DNA break repair: refined rules of an already complicated game

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Abstract: Of the many types of DNA-damage repair, this review concentrates on the aspects of DNA single- and double-strand break repair. Originally considered to represent separate routes based on distinct enzymatic machineries, it has recently been shown that these pathways converge and are interlinked at a number of points. Poly(ADP-ribose) polymerase-1 (PARP-1) is a central player in this complicated game. We present new data and our view on the mechanisms by which PARP-1 is guided to its respective interaction partners to coordinate or participate in repair or apoptosis.

Key words: DNA strand break repair (DSBR), non-homologous end joining (NHEJ), nuclear architecture, nuclear matrix, PARP-1.

Introduction and scope

DNA molecules can be damaged in many ways: spontaneous damage due to replication errors, deamination, depurination, and oxidation occurs, in addition to influences of external forces, such as radiation and environmental chemicals. Because repair systems must be able to recognize and deal with each type of damage, it is not surprising that there is a large number of genes — 130 have been identified in the human genome — participating in DNA repair.

The eukaryotic cell has at least 7 major systems for restoring structural integrity: (i) They directly repair effects such as O6-alkyl guanine formation, which can be fixed by transferring the alkyl group to a specific cysteine residue in the active center of O6-alkyltransferase, using a suicide mechanism; (ii) They use homologous recombination to repair double-strand breaks (DSBs); (iii) They use nonhomologous end-joining (NHEJ) to repair DSBs, a route that involves the DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing group 4 protein (XRCC4), and ligase IV (Fig. 1, route 2b); (iv) They use the novel activity of poly(ADP-ribosyl) polymerase-1 (PARP-1) to repair DSBs (Fig. 1, route 2c merging with 3); (v) They use the PARP-1, XRCC1, and ligase III route to perform single-strand break (SSB) repair or base-excision repair (Fig. 1, route 3) (Caldecott 2001); (vi) They use nucleotide-excision repair to fix products with bulky substituents, such as the pyrimidine dimers that arise from UV irradiation or the products of environmental mutagens (deamination reactions); and (vii) They use mismatch repair, which is the correction of mismatched nucleotides and small loops as they occur during replication, using mechanisms such as the deamination of 5-methyl cytosine to yield thymidine. Nucleases, including a 5′–3′ exonuclease and a Flap endonuclease, contribute to mismatch repair. In addition, several standard replication proteins are needed, such as the clamp protein PCNA (proliferating cell nuclear antigen) (Fig. 1, route 3); replication factor C, which loads PCNA onto DNA; DNA polymerase δ (Pol δ), which is held...
in place by PCNA and a single-stranded DNA-binding protein (RPA). The PARP-1–XRCC1–ligase III route can take over when an appropriate DNA-N-glycosylase is available. If DNA damage cannot be repaired, the decision may be made to enter apoptosis. This review will concentrate on the mechanisms that are activated in the cell in response to DNA-strand breaks, which are triggered, for instance, by ionizing irradiation. Originally, the DNA-PK–XRCC4–ligase IV and the PARP-1–XRCC1–ligase III routes (Fig. 1, routes 2b and 3), were thought to act independently, because the basic steps — break detection, bringing together the broken ends (synapsis), and ligation — contain distinct components (Table 1).

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It is intriguing to note that evidence is now accumulating (Audebert et al. 2004) to show that even these routes are interconnected, through, for instance, in an alternative route for DSB-NHEJ repair (Table 1; Fig. 1, route 2c merging with 3). These facts and the cooperation of these pathways in decisions concerning repair or apoptosis will be major aspects in this review.

Table 1. Major routes and players in DNA single- and double-strand repair.

<table>
<thead>
<tr>
<th>Step</th>
<th>SSBR (3)</th>
<th>DSBR (NHEJ; 2b)</th>
<th>DSBR (alternative route 2c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Break detection</td>
<td>PARP-1</td>
<td>Ku antigen(s)</td>
<td>PARP-1</td>
</tr>
<tr>
<td>Synapsis</td>
<td>PARP-1 dimer</td>
<td>Ku dimer (Ku70/-86)</td>
<td>PARP-1 dimer</td>
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<tr>
<td>End processing</td>
<td>PNK</td>
<td>DNA-PK, Artemis, PNK</td>
<td></td>
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<tr>
<td>Gap filling</td>
<td>PCNA × Pol δ or Pol β alone</td>
<td>Pol λ(?) , Pol μ(?)</td>
<td></td>
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<tr>
<td>Ligation</td>
<td>XRCC1 × Lig III</td>
<td>XRCC4 × Lig IV</td>
<td>XRCC1 × Lig III</td>
</tr>
</tbody>
</table>

Note: SSBR, single-strand break repair; DSBR, double-strand break repair; NHEJ, nonhomologous end-joining; PARP-1, poly(ADP-ribose) polymerase-1; PNK, polynucleotide kinase; DNA-PK, DNA-dependent protein kinase; PCNA, proliferating cell nuclear antigen; Pol, polymerase.

The playground

The question of how the interior of the nucleus is organized has occupied generations of biologists. Major milestones were the first report about the existence of a nuclear matrix by Berezney and Coffey (1977), and the finding that such an entity consists of 3 major components: the nuclear lamina/lamins; an inner fibrogranular network; and residual nucleoli.

The development of high-resolution microscopic approaches, which can be used to observe domains of genomic organization and function (chromatin domains), has provided data that supports the existence of such an entity. This entity now appears as a dynamic structure that can adapt both its composition and DNA attachment points to provide temporal and spatial control of functional complexes in gene expression, replication, and repair (Stein et al. 2003). Attachment points have been characterized as specific DNA elements, so-called scaffold–matrix attachment regions (S/MARs), that are used to support the expression of a given cell type (Bode et al.
Recently halo-FISH strategies have been developed, which clearly demonstrate the existence of this dynamic and cell-specific architecture (Heng et al. 2004). Although virtually all methods used today rely on some extraction steps for matrix isolation, halo-FISH circumvents nuclelease treatments and involves the fixation of DNA loops as they emerge from the nuclear remnant, i.e., the nuclear matrix.

Although the concept of the nuclear matrix has matured considerably, until today it has received little support from those working with established FISH techniques. These methods have unraveled important aspects of nuclear architecture, most notably the fact that chromosomes occupy distinct territories and have preferred nuclear locations during interphase. Three nuclear compartments have been characterized: a higher-order open compartment with active chromatin domains; a closed chromatin compartment made up of inactive genes; and an interchromatin-domain compartment, containing macromolecular complexes for transcription, splicing, DNA replication, and repair. The interchromatin domain compartment is the equivalent of an in vivo nuclear matrix, and a number of present efforts have been applied to firmly establish such a correlation (Cremer and Cremer 2001).

A study by Ma et al. (1999) has lent considerable support to a function of the nuclear matrix in organizing chromosome territories. When cells were treated in situ with certain classic extraction procedures, and the process was followed by chromosome painting, territories remained intact up to the point where a small subset of acidic nuclear matrix proteins was released. Other studies have supported the role of a nuclear skeleton or matrix in immobilizing the sites of DNA replication (called replication factories) or transcription (transcription factories); the synthetic apparatus of such a matrix remains in a fixed position while the DNA is reeled through it (Cook 2002).

A report by Nelms et al. (1998) indicates that this relation may be somewhat different for processes of DNA repair; in the early stages, DSBs are found in a fixed position and repair proteins (exemplified by Mre11) become recruited to the site of damage within 30 min. Mre11 is an abundant small protein with 3′ to 5′ exonuclease activity and functions in telomere maintenance and DNA repair. Future studies will determine if it can serve as a marker for other well-known repair proteins that are recruited to the site of damage. If this is the case, we have to anticipate that a repairosome is divided into early components, which immediately recognize and fix the damaged site, and late components, which are subsequently recruited to perform the individual repair steps. Such a principle will be considered throughout this review, and it will lead to the model illustrated in Fig. 1, which combines the traditional views of the repair process with more recent findings. Evidence will be summarized in accordance with the structure of the nuclear matrix by which all the players are organized, both in a spatial and a temporal way; this matrix serves as the playground.

The players

PARP-1–XRCC1–ligase III: the SSB repair team (route 3)

DNA SSBs are among the most frequent DNA lesions; they are produced during DNA metabolism or by reactive oxygen species, mutagens, and, in particular, certain forms of radiation. Most studies have implicated poly(ADP-ribose) polymerase-1 ((ADP-ribosyl) transferase (NAD⁺-poly (ADP-ribose) polymerase)-1), mostly abbreviated PARP-1, in recognition of nicks by specific Zn-fingers motif (Masson et al. 1998), and suggested that the enzyme is a major determinant in the repair of SSBs but not of DSBs (Noël et al. 2003). PARP-1 is also an established member of the base-excision repair (BER) multienzyme complex, where it recognizes and temporally protects the DNA sites to be repaired.

PARP-1 is an abundant protein that, after binding to SSBs, synthesizes chains of poly(ADP-ribose) (PAR) on itself (aut.modification; Desmarais et al. 1991) and also on other target proteins (heteromodification, Rolli et al. 2000). In the auto.modification reaction, and probably in general, the enzyme functions as a dimer, whereby 2 PARP-1 molecules interact for the mutual transfer of PAR-units to an acceptor site on the neighboring molecule, which consists of 28 glutamic acid side chains (Desmarais et al. 1991). PARP-1 catalyzes its automodification with multiple, variably sized ADP-ribose polymers, which may contain up to 200 residues and several branching points. This process enables or alters interaction with partners and, at high degrees of modification, electrostatic repulsion may result in the dissociation of the complex from DNA. These events can be reversed by either poly (ADP-ribose) glycohydrolase (PARG) or by (ADP-ribosyl) lyase. PARG performs both endo- and exoglycosidase activity; it can directly hydrolyze the linkage to the glutamic acid residue and it is responsible for the short half-life of poly(ADP-ribose) under conditions of DNA breakage (Buerkle 2001).

Moderate levels of PARP-1 automodification help the recruitment of other proteins to the break, where they may be modified, in turn, to modulate their activity. The protein-interaction network arising here is a highly complex one that involves BRCT modules. Named after a breast cancer susceptibility protein C-terminal domain, these modules contain 90–100 amino acids and are widespread in DNA-repair and DNA damage-responsive cell-cycle checkpoint proteins (Lee et al. 2000). Important facets of the resulting network of interacting partners have been outlined by Masson et al. (1998, Fig. 7). The fact that the BRCT-module of PARP-1 overlaps the automodification domain indicates a crossover between 2 modes of interaction. Members of the nick-repair team that may be involved in PAR-mediated interactions are XRCC1, p21cip1/waf1 (p21), and DNA ligase III. Members of the DSB-repair team are also found in this group: the catalytic subunit of DNA-PK; and a component of the DSB-sensor system (Ku70) and DNA polymerase ε (Pol ε), an enzyme with largely unknown implications in DNA replication, DNA repair, and cell-cycle control (Pleschke et al. 2000).

Depending on the presence of PAR chains close to SSBs, XRCC1 is recruited very rapidly and efficiently from distant sites throughout the nucleus (including the nucleolus). XRCC1 has no catalytic activity itself, but it plays a central role as an assembly platform for components involved in the repair of SSBs through at least 3 interacting domains: an N-terminal domain and 2 BRTC modules (BRCT1 and 2, respectively; Masson et al. 1998). While the central BRCT1 domain contributes to the interaction with PARP-1, the BRCT2 module close to the C terminus accommodates DNA ligase III. Other XRCC1 interaction partners that have been established by
biochemical studies are components responsible for DNA-end processing (polynucleotide kinase, PNK) and gap-filling (Pol δ; discussed below), which precede action of the ligase. Still other components are AP endonuclease (an endonuclease involved in nucleotide-excision repair and an activated PARP-variant, PARP-2, which is a component of the BER (Fig. 1, route 3) (Schreiber et al. 2002).

With 2 relevant enzyme activities, PNK makes important contributions to end processing: in its kinase reaction, it transfers phosphate from ATP to 5′-OH groups; and in a phosphatase reaction, it removes 3′-phosphate groups. The consequence of these actions is the conversion of ends that are initially nonligatable to 5′-phosphate/3′-OH nicks that can be further processed. Finally, ligase III, like PARP-1, contains a Zn-finger domain that can serve as a molecular DNA-nick-sensor.

**Pol δ–PCNA pathway**

Before a ligation reaction can take place, the gap has to be filled by a polymerase, either Pol δ × PCNA or Pol β (Table 1). By itself, DNA Pol δ would dissociate from DNA after synthesis of only a few nucleotides, and would have to rebind to continue. However, when coupled with a sliding clamp, it becomes rapid and highly processive. PCNA, the clamp specific for eukaryotes, is loaded onto DNA through the action of replication factor C, where it provides a scaffold for the consecutive attachment of numerous factors, including nucleases, polymerases (DNA Pol β and Pol ε), DNA ligase I, topoisomerases, and even PARP-1 (Paunesku et al. 2001).

PCNA, the “ringmaster of the genome” (Paunesku et al. 2001), is a trimer, and each subunit consists of 2 domains. An interdomain connecting loop permits a variety of interactions. During repair, association with PARP-1 may be among the first steps in shutting off its enzymatic activity. Studies in vivo have shown that the association between PCNA and PARP-1 increases under these conditions (Prosperi and Scovassi 2004). Tethering Pol δ to the complex is a prerequisite of rapid and processive DNA synthesis, which is under the control of the cell-cycle checkpoint processive protein p21NAP1/CIP1. Interestingly, p21 stops replication but not repair. It appears that this factor is either accommodated by the interdomain loop in place of the polymerase, or it leaves the scene with PARP-1, after which DNA synthesis is allowed to proceed (Prosperi and Scovassi 2004). In essence, this means that p21 and PARP-1 will compete with Pol δ for a binding site on PCNA. If concentrations of p21 or PARP-1 are high, the polymerase will be stalled. On the other hand, if concentrations of p21 and PARP-1 are high, these partners will form an independent binary complex and clear the binding site of PCNA to permit the participation of Pol δ in the repair process.

**Pol β pathway**

In addition to the PCNA route, SSBs can also be processed in a DNA Pol β-dependent manner. The N-terminal domain of XRCC1 binds to Pol β and there is evidence that PARP-1 can likewise interact with Pol β (Caldecott et al. 1996). The decision between pathways Pol δ–PCNA and Pol β is thought to be damage- and (or) dose-dependent. In contrast to Pol δ, Pol β is a rather processive enzyme, and does not need a clamp like PCNA.

Taken together, these data suggest that the activity of the PARP-1, XRCC1, PNK, polymerase, and ligase III proteins is coordinated in human cells within a single multiprotein complex, the so-called repairosome. PARP-1 may have additional functions in modulating chromatin structure, such as inhibiting recombination and generating ATP for DNA ligation.

**PARP-1 and the nuclear matrix**

Most published studies have been devoted to the function of PARP-1 in cells responding to DNA damage, but little is known about the enzyme’s subnuclear distribution under normal physiological conditions. There have been indications that a major fraction of the enzyme is associated with the nuclear matrix (Cardenas-Corona et al. 1987; Alvarez-Gonzales and Ringer 1988; Quesada et al. 1994, 2000). We recently confirmed and extended these findings with in vivo crosslinking studies, coimmunoprecipitation experiments, and an examination of protein–protein interactions in a 2-component system (Vidaković et al. 2004, 2005). These experiments clearly showed that a significant portion of nuclear matrix-associated PARP-1 physically interacts with lamin B in the absence of DNA damage, when the enzyme has only basal activity. Using the cis-dichlorodiammineplatinum (II) crosslinking procedure established by Ferraro et al. (1996), we demonstrated that both of the established nuclear matrix constituents are accommodated by S/MAR sequences in vivo. S/MAR binding properties for both components had been shown before: Ludérus et al. (1994) demonstrated 2 S/MAR-binding modes for the lamins: DNA single-strand binding and association with the minor groove. PARP-1, besides its binding to DNA breaks, has been shown to be accommodated by S/MARs (Sastry and Kun 1990; Galande and Kohwi-Shigematsu 1999; Soldatenkov et al. 2002). This property has been ascribed to the pronounced strand-unpairing potential of S/MAR sequences (Bode et al. 1992) and it provides a way for PARP-1 to be stored before its activity is requested. Recent work from our laboratory has confirmed and extended these conclusions, using in situ methods that preserve the nuclear architecture: the halo-FISH procedure and confocal microscopy to inspect a frozen in vivo state (Vidaković 2005). The basal state — PARP-1 associated with lamin B on S/MAR sequences — was found to change significantly upon DNA damage, DNA-repair, and apoptosis. Low doses of irradiation were sufficient to release PARP-1 from lamin and, at the same time, PCNA/p21 and p53 were recruited to the nuclear matrix — possibly as new PARP-1-binding partners (Vidaković 2005).

Work to come will have to address the precise mechanism by which PARP-1 interacts with the lamins. Two options have been discussed before (Vidaković et al. 2004). According to the first, the interaction is driven by the formation of a coiled-coil interaction, which involves the α-helical regions of both partner proteins. Consisting of beta-alpha-beta units, the BRCT domain of PARP-1 may participate in such an interaction. Although this model may be valid for the basic state of PARP-1, the alternative, a poly(ADP-ribosyl)ation-driven interaction, would require PARP-1 activation. We have shown that this alternative cannot be effective above a cer-
tain degree of modification because PARP-1 will be released from the lamina (Vidaković et al. 2004, 2005). Related questions have to be answered for nuclear matrix proteins that are known to associate with PARP-1, for instance SAF-A and topoisomerase(s) (Fig. 1, inset).

DNA-PK/Ku–ligase IV–XRCC4: the DSBR repair team (route 2b)

A DNA DSB is formed when 2 or more breaks occur on opposite strands of DNA within about 10 to 20 base pairs of each other. Although this kind of damage arises from ionizing radiation, DSBs are also normal intermediates in several cellular recombination processes, including meiotic recombination, mating type switching in yeast, and antigen receptor gene rearrangement (V(D)J recombination). Both yeast and mammalian cells have homologous recombinational and NHEJ pathways for repairing DSBs. The mechanism of DSBR repair by homologous recombination is only partially understood and, apparently, of minor relevance in higher eukaryotes where NHEJ is clearly preferred. Although there is always the risk that NHEJ might lead to the random joining of any 2 ends, this is usually prevented through an intricate multistage process. Each step requires a different DNA repair protein, and successful repair demands that all of these proteins work correctly and in the proper order.

For these reasons, the repair of DSBs requires particular measures to recognize the damage and to bring the exposed ends together for ligation. This is the particular responsibility of a protein called Ku, a heterodimer of subunits Ku70 and Ku86. This dimer is a DNA-binding protein with ATPase and possible helicase activity and, in mammalian nuclei, it is so abundant that any DSB is, on average, only about 5 molecular diameters away from the nearest Ku molecule. Binding of Ku, therefore, is unlikely to be the rate-limiting step in repair by NHEJ.

After its binding, Ku recruits DNA-PK. DNA-PK is a ubiquitous enzyme that binds to double-stranded DNA and, consequently, it phosphorylates specific substrates, including Ku. Besides Ku, the complete complex accommodates the catalytic subunit (sometimes called DNA-PKcs), which, according to a model by DiBiase et al. (2000), is a component of the nuclear matrix that is positioned close to other components of the NHEJ complex. Before the ligation of free DNA ends can take place, both the 5′ and 3′ termini of each of the DNA strands must be processed to become a proper ligation substrate (a simple nick with 3′-OH and 5′-phosphate). This is achieved during the next steps, during which XRCC4, a 38-kDa nuclear phosphoprotein, is accommodated. XRCC4, in turn, recruits PNK, a factor called Artemis (Ma et al. 2002), and a Pol β-like polymerase (Pol λ or Pol μ) to assist in end processing. After its association, DNA ligase IV activity is stimulated, leading to the ligation of the 2 strands. Most DNA DSBs can also be fixed in the absence of DNA-PK, although the kinetics for this process are slow. Available data indicate that DNA-PK accelerates repair for a large fraction (80%) of radiation-induced DNA DSBs. For this fraction, the process is accelerated 33-fold, requiring only 12 min instead of 12 h.

XRCC4 resembles XRCC1 in the sense that both factors recruit DNA ligases and processing enzymes, such as PNK, to break sites. In vitro XRCC4 binds only weakly to DNA, but binding is apparently modulated after its modification by the catalytic subunit of DNA-PK. Another component, Artemis, is, by itself, a 5′−3′ exonuclease that nibbles single-stranded DNA from the 5′ end. After modification by DNA-PK, however, Artemis becomes an endonuclease, with a preference for cleaving within DNA at single-stranded–double-stranded DNA junctions, permitting it to cut away single-stranded overhangs and, where present, to open hairpins. This rather recent addition to the team seems to function as a genomic caretaker; it suppresses genomic instability in humans, in particular, preventing translocations and telomeric fusions (Ma et al. 2002).

DNA ligase IV plays an important role in the repair of DSBs, similar to the role DNA ligase III plays in SSB repair and BER; it is also involved in V(D)J recombination. All major human DNA ligases (ligases I, III, and IV) employ the same basic pathway for DNA ligation. The mechanism linking a ligase to a particular process is probably the result of accessory proteins that support their function. Hence, DNA ligase I forms a complex with PCNA, whereas the 103-kDa DNA ligase III and the 96-kDa ligase IV become the partners of XRCC1 or XRCC4, respectively, as mentioned above.

Together they are strong: updated rules for the game

Clearly, the efficient repair of DNA DSBs is critical for the maintenance of genomic integrity. Although the most prominent NHEJ process relies on the DNA-PK and the XRCC4–DNA ligase IV complex, several in vitro and in vivo results have indicated that mammalian cells use more than a single end-joining mechanism.

An ever-increasing amount of evidence indicates that PARP-1 is a candidate for this role. PARP-1 is activated in vitro not only by SSBs but also by DSBs, and it binds to DSBs with an even higher affinity. The inhibition of PARP-1 activity impairs DSBR-rejoining, as does the overexpression of its catalytically inactive DNA-binding domain. XRCC1-deficient cell lines display a significant defect in the rejoicing of radiation-induced DNA DSBs.

There are also major cross-interactions between the components, which have previously been considered to be typical for either SSB repair or NHEJ. PARP-1 and Ku form a complex even in the absence of DNA and the complex, and PARP-1 and Ku individually, are able to bind to S/MARs in a DNA end-independent manner, at least in certain cell types (Galande and Kohwi-Shigematsu 1999; Mauldin et al. 2002). PARP-1 has also been shown to interact with all subunits of the DNA-PK complex and to catalyze their poly(ADP-ribosyl)ation. In addition, the protein kinase activity of DNA-PK can be stimulated by PARP-1 in the presence of NAD+, independent of the Ku70/86 complex (Russetti et al. 1998).

Recently, a DSBR end-joining activity, independent of the DNA-PK/XRCC4–ligase IV complex, has been detected; it revealed novel synopsis activity by PARP-1 and ligation activity by the XRCC1–DNA ligase III complex — proteins otherwise involved in SSB repair and BER pathways. As with repair, XRCC1–DNA ligase III recruitment may be favored at low levels of PARP-1 automodification, whereas at higher levels, the XRCC1–DNA ligase III complex dissociates from DNA, and synopsis activity becomes impaired. Taken together, these results strongly suggest that such a mechanism could act as a route of DSBs repair that comple-
ments the DSB repair team (McElhinny et al. 2000) and become useful when NHEJ becomes saturated or inefficient at a subset of DSBs.

So far, in the context of DSBs, PARP-1 has mainly been associated with an antirecombinogenic function, in the sense that its binding to DNA might transiently prevent the inappropriate initiation of recombination. It now appears that the antirecombinogenic activity of PARP-1 is a prerequisite of its synapsis function; it helps prevent the formation of free DSBs intermediates (Sukhanova et al. 2004).

**The refined rules: a model**

Conventional and new facets of DNA strand break repair have been summarized in Fig. 1. Under basal conditions, in the context of SSB repair, PARP-1 is connected to lamin B (route 3; Vidaković et al. 2004). This association concerns the lamina shell, but may also involve an internal network of lamins (Goldman et al. 2002). As mentioned above, lamins have 2 binding modes that contribute to their association with S/MAR sequences (Ludérs et al. 1994): an association with the minor groove of DNA in heterochromatic areas; and the binding to single strands in transcriptionally potentiated regions. This binding mode may also help PARP-1 to recognize single-strand lesions and to open local chromatin structures.

Because lamins are PARP-1 substrates themselves, these processes might be fine-tuned in a combinatorial way by different degrees of poly(ADP-ribosylation). This means that an extensive modification could conceivably support the detachment of PARP-1 from its binding partners (lamins and DNA) and enable its engagement in other interactions. A PARP-1 partner of particular relevance is PCNA (Frouin et al. 2003), which is a nuclear-matrix-associated protein itself (Gerner and Sauermann 1999; Ivanović-Matić et al. 2000). Today, it appears that PARP-1 operates, in multiple ways, at the interface of chromatin decondensation and transcriptional activation (Althaus 2005). Not only does the poly (ADP-ribosylation) of substrates such as histone H1, the core histones, and high-mobility group proteins initiate chromatin opening (Althaus et al. 1994), it also initiates other activating principles. The latest of these is DNA methyl transferase 1, which is captured and inactivated by the long polymer arms of PARP-1 (Reale et al. 2005).

The well-established facts about DSB repair by NHEJ have been incorporated into route 2b of Fig. 1. This pathway can effectively be supported by the alternative PARP-1-dependent route (Fig. 1, route 2c) (Audebert et al. 2004). These results are nicely supported by the outcome of a model experiment that is reproduced in the inset to Fig. 1: in their efforts to unravel the structural requirements for DNA-PK- and Ku-antigen-binding to DNA ends, Mauldin et al. (2002) performed electromobility-shift assay studies, using an unlabelled circular plasmid as a competitor, in order to capture non-end-specific DNA-binding proteins. They unexpectedly found that in these shift experiments, the presence of the circular plasmid was responsible for the formation of a high-molecular-weight complex, which included DNA-PK and Ku. In accordance with models illustrating the involvement of S/MARs in DNA repair, they subsequently performed a parallel series of experiments, using a circular S/MAR-containing plasmid, which, in fact, forcefully supported complex formation. After careful analysis of the protein components (Fig. 1), these observations were interpreted as follows: the factors either associated with the linear segment (i.e., the model of a damaged DNA loop) or they had been preassembled on the circular supercoiled S/MAR-plasmid (a model of S/MAR-matrix attachment points). Together, all data reveal a new activity for the DNA–PK/Ku complex: it can guide DNA ends to the sites where repair becomes possible these sites include components of routes 2b and 3 in Fig. 1). For a full appreciation of these results, it should be kept in mind that S/MARs are regions that are primed for base-unpairing, which comes into effect under negative superhelical tension (i.e., at the linking number of a plasmid; Bode et al. 1992). In Fig. 1, this property is symbolized with DNA bubbles.

A final note concerns the residence of major repair components at S/MAR-type DNA sequences (Boulikas et al. 1996; Koehler and Hanawalt 1996; Mauldin et al. 2002; Goldman et al. 2002). This situation could well be a strategic advantage because S/MARs represent genomic sites that are particularly sensitive to irradiation (Fernandes and Catapano 1995).
The referee's responsibility: repair or apoptosis

The change of binding partners — PARP-1 leaves the lamins (lamin B) to meet PCNA — can be traced by confocal microscopy. Figures 2 and 3 show the aspects of these events that occur between 5 and 75 Gy doses of irradiation, i.e., under conditions that induce DNA single- and double-strand breaks (Bradley and Dysart 1985). Figure 2A illustrates that the lower dose forcefully activates PARP-1 as part of a nuclear matrix preparation; this process becomes impaired at the higher dose. These measurements were accompanied by immunoblot analyses, using an anti-PARP-1 antibody that reflected the apoptotic degradation of the enzyme (Germain et al. 1999) at 75 Gy (Fig. 2B). Confocal analyses clearly indicate that an interaction with PCNA (Fig. 1, routes 3 and 2c) occurring at 5 Gy becomes limited by PARP-1 degradation, which in turn is accompanied by its appearance in the nucleolus. The nucleolus is considered the primary site of an apoptosis-dependent action of caspase 3 (Desnoyers et al. 1996; Alvarez-Gonzalez et al. 1999).

According to current models, PCNA is the major factor deciding whether a cell lives or dies. When not engaged in DNA replication, PCNA uses its interactions with a plethora of proteins to command cells to arrest in G1 or G2, to repair damaged DNA, or to undergo apoptosis. In this scenario, major instructions relate to p53 (Paunesku et al. 2001): PCNA is induced at low levels and repressed at high levels of p53; if PCNA levels are high, in the absence of p53, DNA is replicated; if PCNA levels are high in the presence of p53, DNA is repaired; and if PCNA levels (activities) are low, cells become apoptotic.

The regulatory relevance of p21 associating with PCNA interdomain connecting loops has already been mentioned. It has been argued that certain effects might also be mimicked by peptides derived from PCNA interaction partners that have already been cleaved by caspase 3. Such a process could provide the final push into apoptosis (Slee et al. 2001).

Decision capacity has also been ascribed to PARP-1, which, by multiple poly(ADP-ribose)ylation, helps the activation of repair enzymes. In the case of massive DNA damage, over-activation of PARP-1 might trigger programmed cell death (apoptosis) or suicide (necrosis) because of a decreased NAD+ concentration, commonly believed to result in a depletion of the ATP pool (Tewari et al. 1995). Such a model, however, has to qualify in light of the recent finding that ATP can be resynthesized from poly(ADP-ribose) during DNA repair. In this view, the synthesis of poly(ADP-ribose) is seen to be a process of energy accumulation that is set aside especially for DNA repair (Oei and Ziegler 2000).

Perspectives

This article was designed to demonstrate the many components contributing to DNA repair and the relevance of the temporal and spatial coordination of appropriate steps. Although we anticipate that novel approaches in systems biology and the development of 4D imaging techniques will continue to make major contributions, we also wanted to show the basic correctness of the model pioneered by Boulakis (1996), which emphasized the relevance of nuclear substructures for DNA repair. Along these lines, we have tried to accommodate the many and sometimes diverse observations within the actual picture of a dynamic nuclear matrix structure. We believe that all central pathways do, in fact, converge on a playground that shares significant features with the traditional model.

Acknowledgements

This work was supported by grants from DAAD to Melita Vidaković (A/03/30128 “Protein-DNA Interactions in the Nuclear Matrix”) and from DFG to Juergen Bode (Bo419–6). We thank all our colleagues in Belgrade, Braunschweig, and Amsterdam for the stimulating atmosphere that accompanied experiments in the authors’ laboratories.

References

Alvarez-Gonzalez, R., Spring, H., Mueller, M., and Buerkle, A. 1999. Selective loss of poly(ADP-ribose) and the 85 kDa-fragment of poly(ADP-ribose) polymerase in nucleoli during alkylation-


