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DNA Replication Accessory Proteins

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INTRODUCTION

DNA replication requires the concerted action of many enzymes, as well as other protein and non-protein cofactors. The DNA, in preparation for DNA synthesis, has to become single-strand to serve as a template for the replicative DNA polymerases (pols). It is this form of the DNA that is especially prone to damage of any kind. Nature has provided a set of proteins that support the replicative pols in performing processive, accurate, and rapid DNA synthesis. Furthermore, such proteins also prevent damage to the transient single-strand (ss) DNA. These proteins are called DNA replication accessory proteins. The three best known are the proliferating cell nuclear antigen (PCNA), replication factor C (RF-C), and replication protein A (RP-A). In this chapter, we focus on these three protein classes and compare them to their selected counterparts in eukaryotic viruses. Additional replication proteins that also assist the proper function of pols, such as the 3′→5′ exonuclease, DNA primase, RNase H, 5′→3′ exonuclease, DNA helicases, DNA ligases, and DNA topoisomerases, are covered in various other chapters.

Early Discovery of Replication Accessory Proteins in Prokaryotes by Genetics and Defined In Vitro Replication Systems

Fifteen years ago it was realized that bacteriophages of *Escherichia coli* provide a window to understand the cellular events of DNA replication (Kornberg and Baker 1992). By using ssDNA from ϕX174, G4, and M13 as model replicons, the requirements for a ssDNA-binding protein (SSB) and a DNA synthesis complex were identified. The latter includes the pol III holoenzyme, which consists of many polypeptides besides the pol III core. For example, it includes the homodimeric β subunit and the heteropentameric γ complex (containing the γ, δ, δ′, χ, and ψ subunits) (for review, see Kuriyan and O'Donnell 1993). Well-established genetics

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facilitated the cloning of all genes and expression of the polypeptides in bacteria. This allowed the study of the prokaryotic replication machinery in detail (see, e.g., Stukenberg et al. 1994). Similarly in bacteriophage T4, the gp45 and gp44/62 complex share analogous functions to the β subunit and γ complex, respectively (see, e.g., Young et al. 1992; Kuriyan and O'Donnell 1993).

Discovery of Replication Accessory Proteins in Eukaryotes Thanks to Defined In Vitro Replication Systems

In eukaryotes, a protein was identified that increased the processivity of pol-δ (Tan et al. 1986). It was later identified in the SV40 in vitro replication system as PCNA (Prelich et al. 1987). The same in vitro replication system brought insight into the requirement of an SSB called HSSB (Wobbe et al. 1987), replication factor A (Fairman and Stillman 1988) or RP-A (Wold and Kelly 1988), the latter name now adopted for this protein. Soon thereafter, a factor that could coordinate the synthesis of leading and lagging strands during SV40 DNA replication was identified and called RF-C (Tsurimoto and Stillman 1989). All three proteins have been found to be functional analogs of prokaryotic accessory proteins (see Table 1).

Why Are Accessory Proteins Required in DNA Replication?

DNA replication accessory proteins provide particular functions that are mandatory for replicative pols. Such functions include the recruitment of particular pols when needed, the facilitation of pol binding to the primer terminus, the increase in pol processivity, the prevention of non-productive binding of the pol to ssDNA, the release of the pol after DNA synthesis, and the bridging of pol interactions to other replication proteins. Thus, it is not surprising that these proteins are universally found in nature (Table 1).

REPLICATION ACCESSORY PROTEINS

PCNA

PCNA is the most extensively studied cellular DNA replication accessory protein (Table 2). It was originally discovered as a cell-cycle-regulated nuclear protein whose rate of synthesis correlates with the proliferative state of normal cells and tissues, and later as a processivity

Table 1 Eukaryotic and prokaryotic DNA replication accessory proteins

	Organism				
Functional component	*E. coli*	bacteriophage T4	eukaryote (yeast to man)	HSV	adenovirus
Processivity factor or sliding clamp	β subunit	gp45	PCNA	UL 42	AdDBP[a]
Clamp loader, brace protein, or matchmaker	γ complex	gp44–gp62 complex	RF-C	?	?
SSB	SSB	SSB (*E. coli*)	RP-A	ICP8[b]	AdDBP

Other DNA replication accessory proteins include 3′→5′ exonuclease, DNA primase, RNase H, 5′→3′ exonuclease, DNA helicases, and DNA ligases. They are reviewed in other chapters in this book.

[a]The AdDBP increases the strand-displacement activity of the adenovirus-encoded DNA polymerase.

[b]ICP8 can also enhance processivity.

Table 2 Proliferating cell nuclear antigen

Number of amino acids	260,[a–i] 365,[i] 275[k]
Molecular size (kD)	29,[a–i] 40,[i] 31[k]
Stoke's radius (A)	36.5,[d] 40[e]
pI	4.8[d]
Sedimentation coefficient (S)	5[d,e]
Structure in solution	ring-shaped homotrimer
Interaction with	pol-δ and pol-ε RF-C cyclins A, B, D; CDC2, CDKs 2, 4, 5; p21 CDK-inhibitor; Gadd45
Functions	sliding clamp, increases primer binding and processivity of pol-δ and pol-ε stimulates ATPase activity of RF-C, and forms together with RF-C and ATP the primer recognition complex regulation of DNA replication (?)

[a]*D. melanogaster* (Yamaguchi et al. 1990); [b]rat (Matsumoto et al. 1987); [c]calf (Tan et al. 1986); [d]human (Almendral et al. 1987); [e]*S. cerevisiae* (Bauer and Burgers 1990); [f]*S. pombe* (Waseem et al. 1992); [g]baculovirus (O'Reilly et al. 1989); [h]*Oxytricha sativa* (Suzuka et al. 1989, 1991); [i]*D. carota* (Hata et al. 1992); [k]*Plasmodium falciparum* (Kilbey et al. 1993).

factor for pol-δ (for review, see Hübscher and Spadari 1994). The gene encoding this protein has been cloned from a variety of sources, including yeast, human, and plants, and shows a highly conserved structure (Table 2). Compared to human PCNA, the amino acid sequence similarity is 32% for lower eukaryotes (yeast and protozoa), 64% for plant, and 70% for *Drosophila melanogaster*. PCNA is a highly acidic protein encoded by a single gene in all the organisms tested, with the one exception of *Dracus carota*, in which two distinct genes encoding two different forms of PCNA were isolated. The human gene has been mapped to chromosome 20, and two pseudogenes have been localized on chromosomes X and 6. The structure of the genomic clone of human PCNA is known: It comprises six exons, five introns, and a short 3′-untranslated region. Introns 1 and 4 have been implicated in the negative regulation of transcription of the PCNA gene (Chang et al. 1990; Alder et al. 1992). The promoter sequence contains binding sites for several transcription factors such as Sp1 and E2F, suggesting that the expression of the PCNA gene may be tightly regulated (Jones et al. 1988; Mudryj et al. 1990).

The extreme structural conservation of PCNA is also seen at a functional level. With synthetic oligonucleotides or poly(dA)/oligo(dT) as

DNA templates, PCNA increases the productive binding to a primer and the processivity of pol-δ. Yeast PCNA interacts with mammalian pol-δ, and mammalian PCNA interacts with yeast pol-δ (Bauer and Burgers 1988). PCNA was also shown to stimulate the primer binding and primer elongation reactions catalyzed by pol-ε on similar templates (Maga and Hübscher 1995). If, however, a singly primed M13 DNA was used as a model for a natural template, at least two other auxiliary proteins, RF-C and RP-A, are required. It has been shown that PCNA forms a complex together with RF-C at the primer in an ATP-dependent manner (Lee and Hurwitz 1990; Burgers 1991; Tsurimoto and Stillman 1991; Podust et al. 1992). This primer recognition complex can efficiently recruit pol-δ or pol-ε (but not pol-α) to the primer terminus. These functional experiments, together with the observation that PCNA is essential for coordinated leading- and lagging-strand synthesis during in vitro SV40 DNA replication (Prelich and Stillman 1988), clearly indicate a role for PCNA in DNA replication. PCNA has also been shown to participate in DNA excision repair and to be required for the gap-filling step (Shivji et al. 1992). Recent results showed that PCNA physically interacts with Gadd45, a protein that stimulates DNA excision repair and inhibits entry into S phase in mammalian cells, thus providing a new link between PCNA and the DNA repair pathway (Smith et al. 1994).

PCNA itself does not bind to DNA. It has been shown that PCNA can be loaded onto DNA in two ways: (1) enzymatically, by RF-C and ATP, and (2) topologically, in an ATP-independent manner (Burgers and Yoder 1993). Despite less than 5% of amino-acid-sequence similarity, PCNA is functionally equivalent to two prokaryotic proteins known to function as auxiliary factors in DNA replication: the β subunit of pol III holoenzyme of *E. coli* and the product of the gene 45 of the bacteriophage T4 (Kuriyan and O'Donnell 1993). Yeast PCNA has recently been crystallized, and its structure has been determined (Krishna et al. 1994). PCNA has been shown to be a homotrimer with a closed circular structure that can encircle duplex DNA with a minimum of specific interactions, similar to the dimeric β subunit of *E. coli* pol III. The current model for the function of PCNA suggests that this protein acts as a sliding clamp that allows pols to move rapidly along the DNA while remaining topologically bound to it.

Recent results have indicated that PCNA can interact with the cell-cycle-regulatory machinery. Isolation of kinase complexes containing cyclins A, B, and D, associated with the cyclin-dependent kinases CDC2, CDK2, CDK4, or CDK5, showed that a complex of PCNA and the cyclin-dependent kinase inhibitor p21 associates with each cyclin/CDK

dimer to form quaternary complexes (Xiong et al. 1992; Zhang et al. 1993). Furthermore, p21 has been shown to inhibit PCNA-dependent DNA replication in the absence of cyclins/CDKs, through a direct protein-protein interaction with PCNA (Flores-Rozas et al. 1994; Li et al. 1994; Waga et al. 1994). It is known that the major tumor suppressor protein p53 regulates the expression of p21. In addition, it has recently been shown that p53 is also implicated in the regulation of the expression of the PCNA gene (Jackson et al. 1994; Yamaguchi et al. 1994), suggesting that, during p53-mediated suppression of cell proliferation, p21 and PCNA may be involved in coordination of the cell-cycle progression, DNA replication, and DNA repair.

RF-C

RF-C as an essential replication factor had first been isolated from human 293 cells in the in vitro SV40 DNA replication system (Tsurimoto and Stillman 1989). This protein was later purified from HeLa cells (Lee et al. 1991a), yeast (Yoder and Burgers 1991; Fien and Stillman 1992), and calf thymus (Podust et al. 1992) by using a complementation assay that enabled pol-δ to replicate a primed ssDNA in the presence of PCNA and RP-A. RF-C binds preferentially to template-primer junctions on the DNA with ATP or ATPγS stimulating binding of RF-C to DNA (Lee et al. 1991a; Tsurimoto and Stillman 1991). Footprinting experiments revealed that RF-C bound at a template-primer junction covers 12 bases of ssDNA, whereas on the double-strand (ds) DNA it binds 8 bases of the template strand and 15 bases of the primer strand (Tsurimoto and Stillman 1991). RF-C possesses a DNA-stimulated ATPase activity (Tsurimoto and Stillman 1990; Yoder and Burgers 1991; Lee et al. 1991a; Podust et al. 1992). In the absence of PCNA, ssDNA stimulates the ATPase, whereas primer-template junctions have little or no effect on the activity. PCNA increases ATP hydrolysis by RF-C on multiple primed ssDNA, whereas on unprimed ssDNA, only a marginal effect was detected. These data suggested that PCNA causes the RF-C ATPase activity to become dependent on a primer terminus. All eight nucleoside triphosphates could be hydrolyzed by RF-C with preference for dATP and ATP. K_m values for ATP in the ATPase assay were 42 μM for human and 15 μM for yeast RF-C, respectively. ATPγS and AMP-PNP are competitive inhibitors of the ATPase of yeast RF-C with K_i values of 1.8 μM and 130 μM, respectively.

RF-C itself had little or no effect on DNA synthesis by pols α, δ, and ε. In contrast, the conjunction of RF-C with PCNA, RP-A, and ATP

strongly inhibited pol-α and stimulated pols δ and ε. Isolation of stable pol δ and ε complexes, formed with DNA in the presence of ATP, RF-C, and PCNA (called holoenzymes), indicated that pols δ and ε directly interact with these auxiliary proteins (Lee and Hurwitz 1990; Lee et al. 1991b; Burgers 1991; Podust et al. 1992).

RF-C is a multiprotein complex composed of five subunits, including one large subunit and four small subunits (Table 3). All subunits are encoded by different genes, and all show an extensive amino-acid-sequence homology in the middle part of the protein (O'Donnell et al. 1993; Bunz et al. 1993; Li and Burgers 1994b). All the genes encoding the subunits of yeast RF-C have been shown to be essential (Howell et al. 1994; Li and Burgers 1994a,b; Noskov et al. 1994; Cullmann et al. 1995). The large subunit of human RF-C could be cross-linked to DNA (Tsurimoto and Stillman 1991), and the DNA-binding properties of this subunit might also be expected from primary sequence data (Bunz et al. 1993; Howell et al. 1994). Interestingly, this subunit was able to be cloned from human and murine cDNA libraries by virtue of its dsDNA-binding properties (Burbelo et al. 1993; Lu and Riegel 1994; Luckow et al. 1994). In yeast, the interaction with PCNA was attributed to the large RF-C subunit (McAlear et al. 1994) and in human, to one of the small subunits, hRFC40 (Chen et al. 1992b; Pan et al. 1993). hRFC40 and hRFC37 interacted directly with pol-δ and pol-ε, respectively, as well as with each other (Pan et al. 1993). Yeast Rfc3p and Rfc4p yielded a stable complex upon coexpression in *E. coli* (Li and Burgers 1994b). Finally, hRFC37 and its yeast homolog Rfc2p showed specific primer end binding (Pan et al. 1993; Noskov et al. 1994), whereas hRFC36 and Rfc3p displayed a DNA-dependent ATPase activity (Li and Burgers 1994a; H. Flores-Rozas et al., pers. comm.).

RF-C is a protein that enzymatically loads PCNA onto DNA. Biochemical studies of this reaction might be generalized by the following model (Fig. 1) (Lee and Hurwitz 1990; Burgers 1991; Stillman 1994; Podust et al. 1995): RF-C first binds to DNA in the presence of ATP. PCNA then binds to the RF-C/DNA complex, forming an unstable intermediate complex. Upon hydrolysis of ATP by RF-C, the protein/DNA intermediate undergoes a conformational change resulting in a stable complex (the PCNA clamp). Current data suggest that RF-C remains as a part of the complex after PCNA loading (Podust et al. 1995). The clamp can interact with pol-δ and pol-ε, resulting in the formation of the corresponding holoenzymes that can efficiently replicate single-strand DNA. Preferential binding of RF-C to the template primer, together with the footprinting data, suggested that assembly of the RF-C/PCNA com-

Table 3 Replication factor C

Subunit[a]	Human sequence	Size (kD)[b]	Proposed function for human subunits	Yeast subunits[c]	Yeast sequence	Size (kD)[b]	Proposed function for yeast subunits	Identity (similarity) between human and yeast
hRFC140	Bunz et al. (1993)	128.3	Nucleotide binding, DNA binding	*RFC1* (Rfc1p)	Howell et al. (1994)[d]	94.9	Nucleotide binding, DNA binding, PCNA binding	35.8% (55.3%)
hRFC40	Chen et al. (1992b)	39	ATP binding, interaction with PCNA, RFC37, and pol-δ	*RFC4* (Rfc4p)	Li and Burgers (1994b)	36.2	Interaction with Rfc3p	60.1% (77.6%)
hRFC38	Cullmann et al. (1995)	40.5	unknown	*RFC5* (Rfc5p)	Cullmann et al. (1995)	39.9	unknown	44.6% (63.4%)
hRFC37	Chen et al. (1992a)	39.6	binds to primer ends and ssDNA, interacts with pol-ε	*RFC2* (Rfc2p)	Noskov et al. (1994)	39.7	binds to primer ends	50.6% (66.6%)
hRFC36	Cullmann et al. (1995)	38.5	ATPase	*RFC3* (Rfc3p)	Li and Burgers (1994a)	38.1	DNA-dependent ATPase	50.5% (72.1%)

For further references, see text.
[a]The subunits of RF-C were ordered and named according to the apparent sizes in SDS-PAGE for the purified human RF-C complex.
[b]Calculated from the sequence.
[c]Gene: italic; protein: in parentheses.
[d]*CDC44* (Howell et al. 1994; McAlear et al. 1994) is identical to *RFC1* (Cullmann et al. 1995).

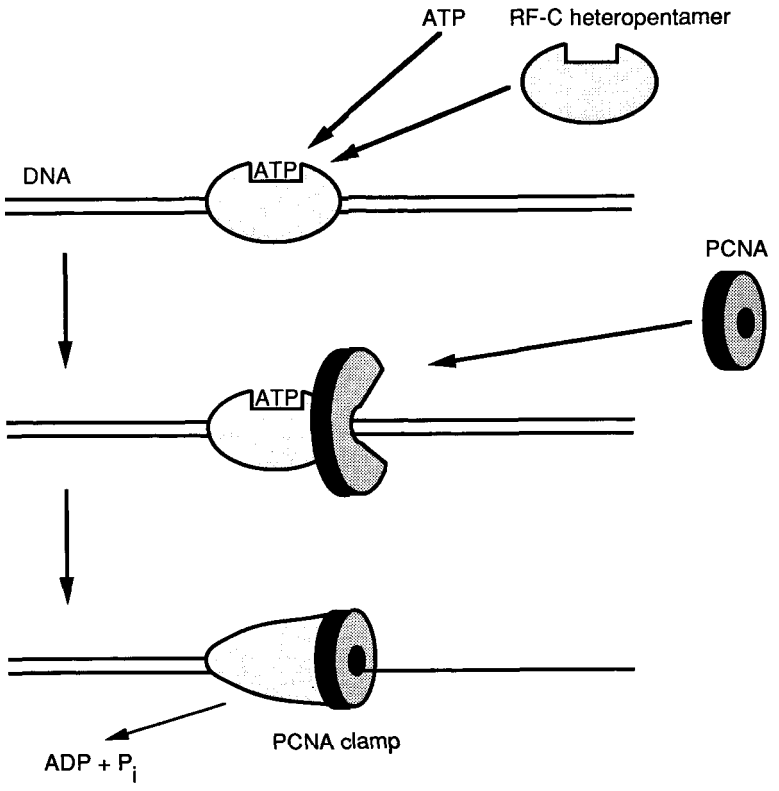

Figure 1 Model for the assembly of the RF-C/PCNA clamp. The heteropentameric RF-C first loads to dsDNA in the presence of ATP. PCNA then binds to the RF-C/DNA complex forming an unstable intermediate. This complex can slide along dsDNA until it encounters a template-primer junction. A 3′-OH group appears to be required for hydrolysis of ATP by the RF-C ATPase activity and for the conversion of the RF-C/PCNA intermediate into a catalytically competent PCNA clamp. The so-formed clamp is able to track along dsDNA. For details see text and citations therein.

plex occurred at the 3′-OH end of the primer. Recent studies showed that the assembly of the primary RF-C/PCNA complex does not require 3′-OH ends and occurs directly on dsDNA, whose topological structure and sequence do not give restriction for loading. The primary salt-sensitive RF-C/PCNA complex was suggested to slide along DNA to the 3′-OH end of a primer, where the final conversion of the intermediate complex to the catalytically competent PCNA clamp takes place (Podust et al. 1995). Using different approaches, it has been shown that the RF-

C/PCNA complex is able to track along the dsDNA (Podust et al. 1994, 1995; Tinker et al. 1994).

RP-A

RP-A is an essential protein that participates in DNA replication, DNA repair, and homologous DNA recombination (Table 4) (for review, see Hübscher and Spadari 1994). Various functional aspects of this protein are also covered in other chapters (see, e.g., Weisshart and Fanning; Brush and Kelly; Friedberg and Wood; Stillman; Borowiec; Newlon; Blow and Chong; all this volume). The protein was first discovered in human cells in the in vitro SV40 replication system (Wobbe et al. 1987) and has a heterotrimeric structure with polypeptides of molecular weights of 70K (called RP-A1), 32–34K (called RP-A2), and 11–14K (called RP-A3). All three subunits of RP-A are essential for viability of the cell (Brill and Stillman 1991) and have functions in DNA replication (Wobbe et al. 1987; Fairman and Stillman 1988; Wold and Kelly 1988), DNA repair (nucleotide excision repair) (Coverley et al. 1991), and homologous DNA recombination (Longhese et al. 1994).

The trimeric RP-A protein has many functional tasks. It is an SSB, partially sensitive to the base content with a preference for thymine-rich stretches in the SV40 origin of replication and has a low affinity to dsDNA (Kim et al. 1992). The binding to ssDNA appears to occur in two modes, since complexes covering 8–10 nucleotides and 30 nucleotides were identified (Blackwell and Borowiec 1994). RP-A assists the SV40 large T antigen in unwinding of the viral origin of replication (Wold and Kelly 1988; Kenny et al. 1989), binds to pol-α:primase (Dornreiter et al. 1992), and appears to suppress nonspecific priming events by the DNA primase activity of pol-α:primase (Collins and Kelly 1991). Furthermore, an important role was postulated in primosome assembly (Melendy and Stillman 1993). RP-A directly interacts with the DNA-binding protein XPA, which is known to be involved in the damage-recognition step of the nucleotide excision repair pathway (Matsuda et al. 1995). Binding to the tumor suppressor protein p53 and to the transcriptional activators VP16 and GAL4 suggested a role in transcription as well (He et al. 1993; Li and Botchan 1993). Finally, RP-A stimulated the four different DNA helicases A, B, C, and D from calf thymus in a species-specific way (Thömmes et al. 1992) and copurified with DNA helicase F (Georgaki et al. 1994).

What is known about the individual subunits? Human RP-A1 is located on chromosome 17q13.3 (Umbricht et al. 1994), is responsible for

Table 4 Replication protein A

Other names	replication factor A (RF-A)
	human ssDNA-binding protein (HSSB)
Structure	heterotrimer of 70 kD (RP-A1), 32–34 kD (RP-A2), and 11–14 kD (RP-A3)
Involvement in DNA transactions	DNA replication
	homologous DNA recombination
	nucleotide excision repair
	transcription?
Properties of the heterotrimeric RP-A	partially sequence-dependent SSB, binds in two different modes
	low affinity to dsDNA
	assists SV40 T antigen in unwinding of the SV40 origin of replication
	interacts with pol-α:primase
	suppresses nonspecific priming events
	functions in primosome assembly
	participates in DNA elongation
	binds to p53, V16, and GAL4
Properties of RP-A1	essential gene
	located on human chromosome 17q13.3
	responsible for binding to ssDNA
	unwinding of DNA at the origin of SV40 replication
Properties of RP-A2	essential gene
	located on human chromosome 1p35
	phosphorylated by a DNA-activated protein kinase
	hyperphosphorylated after treatment with ionizing radiation and UV light
	phosphorylation stimulates nonspecific DNA unwinding
	phosphorylation not required for in vitro replication
	binds to XP-A protein
Properties of RP-A3	essential gene
	located on chromosome 7p22
	possible role in assembly of RP-A

For references, see text.

binding to ssDNA, and assists unwinding of the origin of SV40 replication by viral T antigen (Kenny et al. 1990). Furthermore, RP-A1 unwinds dsDNA nonspecifically (Georgaki and Hübscher 1993). Human RP-A2 is located on chromosome 1p35 (Umbricht et al. 1994). This subunit is phosphorylated in a cell-cycle-dependent manner on Ser-23 and Ser-29

(Din et al. 1990). Hyperphosphorylation of this subunit occurs after treatment of the cell with UV light (Carty et al. 1994) and ionizing radiation (Liu and Weaver 1993). Phosphorylation in the two serines mentioned is not required for in vitro replication (Henricksen and Wold 1994). Phosphorylation of RP-A2 is catalyzed in cyclin-A-activated extracts by a cdk/cyclin A complex and by a DNA-dependent protein kinase (Brush et al. 1994; Pan et al. 1994). Finally, the sequence-independent unwinding of DNA by RP-A1 is stimulated after phosphorylation of RP-A2 in vitro (Georgaki and Hübscher 1993). It is not clear at which step of the heterotrimer assembly the phosphorylation of RP-A2 occurs in vivo (see also Fig. 2). Human RP-A3 is located on chromosome 7p22 (Umbricht et al. 1994). Little is known about its function. A role in the assembly of the heterotrimeric RP-A has been suggested (Fig. 2) (Henricksen et al. 1994; Stigger et al. 1994).

Viral Counterparts of Eukaryotic Accessory Proteins

The two model systems of adenovirus and herpes simplex virus type 1 (HSV-1) rely on their own SSBs for their replication. The adenovirus DNA-binding protein, Ad-DBP, is described in more detail by Hay (this volume). It is a 59-kD zinc metalloprotein and has a triple function in DNA replication (Table 1): (1) binding to ssDNA, (2) helping the viral pol to perform strand-displacement DNA synthesis, and (3) helping in the initiation of DNA replication of the adenovirus. The recently reported crystal structure of the Ad-DBP nucleic-acid-binding domain allows insight into functional details of this protein (Tucker et al. 1994).

ICP8 from HSV-1 is the SSB and is a zinc metalloprotein with a molecular mass of 128 kD (for more details, see Challberg, this volume). The protein is essential for DNA replication, probably by acting as a typical SSB, and may play a key role in initiation of DNA replication by virtue of its helix-destabilizing properties and by its interaction with the initiating DNA helicase UL9 (Boehmer et al. 1994). Finally, ICP8 stimulates the cognate pol, the UL30 gene product (Ruyechan and Weir 1984).

The UL42 protein from HSV-1 is a processivity enhancing factor for the herpetic pol (UL30) and forms a complex with it (Hernandez and Lehman 1990; for more details, see Challberg, this volume). The complex of the pol, the UL42 processivity factor, the ICP8 protein, and the herpes simplex-encoded helicase:primase could be identified. It is shown to replicate dsDNA by a rolling-circle mechanism (Skaliter and Lehman 1994).

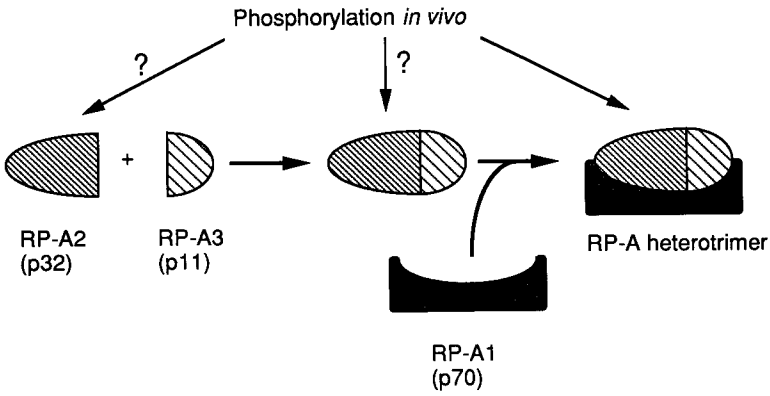

Figure 2 Model for the assembly of the RP-A trimer. RP-A2 and RP-A3 first form a complex, which provides the proper conformation for binding of the large RP-A1 subunit, resulting in a functionally active heterotrimeric RP-A form. (Adapted from Henricksen et al. 1994.)

CONCLUSIONS

The three DNA replication accessory proteins, PCNA, RF-C, and RP-A, possess a variety of essential functions in DNA replication. Their roles provide the replication machinery with physiological properties that allow the achievement of a coordinated action and of an accurate and fast polymerization mode. The functional conservation from bacteria to man is not surprising, due to their universal tasks in DNA replication. Since all subunits of PCNA, RF-C, and RP-A have been cloned and overexpressed, more insights into their biological functions in the DNA replication process will emerge soon.

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