

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

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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 G₁-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 <i>et al.</i> (1996); Chen <i>et al.</i> (1996a)
p57 (Kip2) protein from p21 family, i.e. CDK inhibitor, contains consensus PCNA binding peptide	cell cycle regulation	Watanabe <i>et al.</i> (1998)
CR6 (cytokine response gene 6)	cell cycle regulation	Azam <i>et al.</i> (2001)
GADD45	cell cycle regulation	Smith <i>et al.</i> (1994), Vairapandi <i>et al.</i> (2000)
MyD118 (GADD45 homolog)	cell cycle regulation	Vairapandi <i>et al.</i> (1996, 2000)
Ctf7p chromosome transmission fidelity; contains consensus PCNA binding peptide	chromatid cohesion (established in S-phase)	Skibbens <i>et al.</i> (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 <i>et al.</i> (1996)
Uracil DNA glycosylase (UNG); contains consensus PCNA binding peptide	BER	Otterlei <i>et al.</i> (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 <i>et al.</i> (1997), Warbrick (1998)
RPA (70 KDa subunit) single-strand DNA binding; contains consensus PCNA binding peptide	replication, NER	Dianov <i>et al.</i> (1999), Loor <i>et al.</i> (1997)
RFC 1, RFC 2, RFC 3 contain consensus PCNA-binding peptide	replication, BER, NER	Zhang <i>et al.</i> (1999a), Mossi <i>et al.</i> (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 <i>et al.</i> (1996b), Gary <i>et al.</i> (1997)
XPG 3'-incision endonuclease; contains consensus PCNA binding peptide a.a. 981–1009; particularly important is a.a. 992	NER	Gary <i>et al.</i> (1997), Miura <i>et al.</i> (1996a)
DNA pol δ (p125 subunit); contains consensus PCNA binding peptide	replication, BER, MMR	Zhang <i>et al.</i> (1999b), Kelman <i>et al.</i> (1999)
DNA pol ϵ ; contains consensus PCNA-binding peptide	NER, MMR	Kelman <i>et al.</i> (1999), Maga <i>et al.</i> (1999)
DNA ligase I contains consensus PCNA binding peptide	replication (processing Okazaki fragments), repair	Levin <i>et al.</i> (1997)
Werner syndrome helicase contains consensus PCNA binding peptide a.a. 168–246	repair	Lebel <i>et al.</i> (1999)

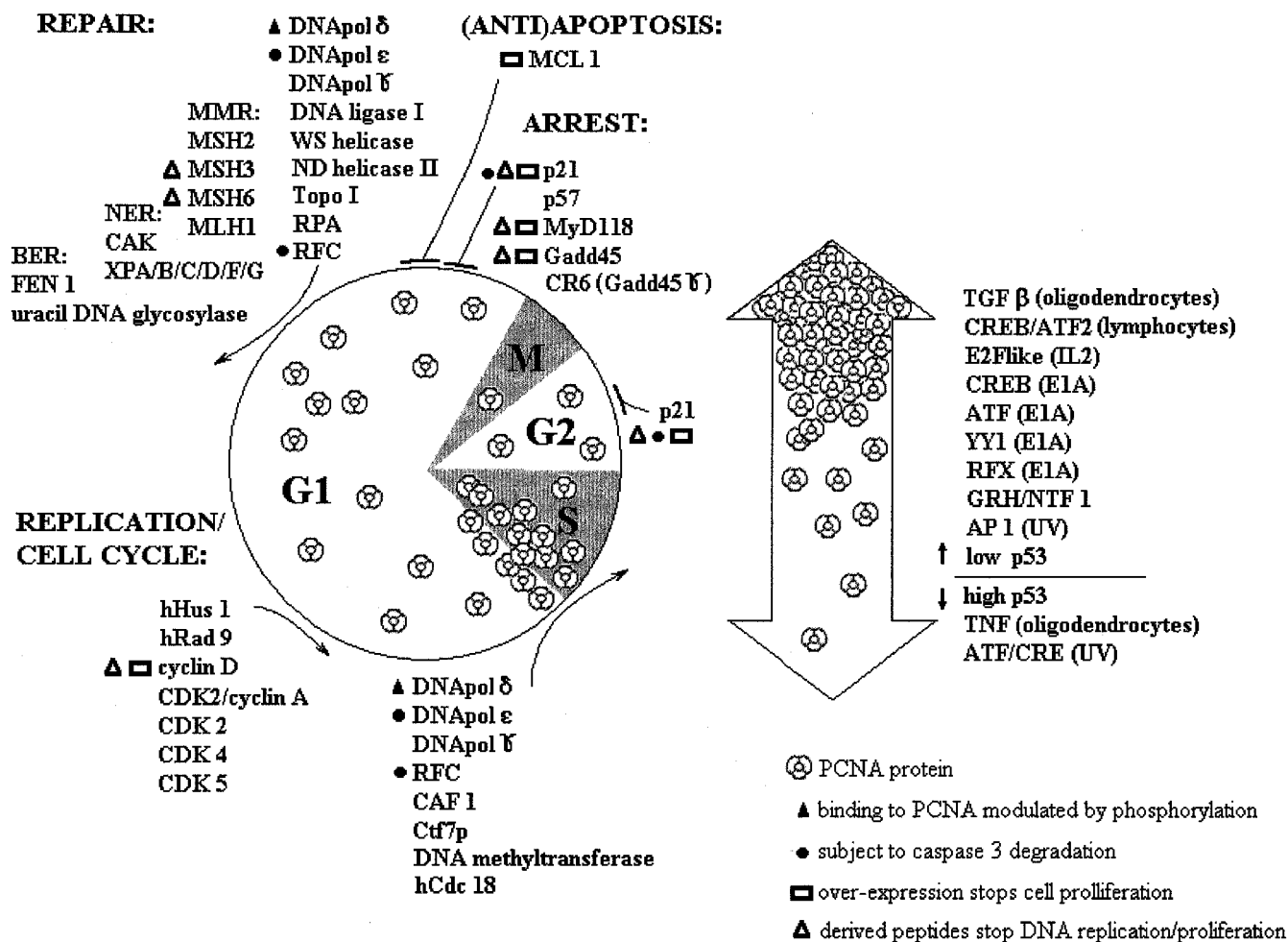


Figure 1. Representation of the numerous protein–protein interactions of PCNA and their roles in replication, repair and cell cycle regulation, accompanied with a brief list of factors controlling PCNA expression.

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 DIMYNLFDWPT
NP_032791.1	origin recognition complex, subunit 2 (Orc2)	LMWDHAK Q SLYN
NP_002543.1	origin recognition complex, subunit 4 (Orc4)	DLFAHHKN Q TLLYNLFD
NP_001245.1	cell division cycle 18 (hCdc18)	MDQLDSK Q QDVLYTLFEWP
NP_013365.1	meiosis-specific protein: chromosome synapsis and chiasmata formation	KKK Q QKKLT NF
CAA66705.1	RADHA protein (chromatin rearrangement)	EREY Q QFR Q NG MSPRY
P09884	DNA-directed DNA polymerase α	K Q DNLITD TQYYLAQQ
AAA17543.1	mitochondrial DNA polymerase	ETLYNVSD YPT
NP_035262.1	DNA polymerase ϵ	VTYNGD FFDWP
AAC98785.1	DNA polymerase zeta	NFADLNH SKRKL
NP_032025.1	flap structure specific endonuclease 1 (FEN1)	G S T Q R L DD FF K V TG S L S S A
NP_035859.1	XPG complementing protein (ERCC-5)	T Q L R IDS FF R L
NP_013928.1	XPA homolog (Rad14)	EEW Q R R EEG
NP_011098.1	XPD homolog (Rad3)	L D EV V W K HK
NP_031525.1	mouse ataxia telangiectasia homolog	T S ED F Y R SCY K IL
JC4019	mutS (<i>E. coli</i>) homolog 3 (MSH3)	L S R FF RSAG S LR S S
NP_035691.1	T:G mismatch specific thymine-DNA glycosylase	KEK Q E K IT D A F K V K R K V
AAC23702.1	BRCA2	I D S F Y K E A E K K
A57514	RNA helicase HEL117	Q T A L T G Y Q T K Q R K L
CAA58247.1	06-methylguanine DNA repair methyltransferase	K Q D T M Y D L
NP_013691.1	uracil DNA glycosylase (UNG)	R K R K Q T T IE D FF G T K K
NP_014647.1	ATP dependent DNA ligase II	K R K R V L IS D S F H Q N R K
NP_012035.1	U5 snRNP and spliceosome component (Prp8p [with prp17 causes G2 arrest])	TE P Q M V L F N I Y D
CAA39830.1	MRS3 protein (suppresses the mitochondrial RNA splicing defects)	Q SE G LA A F Y YS
CAA86151.1	meiosis-specific protein (HO1)	L D T K W S K F Q D L M
NP_033960.1	cyclin E2	D L S W A C S Q E V W Q N M
P38936	p21; CDN1; CIP1; WAF1	R K R R Q T S M T D F Y H S K R R L I F S
P02825	Hsp 70A major heat shock protein	Q S L L Q D FF H G K
AAC72232.1	Bcl-x (apoptosis regulation)	G V N W G R I V A FF S F G G A L
P36776	mitochondrial ATP-dependent protease	G S T Q Q K L C F Y G P P G
AAF21125.1	E3 ubiquitin ligase	Q Q G Q V I F Y H I P
NP_037260.1	carboxypeptidase	L E S F Y E R K
NP_000284.1	phosphorylase kinase β	K Q D D M T S F Y N T P
NP_001735.1	calcium/calmodulin-dependent protein kinase IV	G S N R D A L S D FF E V E S E L
NP_033461.1	serine/threonine kinase 22A	Q G D L L E F I K T R G A L
I48845	lymphocyte-specific protein tyrosine kinase	L D D FF T L T
NP_002820.1	protein tyrosine phosphatase (PTP-H1)	G V D Q Q L L D D D F H R V T
NP_002622.1	phosphogluconate dehydrogenase, decarboxylating (PGD)	L D D FF K
Q08499	cAMP dependent 3' 5'-cyclic phosphodiesterase	Q W T D R I M E E FF R Q G D R E R E
NP_005081.1	cytosolic phospholipase A2 β	S T A G R I A E F F
AAD27760.1	glycogen phosphorylase	L W S A K S P I D F N L
AAD37118.1	SH2-containing inositol phosphatase	S T Q L L L D S D F L K T G S
NP_012461.1	SMC3p chromosomal ATPase	K E L W R K E Q K L Q T V L
NP_006309.1	glioblastoma cell differentiation-related protein	Q T N M R D F Q T E L R K I L V S
AAB58975.1	neurofibromin (neurofibromatosis type 1 tumour suppressor)	L A L H R L L W T H Q E K I G D Y L S S R
NP_0.11318.1	negative regulator of early meiotic genes (MCK1 dosage suppressor 3)	R R S N T L T D Y M H S N K A S P F S
P41139	ID-4 transcription factor for myogenesis, neurogenesis and haematopoiesis	Q C D M N D C Y S R L R R L V
AAD40474.1	doublesex and mab-3 related transcription factor 1	Q G R A G G F G K A S G A L V G A
NP_002606.1	pigment epithelial-differentiating factor	R K T S L E D F Y L D E E R T V
P42284	LOLA longitudinals lacking protein (regulator of axon-target interaction)	L R W N N H Q S T L I S V F D
CAA67753.1	fertilin	Q P R L D P FF K Q Q A V C S N A
CAB08076.1	tenascin-R (restrictin, janusin) (development of astrocytes)	Q R R Q NG Q T D F F
P17789	TTKB tramtrak protein (developmental transcription factor)	L R W N N H Q S N L L S V F D
BAA84069.1	flamingo (cell polarity gene)	R Q G V L Y I F D
AAF02618.1	starry night (tissue polarity gene)	R Q G V L Y I F D

Table 2. (Continued).

Protein ID	Protein (human, mouse, rat, fruit fly or yeast)	Possible PCNA binding sequence
P48679	lamin A	QQSRIRIDS
CAB63111.1	dysferlin (mutations cause myopathy/muscular dystrophy)	QIRIKLWFGLSVDEKE
2203411A	reeler gene (mutants show ataxic gait and trembling)	RQHGLRRHFYNNRRRR
AAC28409.1	small optic lobes (SOL1) (with calpain-like domain)	REGMTAYY
NP_032058.1	fragile X2 homolog (FMR2)	QTRLEDF
CAA76528.1	gap junction protein connexin36	RRQEGISRFF
AAB39720.1	presynaptic protein munc13-3	SDRELWQRKQEGMTALYHSP
P98159	nudel protein precursor, serine protease	ERQLWLKKEFE
NP_013488.1	killer toxin sensitivity	SEKQNVYINNY
P50284	lymphotoxin-beta receptor	SDRKAECRCQPGMSCVY
P51787	voltage dependent K ⁺ channel	THVQGRVYNFLERPT
A39402	potassium channel protein IIIA form 1, shaker-type	WRKLRQPRMWFALFEDP
AAF32521.1	anti-vesicular stomatitis virus single-chain Fv antibody fragment 0B1	GLTAYYRSP
NP_004289.1	nucleoporin 155kD (NUP155)	QEQLKITTFKDLVIRDKE
P16036	mitochondrial phosphate transport protein	KEEGLNAFY
CAB66155.1	sugar transporter	QIESFFRMGRR
NP_012033.1	glucose-6-phosphate dehydrogenase	KQNIMHEIFD
NP_01053.1	galactosyl-transferase	VWEHREQDALEALYEN
NP_005065.1	sodium phosphate transport protein 1	GNLAGQLSDFLTRNLS
BAA11374.1	Orn decarboxylase antizyme	EEQLRADHVIFCFHKNRED
AAD32244.1	microtubule-actin crosslinking factor	QDTLQAMFDW
AAC27437.1	brush border myosin	RKSQJLISSWFRGNMQKK
NP_006748.1	troponin	ELQALIDSHFEARKKEEEE
NP_035927.1	carbonic anhydrase 14 (CA XIV)	QLEQFFRYNGSLTT
NP_013060.1	peripheral vacuolar membrane protein complex	GMTVFFYH
AAA51242.1	T-cell receptor alpha	RLITLFFYLAQGTKEN
AAA52139.1	cytochrome P-450-1	RDTSLKGFYIPKGRCVF
AAC64595.1	olfactory receptor	QDKMYSLF
AAD13333.1	olfactory receptor H12	STQGRIKAF

** 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 G₁ 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

HUMAN PCNA PROMOTER

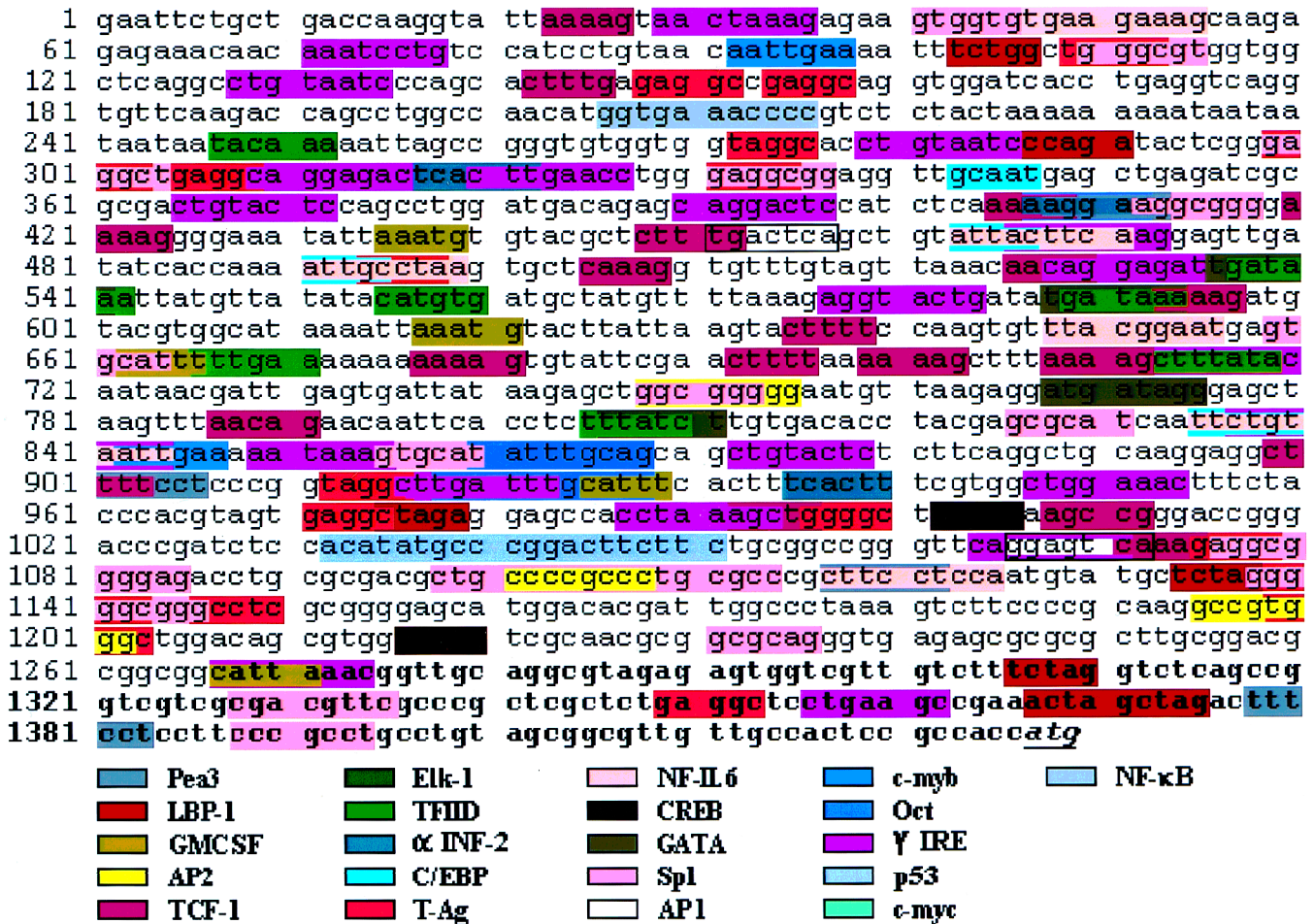


Figure 2. Map of the human PCNA promoter with putative binding sites for transcription factors relevant to radiobiology. Data were derived from the NIH Transfac database (<ftp://ncbi.nlm.nih.gov/repository/TFD/datasets.tfsites.sig.scan>; Wingender *et al.* 2000), except the p53 site (Morris *et al.* 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 E1A 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, Hajhosseini *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 *Mhl1* (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 dimers 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 excises 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 G₁- and G₂-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 G₁- or G₂-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 than 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.

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