090–12098.

41. **Brown, G. W., J. C. Hines, P. Fisher, and D. S. Ray.** 1994. Isolation of the genes encoding the 51–kilodalton and 28–kilodalton subunits of Crithidia fasciculata replication protein A. *Mol. Biochem. Parasitol.* **63:** 135–42.

42. **Ishiai, M., J. P. Sanchez, A. A. Amin, Y. Murakami, and J. Hurwitz.** 1996. Purification, gene cloning, and reconstitution of the heterotrimeric single-stranded DNA-binding protein from Schizosaccharomyces pombe. *J. Biol. Chem.* **271:** 20868–78.

43. **Dong, J., J.-S. Park, and S.-H. Lee.** 1999. *In vitro* analysis of the zinc-finger motif in human replication protein A. *Biochem. J.* **337:** 311–317.

44. **Lao, Y., C. G. Lee, and M. S. Wold.** 1999. Replication protein A interactions with DNA: 2. Characterization of double-stranded DNA binding/helix-destabilization activities and the role of the zinc-finger domain in DNA interactions. *Biochemistry* **38:** 3974–3984.

45. **Stigger, E., R. Drissi, and S.-H. Lee.** 1998. Functional analysis of human replication protein A in nucleotide excision repair. *J. Biol. Chem.* **273:** 9337–43.

46. **Lin, Y. L., M. K. Shivji, C. Chen, R. Kolodner, R. D. Wood, and A. Dutta.** 1998. The evolutionarily conserved zinc finger motif in the largest subunit of human replication protein A is required for DNA replication and mismatch repair but not for nucleotide excision repair. *J. Biol. Chem.* **273:** 1453–61.

47. **Iftode, C. and J. A. Borowiec.** 1998. Unwinding of origin-specific structures by human replication protein A occurs in a two-step process. *Nucl. Acids Res.* **26:** 5636–43.

48. **Lewis, S. A., G. Tian, I. E. Vainberg, and N. J. Cowan.** 1996. Chaperonin-mediated folding of actin and tubulin. *J. Cell Biol.* **132:** 1–4.

49. **Qiu, H., T. Kodadek, and D. P. Giedroc.** 1994. Zinc-free and reduced T4 gene 32 protein binds single-stranded DNA weakly and fails to stimulate UvsX-catalyzed homologous pairing. *J. Biol. Chem.* **269:** 2773–81.

50. **Gomes, X. V., L. A. Henricksen, and M. S. Wold.** 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.

51. **Kenny, M. K., S.-H. Lee, and J. Hurwitz.** 1989. Multiple functions of human single-stranded-DNA binding protein in simian virus 40 DNA replication: single-strand stabilization and stimulation of DNA polymerases α and δ. *Proc. Natl. Acad. Sci. USA* **86:** 9757–9761.

52. **Tsurimoto, T. and B. Stillman.** 1989. Multiple replication factors augment DNA synthesis by the two eukaryotic DNA polymerases, a and d. *EMBO J.* **8:** 3883–3889.

53. **Braun, K. A., Y. Lao, Z. He, C. J. Ingles, and M. S. Wold.** 1997. Role of protein-protein interactions in the function of rep-

lication protein A (RPA): RPA modulates the activity of DNA polymerase a by multiple mechanisms. *Biochemistry* **36:** 8443–54.

54. **Dutta, A., J. M. Ruppert, J. C. Aster, and E. Winchester.** 1993. Inhibition of DNA replication factor RPA by p53. *Nature* **365:** 79–82.

55. **He, Z., B. T. Brinton, J. Greenblatt, J. A. Hassell, and C. J. Ingles.** 1993. The transactivator proteins VP16 and GAL4 bind replication factor A. *Cell* **73:** 1223–1232.

56. **Li, R. and M. Botchan.** 1993. The acidic transcriptional activation domains of VP16 and p53 bind the cellular replication protein A and stimulate in vitro BPV-1 DNA replication. *Cell* **73:** 1207–1221.

57. **Chen, C., K. Umezu, and R. D. Kolodner.** 1998. Chromosomal rearrangements occur in *S. cerevisiae rfa1* mutator mutants due to mutagenic lesions processed by double-strand-break repair. *Mol. Cell* **2:** 9–22.

58. **Firmenich, A. A., M. Elias-Arnanz, and P. Berg.** 1995. A novel allele of *Saccharomyces cerevisiae RFA1* that is deficient in recombination and repair and suppressible by *RAD52*. *Mol. Cell. Biol.* **15:** 1620–31.

59. **Smith, J. and R. Rothstein.** 1995. A mutation in the gene encoding the *Saccharomyces cerevisiae* single- stranded DNA-binding protein Rfa1 stimulates a *RAD52*–independent pathway for direct-repeat recombination. *Mol. Cell. Biol.* **15:** 1632–41.

60. **Smith, J. and R. Rothstein.** 1999. An allele of *RFA1* suppresses *RAD52*–dependent double-strand break repair in *Saccharomyces cerevisiae*. *Genetics* **151:** 447–58.

61. **Din, S.-, S. J. Brill, M. P. Fairman, and B. Stillman.** 1990. Cell-cycle-regulated phosphorylation of DNA replication factor A from human and yeast cells. *Genes Dev.* **4:** 968–977.

62. **Dutta, A. and B. Stillman.** 1992. cdc2 family kinases phosphorylate a human cell DNA replication factor, RPA, and activate DNA replication. *EMBO J.* **11:** 2189–2199.

63. **Lee, S.-H. and D.-K. Kim.** 1995. The role of the 34–kDa subunit of human replication protein A in simian virus 40 DNA replication in vitro. *J. Biol. Chem.* **270:** 12801–12807.

64. **He, Z., L. A. Henricksen, M. S. Wold, and J. C. Ingles.** 1995. RPA involvement in the damage-recognition and incision steps of nucleotide excision repair. *Nature* **374:** 566–569.

65. **Nagelhus, T. A., T. Haug, K. K. Singh, K. F. Keshav, F. Skorpen, M. Otterlei, S. Bharati, T. Lindmo, S. Benichou, R. Benarous, and H. E. Krokan.** 1997. A sequence in the N-terminal region of human uracil-DNA glycosylase with homology to XPA interacts with the C-terminal part of the 34–kDa subunit of replication protein A. *J. Biol. Chem.* **272:** 6561–6.

66. **Santocanale, C., H. Neecke, M. P. Longhese, G. Lucchini, and P. Plevani.** 1995. Mutations in the gene encoding the 34 kDa subunit of yeast replication protein A cause defective S phase progression. *J. Mol. Biol.* **254:** 595–607.

67. **Maniar, H. S., R. Wilson, and S. J. Brill.** 1997. Roles of replication protein-A subunits 2 and 3 in DNA replication fork movement in Saccharomyces cerevisiae. *Genetics* **145:** 891–902.

68. **Kelly, T. J., P. Simancek, and G. S. Brush.** 1998. Identification and characterization of a single-stranded DNA-binding protein from the archaeon Methanococcus jannaschii. *Proc. Natl. Acad. Sci. USA* **95:** 14634–9.

69. **Chédin, F., E. M. Seitz, and S. C. Kowalczykowski.** 1998. Novel homologs of replication protein A in archaea: implications for the evolution of ssDNA-binding proteins. *Trends Biochem. Sci.* **23:** 273–7.

70. **Umbricht, C. B., L. F. Erdile, E. W. Jabs, and T. J. Kelly.** 1993. Cloning, overexpression, and genomic mapping of the 14–kDa subunit of human replication protein A. *J. Biol. Chem.* **268:** 6131–6138.

71. **Ozawa, K., F. B. Dean, M. Chen, S. H. Lee, A. Shiratori, Y. Murakami, T. Sakakura, J. Hurwitz, and T. Eki.** 1993. Mapping of the 70 kDa, 34 kDa, and 11 kDa subunit genes of the human multimeric single-stranded DNA binding protein (hSSB/RPA) to chromosome bands 17p13, 1p35–p36.1, and 7p21–p22. *Cell Struct. Funct.* **18:** 221–30.

72. **Umbricht, C. B., C. A. Griffin, A. L. Hawkins, K. H. Grzeschik, P. O'Connell, R. Leach, E. D. Green, and T. J. Kelly.** 1994. High-resolution genomic mapping of the three human replication protein A genes (RPA1, RPA2, and RPA3). *Genomics* **20:** 249–57.

73. **Brill, S. J. and B. Stillman.** 1991. Replication factor-A from *Saccharomyces cerevisiae* is encoded by three essential genes coordinately expressed at S phase. *Genes Dev.* **5:** 1589–1600.

74. **Alani, E., R. Thresher, J. Griffith, and R. D. Kolodner.** 1992. Characterization of DNA-binding and strand-exchange stimulation properties of y-RPA, a yeast single-strand-DNA-binding protein. *J. Mol. Biol.* **227:** 54–71.

75. **Kim, C., B. F. Paulus, and M. S. Wold.** 1994. Interactions of human replication protein A with oligonucleotides. *Biochemistry* **33:** 14197–14206.

76. **Kim, C. and M. S. Wold.** 1995. Recombinant human replication protein A binds to polynucleotides with low cooperativity. *Biochemistry* **34:** 2058–64.

77. **Carmichael, E. P., J. M. Roome, and A. F. Wahl.** 1993. Binding of a sequence-specific single-stranded DNA-binding factor to the simian virus 40 core origin inverted repeat domain is cell cycle regulated. *Mol. Cell. Biol.* **13:** 408–20.

78. **Iftode, C. and J. A. Borowiec.** 1999. Manuscript in preparation.

79. **Atrazhev, A., S. Zhang, and F. Grosse.** 1992. Single-stranded DNA binding protein from calf thymus. Purification, properties, and stimulation of the homologous DNA-polymerase-alpha-primase complex. *Eur. J. Biochem.* **210:** 855–865.

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

81. **Hurwitz, J., F. B. Dean, A. D. Kwong, and S.-H. Lee.** 1990. The *in vitro* replication of DNA containing the SV40 origin. *J. Biol. Chem.* **265:** 18043–18046.

82. **Luche, R. M., W. C. Smart, T. Marion, M. Tillman, R. A. Sumrada, and T. G. Cooper.** 1993. Saccharomyces cerevisiae BUF protein binds to sequences participating in DNA replication in addition to those mediating transcriptional repression (URS1) and activation. *Mol. Cell. Biol.* **13:** 5749–5761.

83. **Singh, K. K. and L. Samson.** 1995. Replication protein A binds to regulatory elements in yeast DNA repair and DNA metabolism genes. *Proc. Natl. Acad. Sci. USA* **92:** 4907–4911.

84. **Tang, C. M., A. E. Tomkinson, W. S. Lane, M. S. Wold, and E. Seto.** 1996. Replication protein A is a component of a complex that binds the human metallothionein IIA gene transcription start site. *J. Biol. Chem.* **271:** 21637–21644.

85. **Clugston, C. K., K. McLaughlin, M. K. Kenny, and R. Brown.** 1992. Binding of human single-stranded DNA binding protein to DNA damaged by the anticancer drug cis-diamminedichloroplatinum (II). *Cancer Res.* **52:** 6375–9.

86. **Patrick, S. M. and J. J. Turchi.** 1998. Human replication protein A preferentially binds cisplatin-damaged duplex DNA in vitro. *Biochemistry* **37:** 8808–15.

87. **Burns, J. L., S. N. Guzder, P. Sung, S. Prakash, and L. Prakash.** 1996. An affinity of human replication protein A for ultraviolet-damaged DNA. *J. Biol. Chem.* **271:** 11607–10.

88. **Patrick, S. M. and J. J. Turchi.** 1999. Replication protein A (RPA) binding to duplex cisplatin-damaged DNA is mediated through the generation of single-stranded DNA. *J. Biol. Chem.* **274:** 14972–14978.

89. **Blackwell, L. J., J. A. Borowiec, and I. A. Mastrangelo.** 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.

90. **Lavrik, O. I., D. M. Kolpashchikov, H. P. Nasheuer, K. Weisshart, and A. Favre.** 1998. Alternative conformations of human replication protein A are detected by crosslinks with primers carrying a photoreactive group at the 3′-end. *FEBS Lett.* **441:** 186–90.

91. **Bujalowski, W., L. B. Overman, and T. M. Lohman.** 1988. Binding mode transitions of *Escherichia coli* single strand binding protein-single-stranded DNA complexes. Cation, anion, pH, and binding density effects. *J. Biol. Chem.* **263:** 4629–4640.

92. **Sugiyama, T., E. M. Zaitseva, and S. C. Kowalczykowski.** 1997. A single-stranded DNA-binding protein is needed for efficient presynaptic complex formation by the *Saccharomyces cerevisiae* Rad51 protein. *J. Biol. Chem.* **272:** 7940–5.

93. **de Laat, W. L., E. Appeldoorn, K. Sugasawa, E. Weterings, N. G. J. Jaspers, and J. H. J. Hoeijmakers.** 1998. DNA-binding polarity of human replication protein A positions nucleases in nucleotide excision repair. *Genes Dev.* **12:** 2598–2609.

94. **Mass, G., T. Nethanel, and G. Kaufmann.** Unpublished observations.

95. **Georgaki, A. and U. Hübscher.** 1993. DNA unwinding by replication protein A is a property of the 70 kDa subunit and is facilitated by phosphorylation of the 32 kDa subunit. *Nucl. Acids Res.* **21:** 3659–3665.

96. **Georgaki, A., B. Strack, V. Podust, and U. Hübscher.** 1992. DNA unwinding activity of replication protein A. *FEBS Lett.* **308:** 240–244.

97. **Matsumoto, K. and Y. Ishimi.** 1994. Single-stranded-DNA-binding protein-dependent DNA unwinding of the yeast ARS1 region. *Mol. Cell. Biol.* **14:** 4624–4632.

98. **Alberts, B. M. and L. Frey.** 1970. T4 bacteriophage gene 32: a structural protein in the replication and recombination of DNA. *Nature* **227:** 1313–1318.

99. **Monaghan, A., A. Webster, and R. T. Hay.** 1994. Adenovirus DNA binding protein: helix destabilising properties. *Nucl. Acids Res.* **22:** 742–748.

100. **Zijderveld, D. C. and P. C. van der Vliet.** 1994. Helix-destabilizing properties of the adenovirus DNA-binding protein. *J. Virol.* **68:** 1158–1164.

101. **Boehmer, P. E. and I. R. Lehman.** 1993. Herpes simplex virus type 1 ICP8: helix-destabilizing properties. *J. Virol.* **67:** 711–715.

102. **Lee, S. S. and I. R. Lehman.** 1997. Unwinding of the box I element of a herpes simplex virus type 1 origin by a complex of the viral origin binding protein, single-strand DNA binding protein, and single-stranded DNA. *Proc. Natl. Acad. Sci. USA* **94:** 2838–42.

103. **Iftode, C. and J. A. Borowiec.** 1997. Denaturation of the simian virus 40 origin of replication mediated by human replication protein A. *Mol. Cell. Biol.* **17:** 3876–3883.

104. **Dean, F. B., M. Dodson, H. Echols, and J. Hurwitz.** 1987. ATP-dependent formation of a specialized nucleoprotein structure by simian virus 40 (SV40) large

tumor antigen at the SV40 replication origin. *Proc. Natl. Acad. Sci. USA* **84:** 8981–8985.

105. **Deb, S. and P. Tegtmeyer.** 1987. ATP enhances the binding of simian virus 40 large T antigen to the origin of replication. *J. Virol.* **61:** 3649–3654.

106. **Borowiec, J. A. and J. Hurwitz.** 1988. ATP stimulates the binding of simian virus 40 (SV40) large tumor antigen to the SV40 origin of replication. *Proc. Natl. Acad. Sci. USA* **85:** 64–68.

107. **Stahl, H., P. Dröge, and R. Knippers.** 1986. DNA helicase activity of SV40 large tumor antigen. *EMBO J.* **5:** 1939–1944.

108. **Dean, F. B., P. Bullock, Y. Murakami, C. R. Wobbe, L. Weissbach, and J. Hurwitz.** 1987. Simian virus 40 (SV40) DNA replication: SV40 large T antigen unwinds DNA containing the SV40 origin of replication. *Proc. Natl. Acad. Sci. USA* **84:** 16–20.

109. **Wold, M. S., J. J. Li, and T. J. Kelly.** 1987. Initiation of simian virus 40 DNA replication *in vitro*: Large-tumor-antigen- and origin-dependent unwinding of the template. *Proc. Natl. Acad. Sci. USA* **84:** 3643–3647.

110. **Wiekowski, M., M. W. Schwarz, and H. Stahl.** 1988. Simian virus 40 large T antigen DNA helicase. *J. Biol. Chem.* **263:** 436–442.

111. **Goetz, G. S., F. B. Dean, J. Hurwitz, and S. W. Matson.** 1988. The unwinding of duplex regions in DNA by the simian virus 40 large tumor antigen-associated DNA helicase activity. *J. Biol. Chem.* **263:** 383–392.

112. **Wessel, R., J. Schweizer, and H. Stahl.** 1992. Simian virus 40 T-antigen DNA helicase is a hexamer which forms a binary complex during bidirectional unwinding from the viral origin of DNA replication. *J. Virol.* **66:** 804–815.

113. **SenGupta, D. J. and J. A. Borowiec.** 1994. Strand and face: the topography of interactions between the SV40 origin of replication and T antigen during the initiation of replication. *EMBO J.* **13:** 982–992.

114. **Smelkova, N. V. and J. A. Borowiec.** 1997. Dimerization of simian virus 40 T-antigen hexamers activates T-antigen DNA helicase activity. *J. Virol.* **71:** 8766–8773.

115. **Smelkova, N. V. and J. A. Borowiec.** 1998. Synthetic DNA replication bubbles bound and unwound with two-fold symmetry by an SV40 T antigen double hexamer. *J. Virol.* **72:** 8676–8681.

116. **Liu, V. F. and D. T. Weaver.** 1993. The ionizing radiation-induced replication protein A phosphorylation response differs between ataxia telangiectasia and normal human cells. *Mol. Cell. Biol.* **13:** 7222–7231.

117. **Carty, M. P., M. Zernik-Kobak, S. McGrath, and K. Dixon.** 1994. UV light-induced DNA synthesis arrest in HeLa cells is associated with changes in phosphorylation of human single-stranded DNA-binding protein. *EMBO J.* **13:** 2114–2123.

118. **Brush, G. S., D. M. Morrow, P. Hieter, and T. J. Kelly.** 1996. The ATM homologue MEC1 is required for phosphorylation of replication protein A in yeast. *Proc. Natl. Acad. Sci. USA* **93:** 15075–80.

119. **Morgan, S. E. and M. B. Kastan.** 1997. Dissociation of radiation-induced phosphorylation of replication protein A from the S-phase checkpoint. *Cancer Res.* **57:** 3386–9.

120. **Shao, R. G., C. X. Cao, H. Zhang, K. W. Kohn, M. S. Wold, and Y. Pommier.** 1999. Replication-mediated DNA damage by camptothecin induces phosphorylation of RPA by DNA-dependent protein kinase and dissociates RPA:DNA-PK complexes. *EMBO J.* **18:** 1397–1406.

121. **Treuner, K., A. Okuyama, R. Knippers, and F. O. Fackelmayer.** 1999. Hype-

rphosphorylation of replication protein A middle subunit (RPA32) in apoptosis. *Nucl. Acids Res.* **27:** 1499–1504.

122. **Fotedar, R. and J. M. Roberts.** 1992. Cell cycle regulated phosphorylation of RPA-32 occurs within the replication initiation complex. *EMBO J.* **11:** 2177–2187.

123. **Pan, Z.-Q., A. A. Amin, E. Gibbs, H. Niu, and J. Hurwitz.** 1994. Phosphorylation of the p34 subunit of human single-stranded-DNA-binding protein in cyclin A-activated G_1 extracts is catalyzed by cdk-cyclin A complex and DNA-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* **91:** 8343–8347.

124. **Henricksen, L. A. and M. S. Wold.** 1994. Replication protein A mutants lacking phosphorylation sites for $p34^{cdc2}$ kinase support DNA replication. *J. Biol. Chem.* **269:** 24203–24208.

125. **Gibbs, E., Z. Q. Pan, H. Niu, and J. Hurwitz.** 1996. Studies on the in vitro phosphorylation of HSSB-p34 and -p107 by cyclin-dependent kinases. Cyclin-substrate interactions dictate the efficiency of phosphorylation. *J. Biol. Chem.* **271:** 22847–54.

126. **Niu, H., H. Erdjument-Bromage, Z. Q. Pan, S. H. Lee, P. Tempst, and J. Hurwitz.** 1997. Mapping of amino acid residues in the p34 subunit of human single-stranded DNA-binding protein phosphorylated by DNA-dependent protein kinase and Cdc2 kinase in vitro. *J. Biol. Chem.* **272:** 12634–41.

127. **Brush, G. S., C. W. Anderson, and T. J. Kelly.** 1994. The DNA-activated protein kinase is required for the phosphorylation of replication protein A during simian virus 40 DNA replication. *Proc. Natl. Acad. Sci. USA* **91:** 12520–12524.

128. **Boubnov, N. K. and D. T. Weaver.** 1995. *scid* cells are deficient in Ku and Replication Protein A phosphorylation by the DNA-dependent protein kinase. *Mol. Cell. Biol.* **15:** 5700–5706.

129. **Fried, L. M., C. Koumenis, S. R. Peterson, S. L. Green, P. van Zijl, J. Allalunis-Turner, D. J. Chen, R. Fishel, A. J. Giaccia, J. M. Brown, and C. U. Kirchgessner.** 1996. The DNA damage response in DNA-dependent protein kinase-deficient SCID mouse cells: replication protein A hyperphosphorylation and p53 induction. *Proc. Natl. Acad. Sci. USA* **93:** 13825–30.

130. **Gottlieb, T. M. and S. P. Jackson.** 1993. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. *Cell* **72:** 131–142.

131. **Morozov, V. E., M. Falzon, C. W. Anderson, and E. L. Kuff.** 1994. DNA-dependent protein kinase is activated by nicks and larger single-stranded gaps. *J. Biol. Chem.* **269:** 16684–16688.

132. **Gately, D. P., J. C. Hittle, G. K. T. Chan, and T. J. Yen.** 1998. Characterization of ATM Expression, Localization, and Associated DNA-dependent Protein Kinase Activity. *Mol. Biol. Cell.* **9:** 2361–74.

133. **Jeggo, P. A., A. M. Carr, and A. R. Lehmann.** 1998. Splitting the ATM: distinct repair and checkpoint defects in ataxia- telangiectasia. *Trends Genet.* **14:** 312–6.

134. **Plug, A. W., A. H. Peters, Y. Xu, K. S. Keegan, M. F. Hoekstra, D. Baltimore, P. de Boer, and T. Ashley.** 1997. ATM and RPA in meiotic chromosome synapsis and recombination. *Nat. Genet.* **17:** 457–61.

135. **Plug, A. W., A. H. Peters, K. S. Keegan, M. F. Hoekstra, P. de Boer, and T. Ashley.** 1998. Changes in protein composition of meiotic nodules during mammalian meiosis. *J. Cell. Sci.* **111:** 413–23.

136. **Kemp, B. E. and R. B. Pearson.** 1990. Protein kinase recognition sequence motifs. *Trends Biochem. Sci.* **15:** 342–6.

137. **Zernik-Kobak, M., K. Vasunia, M. Connelly, C. W. Anderson, and K. Dixon.** 1997. Sites of UV-induced phos-

phorylation of the p34 subunit of replication protein A from HeLa cells. *J. Biol. Chem.* **272:** 23896–904.

138. **D'Urso, G., R. L. Marraccino, D. R. Marshak, and J. M. Roberts.** 1990. Cell cycle control of DNA replication by a homologue from human cells of the p34cdc2 protein kinase. *Science* **250:** 786–791.

139. **Pan, Z.-Q., C. H. Park, A. A. Amin, J. Hurwitz, and A. Sancar.** 1995. Phosphorylated and unphosphorylated forms of human single-stranded DNA-binding protein are equally active in simian virus 40 DNA replication and in nucleotide excision repair. *Proc. Natl. Acad. Sci. USA* **92:** 4636–4640.

140. **Ariza, R. R., S. M. Keyse, J. G. Moggs, and R. D. Wood.** 1996. Reversible protein phosphorylation modulates nucleotide excision repair of damaged DNA by human cell extracts. *Nucl. Acids Res* **24:** 433–40.

141. **Henricksen, L. A., T. Carter, A. Dutta, and M. S. Wold.** 1996. Phosphorylation of human replication protein A by the DNA-dependent protein kinase is involved in the modulation of DNA replication. *Nucl. Acids Res.* **24:** 3107–3112.

142. **Braun, K. A., L. A. Henricksen, C. G. Lee, T. Carter, S. P. Lees-Miller, and M. S. Wold.** 1999. Manuscript in preparation.

143. **Abramova, N. A., J. Russell, M. Botchan, and R. Li.** 1997. Interaction between replication protein A and p53 is disrupted after UV damage in a DNA repair-dependent manner. *Proc. Natl. Acad. Sci. USA* **94:** 7186–91.

144. **Brenot-Bosc, F., S. Gupta, R. L. Margolis, and R. Fotedar.** 1995. Changes in the subcellular localization of replication initiation proteins and cell cycle proteins during G1– to S-phase transition in mammalian cells. *Chromosoma* **103:** 517–27.

145. **Murti, K. G., D. C. He, B. R. Brinkley, R. Scott, and S.-H. Lee.** 1996. Dynamics of human replication protein A subunit distribution and partitioning in the cell cycle. *Exp. Cell Res.* **223:** 279–289.

146. **Dimitrova, D., Y. Daniely, D. M. Gilbert, and J. A. Borowiec.** Unpublished observations.

147. **Treuner, K., C. Eckerich, and R. Knippers.** 1998. Chromatin association of replication protein A. *J. Biol. Chem.* **273:** 31744–50.

148. **Krude, T.** 1995. Chromatin assembly factor 1 (CAF-1) colocalizes with replication foci in HeLa cell nuclei. *Exp. Cell Res.* **220:** 304–11.

149. **Hozák, P., A. B. Hassan, D. A. Jackson, and P. R. Cook.** 1993. Visualization of replication factories attached to a nucleoskeleton. *Cell* **73:** 361–373.

150. **Newport, J. and H. Yan.** 1996. Organization of DNA into foci during replication. *Curr. Opin. Cell Biol.* **8:** 365–8.

151. **Tanaka, T. and K. Nasmyth.** 1998. Association of RPA with chromosomal replication origins requires an Mcm protein, and is regulated by Rad53, and cyclin- and Dbf4–dependent kinases. *EMBO J.* **17:** 5182–91.

152. **Dimitrova, D. S., I. T. Todorov, T. Melendy, and D. M. Gilbert.** Unpublished observations.

153. **Adachi, Y. and U. K. Laemmli.** 1994. Study of the cell cycle-dependent assembly of the DNA pre-replication centres in Xenopus egg extracts. *EMBO J.* **13:** 4153–64.

154. **Yan, H. and J. Newport.** 1995. An analysis of the regulation of DNA synthesis by cdk2, Cip1, and licensing factor. *J. Cell Biol.* **129:** 1–15.

155. **Yan, H. and J. Newport.** 1995. FFA-1, a protein that promotes the formation of replication centers within nuclei. *Science* **269:** 1883–5.

156. **Yan, H., C. Y. Chen, R. Kobayashi, and J. Newport.** 1998. Replication focus-forming activity 1 and the Werner syndrome gene product. *Nat. Genet.* **19:** 375–8.

157. **Bullock, P. A.** 1997. The initiation of simian virus 40 DNA replication *in vitro*. *Crit. Rev. Biochem. Mol. Biol.* **32:** 503–568.

158. **Gannon, J. V. and D. P. Lane.** 1987. p53 and DNA polymerase α compete for binding to SV40 T antigen. *Nature* **329:** 456–458.

159. **Dornreiter, I., A. Höss, A. K. Arthur, and E. Fanning.** 1990. SV40 T antigen binds directly to the large subunit of purified DNA polymerase α. *EMBO J.* **9:** 3329–3336.

160. **Collins, K. L. and T. J. Kelly.** 1991. Effects of T antigen and replication protein A on the initiation of DNA synthesis by DNA polymerase alpha-primase. *Mol. Cell. Biol.* **11:** 2108–15.

161. **Dornreiter, I., L. F. Erdile, I. U. Gilbert, D. von Winkler, T. J. Kelly, and E. Fanning.** 1992. Interaction of DNA polymerase a-primase with cellular replication protein A and SV40 T antigen. *EMBO J.* **11:** 769–776.

162. **Dornreiter, I., W. C. Copeland, and T. S. Wang.** 1993. Initiation of simian virus 40 DNA replication requires the interaction of a specific domain of human DNA polymerase alpha with large T antigen. *Mol. Cell. Biol.* **13:** 809–820.

163. **Weisshart, K., P. Taneja, and E. Fanning.** 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:** 9771–81.

164. **Longhese, M. P., P. Plevani, and G. Lucchini.** 1994. Replication factor A is required in vivo for DNA replication, repair, and recombination. *Mol. Cell. Biol.* **14:** 7884–7890.

165. **Murakami, Y. and J. Hurwitz.** 1993. Functional interactions between SV40 T antigen and other replication proteins at the replication fork. *J. Biol. Chem.* **268:** 11008–11017.

166. **Matsumoto, T., T. Eki, and J. Hurwitz.** 1990. Studies on the initiation and elongation reactions in the simian virus 40 DNA replication system. *Proc. Natl. Acad. Sci. USA* **87:** 9712–6.

167. **Zhang, D., L. Frappier, E. Gibbs, J. Hurwitz, and M. O'Donnell.** 1998. Human RPA (hSSB) interacts with EBNA1, the latent origin binding protein of Epstein-Barr virus. *Nucl. Acids Res.* **26:** 631–7.

168. **Zhu, F. X., E. E. Biswas, and S. B. Biswas.** 1997. Purification and characterization of the DNA polymerase alpha associated exonuclease: the RTH1 gene product. *Biochemistry* **36:** 5947–54.

169. **Biswas, E. E., F. X. Zhu, and S. B. Biswas.** 1997. Stimulation of *RTH1* nuclease of the yeast *Saccharomyces cerevisiae* by replication protein A. *Biochemistry* **36:** 5955–62.

170. **Ishimi, Y., A. Claude, P. Bullock, and J. Hurwitz.** 1988. Complete enzymatic synthesis of DNA containing the SV40 origin of replication. *J. Biol. Chem.* **263:** 19723–19733.

171. **Harrington, J. J. and M. R. Lieber.** 1994. Functional domains within FEN-1 and RAD2 define a family of structure- specific endonucleases: implications for nucleotide excision repair. *Genes Dev.* **8:** 1344–55.

172. **Waga, S., G. Bauer, and B. Stillman.** 1994. Reconstitution of complete SV40 DNA replication with purified replication factors. *J. Biol. Chem.* **269:** 10923–34.

173. **Murray, J. M., M. Tavassoli, R. al-Harithy, K. S. Sheldrick, A. R. Lehmann, A. M. Carr, and F. Z. Watts.** 1994. Structural and functional conservation of the human homolog of the *Schizosaccharomyces pombe* rad2 gene, which is required for chromosome segregation and recovery from DNA damage. *Mol. Cell. Biol.* **14:** 4878–88.

174. **Sommers, C. H., E. J. Miller, B. Dujon, S. Prakash, and L. Prakash.** 1995. Conditional lethality of null mutations in RTH1 that encodes the yeast counterpart of a mammalian 5'- to 3'-exonuclease required for lagging strand DNA synthesis in reconstituted systems. *J. Biol. Chem.* **270:** 4193–6.

175. **Budd, M. E. and J. L. Campbell.** 1997. A yeast replicative helicase, Dna2 helicase, interacts with yeast FEN-1 nuclease in carrying out its essential function. *Mol. Cell. Biol.* **17:** 2136–42.

176. **Loor, G., S.-J. Zhang, P. Zhang, N. L. Toomey, and M. Y. Lee.** 1997. Identification of DNA replication and cell cycle proteins that interact with PCNA. *Nucl. Acids Res.* **25:** 5041–6.

176a. **Wold, M.** Unpublished observations.

177. **Coverley, D., M. K. Kenny, M. Munn, W. D. Rupp, D. P. Lane, and R. D. Wood.** 1991. Requirement for the replication protein SSB in human DNA excision repair. *Nature* **349:** 538–541.

178. **Mu, D., C. H. Park, T. Matsunaga, D. S. Hsu, J. T. Reardon, and A. Sancar.** 1995. Reconstitution of human DNA repair excision nuclease in a highly defined system. *J. Biol. Chem.* **270:** 2415–8.

179. **Guzder, S. N., Y. Habraken, P. Sung, L. Prakash, and S. Prakash.** 1995. Reconstitution of yeast nucleotide excision repair with purified Rad proteins, replication protein A, and transcription factor TFIIH. *J. Biol. Chem.* **270:** 12973–6.

180. **Moggs, J. G., K. J. Yarema, J. M. Essigmann, and R. D. Wood.** 1996. Analysis of incision sites produced by human cell extracts and purified proteins during nucleotide excision repair of a 1,3–intrastrand d(GpTpG)-cisplatin adduct. *J. Biol. Chem.* **271:** 7177–86.

181. **Mu, D., D. S. Hsu, and A. Sancar.** 1996. Reaction mechanism of human DNA repair excision nuclease. *J. Biol. Chem.* **271:** 8285–94.

182. **Matsuda, T., M. Saijo, I. Kuraoka, T. Kobayashi, Y. Nakatsu, A. Nagai, T. Enjoji, C. Masutani, K. Sugasawa, F. Hanaoka, A. Yasui, and K. Tanaka.** 1995. DNA repair protein XPA binds replication protein A (RPA). *J. Biol. Chem.* **270:** 4152–4157.

183. **Li, L., X. Lu, C. A. Peterson, and R. J. Legerski.** 1995. An interaction between the DNA repair factor XPA and replication protein A appears essential for nucleotide excision repair. *Mol. Cell. Biol.* **15:** 5396–402.

184. **Lee, S.-H., D.-K. Kim, and R. Drissi.** 1995. Human xeroderma pigmentosum group A protein interacts with human replication protein A and inhibits DNA replication. *J. Biol. Chem.* **270:** 21800–21805.

185. **Saijo, M., I. Kuraoka, C. Masutani, F. Hanaoka, and K. Tanaka.** 1996. Sequential binding of DNA repair proteins RPA and ERCC1 to XPA in vitro. *Nucl. Acids Res.* **24:** 4719–24.

186. **Matsunaga, T., C.-H. Park, T. Bessho, D. Mu, and A. Sancar.** 1996. Replication protein A confers structure-specific endonuclease activities to the XPF-ERCC1 and XPG subunits of human DNA repair excision nuclease. *J. Biol. Chem.* **271:** 11047–11050.

187. **Evans, E., J. G. Moggs, J. R. Hwang, J. M. Egly, and R. D. Wood.** 1997. Mechanism of open complex and dual incision formation by human nucleotide excision repair factors. *EMBO J.* **16:** 6559–73.

188. **Evans, E., J. Fellows, A. Coffer, and R. D. Wood.** 1997. Open complex formation around a lesion during nucleotide excision repair provides a structure for cleavage by human XPG protein. *EMBO J.* **16:** 625–638.

189. **Aboussekhra, A., M. Biggerstaff, M. K. Shivji, J. A. Vilpo, V. Moncollin, V. N.**

Podust, M. Protic, U. Hubscher, J. M. Egly, and R. D. Wood. 1995. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell* **80:** 859–68.

190. **Kazantsev, A., D. Mu, A. F. Nichols, X. Zhao, S. Linn, and A. Sancar.** 1996. Functional complementation of xeroderma pigmentosum complementation group E by replication protein A in an in vitro system. *Proc. Natl. Acad. Sci. USA* **93:** 5014–8.

191. **Rapic Otrin, V., I. Kuraoka, T. Nardo, M. McLenigan, A. P. Eker, M. Stefanini, A. S. Levine, and R. D. Wood.** 1998. Relationship of the xeroderma pigmentosum group E DNA repair defect to the chromatin and DNA binding proteins UV-DDB and replication protein A. *Mol. Cell. Biol.* **18:** 3182–90.

192. **Vrieling, H., A. A. van Zeeland, and L. H. Mullenders.** 1998. Transcription coupled repair and its impact on mutagenesis. *Mutat. Res.* **400:** 135–42.

193. **Teng, Y., M. Longhese, G. McDonough, and R. Waters.** 1998. Mutants with changes in different domains of yeast replication protein A exhibit differences in repairing the control region, the transcribed strand and the non-transcribed strand of the Saccharomyces cerevisiae MFA2 gene. *J. Mol. Biol.* **280:** 355–63.

194. **DeMott, M. S., S. Zigman, and R. A. Bambara.** 1998. Replication protein A stimulates long patch DNA base excision repair. *J. Biol. Chem.* **273:** 27492–8.

195. **Umezu, K., N. Sugawara, C. Chen, J. E. Haber, and R. D. Kolodner.** 1998. Genetic analysis of yeast RPA1 reveals its multiple functions in DNA metabolism. *Genetics* **148:** 989–1005.

196. **Petrini, J. H., D. A. Bressan, and M. S. Yao.** 1997. The RAD52 epistasis group in mammalian double strand break repair. *Semin. Immunol.* **9:** 181–8.

197. **Bianco, P. R., R. B. Tracy, and S. C. Kowalczykowski.** 1998. DNA strand exchange proteins: a biochemical and physical comparison. *Front. Biosci.* **3:** D570–603.

198. **Baumann, P. and S. C. West.** 1998. Role of the human RAD51 protein in homologous recombination and double- stranded-break repair. *Trends Biochem. Sci.* **23:** 247–51.

199. **Shinohara, A., H. Ogawa, and T. Ogawa.** 1992. Rad51 protein involved in repair and recombination in S. cerevisiae is a RecA-like protein. *Cell* **69:** 457–70.

200. **Sung, P.** 1994. Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. *Science* **265:** 1241–3.

201. **Sung, P. and D. L. Robberson.** 1995. DNA strand exchange mediated by a RAD51–ssDNA nucleoprotein filament with polarity opposite to that of RecA. *Cell* **82:** 453–61.

202. **Mortensen, U. H., C. Bendixen, I. Sunjevaric, and R. Rothstein.** 1996. DNA strand annealing is promoted by the yeast Rad52 protein. *Proc. Natl. Acad. Sci. USA* **93:** 10729–34.

203. **Milne, G. T. and D. T. Weaver.** 1993. Dominant negative alleles of RAD52 reveal a DNA repair/recombination complex including Rad51 and Rad52. *Genes Dev.* **7:** 1755–65.

204. **Hays, S. L., A. A. Firmenich, and P. Berg.** 1995. Complex formation in yeast double-strand break repair: participation of Rad51, Rad52, Rad55, and Rad57 proteins. *Proc. Natl. Acad. Sci. USA* **92:** 6925–9.

205. **Shen, Z., K. G. Cloud, D. J. Chen, and M. S. Park.** 1996. Specific interactions between the human RAD51 and RAD52 proteins. *J. Biol. Chem.* **271:** 148–52.

206. **Park, M. S., D. L. Ludwig, E. Stigger, and S.-H. Lee.** 1996. Physical interaction

between human RAD52 and RPA is required for homologous recombination in mammalian cells. *J. Biol. Chem.* **271:** 18996–9000.

207. **Hays, S. L., A. A. Firmenich, P. Massey, R. Banerjee, and P. Berg.** 1998. Studies of the interaction between Rad52 protein and the yeast single-stranded DNA binding protein RPA. *Mol. Cell. Biol.* **18:** 4400–6.

208. **Baumann, P. and S. C. West.** 1997. The human Rad51 protein: polarity of strand transfer and stimulation by hRP-A. *EMBO J.* **16:** 5198–206.

209. **Golub, E. I., R. C. Gupta, T. Haaf, M. S. Wold, and C. M. Radding.** 1998. Interaction of human rad51 recombination protein with single-stranded DNA binding protein, RPA. *Nucl. Acids Res* **26:** 5388–93.

210. **Sung, P.** 1997. Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. *J. Biol. Chem.* **272:** 28194–7.

211. **Shinohara, A. and T. Ogawa.** 1998. Stimulation by Rad52 of yeast Rad51–mediated recombination. *Nature* **391:** 404–7.

212. **New, J. H., T. Sugiyama, E. Zaitseva, and S. C. Kowalczykowski.** 1998. Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. *Nature* **391:** 407–10.

213. **White, C. I. and J. E. Haber.** 1990. Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae. EMBO J.* **9:** 663–73.

214. **Gasior, S. L., A. K. Wong, Y. Kora, A. Shinohara, and D. K. Bishop.** 1998. Rad52 associates with RPA and functions with rad55 and rad57 to assemble meiotic recombination complexes. *Genes Dev.* **12:** 2208–21.

215. **Sugawara, N. and J. E. Haber.** 1992. Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. *Mol. Cell. Biol.* **12:** 563–75.

216. **Prives, C.** 1998. Signaling to p53: breaking the MDM2–p53 circuit. *Cell* **95:** 5–8.

217. **Kühn, C., F. Müller, C. Melle, H.-P. Nasheuer, F. Janus, W. Deppert, and F. Grosse.** 1999. Surface plasmon resonance measurements reveal stable complex formation between p53 and DNA polymerase α. *Oncogene* **18:** 769–74.

218. **Leiter, L. M., J. Chen, T. Marathe, M. Tanaka, and A. Dutta.** 1996. Loss of transactivation and transrepression function, and not RPA binding, alters growth suppression by p53. *Oncogene* **12:** 2661–8.

219. **Miller, S. D., K. Moses, L. Jayaraman, and C. Prives.** 1997. Complex formation between p53 and replication protein A inhibits the sequence-specific DNA binding of p53 and is regulated by single-stranded DNA. *Mol. Cell. Biol.* **17:** 2194–2201.

220. **Fortunato, E. A. and D. H. Spector.** 1998. p53 and RPA are sequestered in viral replication centers in the nuclei of cells infected with human cytomegalovirus. *J. Virol.* **72:** 2033–9.

221. **Amariglio, F., F. Tchang, M.-N. Prioleau, T. Soussi, C. Cibert, and M. Méchali.** 1997. A functional analysis of p53 during early development of *xenopus laevis*. *Oncogene* **15:** 2191–9.

222. **Longhese, M. P., H. Neecke, V. Paciotti, G. Lucchini, and P. Plevani.** 1996. The 70 kDa subunit of replication protein A is required for the G1/S and intra-S DNA damage checkpoints in budding yeast. *Nucl. Acids Res* **24:** 3533–7.

223. **Sandell, L. L. and V. A. Zakian.** 1993. Loss of a yeast telomere: arrest, recovery, and chromosome loss. *Cell* **75:** 729–39.

224. **Lee, S. E., J. K. Moore, A. Holmes, K. Umezu, R. D. Kolodner, and J. E. Haber.**

1998. Saccharomyces Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* **94:** 399–409.

225. **O'Neill, E. A., C. Fletcher, C. R. Burrow, N. Heintz, R. G. Roeder, and T. J. Kelly.** 1988. Transcription factor OTF-1 is functionally identical to the DNA replication factor NF-III. *Science* **241:** 1210–1213.

226. **Cheng, L. and T. J. Kelly.** 1989. Transcriptional activator nuclear factor I stimulates the replication of SV40 mini-chromosomes in vivo and in vitro. *Cell* **59:** 541–551.

227. **Murakami, Y., M. Satake, Y. Yamaguchi-Iwai, M. Sakai, M. Muramatsu, and Y. Ito.** 1991. The nuclear protooncogenes c-jun and c-fos as regulators of DNA replication. *Proc. Natl. Acad. Sci. USA* **88:** 3947–51.

228. **Luche, R. M., W. C. Smart, and T. G. Cooper.** 1992. Purification of the heteromeric protein binding to the URS1 transcriptional repression site in Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. USA* **89:** 7412–6.

229. **Gailus-Durner, V., C. Chintamaneni, R. Wilson, S. J. Brill, and A. K. Vershon.** 1997. Analysis of a meiosis-specific URS1 site: sequence requirements and involvement of replication protein A. *Mol. Cell. Biol.* **17:** 3536–46.

230. **Borowiec, J. A.** 1996. DNA helicases, p. 545–574. In: DePamphilis, M. L., Ed. *DNA Replication in Eukaryotic Cells*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

231. **Hughes, P. and G. Baldacci.** 1997. A DNA helicase purified by replication protein A (RPA) affinity chromatography from mouse FM3A cells. *Nucl. Acids Res* **25:** 3881–8.

232. **Johnson, F. B., D. A. Sinclair, and L. Guarente.** 1999. Molecular biology of aging. *Cell* **96:** 291–302.

233. **Sinclair, D. A. and L. Guarente.** 1997. Extrachromosomal rDNA circles—a cause of aging in yeast. *Cell* **91:** 1033–42.

234. **Sinclair, D. A., K. Mills, and L. Guarente.** 1997. Accelerated aging and nucleolar fragmentation in yeast *sgs1* mutants. *Science* **277:** 1313–6.