INT. J. RADIAT. BIOL 2001, VOL. 77, NO. 10, 1007–1021

Proliferating cell nuclear antigen (PCNA): ringmaster of the genome

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(Received 10 November 2000; accepted 1 June 2001)

Abstract.
Proliferating cell nuclear antigen (PCNA) protein is one of the central molecules responsible for decisions of life and death of the cell. The *PCNA* gene is induced by p53, while PCNA protein interacts with p53-controlled proteins Gadd45, MyD118, CR6 and, most importantly, p21, in the process of deciding cell fate. If PCNA protein is present in abundance in the cell in the absence of p53, DNA replication occurs. On the other hand, if PCNA protein levels are high in the cell in the presence of p53, DNA repair takes place. If PCNA is rendered non-functional or is absent or present in low quantities in the cell, apoptosis occurs. The evolution from prokaryotes to eukaryotes involved a change of function of PCNA from a 'simple' sliding clamp protein of the DNA polymerase complex to an executive molecule controlling critical cellular decision pathways. The evolution of multicellular organisms led to the development of multicellular processes such as differentiation, senescence and apoptosis. PCNA, already an essential molecule in the life of single cellular organisms, then became a protein critical for the survival of multicellular organisms.

1. Introduction

Proliferating cell nuclear antigen (PCNA) was originally defined as a cyclin as it was found to be expressed at high levels in cycling cells (Almendral *et al.* 1987). More precisely, PCNA expression was found to occur during the last 5% of G_1-phase and the first 35% of S-phase of the cell cycle (Takahashi and Caviness 1993). As the sequences of the various cyclin genes became known, it was clear that PCNA, while necessary for proliferation, is neither structurally nor evolutionarily related to the cyclin proteins, so the name inspired by its pattern of expression—proliferating cell nuclear antigen—is used. PCNA is a ring-like protein involved in the major DNA replication and repair machinery of the cell. In its role as the sliding clamp of DNA polymerases, PCNA is related to sliding clamps of both eukaryotes and prokaryotes (Krishna *et al.* 1994, Kelman and O'Donnell 1995b, Matsumiya *et al.* 2001), and it retains similar functions throughout the eukaryotic phylogenetic tree (Almendral *et al.* 1987, Kelman 1997, Shibahara and Stillman 1999). PCNA has been well studied in the literature since its discovery in 1985, but its many functions and diverse expression patterns have made it difficult to uncover each PCNA-related pathway. Now, many of these pathways are known, and it is the purpose of this review to attempt to link these pathways just as PCNA binds them in the life of the cell.

PCNA has a triple function in life and death of the cells. When not engaged in DNA replication, PCNA (most often under the control of p53) commits cells to cell cycle arrest and repair of DNA damage, or, when repair is not possible, absence or low levels of functional PCNA may drive cells into apoptosis.

2. PCNA: structural studies

Most cellular processes that include DNA synthesis depend on and include PCNA in the process. Eukaryotic PCNA is homologous to the β-subunit of DNA polymerase III in *E. coli*, and both act as sliding clamps needed for activity of DNA polymerase(s) and other enzymes from the battery used in DNA synthesis. Both the prokaryotic (β-subunit of DNA polymerase III) and eukaryotic (PCNA) DNA sliding clamp proteins are circular and ring shaped, with six domains (Krishna *et al.* 1994, Kelman and O'Donnell 1995b). The functional eukaryotic sliding clamp (PCNA) is a trimeric protein of ~780 amino acids (a.a.) total, with two domains per subunit, while the prokaryotic protein of ~730 a.a. total is a dimer with three domains per subunit. Similar sliding clamp proteins have been found in *Archaea* and one structure has recently been determined (Matsumiya *et al.* 2001). Venclovas and Thelen (2000) showed that at least four proteins in fission yeast (Rad 1, Aus 1, Rad 9, Rad 17) have a protein fold in common with PCNA and may participate in the sliding clamp structures, demonstrating the presence of multiple types of sliding clamp proteins in eukaryotes. Several human PCNA-like DNA damage sensors (yeast homologs) have been discovered recently, including

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RAD1, RAD9 and HUS1 (Wood *et al.* 2001); their functional significance for cell proliferation is unclear.

Trimeric ring configuration is necessary for PCNA protein function, for its interactions with DNA and with other proteins. A human PCNA a.a. 114-point mutation interferes with the formation of PCNA trimers, and is lethal in PCNA$^-$ yeast (Jónsson *et al.* 1995). In that system, loading of PCNA onto DNA is disabled. The trimeric conformation is critical for the interaction between PCNA and p21 (Knibiehler *et al.* 1996), and most other PCNA-interacting proteins (table 1). The interdomain connecting loop of the PCNA trimer is the primary interfacing surface for interactions of PCNA with other proteins.

3. PCNA protein–protein interactions

In the role of the sliding clamp of DNA polymerases, PCNA loads onto DNA through the action of the replication factor C (RFC) complex and provides a scaffold for consecutive attachment of various DNA nucleases (such as FEN1 or XPG), DNA polymerases (such as DNA polymerases δ and ε, and mitochondrial DNA polymerase-γ), DNA ligases (such as DNA ligase-I), and others (such as nuclear DNA helicase II, RPA, and Topo I (Loor *et al.* 1997), or hCdc18 (Saha *et al.* 1998)) (table 1 and figure 1), thus being involved in DNA replication, recombination and repair. PCNA also has a function in post-replicative DNA processing such as methylation (PCNA interacts with DNA methyltransferase) or chromosome assembly (interactions with chromatin assembly factor 1 (CAF1), and chromosome transmission fidelity protein (Ctf7p)) (table 1 and figure 1).

Many cell cycle control proteins influence cell cycle progression through their interactions with PCNA. Numerous positive regulators of the cell cycle

Table 1. PCNA protein interactions across the domain connecting loop amino acids (a.a.) 119–133.

Interacting protein	Function	Reference
p21 CDK inhibitor ($K_d = 10$ nM) contains consensus PCNA binding peptide: QXX (I/L/M)XX(F/Y)(F/Y)	cell cycle regulation	Gulbis *et al.* (1996); Chen *et al.* (1996a)
p57 (Kip2) protein from p21 family, i.e. CDK inhibitor, contains consensus PCNA binding peptide	cell cycle regulation	Watanabe *et al.* (1998)
CR6 (cytokine response gene 6)	cell cycle regulation	Azam *et al.* (2001)
GADD45	cell cycle regulation	Smith *et al.* (1994), Vairapandi *et al.* (2000)
MyD118 (GADD45 homolog)	cell cycle regulation	Vairapandi *et al.* (1996, 2000)
Ctf7p chromosome transmission fidelity; contains consensus PCNA binding peptide	chromatid cohesion (established in S-phase)	Skibbens *et al.* (1999)
CAF1 (p150 subunit) chromatin assembly factor 1; contains consensus PCNA binding peptide	chromatin assembly (maintains epigenetic inheritance)	Shibahara and Stillman (1999)
MSH2 and MLH1	MMR	Umar *et al.* (1996)
Uracil DNA glycosylase (UNG); contains consensus PCNA binding peptide	BER	Otterlei *et al.* (1999)
DNA (cytosine-5) methyltransferase, a DNA methylation protein; contains consensus PCNA binding peptide a.a. 163–174	methylation post-mismatch repair and post-replication (maintains epigenetic inheritance)	Chuang *et al.* (1997), Warbrick (1998)
RPA (70 KDa subunit) single-strand DNA binding; contains consensus PCNA binding peptide	replication, NER	Dianov *et al.* (1999), Loor *et al.* (1997)
RFC 1, RFC 2, RFC 3 contain consensus PCNA-binding peptide	replication, BER, NER	Zhang *et al.* (1999a), Mossi *et al.* (1997)
FEN1 flap 5′-3′ endonuclease ($K_d = 60$ nM); contains consensus PCNA binding peptide a.a. 328–355; particularly important are a.a. 343, 344, and 339	replication (processing Okazaki fragments), BER	Chen *et al.* (1996b), Gary *et al.* (1997)
XPG 3′-incision endonuclease; contains consensus PCNA binding peptide a.a. 981–1009; particularly important is a.a. 992	NER	Gary *et al.* (1997), Miura *et al.* (1996a)
DNA pol δ (p125 subunit); contains consensus PCNA binding peptide	replication, BER, MMR	Zhang *et al.* (1999b), Kelman *et al.* (1999)
DNA pol ε; contains consensus PCNA-binding peptide	NER, MMR	Kelman *et al.* (1999), Maga *et al.* (1999)
DNA ligase I contains consensus PCNA binding peptide	replication (processing Okazaki fragments), repair	Levin *et al.* (1997)
Werner syndrome helicase contains consensus PCNA binding peptide a.a. 168–246	repair	Lebel *et al.* (1999)

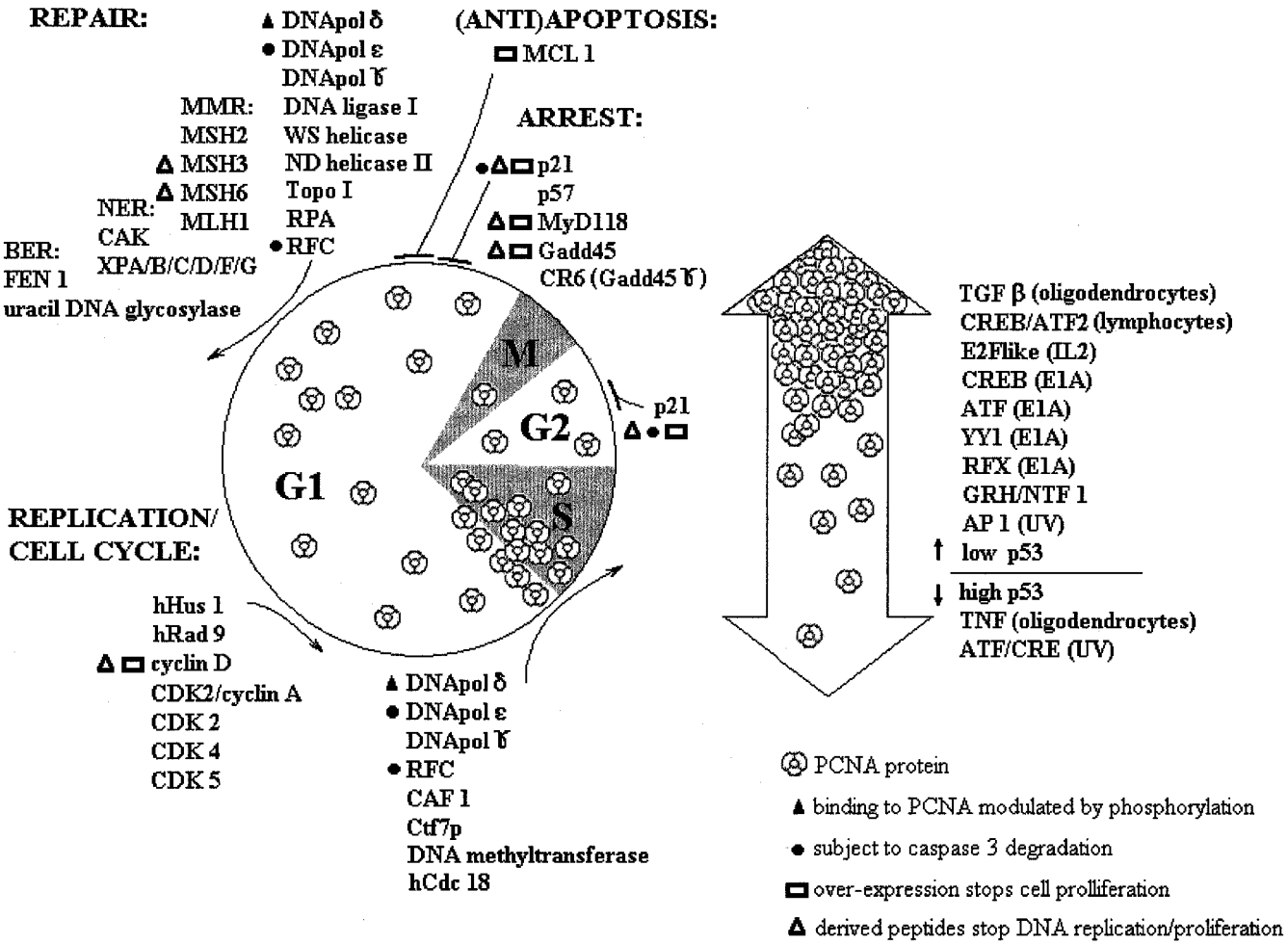

Figure 1. Representation of the numerous protein–protein interactions of PCNA and their roles in replication, repair and cell cycle regulation, accompanied with a brief list of factors controlling PCNA expression.

interact with PCNA, such as cyclin-dependent kinases, CDK2, CDK4 and CDK5 (Loor *et al.* 1997), and cyclins D and A (Fukami-Kobayashi and Mitsui 1999, Koundrioukoff *et al.* 2000). Checkpoint proteins hHus1 and hRad9 interact with PCNA (Komatsu *et al.* 2000) as does the anti-apoptotic protein myeloid cell leukaemia 1 (MCL1) (from B-cell lymphoma leukaemia 2 (Bcl2) gene family) (Fujise *et al.* 2000). Leading to cell cycle arrest, probably the best known and most studied interaction of PCNA is with p21 (Chen *et al.* 1996a, Gulbis *et al.* 1996). Binding of p21 occurs at the same location on PCNA (in the interdomain connector loop) as does the binding of most other PCNA-interacting proteins.

The PCNA interdomain connector loop (a.a. 119–133) binds the consensus PCNA-binding peptide sequence QXX(I/L/M)XX(F/Y)(F/Y) present in the majority of PCNA-interacting proteins (table 1). It must be mentioned, however, that many PCNA-interacting proteins use additional a.a. residues for interactions with PCNA (outside of the interdomain connector loop). For example, Vairapandi *et al.* (2000) showed that proteins encoded by *My118* and *Gadd45* interact by their C-terminal regions with both the N-terminal (a.a. 1–46) and interdomain connector loop (a.a. 119–133) regions of PCNA. PCNA mutation analyses determined the PCNA a.a. involved in protein–protein interactions outside of the interdomain connector loop domain (Warbrick 1998, Tsurimoto 1999). These additional interactions provide a mechanism to toggle PCNA partner proteins and allow them to replace each other, which leads to the fine regulation of cellular processes, e.g. binding of p21 to PCNA does not inhibit DNA repair in mammalian cells while it does stop DNA replication (Cox and Lane 1995, Shivji *et al.* 1998). In addition, the C-terminal domain of p21 shows modulated binding to PCNA dependent on reversible phosphorylation (Scott *et al.* 2000).

We performed BLAST analysis (http://www.

Table 2. Proteins containing consensus or near consensus PCNA binding sequence.

Protein ID	**Protein** (human, mouse, rat, fruit fly or yeast)	**Possible PCNA binding sequence**
(GenBank)	**consensus**	**QXX(I/L/M)XX(F/Y)(F/Y)****
NP_004144.1	origin recognition complex, subunit 1 (Orc1); replication control protein 1	LDLLWTHK**Q**DI**M**YNL**F**DWPT
NP_032791.1	origin recognition complex, subunit 2 (Orc2)	LMWDHAK**Q**SLYN
NP_002543.1	origin recognition complex, subunit 4 (Orc4)	DLFAHHKN**Q**TL**L**YNL**F**D
NP_001245.1	cell division cycle 18 (hCdc18)	MDQLDSKG**Q**DV**L**YTL**F**EWP
NP_013365.1	meiosis-specific protein: chromosome synapsis and chiasmata formation	KKKQ**Q**KK**L**TN**F**
CAA66705.1	RADHA protein (chromatin rearrangement)	EREYGQFR**Q**NG**M**SPR**Y**
P09884	DNA-directed DNA polymerase α	KQDNLTIDTQ**YY**LAQQ
AAA17543.1	mitochondrial DNA polymerase	ETLYNVSD**Y**PT
NP_035262.1	DNA polymerase ε	VTYNGD**FF**DWP
AAC98785.1	DNA polymerase zeta	NFADLNHSKRKL
NP_032025.1	flap structure specific endonuclease 1 (FEN1)	GST**Q**GR**L**DD**FF**KVTGSLSSA
NP_035859.1	XPG complementing protein (ERCC-5)	T**Q**LR**I**DS**FF**RL
NP_013928.1	XPA homolog (Rad14)	EEW**Q**RREEG
NP_011098.1	XPD homolog (Rad3)	**L**DEVWKHK
NP_031525.1	mouse ataxia telangiectasia homolog	TS**I**ED**FY**RSCYKIL
JC4019	mutS (*E. coli*) homolog 3 (MSH3)	**L**SR**FF**RSAGSLRSS
NP_035691.1	T:G mismatch specific thymine-DNA glycosylase	KEK**Q**EK**I**TDA**F**KVKRKV
AAC23702.1	BRCA2	**I**DS**FY**KEAEKK
A57514	RNA helicase HEL117	**Q**TA**L**TG**Y**QTKQRKLL
CAA58247.1	06-methylguanine DNA repair methyltransferase	K**Q**DT**M**YDL
NP_013691.1	uracil DNA glycosylase (UNG)	RKRK**Q**TT**I**ED**FF**GTKK
NP_014647.1	ATP dependent DNA ligase II	KKRKRVLISDSFH **Q**NRK
NP_012035.1	U5 snRNP and spliceosome component (Prp8p [with prp17 causes G2 arrest])	TEP**Q**MV**L**FNI**Y**D
CAA39830.1	MRS3 protein (suppresses the mitochondrial RNA splicing defects)	**Q**SEG**L**AA**FY**YS
CAA86151.1	meiosis-specific protein (HOP1)	LDTKWSKF**Q**DL**M**
NP_033960.1	cyclin E2	DLSWACS**Q**EVWQNM
P38936	p21; CDN1; CIP1; WAF1	RKRR**Q**TS**M**TD**FY**HSKRRLIFS
P02825	Hsp 70A major heat stock protein	**Q**SL**L**QD**FF**HGK
AAC72232.1	Bcl-x (apoptosis regulation)	GVNWGR**I**VA**FF**SFGGAL
P36776	mitochondrial ATP-dependent protease	GST**Q**GK**I**LC**FY**GPPG
AAF21125.1	E3 ubiquitin ligase	**Q**QGQVY**FY**HIP
NP_037260.1	carboxypeptidase	**L**ES**FY**ERK
NP_000284.1	phosphorylase kinase β	K**Q**DD**M**TS**FY**NTP
NP_001735.1	calcium/calmodulin-dependent protein kinase IV	GSNRDA**L**SD**FF**EVESEL
NP_033461.1	serine/threonine kinase 22A	**Q**GD**L**LE**F**IKTRGAL
I48845	lymphocyte-specific protein tyrosine kinase	**L**DD**FF**TAT
NP_002820.1	protein tyrosine phosphatase (PTP-H1)	GVD**Q**QL**L**DD**F**HRVT
NP_002622.1	phosphogluconate dehydrogenase, decarboxylating (PGD)	**L**DD**FF**K
Q08499	cAMP dependent 3′ 5′-cyclic phosphodiesterase	QWTDRI**M**EE**FF**RQGDRERE
NP_005081.1	cytosolic phospholipase A2 β	STAGR**I**AE**FF**
AAD27760.1	glycogen phosphorylase	LWSAKSPIDFNL
AAD37118.1	SH2-containing inositol phosphatase	ST**Q**LL**L**DSD**F**LKTGS
NP_012461.1	SMC3p chromosomal ATPase	KELWRKE**Q**KLQTVL
NP_006309.1	glialblastoma cell differentiation-related protein	**Q**TN**M**RD**F**QTELRKILVS
AAB58975.1	neurofibromin (neurofibromatosis type 1 tumour suppressor)	**L**ALHRL**L**WTH**Q**EK**I**GD**Y**LSSR
NP_0.11318.1	negative regulator of early meiotic genes (MCK1 dosage suppressor 3)	RRSNT**L**TD**Y**MHSNKASPFS
P41139	ID-4 transcription factor for myogenesis, neurogenesis and haematopoiesis	**Q**CD**M**NDC**Y**SRLRRLV
AAD40474.1	doublesex and mab-3 related transcription factor 1	**Q**GRAGG**F**GKASGALVGA
NP_002606.1	pigment epithelial-differentiating factor	RKTS**L**ED**FY**LDEERTV
P42284	LOLA longitudinals lacking protein (regulator of axon-target interaction)	LRWNNH**Q**ST**L**ISV**F**D
CAA67753.1	fertilin	**Q**PR**L**DP**FF**KQQAVCSNA
CAB08076.1	tenascin-R (restrictin, janusin) (development of astrocytes)	QRR**Q**NGQTD**FF**
P17789	TTKB tramtrak protein (developmental transcription factor)	LRWNNH**Q**SN**L**LSV**F**D
BAA84069.1	flamingo (cell polarity gene)	R**Q**GV**L**YYI**F**D
AAF02618.1	starry night (tissue polarity gene)	R**Q**GV**L**YYI**F**D

Table 2. (*Continued*).

Protein ID	**Protein** (human, mouse, rat, fruit fly or yeast)	**Possible PCNA binding sequence**
P48679	lamin A	QQSR**I**RIDS
CAB63111.1	dysferlin (mutations cause myopathy/muscular dystrophy	**Q**IR**I**KLW**F**GLSVDEKE
2203411A	reeler gene (mutants show ataxic gait and trembling)	R**Q**HG**L**RH**FY**NRRRR
AAC28409.1	small optic lobes (SOL1) (with calpain-like domain)	REG**M**TA**YY**
NP_032058.1	fragile X2 homolog (FMR2)	**Q**TR**L**ED**FF**
CAA76528.1	gap junction protein connexin36	RR**Q**EG**I**SR**FY**
AAB39720.1	presynaptic protein munc13-3	SDRELWQRK**Q**EG**M**TAL**Y**HSP
P98159	nudel protein precursor, serine protease	ER**Q**LW**L**KK**F**E
NP_013488.1	killer toxin sensitivity	SEK**Q**NV**I**YNI**Y**
P50284	lymphotoxin-beta receptor	SDRKAECRC**Q**PG**M**SCV**Y**
P51787	voltage dependent K^+ channel	THV**Q**GRVYN**F**LERPT
A39402	potassium channel protein IIIA form 1, shaker-type	WRKL**Q**PR**M**WAL**F**EDP
AAF32521.1	anti-vesicular stomatitis virus single-chain Fv antibody fragment 0B1	G**L**TA**YY**RSP
NP_004289.1	nucleoporin 155kD (NUP155)	QE**Q**LK**I**TT**F**KDLVIRDKE
P16036	mitochondrial phosphate transport protein	KEEG**L**NA**FY**
CAB66155.1	sugar transporter	Q**I**ES**FF**RMGRR
NP_012033.1	glucose-6-phosphate dehydrogenase	K**Q**NI**M**HEI**F**D
NP_01053.1	galactosyl-transferase	VWEHRE**Q**DA**L**EAL**Y**EN
NP_005065.1	sodium phosphate transport protein 1	GNLAGQ**L**SD**FF**LTRNILS
BAA11374.1	Orn decarboxylase antizyme	EE**Q**LRADHV**F**ICFHKNRED
AAD32244.1	microtubule-actin crosslinking factor	**Q**DT**L**QAM**F**DW
AAC27437.1	brush border myosin	RKS**Q**IL**I**SSW**F**RGNMQKK
NP_006748.1	troponin	EL**Q**AL**I**DSH**F**EARKKEEEE
NP_035927.1	carbonic anhydrase 14 (CA XIV)	Q**L**EQ**FF**RYNGSLTT
NP_013060.1	peripheral vacuolar membrane protein complex	G**M**TV**FY**H
AAA51242.1	T-cell receptor alpha	RL**I**TL**FY**LAQGTKEN
AAA52139.1	cytochrome P-450-1	RDTS**L**KG**FY**IPKGRCVF
AAC64595.1	olfactory receptor	**Q**DK**M**YSL**F**
AAD13333.1	olfactory receptor H12	ST**Q**GR**I**KA**F**

Amino acids in **bold exactly match the PCNA binding consensus.

ncbi.nlm.nih.gov/BLAST; Altschul *et al.* 1990) looking for the proteins that contain consensus or near consensus PCNA binding sequence. In addition to proteins already published to be interacting with the PCNA interdomain connecting loop, we found several additional proteins (table 2). These proteins include proteins involved in replication: origin recognition complex subunits 1, 2 and 4 (homologs of hCdc18) and DNA polymerase-α; proteins linked with repair and radiosensitivity such as BRCA2 and ATM (however, Balajee and Geard 2001 found that ATM is not required for PCNA complex assembly); proteins involved in control of apoptosis such as Bcl-x and different proteases; and several kinases that were not previously mentioned in conjunction with PCNA such as calcium/calmodulin-dependent kinase, SNF-1-dependent kinase; and others.

4. PCNA gene regulation

The promoter and introns of the *PCNA* gene contain numerous control elements responsible for the tightly controlled expression of this gene during the cell cycle, during development, with tissue specificity, and in response to external stimuli. Negative regulatory regions are found not only in the promoter, but also in introns 1 and 4 of the *PCNA* gene (Ottavio *et al.* 1990, Alder *et al.* 1992, Huang *et al.* 1994). Figure 2 shows the sequence of the human PCNA promoter, with putative binding sites of transcription factors relevant to radiobiology labelled.

Using *PCNA* promoter-reporter gene constructs, Kannabiran *et al.* (1999) demonstrated that E1A can repress expression from the PCNA promoter when transfected into rat embryo fibroblasts and primary baby rat kidney cells, but not when transfected into HeLa cells. *PCNA* repression in primary baby rat kidney cells was shown to be mediated by p53 and was relieved by p53 inactivation that occurs in HeLa cells due to E1B protein expression and binding to p53. E1A was found to reactivate successfully terminally differentiated cells by bypassing early G_1 and activating cyclins A and E, cdk2, B-*myb*, and PCNA (Tiainen *et al.* 1996). Adenovirus E1A induces PCNA through a *cis* element that binds activating transcription factor ATF, transcription factor YY1, and the

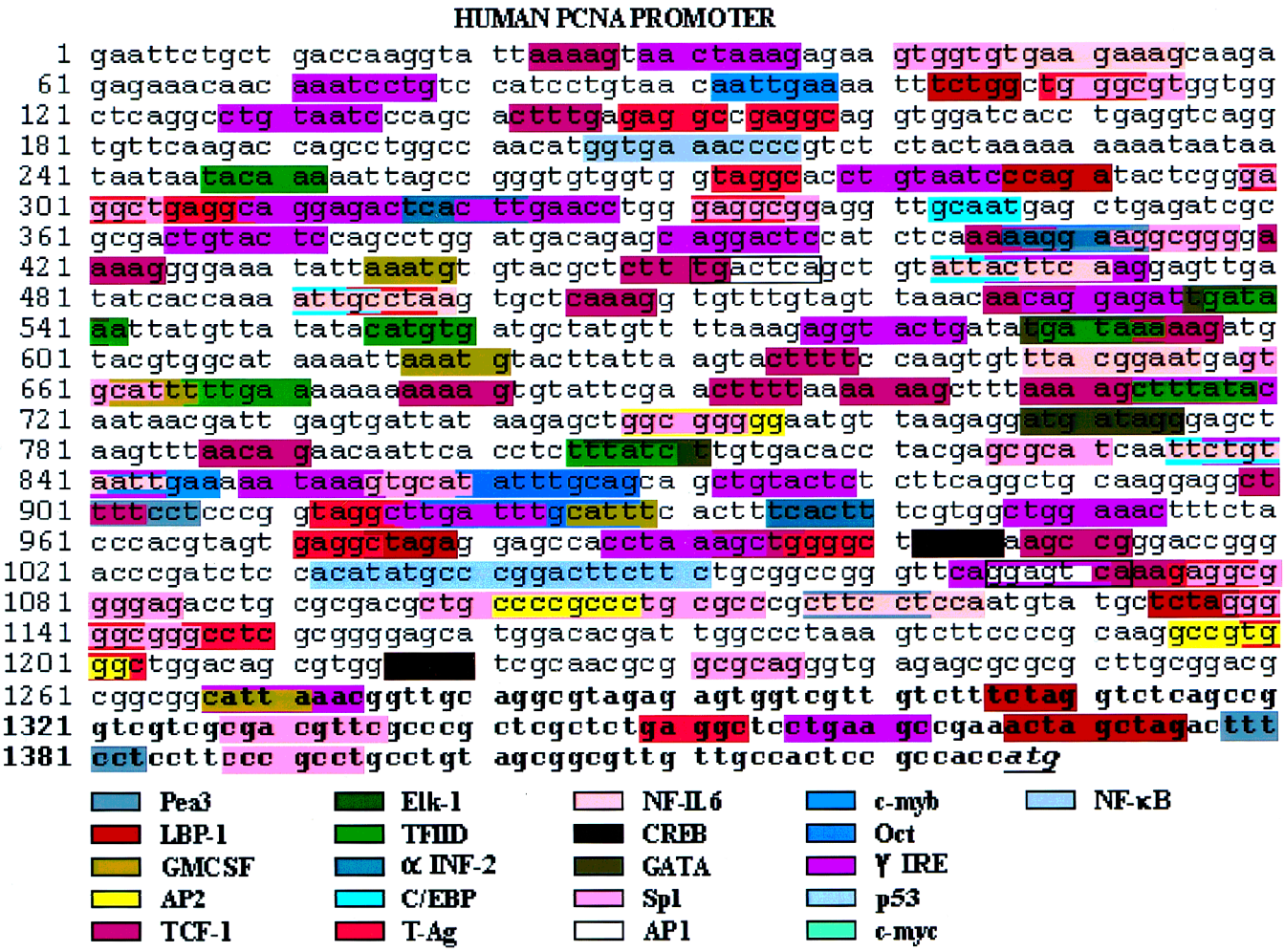

Figure 2. Map of the human PCNA promoter with putative binding sites for transcription factors relevant to radiobiology. Data were derived from the NIH Transfac database (ftp://ncbi.nlm.nih.gov/repository/TFD/datasets.tfsites.sig.scan; Wingender *et al.* 2000), except the p53 site (Morris *et al.* 1996). **Bold** letters indicate transcribed sequences; underlined letters indicate the translated sequence. Coloured boxes around letters indicate binding sites for transcription factors: Pea3 (oncogene responsive polyoma enhancer activator 3), LBP-1 (LTR binding protein), GMCSF (granulocyte-macrophage colony-stimulating factor), AP2 (activating protein 2), TCF-1 (T-cell-specific factor 1, from the family of high-mobility-group-proteins), Elk-1 (member of protooncogene family *ets*), TFIID (transcription factor II), α IFN2 (α interferon response element binding protein), C/EBP (CAT box/enhancer binding protein), T-Ag (SV-40 large T antigen), NF-IL6 (nuclear factor regulating IL-6 gene expression), CREB (cAMP response element binding protein), GATA (enhancer binding protein), Sp1 (transcription factor Sp1), AP-1 (activating protein 1, c-fos/c-jun heterodimer), c-myb (Myb oncogene), Oct (proteins from octamer binding protein family), γ IRE (γ-interferon response element binding protein), p53 (tumour suppressor p53), c-myc (Myc/Max heterodimers), and NF-κB (nuclear factor-κB) (reviewed by Boulikas 1994).

hepatitis B-virus enhancer-associated protein RFX1 (Labrie *et al.* 1995). Hayashi *et al.* (1999) demonstrated a role for the *Drosophila* Grainyhead/nuclear transcription factor-1 (GRH/NTF-1) protein in the regulation of the fly *PCNA* gene, although involvement of the human GRH/NTF-1 homolog in the regulation of the human *PCNA* gene has not yet been established.

Induction of *PCNA* transcription in response to radiation and other types of stress is regulated by p53 (Xu and Morris 1999), as p53-binding sites are present in the *PCNA* gene promoter (Shivakumar *et al.* 1995, Morris *et al.* 1996). Low and moderate cellular quantities of p53 positively stimulate transcription of PCNA, while high levels of p53 inhibit PCNA expression (Yamaguchi *et al.* 1994, Shivakumar *et al.* 1995, Morris *et al.* 1996). Chang *et al.* (1999) determined that the UV-inducibility of the PCNA promoter is (1) linked to the AP-1 site, (2) enhanced if the ATF/CRE site is mutated and (3) not dependent on p53. p53-independent PCNA expression regulation can also be achieved in the presence of ElA oncoprotein and CREB binding protein (Karuppayil *et al.* 1998). Ise *et al.* (1999)

showed that the Y-box binding protein, critical in control of TATA-less genes such as *PCNA*, also binds to damaged DNA, which makes it an additional avenue for radiation-mediated *PCNA* gene regulation.

In lymphocytes, the CREB site of the *PCNA* gene binds an inducible factor made up of proteins CREB and ATF2 (Huang *et al.* 1994, Feuerstein *et al.* 1995). IL2-stimulated T-cell proliferation depends on an E2F-like element at the very 5′ end of the transcript (Huang and Prystowsky 1996, Hayashi *et al.* 1999). PCNA expression in the brain and thymus is non-uniform (Turka *et al.* 1993, Hajihosseini *et al.* 1996) and not always in direct coordination with cell cycle progression. In healthy lymphoid tissues, it has been shown that immature, $CD4^+CD8^+$ double-positive thymocytes have 6-fold more PCNA protein and significantly higher levels of PCNA mRNA when compared with more mature, single-positive thymocytes. In comparison with peripheral blood T-cells, $CD4^+CD8^+$ cells expressed 30 times more PCNA. Most interestingly, this level of PCNA was entirely independent of stimulation by mitogens (Turka *et al.* 1993). Butyrate used in many systems as a powerful differentiating agent has been shown to inhibit proliferation by inhibiting PCNA expression in smooth muscle cells (Ranganna *et al.* 2000). Transforming growth factor-β (TGF-β) has also been shown to induce PCNA expression in cells of oligodendroglial lineage, while tumour necrosis factor (TNF) has been shown to reduce the amounts of the protein (Yu *et al.* 2000).

Post-transcriptional regulation was also reported for PCNA mRNA (Chang *et al.* 1990, 1999). Post-translational regulation of PCNA was studied as well, mostly in conjunction with stress. A direct response of PCNA to cellular damage was observed by Miura *et al.* (1996b) in studies showing localization of PCNA to DNA in X-ray-damaged human fibroblasts. Savio *et al.* (1998) showed that PCNA localizes to DNA in fibroblasts following exposure to alkylating agents and to oxidative damage. Similarly, DNA repair upon UV irradiation coincides with nuclear PCNA accumulation in human and monkey cells (Celis and Madsen 1986, Otrin *et al.* 1997). Wenz *et al.* (1998) and Lohr *et al.* (1998) documented ionizing radiation-induced changes in PCNA distribution in transformed human and hamster cells with enhanced levels in the nucleus. This post-translational regulation could be either p53-dependent or p53-independent (Lohr *et al.* 1998).

5. PCNA and DNA repair

The importance of PCNA for eukaryotic DNA repair emerged from numerous observations.

1. Yeast PCNA mutants show an increased rate of instability of simple repetitive DNA sequences, an elevated level of spontaneous mutations, and an increased sensitivity to DNA damage (Ayyagari *et al.* 1995, Johnson *et al.* 1996, Eissenberg *et al.* 1997, Arroyo *et al.* 1998). Different yeast PCNA (pol 30) mutant strains have been shown to have mismatch repair (MMR) abnormalities. One yeast PCNA mutant shows dinucleotide tract instability at levels equivalent to those found in strains deficient in *Mlh*1 (Umar *et al.* 1996). Kokoska *et al.* (1999) demonstrated that some yeast PCNA (pol 30) mutants have destabilized microsatellites (1–8 bp) and minisatellites (20 bp) repeat sequences. Since both short and longer repeat sequences are affected, the PCNA mutation likely affects both MMR and DNA polymerase slippage. Torres-Ramos *et al.* (1996) identified a yeast UV-sensitive PCNA mutant deficient in error-free bypass repair (post-replication repair). Arroyo and Wang (1998) analysed yeast PCNA mutants with increased sensitivity to γ-rays, UV and hydroxyurea. Many more PCNA mutant strains deficient in repair show normal DNA replication, suggesting that the role of PCNA in DNA repair is separable from its role in DNA replication (Ayyagari *et al.* 1995).
2. Non-lethal *Drosophila* PCNA mutants show an increased sensitivity to mutagens including ionizing radiation and an inability to repair double-strand breaks (Henderson *et al.* 1994, Henderson and Glover 1998). Heat-sensitive lethal mutants of *D. melanogaster* grown at permissive temperatures were hypersensitive to DNA-damaging agents, and females were sterile—their germ-line cells were unable to proliferate (Henderson *et al.* 2000).
3. In mammalian cells, as reviewed by Tsurimoto (1999) and Kelman (1997), PCNA, together with polymerases δ and ε, is indispensable for nucleotide excision repair (NER) (Nichols and Sancar 1992, Shivji *et al.* 1992), base excision repair (BER) (Kessis *et al.* 1993, Gary *et al.* 1997, Fortini *et al.* 1998) and mismatch repair (MMR) (Umar *et al.* 1996, Longley *et al.* 1997), as well as repair initiation (Shivji *et al.* 1992, Umar *et al.* 1996). While PCNA point mutations in mammalian systems were not investigated, over-expression of PCNA was linked with increased radioresistance in tumours and cell lines. On the other hand, the *wasted* mutant mouse with diminished PCNA expression in selective tissues shows an increased radiosensitivity (Woloschak *et al.* 1996a, b). Goukassian

et al. (2000) examined the amount of PCNA and other repair proteins present in dermal fibroblasts from aged individuals. Their studies demonstrated that ageing caused a decrease in the protein and mRNA levels for PCNA and that this decrease is associated with a significantly reduced rate of removal of thymine dimmers and (6-4) photoproducts in UV-irradiated skin, suggesting a relationship between reduced PCNA protein and reduced repair capacity in aged human fibroblasts.

Using human cell extracts, Gu *et al.* (1998) found the need for PCNA in an *in vitro* mismatch repair (MMR) assay. Eukaryotic MMR requires two different complexes of MMR proteins (MSH2-MSH3 and MSH2-MSH6) and two complexes of Mut-L proteins (MLH1-PMS1/PMS2 and MLH1-MLH3/PMS1). MLH1-PMS1 and MSH2-MSH6 interact with each other and with PCNA (Bowers *et al.* 2001). MSH3 and MSH6 directly interact with PCNA and peptides derived from these proteins inhibit *in vitro* MMR at the step preceding DNA synthesis (Clark *et al.* 2000). MLH1-PMS1 and MSH2-MSH6, DNA polymerases δ and ε, which each use PCNA as a subunit, have also been implicated in MMR pathways, perhaps working in conjunction with NER processes.

Two distinct BER pathways have been identified: a single nucleotide insertion pathway (PCNA-independent) and a patch insertion pathway—a PCNA-dependent pathway that resynthesizes two to 10 nucleotides 3′ to the lesion (Pascucci *et al.* 1999). Many components of the PCNA-dependent BER pathway are shared with the NER pathway induced by UV damage; polymerization and ligation may be carried out by the same enzymes, but recent studies have suggested that the helicase activities (XPD, XPB members of NER pathway) are not involved in the PCNA-dependent phase of the pathway (Cappelli *et al.* 1999). PCNA binds to FEN-1 (flap endonuclease-1) and stimulates its nuclease activity, which excizes a flap of oligonucleotides containing damaged bases (Tom 2000). Studies of PCNA mutants that can no longer bind to FEN-1 have demonstrated that the presence of PCNA can enhance FEN-1 activity, even in the absence of specific binding (Gomes and Burgers 2000). In reconstitution experiments, omission of PCNA leads to the accumulation of pre-excision reaction intermediates, suggesting a role for PCNA in excision during patch insertion BER (Gary *et al.* 1999). Disruption of the PCNA binding site of either FEN-1 or DNA ligase-I significantly reduced the efficiency of BER in reconstituted systems but did not affect repair patch size (Matsumoto *et al.* 1999).

Damage to DNA induced by UV light is repaired primarily by the nuclear excision repair (NER) pathway, which is a cellular repair pathway highly dependent on PCNA. NER shares several enzymatic steps with BER, and it is hypothesized that both require DNA polymerase-δ/ε and DNA ligase-I (Cappelli *et al.* 1999). Following UV irradiation of quiescent cells, PCNA forms an insoluble complex with nuclear structures. This complex appears essential for the formation of repairosome in both NER and transcription-coupled repair (TCR) pathways (Balajee *et al.* 1998). Hamster cells deficient in either TCR (CSB-deficient; gene linked to Cockayne's syndrome) or NER (XPD- or XPB-deficient; genes linked with xeroderma pigmentosum) do not have such complex formation following UV-induced DNA damage. Ehrenhofer-Murray *et al.* (1999) showed a role for PCNA in transcriptional silencing in *Saccharomyces cerevisiae*, implicating PCNA as a putative TCR protein.

Recent studies examining the minimal set of factors required for NER *in vitro* have identified complexes of RPA, XPA, XPC, XPG, XPF, TFIIH (composed of helicases XPB and XPD, and several other proteins); three-subunit kinase (CAK); RFC; DNA polymerases δ and ε; DNA ligase-I; and PCNA as being essential to reconstitute DNA adduct repair by using recombinant incision factors and human replication proteins *in vitro* (Araujo *et al.* 2000).

6. PCNA and radiation responses

PCNA is one of the key molecules in determining the cellular response to radiation damage: on one hand it is one of the few inducible repair genes following radiation exposure (low and high doses); on the other hand PCNA executes cellular responses to stress-repair or apoptosis. Several types of repair pathways have been identified following ionizing radiation exposure, including non-homologous end-joining (Jeggo 1998, Tuskamoto and Ikeda 1998), homologous recombination (Takata *et al.* 1998), and BER resulting from oxidative damage to DNA (Wallace 1998). PCNA is involved in all of these processes as is repeatedly proven by its numerous interactions with proteins representing each one of these pathways (table 1 and figure 1).

The precise role of PCNA in radiation sensitivity has not been defined in higher eukaryotes because knockout mutants of the gene (at cellular and whole-animal levels) are lethal. However, PCNA was linked with radiosensitivity on numerous occasions. Diminished PCNA expression was found in *wasted* mice which are radiosensitive (Woloschak *et al.* 1996a, b), while PCNA over-expressing cell lines have a

capacity for adaptive survival response to low-dose radiation (Boothman *et al.* 1996, Yang and Boothman, personal communication). Overexpression of PCNA is used as a monitor of tumour cell cycle time and is the most frequent change found in tumour cells relative to controls, particularly in those tumour cells showing enhanced radioresistance.

PCNA responses to irradiation in different cell types show differences that may be ascribed to cell type-specific differences, kinetics/dose–response differences, and/or a variety of other factors. PCNA induction by ionizing radiation was found in many cell types (Miura *et al.* 1996b, Velicky *et al.* 1997, Xu and Morris 1999). On the other hand, our own data done *in vitro* with Syrian hamster embryo (SHE) cells have not shown induction of PCNA following ionizing radiation (Chang-Liu and Woloschak 1997). These SHE cells may have acquired p53 mutations in culture, as is common for SHE cells (Chang-Liu and Woloschak, unpublished data), and thus may have a dysregulated PCNA expression. Likewise, after ionizing irradiation Wenz *et al.* (1998) found no change in PCNA expression in several human lymphoblastoid cell lines, while Sendler *et al.* (1993) have shown a decrease in PCNA expression in X-ray irradiated HL60 cells induced to differentiate to the granulocyte lineage with 1.25% dimethyl sulphoxide.

7. PCNA and cell survival

The presence of functional PCNA is critical for cell survival because of all of its functions: DNA replication, DNA repair and probably DNA recombination processes. Hegyi and Skepper (2000) postulated a role for PCNA in the repair of DNA in cardiovascular cells that do not undergo DNA synthesis. In retinal cells, Ju *et al.* (2000) similarly demonstrated an induction of PCNA mRNA in response to transient ischaemia, possibly to permit repair and cell survival. Ino and Chiba (2000) detected high-level PCNA protein expression in non-proliferating neurons of the brain, further supporting a repair-related role for PCNA in post-mitotic cells. One possibility is that PCNA is required for the repair not only of nuclear DNA, but also of mitochondrial DNA. Wang *et al.* (2000) demonstrated that activation of DNA repair processes including PCNA induction is associated with the presence of mitochondrial DNA damage in rat neuronal cells.

Lack of functional PCNA protein is lethal in yeast (Bauer and Burgers 1990, Jónsson *et al.* 1995). Two recently characterized point mutations of PCNA in *D. melanogaster* that interfere with protein–protein interactions are heat-sensitive lethal (Henderson *et al.* 2000). PCNA knockout animals were never obtained (Kelman and O'Donnell 1995a), while in the *wasted* mouse, PCNA protein is absent from thymus and spleen (Woloschak *et al.* 1996b). At the time of death, thymus and spleen tissues of these animals are only one-tenth of the size of those in healthy littermates, and markers of apoptosis were observed in these tissues (Libertin *et al.* 1994, Woloschak *et al.* 1996a).

PCNA is essential for progression through the cell cycle (figure 1). Antisense PCNA oligonucleotides prevent the cells from entering the S-phase of the cell cycle (Jaskulski *et al.* 1988, Liu *et al.* 1989, Morita *et al.* 1997), and the same effect was achieved by using PCNA antibodies (Zuber *et al.* 1989). In those cases when cell cycle proteins are expressed at 'natural' cellular levels PCNA is balanced between DNA replication and repair. For example, binding of p21 to PCNA stops DNA replication but it does not inhibit repair (Cox and Lane 1995, Shivji *et al.* 1998). On the other hand, binding of p21 to PCNA induces arrest in the G_1- and G_2-phases of the cell cycle even in cells deficient in p53 (Cayrol *et al.* 1998). Overexpression of Gadd45 and MyD118 also inhibits progression through the cell cycle (Zhan *et al.* 1994). However, overexpression of any of the PCNA binding proteins stops progression through the cell cycle, as is evidenced by the fact that accumulation of the anti-apoptotic protein myeloid cell leukaemia 1 (MCL1) (Fujise *et al.* 2000) inhibits proliferation. In addition, Fukami-Kobayashi and Mitsui (1999) showed that cyclin D1 accumulation inhibits DNA synthesis through direct binding to PCNA and CDK2 (multiprotein complexes formed by this interaction abound in senescent cells). Furthermore, a number of synthetic peptides carrying the consensus sequence for binding PCNA interdomain connecting loop stop progression through the cell cycle and induce apoptosis when transfected into cells in culture. These peptides can be derived from a number of PCNA-interacting proteins: the N-terminal domain of p21 (Chen *et al.* 1996a), or C-terminal domains of Gadd45 and MyD118 (Vairapandi *et al.* 2000). Ling *et al.* (2000) showed that even an HLA-derived peptide blocks T-cell proliferation by binding to PCNA. As mentioned above, MSH3 and MSH6 derived peptides inhibit beginning of DNA synthesis in an *in vitro* mismatch repair assay (Clark *et al.* 2000).

These findings lead us to postulate that interactions between PCNA and peptides binding to the interdomain connector loop gives cells the 'final push' into apoptosis. A source of such peptides could be degradation of PCNA-interacting proteins. Such degradation is promoted by caspase-3, which is known to cut proteins 'between the regions' dedicated to different functions. Caspase 3 is expressed during

the earliest stages of apoptosis, and some of the proteins subject to caspase-3 cleavage that are known to interact with PCNA include: DSEB/RF-C140 subunit of the replication factor C complex (Ubeda and Habener 1997); myeloid cell leukaemia 1(MCL1) (Fujise *et al.* 2000); DNA polymerase-ε (Lin and Linn 2000); and p21 (Gervais *et al.* 1998). We hypothesize that the short PCNA binding peptides created by proteolytic cleavage functionally inactivate PCNA and make it unavailable for either DNA repair or replication, and push the affected cells down the apoptotic path.

8. Conclusions

PCNA is a multifunctional protein with roles in DNA replication synthesis, DNA repair synthesis and recombination-driven DNA synthesis. It is a subunit of DNA polymerases δ and ε, both of which have been associated with repair including recombination-driven DNA synthesis, NER, BER, mismatch repair and post-replication repair. PCNA also binds other proteins important in DNA replication and proteins involved in non-homologous end-joining and homologous recombination. PCNA is also a subunit of mitochondrial DNA polymerase-γ that is reported to have a repair function.

PCNA expression is tightly regulated by a vast array of transcription factors (including TGF-β, TNF, YY1 and others) during every stage of cell life: resting, proliferation, differentiation and programmed cell death. *PCNA* transcription in response to stress is in the first place controlled by p53 and also by AP1, CRE binding protein and others.

PCNA interacts with a variety of proteins important in cell cycle regulation (cyclins, cyclin-dependent kinases, etc.), and these interactions do not interfere with its involvement in DNA replication. During cellular proliferation, PCNA interacts with proteins that regulate chromatin assembly (CAF1, Ctf7p) as well as with those involved in DNA 'processing' (from DNA polymerases to methyltransferases to origin recognition complex proteins). During cell cycle arrest, PCNA protein forms complexes with several p53-regulated proteins (p21, p57, Gadd45, MyD118, CR6), particularly after exposure of cells to DNA-damaging agents.

Cells lacking PCNA die, as was shown in cases when an antagonistic oligonucleotide, peptide or anti-PCNA antibody was added to cells in culture, and cells and organisms without functional PCNA do not survive. We hypothesize that caspase-3 promoted massive degradation of PCNA-interacting proteins at an early stage of apoptosis releases PCNA binding peptides that interfere with PCNA function. This may, in turn, finally commit the cells to apoptosis.

Studies reviewed here suggest that PCNA is the executive molecule in life of the cell, committing it to replicate DNA, arrest the cell in G_1- or G_2-phase, repair damaged DNA, or commit the cell to apoptosis, based on the interactions with a plethora of proteins, 'instructions' coming from p53 and the presence of the PCNA-inactivating peptides.

In conclusion, there are many more questions about PCNA that remain unanswered. How does it fulfil its function as an executor of apoptosis? How is the lack of functional PCNA recognized by the cell? What is the precise role of PCNA in mitochondria? Mitochondria, organelles descending from prokaryotes, use eukaryotic PCNA as a sliding clamp while they still retain a mitochondrially encoded DNA polymerase-γ as their DNA polymerase. Is PCNA in mitochondria therefore used in a prokaryotic fashion—as a sliding clamp only—or in the eukaryotic way, for both replication and repair? Is it possible that the mitochondrial genome 'lost' prokaryotic SOS genes because the eukaryotic sliding clamp PCNA could interface DNA polymerase-γ with nuclear-encoded repair proteins?

Further studies of PCNA promise to be no less exciting then they have been so far. As a paradigm of a pleiotropic protein, PCNA may provide insight in the mechanism and purpose of sequential protein–protein interactions for global cell life regulation.

Acknowledgements

The work was supported by NIH Grants CA81375, CA73042 and NS 21442 at the US Department of Energy, Office of Health and Environmental Research, under Contract No. W-32-109-ENG-38.

References

ALDER, H., YOSHINOUCHI, M., PRYSTOWSKY, M. B., APPASAMY, P. and BASERGA, R., 1992, A conserved region in intron 1 negatively regulates the expression of the PCNA gene. *Nucleic Acids Research*, **20,** 1769–1775.

ALMENDRAL, J., HUEBSCH, M., BLUNDELL, P. A., MACDONALD-BRAVO, H. and BRAVO, R., 1987, Cloning and sequence of the human nuclear protein cyclin: homology with DNA-binding proteins. *Proceedings of the National Academy of Sciences, USA*, **84,** 1575–1579.

ALTSCHUL, S. F., GISH, W., MILLER, W., MYERS, E. W. and LIPMAN, D. J., 1990, Basic local alignment search tool. *Journal of Molecular Biology*, **215,** 403–410.

ARAUJO, S. J., TIRODE, F., COIN, F., POSPIECH, H., SYVAOJA, J. E., STUCKI., M., HUBSCHER, U., EGLY, J. M. and WOOD, R. D., 2000, Nucleotide excision repair of DNA with recombinant human proteins: definition of the minimal set of factors, active forms of TFIIH, and modulation by CAK. *Genes and Development*, **14,** 349–359.

ARROYO, M. P., DOWNEY, K. M., SO, A. G. and WANG, T. S. F., 1996, *Schizosaccharomyces pombe* proliferating cell nuclear antigen mutations affect DNA polymerase delta processivity. *Journal of Biological Chemistry*, **271,** 15971–15980.

ARROYO, M. P. and WANG, T. S., 1998, Mutant PCNA alleles are associated with cdc phenotypes and sensitivity to DNA damage in fission yeast. *Molecular and General Genetics*, **257,** 505–518.

AYYAGARI, R., IMPELLIZZERI, K. J., YODER, B. L., GARY, S. L. and BURGERS, P. M., 1995, A mutational analysis of the yeast proliferating cell nuclear antigen indicates distinct roles in DNA replication and DNA repair. *Molecular and Cellular Biology*, **15,** 4420–4429.

AZAM, N., VAIRAPANDI, M., ZHANG, W., HOFFMAN, B. and LIEBERMANN, D. A., 2001, Interaction of CR6 (GADD45γ) with Proliferating Cell Nuclear Antigen impedes negative growth control. *Journal of Biological Chemistry*, **276,** 2766–2774.

BALAJEE, A. S. and GEARD, C. R., 2001, Chromatin-bound PCNA complex formation triggered by DNA damage occurs independent of the ATM gene product in human cells. *Nucleic Acids Research*, **29,** 1341–1351.

BALAJEE, A. S., MAY, A., DIANOVA, I. and BOHR, V. A., 1998, Efficient PCNA complex formation is dependent upon both transcription coupled repair and genome overall repair. *Mutation Research-DNA Repair*, **409,** 135–146.

BAUER, G. A. and BURGERS, P. M. J., 1990, Molecular cloning, structure and expression of the yeast proliferating cell nuclear antigen gene. *Nucleic Acids Research*, **18,** 261–265.

BOOTHMAN, D. A., MEYERS, N., ODEJAARD, Z. and WANG, M., 1996, Altered G_1 checkpoint control determines adaptive survival responses to ionizing radiation. *Mutation Research*, **358,** 143–153.

BOULIKAS, T., 1994, A compilation and classification of DNA binding sites for protein transcription factors from vertebrates. *Critical Reviews in Eukaryotic Gene Expression*, **4,** 117–321.

BOWERS, J., TRAN, P. T., JOSHI, A., LISKAY, R. M. and ALANI, E., 2001, MSH-MLH complexes formed at a DNA mismatch are disrupted by the PCNA sliding clamp. *Journal of Molecular Biology*, **306,** 957–968.

CAPPELLI, E., CARROZZINO, F., ABVONDANDOLO, A. and FROSINA, G., 1999, The DNA helicases acting in nucleotide excision repair, XPD, CSB and XPB, are not required for PCNA-dependent repair of abasic sites. *European Journal of Biochemistry*, **259,** 325–330.

CAYROL, C., KINBIEHLER, M. and DUCOMMUN, B., 1998, p21 binding to PCNA causes G_1 and G_2 cell cycle arrest in p53-deficient cells. *Oncogene*, **16,** 311–320.

CELIS, J. E. and MADSEN, P., 1986, Increased nuclear cyclin/PCNA antigen staining of non S-phase transformed human amnion cells engaged in nucleotide excision DNA repair. *FEBS Letters*, **209,** 277–283.

CHANG, C. D., OTTAVIO, L., TRAVALI, S., LIPSON, K. E. and BASERGA, R., 1990, Transcript posttranscriptional regulation of proliferating cell nuclear antigen gene. *Molecular and Cellular Biology*, **10,** 3289–3296.

CHANG, H. W., LAI, Y. C., CHENG, C. Y., HO, J. L., DING, S. T. and LIU, Y. C., 1999, UV inducibility of rat proliferating cell nuclear antigen gene promoter. *Journal of Cellular Biochemistry* **73**, 423–432.

CHANG-LIU, C. M. and WOLOSCHAK, G. E., 1997, Effect of passage number on cellular response to DNA-damaging agents: cell survival and gene expression. *Cancer Letters*, **113,** 77–86.

CHEN, J., PETERS, R., SAHA, P., LEE, P., THEODORAS, A., PAGANO, M., WAGNER, G. and DUTTA, A., 1996a, A 39 a.a. fragment of the cell cycle regulator p21 is sufficient to bind PCNA and partially inhibit DNA replication *in vivo*. *Nucleic Acids Research*, **24,** 1727–1733.

CHEN, J., CHEN, S., SAHA, P. and DUTTA, A., 1996b, p21 Cip1/Waf1 disrupts the recruitment of human Fen1 by proliferating-cell nuclear antigen into the DNA replication complex. *Proceedings of the National Academy of Sciences, USA*, **93,** 11597–11602.

CHUANG, L. S.-H., IAN, H.-I., KOH, T.-W., NG, H.-H., XU, G. and LI, B. F. L., 1997, Human DNA- (cytosine-5) methyltransferase–PCNA complex as a target for p21WAF1. *Science*, **277,** 1996–2000.

CLARK, A. B., VALLE, F., DROTSCHMANN, K., GARY, R. K. and KUNKEL, T. A., 2000, Functional interaction of proliferating cell nuclear antigen with MSH2–MSH6 and MSH2–MSH3 complexes. *Journal of Biological Chemistry*, **275,** 36498–36501.

COX, L. S. and LANE, D. P., 1995, Tumour suppressors, kinases and clamps: how p53 regulates the cell cycle in response to DNA damage. *Bioessays*, **17,** 501–508.

DIANOV, G., L., JENSEN, B., R., KENNY, M., K. and BOHR, V., A., 1999, Replication protein A stimulates proliferating cell nuclear antigen-dependent repair of abasic sites in DNA by human cell extracts. *Biochemistry*, **38,** 11021–11025.

EISSENBERG, J. C., AYYAGARI, R., GOMES, X. V. and BURGERS, P. M., 1997, Mutations in yeast proliferating cell nuclear antigen define distinct sites for interaction with DNA polymerase delta and DNA polymerase epsilon. *Molecular and Cellular Biology*, **17,** 6367–6378.

EHRENHOFER-MURRAY, A. E., KAMAKAKA, R. T. and RINE, J., 1999, A role for the replication proteins PCNA, RF-C, polymerase epsilon and Cdc45 in transcriptional silencing in *Saccharomyces cerevisiae*. *Genetics*, **153,** 1171–1182.

FEUERSTEIN, N., HUANG, D. and PRYSTOWSKY, M. B., 1995, Rapamycin selectively blocks interleukin-2-induced proliferating cell nuclear antigen gene expression in T-lymphocytes. *Journal of Biological Chemistry*, **270,** 9454–9458.

FORTINI, P., PASCUCCI, B., PARLANTI, E., SOBOL, R. W., WILSON, S. H. and DOGLIOTTI, E., 1998, Different DNA polymerases are involved in the short- and long-patch base excision repair in mammalian cells. *Biochemistry*, **37,** 3575–3580.

FUJISE, K., ZHANG, D., LIU, J. and YEH, E. T., 2000, Regulation of apoptosis and cell cycle progression by MCL1. Differential role of proliferating cell nuclear antigen. *Journal of Biological Chemistry*, **275,** 39 458–39 465.

FUKAMI-KOBAYASHI, J. and MITSUI, Y., 1999, Cyclin D1 inhibits cell proliferation through binding to PCNA and Cdk2. *Experimental Cell Research*, **246,** 338–347.

GARY, R., KIM, K., CORNELIUS, H. L., PARK, M. S. and MATSUMOTO, Y., 1999, Proliferating cell nuclear antigen facilitates excision in long-patch base excision repair. *Journal of Biological Chemistry*, **274,** 4354–4363.

GARY, R., LUDWIG, D. L., CORNELIUS, H. L., MACINNES, M. A. and PARK, M. S., 1997, The DNA repair endonuclease XPG binds to proliferating cell nuclear antigen (PCNA) and shares sequence elements with the PCNA binding regions of FEN-1 and cyclin-dependent kinase inhibitor p21. *Journal of Biological Chemistry*, **272,** 24 522–24 529.

GERVAIS, J. L., SETH, P. and ZHANG, H., 1998, Cleavage of CDK inhibitor p21(Cip1/Waf1) by caspases is an early event during DNA damage-induced apoptosis. *Journal of Biological Chemistry*, **273,** 19207–19212.

GOUKASSIAN, D., GAD, F., YAAR, M., ELLER, M. S., NEHAL, U. S. and GILCHREST, B. A., 2000, Mechanisms and implications of age-associated decrease in DNA repair capacity. *FASEB Journal*, **14,** 1325–1334.

GOMES, X. V. and BURGERS, P. M. J., 2000, Two modes of FEN1 binding to PCNA regulated by DNA. *EMBO Journal*, **19,** 3811–3821.

GU, L. Y., HONG, Y., MCCULLOCH, S., WATANABE, H. and LI, G. M., 1998, ATP-dependent interaction of human mismatch repair proteins and dual role of PCNA in mismatch repair. *Nucleic Acids Research*, **26,** 1173–1178.

GULBIS, J. M., KELMAN, Z., HURWITZ, J., O´DONNELL, M. and KURIYAN, J., 1996, Structure of the C-terminal region of p21(WAF1/CIP1) complexed with human PCNA. *Cell*, **87,** 297–306.

HAJIHOSSEINI, M., THAM, T. N. and DUBOIS-DALCQ, M., 1996, Origin of oligodendrocytes within the human spinal cord. *Journal of Neuroscience*, **16,** 7981–7994.

HAYASHI, Y., YAMAGISHI, M., NISHIMOTO, Y., TAGUCHI, O., MATSUKAGE, A. and YAMAGUCHI, M., 1999, A binding site for the transcription factor Grainyhead/nuclear transcription factor-1 contributes to regulation of the *Drosophila* proliferating cell nuclear antigen gene promoter. *Journal of Biological Chemistry*, **274,** 35080–35088.

HEGYI, L. and SKEPPER, J. N., 2000, The significance of the expression of proliferating cell nuclear antigen in the cardiovascular system: mitosis or DNA repair? *Circulation*, **101,** E240–241.

HENDERSON, D. S., BANGA, S. S., GRIGLIATTI, T. A. and BOYD, J. B., 1994, Mutagen sensitivity and suppression of position-effect variation results from mutations in mus209, the *Drosophila* gene encoding PCNA. *EMBO Journal*, **13,** 1450–1459.

HENDERSON, D. S. and GLOVER, D. M., 1998, Chromosome fragmentation resulting from an inability to repair transposase-induced DNA double-strand breaks in PCNA mutants of *Drosophila*. *Mutagenesis*, **13,** 57–60.

HENDERSON, D. S., WIEGAND, U. K., NORMAN, D. G., GLOVER, D. M., 2000, Mutual correction of faulty PCNA subunits in temperature-sensitive lethal mus209 mutants of *Drosophila melanogaster*. *Genetics*, **154,** 1721–1733.

HUANG, D., SHIPMAN-APPASAMY, P. M., ORTEN, D. J., HINRICHS, S. H. and PRYSTOWSKY, M. B., 1994, Promoter activity of the proliferating-cell nuclear antigen gene is associated with inducible CRE-binding proteins in interleukin 2-stimulated T lymphocytes. *Molecular and Cellular Biology*, **14,** 4233–4243.

HUANG, D. Y. and PRYSTOWSKY, M. B., 1996, Identification of an essential cis element near the transcription start site for transcriptional activation of the proliferating cell nuclear antigen gene. *Journal of Biological Chemistry*, **271,** 1218–1225.

INO, H. and CHIBA, T., 2000, Expression of proliferating cell nuclear antigen (PCNA) in the adult and developing mouse nervous system. *Molecular Brain Research*, **78,** 183–174.

ISE, T., NAGATANI, G., IMAMURA, T., KATO, K., TAKANO, H., NOMOTO, M., IZUMI, H., OHMORI, H., OKAMOTO, T., UCHIUMI, T., KUWANO, M. and KOHNO, K., 1999, Transcription factor Y-box binding protein 1 binds preferentially to cisplatin-modified DNA and interacts with proliferating cell nuclear antigen. *Cancer Research*, **59,** 342–346.

JASKULSKI, D., GATTI, C., TRAVALI, S., CALABRETTA, B. and BASERGA, R., 1988, Regulation of the proliferating cell nuclear antigen cyclin and thymidine kinase mRNA levels by growth factors. *Journal of Biological Chemistry*, **263,** 10 175–10 179.

JEGGO, P. A., 1998, DNA breakage and repair. *Advances in Genetics*, **38,** 185–218.

JOHNSON, R. E., KOVVALI, G. K., GUZDER, S. N., AMIN, N. S., HOLM, C., HABRAKEN, Y., SUNG, P., PRAKASH, L. and PRAKASH, S., 1996, Evidence for involvement of yeast proliferating cell nuclear antigen in DNA mismatch repair. *Journal of Biological Chemistry*, **271,** 27987–27990.

JÓNSSON, Z. O., PODUST, V. N., PODUST, L. M. and HÜBSCHER, U., 1995, Tyrosine 114 is essential for the trimeric structure and the functional activities of human proliferating cell nuclear antigen. *EMBO Journal*, **14,** 5745–5451.

JU, W. K., KIM. K. Y., HOFMANN, H. D., KIM, I. B., LEE, M. Y., OH, S. J. and CHUN, M. H., 2000, Selective neuronal survival and upregulation of PCNA in the rat inner retina following transient ischemia. *Journal of Neuropathology and Experimental Neurology*, **59,** 241–250.

KANNABIRAN, C., MORRIS, G. F. and MATHEWS, M. B., 1999, Dual action of the adenovirus E1A 243 R oncoprotein on the human proliferating cell nuclear antigen promoter: repression of transcriptional activation by p53. *Oncogene*, **18,** 7825–7833.

KARUPPAYIL, S. M., MORAN, E. and DAS, G. M., 1998, Differential regulation of p53-dependent and -independent proliferating cell nuclear antigen gene transcription by 12 S E1A oncoprotein requires CBP. *Journal of Biological Chemistry*, **273,** 17 303–17 306.

KELMAN, Z., 1997, PCNA: structure, functions and interactions. *Oncogene* **14**, 629–640.

KELMAN, Z. and O´DONNELL, M. N., 1995a, Embryonic PCNA: a missing link? *Current Biology*, **5,** 814.

KELMAN, Z. and O´DONNELL, M., 1995b, Structural and functional similarities of prokaryotic and eukaryotic DNA polymerase sliding clamps. *Nucleic Acids Research*, **23,** 3613–3620.

KELMAN, Z., ZUO, S., ARROYO, M. P., WANG, T. S.-F. and HURWITZ, J., 1999, The C-terminal region of *Schizosaccaromyces pombe* proliferating cell nuclear antigen is essential for DNA polymerase activity. *Proceedings of the National Academy of Sciences, USA*, **96,** 9515–9520.

KESSIS, T. D., SLEBOS, R. J., NELSON, W. G., KASTAN, M. B., PLUNKETT, B. S., HAN, S. M., LORINCZ, A. T., HEDRICK, L. and CHO, K. R., 1993, Human papillomavirus 16 E6 expression disrupts the p53-mediated cellular response to DNA damage. *Proceedings of the National Academy of Sciences, USA*, **90,** 3988–3992.

KNIBIEHLER, M., GOUBIN, F., ESCALAS, N., JIONSSON, Z. O., MAZARGUIL, H., HIUBSCHER, U. and DUCOMMUN, B., 1996, Interaction studies between the p21 Cip1/Waf1 cyclin-dependent kinase inhibitor and proliferating cell nuclear antigen (PCNA) by surface plasmon resonance. *FEBS Letters*, **391,** 66–70.

KOKOSKA, R.. J., STEFANOVIC, L., BUERMEYER, A. B., LISKAY, R. M. and PETES, T. D., 1999, A mutation of the yeast gene encoding PCNA destabilizes both microsatellite and minisatellite DNA sequences. *Genetics*, **151,** 511–519.

KOMATSU, K., WHARTON, W., HANG, H., WU, C., SINGH, S., LIEBERMAN, H. B., PLEDGER, W. J. and WANG, H. G., 2000, PCNA interacts with hHus1/hRad9 in response to DNA damage and replication inhibition. *Oncogene*, **19,** 5291–5297.

KOUNDRIOUKOFF, S., JÓNSSON, Z. O., HASAN, S., DE JONG, R. N., VAN DER VLIET, P. C., HOTTIGER, M. O. and HÜBSCHER, U., 2000, A direct interaction between proliferating

cell nuclear antigen (PCNA) and Cdk2 targets PCNA-interacting proteins for phosphorylation. *Journal of Biological Chemistry*, **275,** 22 882–22 887.

KRISHNA, T. S. R., KONG, X.-P, GARY, S., BURGER, P. M. and KURIYAN, J., 1994, Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. *Cell*, **79,** 1233–1243.

LABRIE, C., LEE, B. H. and MATHEWS, M. B., 1995, Transcription factors RFX1/EF-C and ATF-1 associate with the adenovirus E1A-responsive element of the human proliferating cell nuclear antigen promoter. *Nucleic Acids Research*, **23,** 3732–3741.

LEBEL, M., SPILLARE, E. A., HARRIS, C. C. and LEDER, P. J., 1999, The Werner syndrome gene product co-purifies with the DNA replication complex and interacts with PCNA and topoisomerase I. *Journal of Biological Chemistry*, **274,** 37 795–37 799.

LEVIN, D. S., BAI, W., YAO, N., O´DONNELL, M. and TOMKINSON, A. E., 1997, An interaction between DNA ligase I and proliferating cell nuclear antigen: Implications for Okazaki fragment synthesis and joining. *Proceedings of the National Academy of Sciences, USA*, **94,** 12 863–12 868.

LIBERTIN, C. R., WEAVER, P., MOBARHAN, S. and WOLOSCHAK, G. E., 1994, Subnormal albumin gene expression is associated with weight loss in immunodeficient/DNA-repair-impaired wasted mice. *Journal of the American College of Nutrition*, **13,** 149–153.

LING, X., KAMANGAR, S., BOYTIM, M. L., KELMAN, Z., HUIE, P., LYU, S. C., SIBLEY, R. K., HURWITZ, J., CLAYBERGER, C. and KRENSKY, A. M., 2000, Proliferating cell nuclear antigen as the cell cycle sensor for an HLA-derived peptide blocking T cell proliferation. *Journal of Immunology*, **164,** 6188–6192.

LIU, W. and LINN, S., 2000, Proteolysis of the human DNA polymerase epsilon catalytic subunit by caspase-3 and calpain specifically during apoptosis. *Nucleic Acids Research*, **28,** 4180–4188.

LIU, Y. C., MARRACCINO, R. L., KENG, P. C., BAMBARA, R. A., LORD, E. M., CHOU, W. G. and ZAIN, S. B., 1989, Requirement for proliferating cell nuclear antigen expression during stages of the Chinese hamster ovary cell cycle. *Biochemistry*, **28,** 2967–2974.

LOHR, F., HOF, H., WEBER, K. J., LATZ, D. and WENZ, F., 1998, X-ray induced changes in immunostaining of proliferating cell nuclear antigen (PCNA) in V79 hamster fibroblasts. *Strahlenther Onkologie*, **174,** 575–579.

LONGLEY, M. J., PIERCE, A. J. and MODRICH, P., 1997, DNA polymerase delta is required for human mismatch repair *in vitro*. *Journal of Biological Chemistry*, **272,** 10 917–10 921.

LOOR, G., ZHANG, S. J., ZHANG, P., TOOMEY, N. L. and LEE, M. Y. W. T., 1997, Identification of DNA replication and cell cycle proteins that interact with PCNA. *Nucleic Acids Research*, **25,** 5041–5046.

MAGA, G., STUCKI, M., SPADARI, S. and HUBSCHER, U., 2000, DNA polymerase switching: I. Replication factor C displaces DNA polymerase alpha prior to PCNA loading. *Journal of Molecular Biology*, **295,** 791–801.

MATSUMIYA, S., ISHINO, Y. and MORIKAWA, K., 2001, Crystal structure of an archaeal DNA sliding clamp: proliferating cell nuclear antigen from *Pyrococcus furiosus*. *Protein Science*, **10,** 17–23.

MATSUMOTO, Y., KIM, K., HURWITZ, J., GARY, R., LEVIN, D. S., TOMKINSON, A. E. and PARK, M. S., 1999, Reconstitution of proliferating cell nuclear antigen-dependent repair of apurinic/apyrimidinic sites with purified human proteins. *Journal of Biological Chemistry*, **274,** 33 703–33 708.

MIURA, M., NAKAMURA, S., SASAKI, T., TAKASAKI, Y., SHIOMI, T. and YAMAIZUMI, M., 1996a, Roles of XPG and XPF/ERCC1 endonucleases in UV-induced immunostaining of PCNA in fibroblasts. *Experimental Cell Research*, **226,** 126–132.

MIURA, M., SASAKI, T. and TAKASAKI, Y., 1996b, Characterization of x-ray-induced immunostaining of proliferating cell nuclear antigen in human diploid fibroblasts. *Radiation Research*, **145,** 75–80.

MORITA, Y., KASHIHARA, N., YAMAMURA, M., OKAMOTO, H., HARADA, S., MAESHIMA, Y., OKAMOTO, K. and MAKINO, H., 1997, Inhibition of rheumatoid synovial fibroblast proliferation by antisense oligonucleotides targeting proliferating cell nuclear antigen messenger RNA. *Arthritis Rheumatoides*, **40,** 1292–1297.

MORRIS, G. F., BISCHOFF, J. R. and MATHEWS, M. B., 1996, Transcriptional activation of the human proliferating-cell nuclear antigen promoter by p53. *Proceedings of the National Academy of Sciences, USA*, **93,** 895–899.

MOSSI, R., JÓNSSON, Z. O., ALLEN, B. L., HARDIN, S. H. and HÜBSCHER, U., 1997, Replication factor C interacts with the C-terminal side of proliferating cell nuclear antigen. *Journal of Biological Chemistry*, **17,** 1769–1776.

NICHOLS, A. F. and SANCAR, A., 1992, Purification of PCNA as a nucleotide excision repair protein. *Nucleic Acids Research*, **20,** 2441–2446.

OTRIN, V. R., MCLENIGAN, M., TAKAO, M., LEVINE, A. S. and PROTIĆ, M., 1997, Translocation of a UV-damaged DNA binding protein into a tight association with chromatin after treatment of mammalian cells with UV light. *Journal of Cellular Sciences*, **110,** 1159–1168.

OTTAVIO, L., CHANG, C.-D., RIZZO, M.-G., TRAVALI, S., CASADEVALL, C. and BASERGA, R., 1990, Importance of introns in the growth regulation of mRNA levels of the proliferating cell nuclear antigen gene. *Molecular and Cellular Biology*, **10,** 303–309.

OTTERLEI, M., WARBRICK, E., NAGELHUS, T. A., HAUG, T., SLUPPHAUG, G., AKBARI, M., AAS, P. A., STEINSBEKK, K., BAKKE, O. and KROKAN, H. E., 1999, Post-replicative base excision repair in replication foci. *EMBO Journal*, **18,** 3834–3844.

PASCUCCI, B., STUCKI, M., JÓNSSON, Z. O., DOGLIOTTI, E. and HÜBSCHER, U., 1999, Long patch base excision repair with purified human proteins. DNA ligase I as patch size mediator for DNA polymerases delta and epsilon. *Journal of Biological Chemistry*, **274,** 33 696–33 702.

RANGANNA, K, YATSU, F. M., HAYES, B. E., MILTON, S. G. and JAYAKUMAR, A., 2000, Butyrate inhibits proliferation-induced proliferating cell nuclear antigen expression (PCNA) in rat vascular smooth muscle cells. *Molecular and Cellular Biochemistry*, **205,** 149–161.

SAHA, P., CHEN, J., THOME, K. C., LAWLIS, S. J., HOU, Z. H., HENDRICKS, M., PARVIN, J. D. and DUTTA, A., 1998, Human CDC6/Cdc18 associates with Orc1 and cyclin-cdk and is selectively eliminated from the nucleus at the onset of S-phase. *Molecular and Cellular Biology*, **18,** 2758–2767.

SAVIO, M., STIVALA, L. A., BIANCHI, L., VANNINI, V. and PROSPERI, E., 1998, Involvement of the proliferating cell nuclear antigen (PCNA) in DNA repair induced by alkylating agents and oxidative damage in human fibroblasts. *Carcinogenesis*, **19,** 591–596.

SCOTT, M. T., MORRICE, N. and BALL, K. L., 2000, Reversible phosphorylation at the C-terminal regulatory domain of p21(Waf1/Cip1) modulates proliferating cell nuclear

antigen binding. *Journal of Biological Chemistry*, **275,** 11 529–11 537.

SENDLER, A., KAFFENBERGER, W., NUYKEN, I. and VAN BEUNINGEN, D., 1993, Proliferation kinetics and PCNA expression of HL-60 cells following ionizing irradiation and granulocytic differentiation. *Cell Proliferation*, **26,** 531–543.

SHIBAHARA, K. and STILLMAN, B., 1999, Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. *Cell*, **96,** 575–585.

SHIVAKUMAR, C. V., BROWN, D. R., DEB, S. and DEB, S. P., 1995, Wild-type human p53 transactivates the human proliferating cell nuclear antigen promoter. *Molecular and Cellular Biology*, **15,** 6785–6793.

SHIVJI, M. K., FERRARI, E., BALL, K., HUBSCHER, U. and WOOD, R. D., 1998, Resistance of human nucleotide excision repair synthesis *in vitro* to p21(Cdn1). *Oncogene*, **17,** 2827–2838.

SHIVJI, M. K. K., KENNY, M. K. and WOOD, R. D., 1992, Proliferating cell nuclear antigen is required for DNA excision repair. *Cell*, **69,** 367–374.

SKIBBENS, R. V., CORSON, L. B., KOSHLAND, D. and HIETER, P., 1999, Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. *Genes and Development*, **13,** 307–319.

SMITH, M. L., CHEN, I.-T., ZHAN, Q., BAE, I., CHEN, C.-Y., GILMER, T. M., KASTAN, M. B., O´CONNOR, P. M. and FORNACE, A. J., JR, 1994, Interaction of the p53-regulated protein Gadd 45 with proliferating cell nuclear antigen. *Science*, **266,** 1376–1380.

TAKAHASHI, T. and CAVINESS, V. S., JR, 1993, PCNA-binding to DNA at the G_1/S transition in proliferating cells of the developing cerebral wall. *Journal of Neurocytology*, **22,** 1096–1102.

TAKATA, M., SASAKI, M. S., SONODA, E., MORRISON, C., HASHIMOTO, M., UTSUMI, H., YAMAGUCHI-IWAI, Y., SHINOHARA, A. and TAKEDA, S., 1998, Homologous recombination and non homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO Journal*, **17,** 5497–5508.

TIAINEN, M., SPITKOVSKY, D., JANSEN-DÜRR, P., SACCHI, A. and CRESCENZI, M., 1996, Expression of E1A in terminally differentiated muscle cells reactivates the cell cycle and suppresses tissue-specific genes by separable mechanisms. *Molecular and Cellular Biology*, **16,** 5302–5312.

TOM, S., HENRICKSEN, L. A. and BAMBARA, R. A., 2000, Mechanism whereby proliferating cell nuclear antigen stimulates flap endonuclease 1. *Journal of Biological Chemistry*, **275,** 10498–10505.

TORRES-RAMOS, C. A., YODER, B. L., BURGERS, P. M., PRAKASH, S. and PRAKASH, L., 1996, Requirement of proliferating cell nuclear antigen in RAD6-dependent postreplicational DNA repair. *Proceedings of the National Academy of Sciences, USA*, **93,** 9676–9681.

TSUKAMOTO, Y. and IKEDA, H., 1998, Double-strand break repair mediated by DNA end-joining. *Genes Cells*, **3,** 135–144.

TSURIMOTO, T., 1999, PCNA binding proteins. *Frontiers of Biosciences*, **4,** D849–858.

TURKA, L. A., GRATIOT-DEANS, J., KEIM, D., BANDUKWALA, R., GREEN, J., STRAHLER, J. and HANASH, S. M., 1993, Elevated proliferating cell nuclear antigen levels in immature thymocytes: dissociation from cell cycle progression. *Journal of Immunology*, **150,** 2746–2752.

UBEDA, M. and HABENER, J. F., 1997, The large subunit of the DNA replication complex C (DSEB/RF-C140) cleaved and inactivated by caspase-3 (CPP32/YAMA) during Fas-induced apoptosis. *Journal of Biological Chemistry*, **272,** 19562–19568.

UMAR, A., BUERMEYER, A. B., SIMON, J. A., THOMAS, D. C., CLARK, A. B., LISKAY, R. M. and UNKEL, T. A., 1996, Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. *Cell*, **87,** 65–73.

VAIRAPANDI, M., AZAM, N., BALLIET, A. G., HOFFMAN, B., LIEBERMANN, D. A., 2000, Characterization of MyD118, Gadd45, and proliferating cell nuclear antigen (PCNA) interacting domains. PCNA impedes MyD118 and Gadd45-mediated negative growth control. *Journal of Biological Chemistry*, **275,** 16 810–16 819.

VAIRAPANDI, M., BALLIET, A. G., FORNACE, A. J., JR, HOFFMAN, B. and LIEBERMANN, D. A., 1996, The differentiation primary response gene MyD118, related to GADD45, encodes for a nuclear protein which interacts with PCNA and p21WAF1/CIP1. *Oncogene*, **12,** 2576–2594.

VELICKY, J., TITLBACH, M., LOJDA, Z., JELINEK, F., VOBECKY, M. and RASKA, I., 1997, Expression of the proliferating cell nuclear antigen (PCNA) in the rat thyroid gland after exposure to bromide. *Acta Histochemica*, **99,** 391–399.

VENCLOVAS, C., Thelen, M. P., 2000, Structure-based predictions of Rad1, Rad9, Hus1 and Rad17 participation in sliding clamp and clamp-loading complexes. *Nucleic Acids Research*, **28,** 2481–2493.

WALLACE, S. S., 1998, Enzymatic processing of radiation-induced free radical damage in DNA. *Radiation Research*, **150,** S60–S79.

WANG, G., TAPAS, K. H., MITRA, S., LEE, H.-M. and ENGLANDER, E. W., 2000, Mitochondrial DNA damage and a hypoxic response are induced by $CoCl_2$ in rat neuronal PC12 cells. *Nucleic Acids Research*, **28,** 2135–2140.

WARBRICK, E., 1998, PCNA binding through a conserved motif. *Bioessays*, **20,** 195–199.

WATANABE, H., PAN, Z. Q., SCHREIBER-AGUS, N., DEPINHO, R. A., HURWITZ, J. and XIONG, Y., 1998, Suppression of cell transformation by the cyclin-dependent kinase inhibitor p57KIP2 requires binding to proliferating cell nuclear antigen. *Proceedings of the National Academy of Sciences, USA*, **95,** 1392–1397.

WENZ, F., AZZAM, E. I. and LITTLE, J. B., 1998, The response of proliferating cell nuclear antigen to ionizing radiation in human lymphoblastoid cell lines is dependent on p53. *Radiation Research*, **149,** 32–40.

WINGENDER, E., CHEN, X., HEHL, R., KARAS, H., LIEBICH, I., MATYS, V., MEINHARDT, T., PRUSS, M., REUTER, I. and SCHACHERER, F., 2000, TRANSFAC: an integrated system for gene expression regulation. *Nucleic Acids Research*, **28,** 316–319.

WOLOSCHAK, G. E., CHANG-LIU, C. M., CHUNG, J. and LIBERTIN, C. R., 1996a, Expression of enhanced spontaneous and gamma-ray-induced apoptosis by lymphocytes of the wasted mouse. *International Journal of Radiation Biology*, **69,** 47–55.

WOLOSCHAK, G. E., PAUNESKU, T., LIBERTIN, C. R., CHANG-LIU, C. M., CHURCHILL, M., PANOZZO, J., GRDINA, D., GEMMELL, M. A. and GIOMETTI, C., 1996b, Regulation of thymus PCNA expression is altered in radiation-sensitive wasted mice. *Carcinogenesis*, **17,** 2357–2365.

WOOD, R. D., MITCHELL, M., SGOUROS, J. and LINDAHL, T., 2001, Human DNA repair genes. *Science*, **291,** 1284–1289.

XU, J. and MORRIS, G. F., 1999, p53-mediated regulation of proliferating cell nuclear antigen expression in cells exposed to ionizing radiation. *Molecular and Cellular Biology*, **19,** 12–20.

YAMAGUCHI, M., HAYASHI, Y., MATSUOKA, S., TAKAHASHI, T. and MATSUKAGE, A., 1994, Differential effect of p53 on the promoters of mouse DNA polymerase beta gene and proliferating-cell-nuclear-antigen gene. *European Journal of Biochemistry*, **221,** 227–237.

YU, C., TAKEDA, M. and SOLIVEN, B., 2000, Regulation of cell cycle proteins by TNF-alpha and TGF-beta in cells of oligodendroglial lineage. *Journal of Neuroimmunology*, **108,** 2–10.

ZHAN, Q., LORD, K. A., ALAMO, I. JR, HOLLANDER, M. C., CARRIER, F., RON, D., KOHN, K. W., HOFFMAN, B., LIEBERMANN, D. A. and FORNACE, A. J., JR, 1994, The gadd and MyD genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth. *Molecular and Cellular Biology*, **14,** 2361–2371.

ZHANG, G., GIBBS, E., KELMAN, Z., O´DONNELL, M. and HURWITZ, J., 1999a, Studies on the interactions between human replication factor C and human proliferating cell nuclear antigen. *Proceedings of the National Academy of Sciences, USA*, **96,** 1869–1874.

ZHANG, P., MO, J.-Y., PEREZ, A., LEON, A., LIU, L., MAZLOUM, N., XU, H. and LEE, M. Y. W. T., 1999b, Direct interaction of Proliferating Cell Nuclear Antigen with the p125 catalytic subunit of mammalian DNA Polymerase δ. *Journal of Biological Chemistry*, **274,** 26 647–26 653.

ZUBER, M. E., TAN, M. and RYOJI, M., 1989, Involvement of proliferating cell nuclear antigen (PCNA) in DNA replication in living cells. *Molecular and Cellular Biology*, **9,** 57–66.