Protein–protein interactions during mammalian DNA single-strand break repair

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Abstract
The genetic stability of living cells is continually threatened by endogenous reactive oxygen species and other genotoxic molecules. Of particular threat are the thousands of single-strand breaks that arise in each cell every day. If left unrepaired, such breaks can give rise to potentially clastogenic or lethal chromosomal double-strand breaks. This article summarizes our current understanding of how mammalian cells detect and repair single strand breaks, and provides insights into novel polypeptide components of this process.

Origin and structure of DNA single-strand breaks (SSBs)
SSBs are discontinuities in the sugar–phosphate backbone of one strand of a DNA duplex. Hundreds of thousands of cellular SSBs arise from DNA damage each day, and if these are not repaired, they can be converted into potentially clastogenic and/or lethal DNA double-strand breaks (DSBs). The two major sources of endogenous SSBs are sugar damage and DNA base damage arising primarily from attack by reactive oxygen species (ROS) and other electrophilic molecules, and from the intrinsic instability of DNA [1,2]. SSBs can also be induced by exposure to environmental genotoxins, UV light or ionizing radiation (e.g. X-rays). While SSBs arise directly from sugar damage, by disintegration of deoxyribose, those from base damage arise indirectly, through enzymic removal of the damaged base or nucleotide by DNA base excision repair (BER). Another source of SSBs is topoisomerase I (Topo1), which nicks and resales DNA as part of its catalytic activity. Under certain conditions, such SSBs can be ‘uncoupled’ from Topo1 activity and converted into proper single-stranded or double-stranded breaks during transcription or DNA replication. Most SSBs are accompanied by the loss of a single nucleotide at the site of the break and are thus actually single-stranded gaps. In the case of direct SSBs induced by endogenous ROS or ionizing radiation, base loss arises when the damaged sugar becomes fragmented. In the case of indirect SSBs, base loss occurs when the damaged base is excised during BER. SSBs typically possess ‘damaged’ termini that lack the conventional 5′-phosphate or 3′-hydroxy moieties. The 3′-termini of direct SSBs induced by endogenous ROS or ionizing radiation possess primarily monophosphate (approx. 70%) or phosphoglycolate (approx. 30%) end-groups [2]. Although most 5′-termini at such breaks are of the conventional 5′-phosphate format, some may possess a hydroxy end-group [3,4]. Topo1-induced breaks possess both 5′-phosphate and 3′-hydroxy damaged termini, i.e. the reverse format to that present at conventional DNA ends [5]. Indirect SSBs arising during BER occur in one of two formats, depending on the mechanism of base excision: a normal 3′-terminus and ‘damaged’ 5′-deoxyribose phosphate terminus, or a normal 5′-terminus and damaged 3′-α,β-unsaturated aldehyde (αβ) terminus [6,7].

Effect of SSBs on genetic stability and cell death
It is generally accepted that SSBs are far less toxic to cells than their double-stranded counterparts, however, this largely reflects the ability of cells to rapidly repair large numbers of SSBs, rather than an intrinsic lack of toxicity of these lesions. Indeed, any SSB not repaired prior to DNA replication or actively ‘tolerated’ in the appropriate way during DNA replication will become a DSB. Given the frequency of ‘spontaneous’ SSBs, it is thus not surprising that cells have evolved highly efficient mechanisms to minimize their impact. Perhaps the most compelling experimental evidence for this emerges from mammalian cells in which there is a defect in SSB repair (SSBR). Rodent cells lacking the molecular scaffold protein X-ray repair cross complementing group 1 (XRCC1) [8] exhibit elevated cellular sensitivity to a range of agents that induce the SSB directly or indirectly, including alkylating agents, ionizing radiation, hydrogen peroxide, and camptothecin [9]. Moreover, XRCC1−/− embryos fail to develop beyond embryonic day 7.5/8.5, suggesting that endogenous levels of SSBs are incompatible with embryonic development in the absence of efficient SSBR [10]. Un-rejoined SSBs also threaten genetic stability. XRCC1-mutant rodent cells exhibit increased frequencies of ‘spontaneous’ and induced chromosome aberrations, including translocations.
and genetic deletion [9,11]. The simplest explanation for this is that un-rejoined SSBs are converted into DSBs during chromosomal replication and thereby increase the risk of unwanted genetic rearrangement. In addition to gross genetic changes, SSBs can also result in point mutations, due presumably to occasional DNA polymerase mis-incorporation during chromosome replication. Finally, SSBs may also affect transcription. In one study in which transcription by T7 polymerase was examined in vitro, a single nucletide gap in the template strand failed to stop elongation, resulting rather in a transcript shortened by one nucleotide [12]. However, SSBs possessing 'damaged' 3′-phosphate termini did block SSBR, due apparently to effects of charge repulsion between the neighbouring 3′- and 5′-terminal phosphates. Thus, whereas SSBs with normal termini may result in mutant transcripts, SSBs with 3′-phosphate termini, such as those caused by endogenous ROS or ionizing radiation, may block transcription.

**Detection and processing of SSBs**

An early step in the repair of SSBs appears to be the rapid binding by poly(ADP-ribose) polymerase-1 (PARP-1), a molecular ‘nick sensor’ that is activated at SSBs and that synthesizes negatively charged polymers of ADP-ribose [7,13]. A model for the repair of mammalian SSBs is presented in Figure 1. The role of PARP-1 is unclear, but may include inhibiting unwanted recombination at SSBs by charge repulsion [14] or synthesizing ATP cofactor for the DNA ligation step of SSBR [15]. PARP-1 may help sequester other repair proteins to the sites of SSBs, a notion supported by its ability to interact with XRCC1 [16–19]. As discussed above, the termini of many SSBs are ‘damaged’ as they lack conventional 3′-hydroxy and/or 5′-phosphate termini. Such termini are often described as ‘blocked’, because they are unable to serve as primers for DNA polymerases or support the activity of DNA ligases. After the dissociation of PARP-1 from the SSB, the damaged DNA ends must thus be restored to conventional 3′-hydroxy and 5′-phosphate end-groups to enable gap filling and DNA ligation. The removal of 5′-deoxyribose phosphate termini from indirect SSBs created during BER is achieved primarily by the apurinic/apyrimidinic (AP)-lyase activity of DNA polymerase-β (Polβ) [20]. This is an attractive scenario because, as discussed below, Polβ is also the DNA polymerase most commonly employed for gap filling at SSBs. The 3′-phosphoglycolate or 3′-monophosphate termini present at directly induced SSBs, and the 3′-α,β-unsaturated aldehyde termini at indirectly induced SSBs, are substrates for the diesterase activity of mammalian AP endonuclease 1 (APE1/HAP1). However, this activity is relatively weak and it is possible that other enzymes can process these termini. One possible candidate is the mammalian homologue of T4 polynucleotide kinase. Polynucleotide kinase possesses both 5′-hydroxyl and 3′-phosphatase activity, and could thus remove both 5′-hydroxy and 3′-phosphate damaged termini from SSBs [21,22]. XRCC1 interacts with polynucleotide kinase and stimulates both the DNA kinase and DNA phosphatase activity of this enzyme, and in so doing accelerates SSBR reactions [8].

**DNA repair synthesis**

Once the damaged 3′-termini at SSBs have been restored to their conventional (hydroxy) configuration, gap filling can occur. Since a single nucleotide is lost at most sites of SSB, gap filling need only replace a single nucleotide; however, several nucleotides can be replaced if strand displacement occurs downstream of the gap followed by removal of the resulting single-stranded flap, by flap endonuclease 1 (FEN1) [23]. These two distinct scenarios are defined as ‘short-patch’ or ‘single-nucleotide’ repair, and ‘long-patch’ repair respectively. Unfortunately, with the exception of rodent cells lacking Polβ, cell lines that lack the ‘error-free’ polymerases that are candidates for gap filling are unavailable, due presumably to essential roles in chromosome replication. Consequently, most of our understanding of polymerase utilization during DNA repair has emerged from DNA repair reactions conducted in vitro using permeabilized cells, cell extract, or purified proteins. Many studies have implicated Polβ in gap filling at both directly induced SSBs and those arising during BER [24–26]. Furthermore, both genetic and biochemical analyses reveal that human Polβ can interact with APE1 [27] and XRCC1 [16,28]. These interactions may serve to recruit Polβ to SSBs for the process of gap filling. Indeed, in the case of APE1, the interaction appears to occur only in the presence of DNA substrate. An attractive model of how the N-terminal domain of XRCC1 might interact with Polβ at a single-nucleotide gap has also been presented [29]. Polβ induces a 90° kink in DNA at a SSB and binds the outside bend of this kink, and the model of Marintchev et al. [29] suggests that XRCC1 may simultaneously bind both the inside of this DNA bend and the palm-thumb domains of Polβ. It is possible that by encompassing the SSB in this way, the single-strand region is protected from nucleolytic attack, which might otherwise create a DSB.

In addition to Polβ, most in vitro studies also imply a role for DNA polymerase δ and/or DNA polymerase ε (Polδ/ε) in the repair of direct and indirect SSBs [23,30–35]. This has been suggested in part by reactions conducted with cell-free extracts, in which some SSBR events are dependent upon proliferating cell nuclear antigen (PCNA), an accessory protein that is both bound by Polδ/ε and required for their activity. However, recent experiments have raised another possible role for PCNA during SSBR, in stimulating FEN1 endonuclease activity during long-patch repair. Nevertheless, there is much evidence, from inhibitor studies employing compounds such as aphidicolin, that Polδ/Polε can contribute to SSBR reactions. Polδ appears to conduct most (50–80%) of the gap filling during SSBR, and primarily inserts a single nucleotide. In addition to this ‘short-patch’ repair, a smaller fraction of BER events appear to involve the insertion of multiple nucleotides (approx. 2–10) in a long-patch process that can involve either Polβ...
SSBs arise either directly (e.g. sugar damage or ‘spontaneous’ disintegration of abasic sites), or from Topo1 cleavage, or indirectly (e.g. during BER of base damage). (a) PARP-1 binds to SSBs and is activated, thereby synthesizing chains of poly(ADP-ribose). Poly(ADP-ribose) recruits XRCC1-Lig3α (L3) complex to the SSB via the affinity of these polypeptides for this polymer. PARP-1 dissociates from the SSB and is replaced by XRCC1 to establish a molecular scaffold. (b) AP sites created during BER are bound by APE1. Note that while APE1 can couple the creation of the SSB by its own endonuclease activity to the following steps of SSBR by molecular ‘hand-off’ to subsequent enzymes, it is possible that some cleaved abasic sites are not ‘coupled’ in this way. The resulting ‘exposed’ breaks may be channeled into the PARP-1-dependent process (broken arrow). (c) Damaged 5′- and 3′-termini (open unlabelled circles) are converted back into 5′-phosphate and 3′-hydroxy termini by recruitment of the enzyme appropriate for the type of damaged terminus, e.g. APE1, tyrosylphosphodiesterase 1 (TDP1), polynucleotide kinase (PNK), Polβ. Note that the affinity of Polβ, PNK and APE1 for the SSB, and the enzymic activity of the latter two, may be increased by binding to XRCC1. (d) AP sites during BER, APE1 recruits Polβ and possibly XRCC1 via physical interaction, and cleaves the AP site, creating an SSB. This reaction may be stimulated by XRCC1. Polβ then removes the deoxyribose phosphate moiety from the 5′-terminus of the break in a reaction that may be stimulated by APE1. (e) in the case of most (approx. 80%) of direct and indirect SSBs, Polβ binds the gapped substrate and XRCC1, and gap filling inserts one nucleotide (short-patch/single-nucleotide repair). (f) Under some circumstances (e.g. during BER if the 5′-terminus is an oxidized deoxyribose phosphate moiety that Polβ cannot remove) gap filling extends beyond one nucleotide in the long-patch mode (2–10 nucleotides). Polβ or Pol δ/ε can conduct this process. After long-patch synthesis, the displaced single-stranded flap is removed by FEN1 in a reaction that can be stimulated by PCNA. DNA ligation of short-repair patches is conducted by Lig3α (g) and long-repair patches by Lig1 (h).

or Polδ/ε. Recent studies with extracts from wild-type and Polβ−/− cells suggest that most long-patch repair events are conducted by Polβ−/−, with an aphidicolin-sensitive polymerase (presumably Polδ/ε) fulfilling a ‘back-up’ role. In addition to long patch repair, Polδ/ε may also be able to fulfil some ‘back-up’ repair in a single-nucleotide, short-patch process. A major challenge for the future is to determine how polymerase choice is made in living cells; for example, it is possible that Polβ operates globally throughout the genome, whereas the replicative polymerases, Polδ/Polε, have a specific role at SSBs encountered by a replication fork.

DNA ligation

In addition to multiple DNA polymerases, mammalian cells also possess multiple DNA ligases. So far, three
human DNA ligase genes have been identified, which encode at least five different polypeptides [36]. A number of studies have implicated DNA ligase IIIα (Lig3α) in the repair of both direct SSBs and indirect SSB during BER. Moreover, these studies suggest that Lig3α is involved primarily in short-patch repair events involving Polβ; for example, during BER of abasic sites in cell extracts, Lig3α appears to conduct ligation specifically following short-patch gap filling [37], which is mediated primarily by Polβ, and a preference for Lig3α during Polβ-mediated repair has been observed at direct SSBs [38]. Moreover, cellular Lig3α is bound and stabilized by the molecular scaffold protein, XRCC1 [39,40], providing a physical link between this DNA ligase and Polβ. In a similar situation that was observed during gap filling, DNA ligation during SSBR does not appear to be restricted to a single enzyme. In addition to Lig3α, DNA ligase I (Lig1) is implicated in the repair of direct SSBs and of indirect SSBs during BER, as determined by biochemical experiments and by the cellular phenotype of SSBR processes on human disease may thus be more significant than previously thought.

### Perspectives

A number of critical questions remain to be answered. For example, how is the choice of polymerase and choice of long-patch versus short-patch repair made in living cells? Is there any cell cycle specificity for the different mechanisms of SSBR? What is the relationship of these processes to human disease? Intriguingly, with respect to the latter question, a recent two-hybrid screen for novel candidate SSBR proteins that interact with XRCC1 recovered two independent cDNA clones encoding aprataxin, the protein mutated in the ataxia telangiectasia-like neurodegenerative disorder ataxia ocular apraxia (Table 1). The validity of this screen was demonstrated by the recovery from the same screen of multiple cDNA clones of Lig3α, Polβ, and polynucleotide kinase. The impact of SSBR processes on human disease may thus be more significant than previously thought.

### References

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