Single-strand break repair and genetic disease

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Abstract | Hereditary defects in the repair of DNA damage are implicated in a variety of diseases, many of which are typified by neurological dysfunction and/or increased genetic instability and cancer. Of the different types of DNA damage that arise in cells, single-strand breaks (SSBs) are the most common, arising at a frequency of tens of thousands per cell per day from direct attack by intracellular metabolites and from spontaneous DNA decay. Here, the molecular mechanisms and organization of the DNA-repair pathways that remove SSBs are reviewed and the connection between defects in these pathways and hereditary neurodegenerative disease are discussed.

Single-strand breaks (SSBs) are discontinuities in one strand of the DNA double helix and are usually accompanied by loss of a single nucleotide and by damaged 5′- and/or 3′-termini at the site of the break. If not repaired rapidly or appropriately, chromosomal SSBs pose a serious threat to genetic stability and cell survival. Consequently, cells have evolved rapid and efficient mechanisms for their repair. The likely importance of SSB repair (SSBR) is highlighted by the observation that two of the proteins involved in this process are mutated in hereditary neurodegenerative disease.

Here, I describe the source and structure of endogenous cellular SSBs, and outline the ways in which these lesions can have an impact on cell fate. I also present our current working models for SSBR and review the association between defects in these processes and human disease.

Source and structure of endogenous SSBs

One of the most common sources of SSBs is oxidative attack by endogenous reactive oxygen species (ROS). In the case of free radicals from hydrogen peroxide (H_2O_2), which is a physiologically relevant source of ROS, SSBs occur three orders of magnitude more frequently than double-strand breaks (DSBs)[1]. SSBs can occur directly by disintegration of the oxidized sugar or indirectly during the DNA base-excision repair (BER) of oxidized bases, abasic sites, or bases that are damaged or altered in other ways[2–4]. SSBs can also arise as a result of erroneous or abortive activity of cellular enzymes such as DNA topoisomerase 1 (TOP1). TOP1 creates a 'cleavage complex' intermediate containing a DNA nick in order to relax DNA during transcription

and DNA replication[5]. These intermediates are normally transient and are rapidly resealed by TOP1. However, collision with RNA or DNA polymerases, or close proximity to other types of DNA lesion, can convert cleavage complexes into TOP1-linked SSBs (TOP1–SSBs) or TOP1-linked DSBs (TOP1–DSBs), in which TOP1 is covalently linked to the 3′-terminus of the DNA strand break[6]. This type of break has become the focus of much attention in recent years, because defects in its repair are associated with the human genetic disease spinocerebellar ataxia with axonal neuropathy 1 (SCAN1)[7].

SSBs and cell fate

Chromosomal SSBs can have an impact on cell fate in a number of ways if they are not repaired rapidly or appropriately (FIG. 1). The most likely consequence of unrepaired SSBs in proliferating cells is the blockage or collapse of DNA replication forks during the S phase of the cell cycle, possibly leading to the formation of DSBs[8,9]. Even though cells possess a remarkable ability to accurately repair this type of DSB using homologous recombination (HR) (see below), acute increases in cellular SSB levels might saturate this pathway, leading to genetic instability and/or cell death. In non-proliferating cells, such as post-mitotic neurons, cell death induced by SSBs might involve stalling of RNA polymerases during transcription; this is because SSBs can block RNA polymerase progression *in vitro*, particularly if they possess damaged termini[10–13]. Alternatively, under some physiological situations, high levels of single-strand breakage might induce cell death through excessive activation of the SSB sensor

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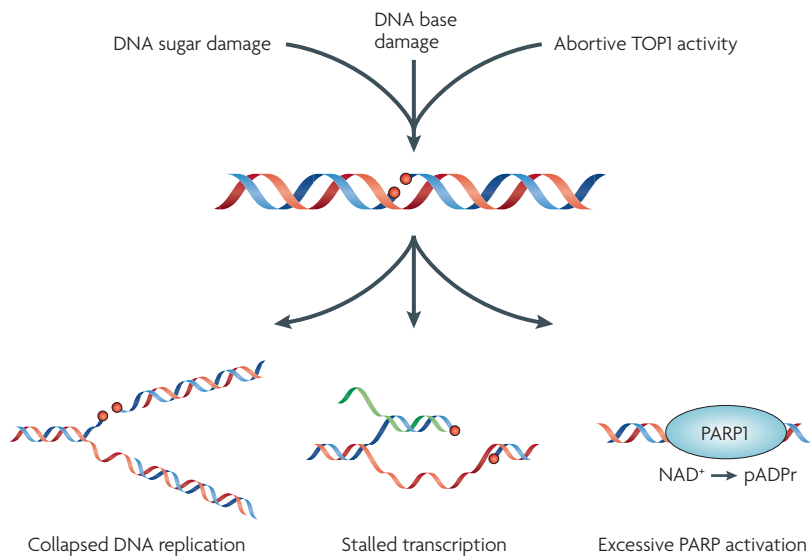e-mail:
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Figure 1 | **Single-strand breaks and cell fate.** Single-strand breaks (SSBs) can arise in a variety of ways, including directly from disintegration of deoxyribose, indirectly as normal intermediates of base-excision repair (BER), or as abortive intermediates of topoisomerase 1 (TOP1) activity. If they are not repaired rapidly or appropriately, SSBs can collapse replication forks, block transcription or promote excessive activation of the SSB sensor protein poly(ADP-ribose) polymerase 1 (PARP1). Red circles denote damaged DNA termini. pADPr, poly(ADP-ribose).

protein poly(ADP-ribose) polymerase 1 (PARP1)[14,15]. Under these conditions, prolonged activation of PARP1 leads to depletion of cellular NAD$^+$ and ATP and/or release of apoptosis-inducing factor (AIF) from mitochondria. This type of cell death is relevant to a number of pathological conditions involving oxidative stress, including diabetes, arthritis, and post-ischaemic brain or heart damage resulting from stroke or heart attack, respectively.

Mechanisms of chromosomal SSBR

Although endogenous SSBs arise from a variety of different sources, there is extensive overlap between the enzymes used to remove these breaks. Consequently, the different mechanisms of repairing SSBs from different sources (for example, those arising as normal intermediates of BER versus those arising directly from sugar disintegration) are considered sub-pathways of SSBR. Most SSBs are repaired by a rapid global SSBR process that can be divided into four basic steps: SSB detection, DNA end processing, DNA gap filling and DNA ligation (FIG. 2).

SSB detection. SSBs arising directly from disintegration of oxidized deoxyribose are primarily detected by PARP1, although contributions from other members of the PARP superfamily are possible[16–18]. PARP1 rapidly binds to and is activated by DNA strand breaks, and subsequently modifies itself and other target proteins with branched chains of poly(ADP-ribose) (pADPr) of up to several hundred ADP-ribose units in length. The binding and activity of PARP1 at DNA breaks is transient, because poly(ADP-ribosylated)

PARP1 rapidly dissociates from DNA and pADPr is rapidly degraded by poly(ADP-ribose) glycohydrolase (PARG), thereby restoring PARP1 and other poly(ADP-ribosylated) proteins to their de-ribosylated state in preparation for subsequent rounds of SSB detection and signalling[19]. PARP1 is also activated at SSBs that arise indirectly during BER[20–22], but it is less clear why such breaks require 'detecting' as they arise as part of a co-ordinated repair process in which DNA intermediates might be passed from one enzyme to another in a molecular relay[23–25]. Perhaps some SSB intermediates become uncoupled from this relay and so require PARP1 to re-engage the repair machinery. Alternatively, perhaps PARP1 fulfils a different role during BER, downstream of SSB detection (see below). It is similarly unclear whether PARP1 is involved in detecting abortive TOP1–SSBs.

A role for PARP activity in accelerating chromosomal SSBR is suggested both by inhibitor studies[20,21,26–29] and by experiments using cells that are selectively depleted or deleted of PARP1 (REFS 30–33). PARG is also required for rapid rates of SSBR, suggesting that optimal SSBR rates require that levels of pADPr are dynamic and tightly regulated[30,34]. PARP1 might accelerate SSBR by promoting the focal accumulation or stability of SSBR protein complexes at chromosomal SSBs through interaction with dedicated pADPr-binding motifs[35–40]. Arguably the most important of these is X-ray repair cross-complementing protein 1 (XRCC1), which functions as a molecular scaffold that interacts with, stabilizes, and stimulates multiple enzymatic components of the SSBR process[41]. Note that the impact of PARP1 on focal accumulation of SSBR proteins at chromosomal SSBs does not necessarily reflect their initial recruitment, which mechanistically might be different and might require recognition of their cognate DNA substrates[42]. PARP1 might also accelerate chromosomal SSBR by regulating chromatin structure: histone proteins are targets for poly(ADP-ribosylation), and pADPr synthesis can disrupt nucleosomes and can regulate higher-order chromatin compaction[43–47]. Other possible roles include promoting DNA gap filling during long-patch repair (see below)[48–50], generating ATP for the final step of DNA ligation[51,52], and inhibiting illegitimate recombination events and/or unwanted nucleolytic activity[22,53]. Finally, because PARP1 is also a transcriptional regulator, it remains possible that it accelerates SSBR rates indirectly, by affecting the level of one or more SSBR proteins[50].

DNA end processing. The 3′- and/or 5′-termini of most, if not all, SSBs are 'damaged' and must be restored to conventional 3′-hydroxyl (3′-OH) and 5′-phosphate moieties for gap filling and DNA ligation to occur. This is the most enzymatically diverse step of SSBR, as indicated by the large number of enzymes that are available for this process (FIG. 2). This, in turn, reflects the variety of damaged termini that can arise (FIG. 3). End processing is a crucial stage of SSBR because DNA breaks with abnormal termini are particularly
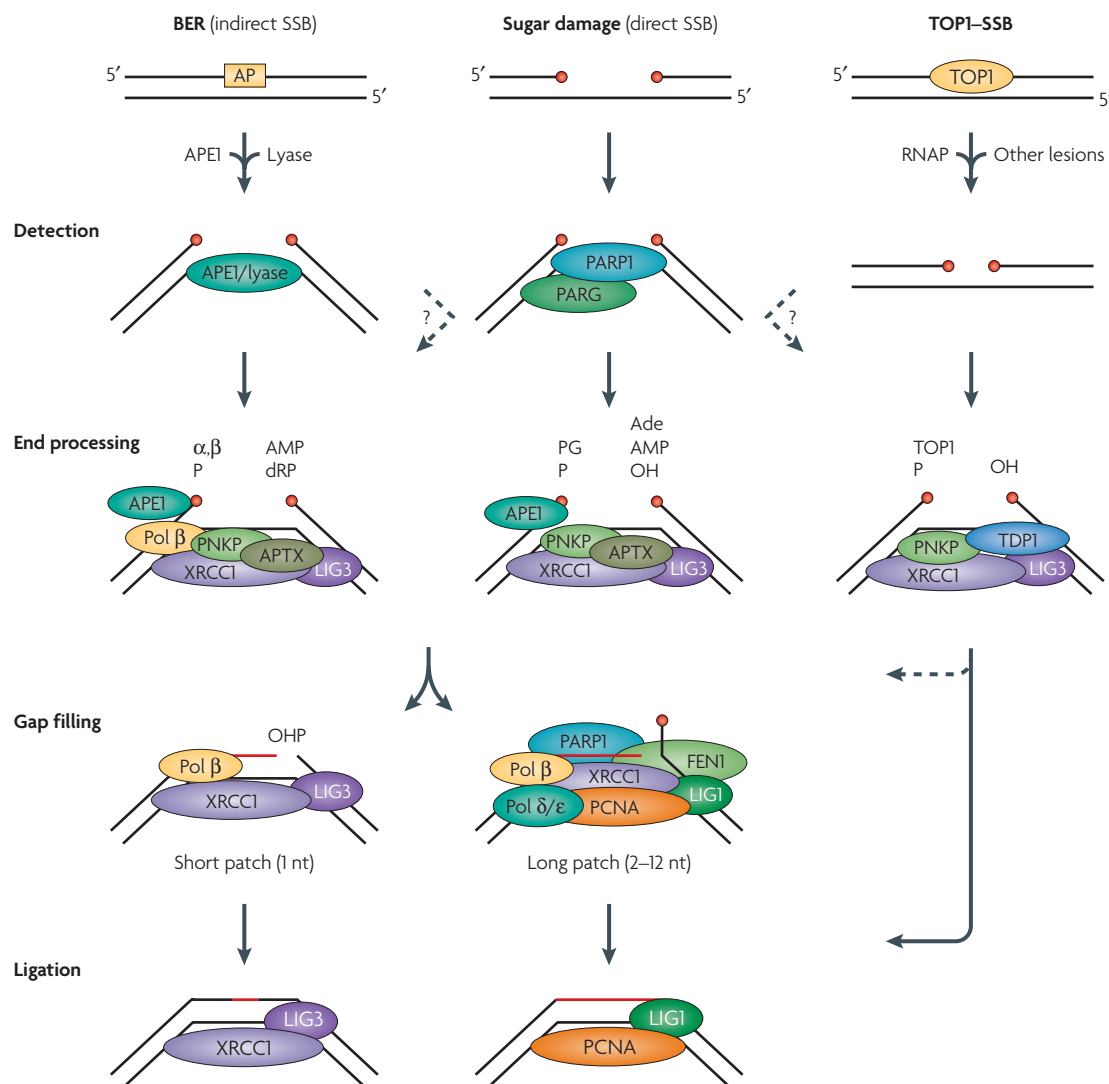
Figure 2 | **A model for global single-strand break repair.** Single strand breaks (SSBs) can arise indirectly during base-excision repair (BER) by enzymatic incision at an apurinic–apyrimidinic (AP) site by AP endonuclease I (APE1) or by the lyase activity of a bifunctional DNA glycosylase. AP sites arise by spontaneous or induced (for example, at oxidized deoxyribose) base loss or by enzymatic excision of damaged (for example, alkylated, oxidized or deaminated) bases. SSBs can also arise directly by reactive oxygen species (ROS)-induced disintegration of oxidized deoxyribose (sugar damage), and during abortive topoisomerase 1 (TOP1) activity involving collision with RNA polymerase (RNAP) or close proximity to other types of DNA lesion, which creates a TOP1-linked SSB (TOP1–SSB). Direct breaks are detected by poly(ADP-ribose) polymerase 1 (PARP1) binding and activation, which promotes rapid access by, and accumulation of, downstream repair factors (see text for details). Poly(ADP-ribose) glycohydrolase (PARG) restores PARP1 to its pre-activated state in preparation for subsequent rounds of SSB detection. Note that SSBs arising during BER or induced by TOP1 might not require PARP1 for detection because these breaks are created in a scheduled fashion by cellular enzymes (APE1 in BER) or might be 'detected' by collision with RNAP (TOP1–SSBs). Once a SSB has been detected, it undergoes end processing. Damaged termini (red circles) that are present at indirect, BER-induced SSBs are repaired by APE1, DNA polymerase (Pol) β, polynucleotide kinase 3′-phosphatase (PNKP) and aprataxin (APTX). Direct, sugar-damage induced SSBs are repaired by APE1, PNKP and APTX. TOP1–SSBs are repaired by tyrosyl-DNA phosphodiesterase 1 (TDP1), which removes TOP1 from the 3′-termini at such breaks, resulting in a 3′-phosphate terminus, which is subsequently repaired by PNKP. PNKP also repairs the 5′-hydroxy termini present at TOP1 breaks. The end-processing mechanism is followed by gap filling. At most SSBs, Pol β inserts the missing nucleotide, this is termed short-patch repair. Under some circumstances (for example, an oxidized deoxyribose phosphate that cannot be repaired by Pol β) gap filling might be extended for ~2–12 nucleotides (nt) by Pol β, Pol δ and/or Pol ε (Pol δ/ε) during long-patch repair. Note that long-patch repair involves the removal of the damaged 5′-terminus as a flap of two or more displaced nucleotides by flap endonuclease 1 (FEN1), in a reaction stimulated by PARP1 and proliferating cell nuclear antigen (PCNA). Also note that TOP1–SSBs are DNA nicks and therefore might not require a gap-filling step. DNA ligation is the final step in SSB repair. Short-patch repair sites are mainly ligated by DNA ligase 3 (LIG3), and long-patch repair sites are mainly ligated by DNA ligase 1 (LIG1). α,β, 3′-α,β unsaturated aldehyde; Ade, aldehyde; AMP, 5′-AMP; dRP, 5′-deoxyribose phosphate; OH, 5′-hydroxyl; P, 3′-phosphate; PG, 3′-phosphoglycolate.

a Types of damaged 3′ termini
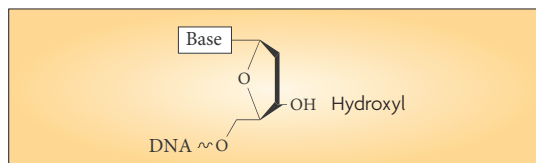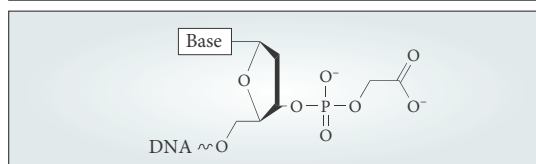
- Undamaged 3′ terminus

Enzyme
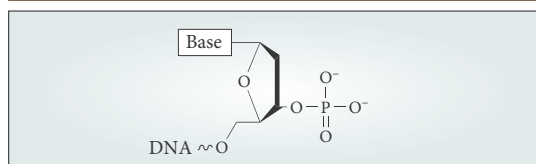
Phosphate
- Direct break; sugar disintegration (ROS)
- Indirect break; BER (ROS, alkylators, spontaneous base damage and/or loss)
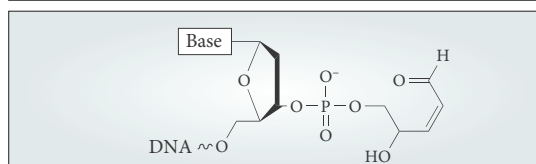- TOP1–SSBs (TOP1 inhibitors, ROS, other genotoxins)

PNKP
(3′-phosphatase)

Phosphoglycolate
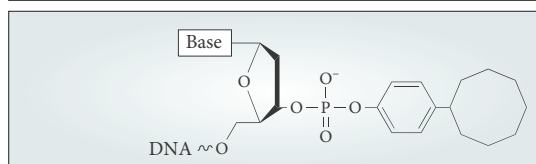- Direct break; sugar disintegration (ROS)

APE1

α,β unsaturated aldehyde
- Indirect break; BER (ROS, alkylators, spontaneous base damage and/or loss)

APE1

TOP1 peptide
- TOP1–SSBs (TOP1 inhibitors, ROS, other genotoxins)

TDP1

b Types of damaged 5′ termini

- Undamaged 5′ terminus

Enzyme
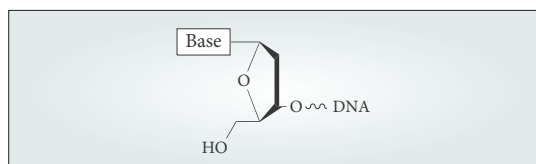
5′-hydroxyl
- TOP1–SSBs (TOP1 inhibitors, ROS, other genotoxins)

PNKP
(5′-kinase)

5′-aldehyde
- Direct break; sugar disintegration (ROS)

?

5′-dRP (AP site)
- Indirect break; BER (ROS, alkylators, spontaneous base damage and/or loss)
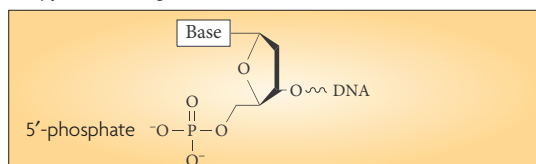- Following ROS, this damage can be an oxidized dRP that might require LPR for removal
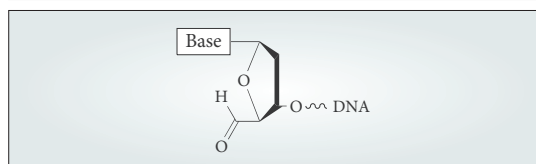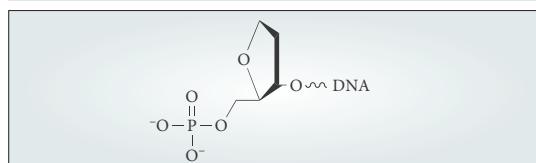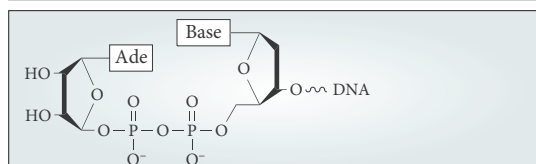
Pol β

5′-AMP
- Abortive ligase activity (can form at breaks with 5′-P)

APTX

◄ Figure 3 | **Common types of damaged single-strand break termini and the enzymes that process them.** For each type of single-strand break (SSB) the mechanism of damage is summarized, with the causative agent(s) in parentheses. Ade, aldehyde: AP, apurinic–apyrimidinic; APE1, AP endonuclease I; APTX, aprataxin; BER, base-excision repair; dRP, 5′-deoxyribose phosphate; FEN1, flap endonuclease 1; LPR, long-patch repair; P, phosphate; PNKP, polynucleotide kinase 3′-phosphatase; Pol β, DNA polymerase β; ROS, reactive oxygen species; TDP1, tyrosyl-DNA phosphodiesterase 1; TOP1, topoisomerase 1.

cytotoxic. For example, it is the removal of 5′-deoxyribose phosphate (dRP) residues by DNA polymerase (Pol) β that accounts for its importance in maintaining cellular resistance to DNA base damage, rather than its role as a DNA polymerase during DNA gap filling (see below)[54]. In addition, over-expression of DNA glycosylase enzymes that initiate BER can actually increase the sensitivity of cells to DNA base damage, and their deletion can decrease this sensitivity, most probably because of changes in steady-state levels of SSB intermediates[55–57]. The cytotoxicity of damaged termini might in part reflect a greater ability to block progression of RNA polymerases, and/or difficulties encountered by cells in dealing with blocked termini at collapsed replication forks.

The common types of damaged termini and the principal SSBR enzymes involved in their repair are summarized above (FIGS 2,3), but several aspects merit further attention. 3′-phosphate and 3′-phosphoglycolate termini are noteworthy because together they constitute the majority of direct SSBs induced by ROS, and because 3′-phosphate is a major substrate for polynucleotide kinase 3′-phosphatase (PNKP)[58–60] and 3′-phosphoglycolate for apurinic–apyrimidinic (AP) endonuclease I (APE1; also known as APEX1)[61–65]. By contrast, SSBs arising indirectly during BER typically harbour 5′-dRP termini created by cleavage of abasic sites by APE1. As discussed above, 5′-dRP termini are substrates for the AP lyase activity of Pol β[54,66]. However, if Pol β cannot remove the 5′-dRP moiety (for example, if the dRP is oxidized[2,67]) then the 5′-terminus can be displaced during long-patch gap filling and removed by flap endonuclease 1 (FEN1) as a single-strand flap. It is worth noting that SSBs that arise during BER might alternatively possess a 3′-phosphate or 3′-α,β-unsaturated aldehyde terminus, rather than a 5′-dRP terminus, if they are created by cleavage of an abasic site through DNA glycosylase AP lyase activity rather than through APE1 activity. These termini are substrates for PNKP and APE1, respectively[63,64,68].

Two other types of damaged terminus that warrant additional attention are 3′- and 5′-termini linked covalently to TOP1 and AMP, respectively. TOP1–SSBs arise from abortive TOP1 activity and are processed by tyrosyl-DNA phosphodiesterase 1 (TDP1)[69,70]. 5′-AMP–SSBs, at which AMP is covalently linked to 5′-phosphate through a pyrophosphate bond, arise from abortive DNA-ligase activity at existing SSBs and are processed by aprataxin (APTX)[71,72]. Although the occurrence of 5′-AMP termini *in vivo* remains to

be demonstrated, it is intriguing that both TDP1 and APTX seem to process SSBs that arise as a result of failed or abortive activity of endogenous enzymatic activities, particularly because both TDP1 and APTX are mutated in hereditary neurodegenerative diseases (see below).

Finally, it is important to note that XRCC1 has a particularly important role during DNA end processing. XRCC1 directly interacts with PNKP[58,73], APTX[74–77], and Pol β[39,78], and indirectly, through DNA ligase IIIα, with TDP1 (REFS 7,79). The repair of 5′-hydroxyl and/or 3′-phosphate termini is rate limiting for SSBR in XRCC1-mutant Chinese hamster ovary (CHO) cells and cell extracts[58,80]. In the case of PNKP, it is clear that the interaction with XRCC1 stimulates both DNA kinase and DNA phosphatase activity *in vitro*, possibly by increasing damage discrimination by PNKP and by displacing the enzyme from its reaction product[58,73,81]. The interaction of XRCC1 with PNKP, APTX and Pol β also promotes the accumulation of these enzymes at oxidative chromosomal breaks induced by H_2O_2 and ultraviolet A (UVA) laser *in vivo*[37,73,82].

DNA gap filling. After damaged 3′-termini at SSBs have been restored to their conventional hydroxyl configuration, gap filling can occur (FIG. 2). This often involves insertion of the single nucleotide that is missing (short-patch repair), but at some SSBs gap filling might continue for two or more nucleotides, with FEN1 removing the displaced 5′-residue either one at a time or as a single-stranded flap (long-patch repair)[47] (FIG. 1). Early studies using permeabilized cells suggest that multiple DNA polymerases, including Pol β, are involved in the repair of SSBs, with the choice of DNA polymerase influenced by the source and type of SSB. *In vitro* studies using DNA molecules with defined types of break similarly implicate Pol β in gap filling, both at direct SSBs arising from oxidative damage and at indirect SSBs during BER, and show that Pol δ and/or Pol ε (Pol δ/ε) can also conduct this role[47]. Direct measurements of chromosomal repair rates in mouse embryonic fibroblasts (MEFs) confirm that Pol β is required for rapid repair of methyl methanesulphonate (MMS)-induced SSBs during BER[83,84]. By contrast, a requirement for Pol β for rapid SSBR rates at oxidative SSBs has not been observed[83]. This might reflect the availability of an alternative short-patch DNA polymerases such as Pol λ or Pol ι, which possess dRP lyase activity, for repair of oxidative SSBs, or alternatively the use of long-patch DNA polymerases[85–88]. A number of accessory proteins might also be important for gap filling during SSBR. For example, PARP1 and FEN1 stimulate long-patch gap filling by Pol β *in vitro*[49,89], and the RFC–PCNA complex — made up of the replication factor C (RFC) clamp loader and the proliferating cell nuclear antigen (PCNA) clamp — might promote long-patch gap filling by Pol δ/ε[90–92]. In addition, XRCC1 interacts with PCNA[93,94] and Pol β[39,78] and is important for the accumulation of RFC, PCNA and Pol β at direct SSBs induced by UVA laser damage in cells[37,90].

DNA ligation. The final step of SSBR is DNA ligation (FIG. 2). Three human DNA ligase genes have been identified (*LIG1*, *LIG3*, and *LIG4*), which encode five different polypeptides (see REF. 95 for a recent review). *LIG3* encodes three polypeptides, denoted DNA ligase IIIα (LIG3α), DNA ligase IIIβ (LIG3β), and a mitochondrial isoform (mtLIG3). Of these, LIG1, LIG3α, and mtLIG3 are implicated in SSBR. mtLIG3 interacts with Pol γ and is required for integrity of the mitochondrial genome[96,97], whereas LIG1 and LIG3α seem to be the enzymes of choice during nuclear long-patch and short-patch SSBR, respectively[47]. LIG3α is stable and active as a recombinant protein *in vitro*, but the nuclear enzyme requires constitutive interaction with XRCC1 for stability and for accumulation at sites of oxidative chromosome damage[98,99]. By contrast, LIG1 requires interaction with PCNA for accumulation at such sites[98], although one might also expect a dependency on XRCC1 because XRCC1 is required for PCNA accumulation at sites of oxidative damage[37]. It is important to note that our understanding of the requirement for specific DNA ligases during SSBR is derived largely from *in vitro* assays, and that the level of functional overlap between LIG1 and LIG3α during SSBR in living cells remains to be determined.

All of the proteins encoded by *LIG3* possess an amino-terminal zinc finger (ZNF) homologous to the two that are present in PARP1 (REF. 100). The LIG3 ZNF is largely dispensable for binding and ligation of simple nicked DNA substrates *in vitro*, although a weak effect (~2-fold) on these activities is observed under some experimental conditions[39,95,101–103]. The dispensability of the ZNF for simple-nick ligation most probably reflects the presence of an efficient nick-binding activity within the conserved catalytic core of the enzyme. However, the ZNF has a major impact on binding and ligation of DNA breaks that are located near to unusual secondary structure or to other intermediates of DNA repair, suggesting that one function of the ZNF is to broaden the range of substrates targeted by LIG3α[95,101,102]. The ZNF might thus be important for repair of chromosomal breaks in repetitive regions of the nuclear and/or mitochondrial genome, or at multiply-damaged sites or clustered lesions. Intriguingly, the ZNF is required for efficient DSB end joining even in the absence of proximal secondary structure, suggesting that this domain might have a major role in alternative non-homologous end joining, in which LIG3 is believed to participate[104,105].

Organization and regulation of SSBR

As indicated in the text above and in FIG. 2, each of the four basic steps of SSBR involves multiple protein–protein interactions. Arguably the most important of these are the interactions between XRCC1 and enzymatic components of the repair process. XRCC1 accelerates the overall process of SSBR, which it does by stabilizing (for example, LIG3α)[99,106] and/or stimulating (for example, PNKP)[58,81] its protein partners. XRCC1 also facilitates the accumulation of its protein partners at sites of chromosomal DNA damage, although it remains to

be determined whether or not this reflects the initial recruitment of these proteins to DNA breaks, which mechanistically might occur by a different process.

It is not obvious why higher eukaryotes use XRCC1 to accelerate global rates of SSBR — lower eukaryotes such as yeast do not need this protein. One possibility is that this factor has arisen in response to dramatic increases in genome size. Because the frequency of SSBs arising per cell per day is intrinsically related to DNA content, it is possible that higher eukaryotes require faster global rates of SSBR to maintain their steady-state level of SSBs below a certain threshold. The organism with the smallest genome that contains XRCC1 is the social amoeba *Dictyostelium*, the genome of which is three times larger (34 Mb) than that of budding yeast, which lacks a recognizable XRCC1.

It remains to be established whether SSBR proteins are organized into one or more constitutive protein complexes or whether SSBR is organized into a sequential series of rapid but transient protein–protein interactions at SSBs. Both are most probably true. For example, the interactions between XRCC1 and PNKP[73], APTX[74,76], and aprataxin and PNK-like factor (APLF)[107,108] are largely constitutive because they are mediated by forkhead-associated (FHA) domain-mediated interactions with threonine and/or serine residues that are phosphorylated by the constitutively active protein kinase, CK2. Similarly, levels of LIG3α are reduced ~5-fold in cells lacking XRCC1 or in those that express a form of XRCC1 that cannot bind LIG3α, suggesting that at least 80% of cellular LIG3α is constitutively bound to XRCC1 (REF. 99). Moreover, the level of these complexes increases little, if at all, in response to acute increases in SSB levels. By contrast, the interaction between XRCC1 and PARP1 clearly increases after DNA damage, consistent with the preferential interaction of XRCC1 with auto-modified PARP1 (REFS 40,48,109). This interaction thus enables the accumulation of XRCC1, and, consequently, those SSBR enzymes that interact with this scaffold protein, at chromosomal breaks.

Although SSBR is a housekeeping process, cellular SSBR capacity might still be regulated. For example, some cell types moderately increase the levels of SSBR proteins (for example XRCC1 and APE1) in response to genotoxins, oxidative stress, or ischemia injury in the brain, probably by changes in gene expression[110–112]. SSBR enzymes might also be regulated post-translationally[113] or even proteolytically as indicated by reduced steady-state levels of LIG3α in cells that lack XRCC1 (REF. 99). This might reflect a general cellular mechanism for removing excess (that is, unbound) amounts of specific subunits of a multi-protein complex, thereby maintaining the stoichiometry of those subunits. This might be particularly important for proteins that could engage in spurious or non-specific DNA metabolic activity if not chaperoned by a protein partner. Alternatively, the degradation of SSBR proteins by the proteosome might reflect the removal of those SSBR proteins that are not actively engaged in chromosomal SSBR[114].

Alternative non homologous end joining
Also known as back-up non homologous end joining. A subpathway of non homologous end joining (NHEJ) that does not require classical NHEJ proteins and which might rejoin DSBs located within short regions of microhomology.
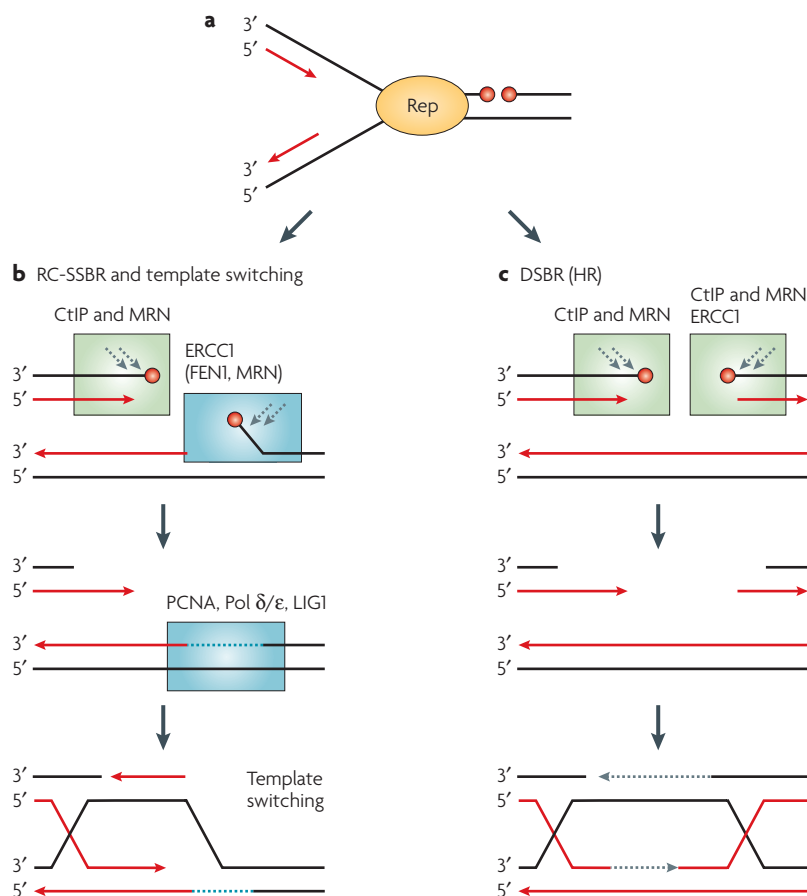
Figure 4 | A model for replication-coupled single-strand break repair (SSBR).
a | A replication fork (Rep) approaches a single-strand break (SSB) with damaged termini (red lines denote nascent strands, red ovals denote damaged termini). Note that in this example the SSB is in the leading-strand template, but similar outcomes are applicable to a SSB in the lagging-strand template. **b** | RC-SSBR and template switching. The replication fork collapses by replication 'run-off' creating a one-ended double-strand break (DSB; green box) in one sister chromatid and leaving a residual SSB in the other (blue box). Note that the SSB shown here possesses a single-strand gap but alternatively might possess a nick or a single-strand flap, depending on the proximity of the terminus of the adjacent nascent strand (in the example depicted this is the 5′-terminus of the nearest Okazaki fragment). The 5′-terminus of the DSB is processed and resected by CtIP together with MRN in preparation for template switching. End processing of the SSB terminus is conducted by global SSBR factors (TDP1, PNKP, APTX and DNA polymerase (Pol) β) or alternatively by structure-specific nucleases such as ERCC1–XPF at 3′-termini (as shown here), FEN1 at 5′-flapped termini, or the MRN complex at 5′- and possibly 3′-termini. DNA gap filling during RC-SSBR involves PCNA, the replicative polymerases Pol δ and/or Pol ε (Pol δ/ε) and associated factors, and DNA ligation by LIG1. Following RC-SSBR, the replication fork is reformed by RAD51-mediated template switching. Note that, following RC-SSBR and homologous recombination (HR), proliferating cells might be more able to tolerate loss of certain global SSBR end-processing factors (for example, TDP1 or APTX). Also note PARP1 and XRCC1 might fulfil an important but undefined role during RC-SSBR. **c** | The SSB is converted into a two-ended DSB by re-initiation of DNA synthesis downstream of the SSB or by approach of a replication fork from the other direction. Note that in this scenario, RC-SSBR is not required because the two-sided DSB can be repaired by HR (as shown) or by non-homologous end joining DSB repair (DSBR) pathways. APTX, aprataxin; CtIP, CTBP-interacting protein; ERCC1, excision repair cross-complementing rodent repair deficiency, complementation group 1; FEN1, flap endonuclease 1; LIG1, DNA ligase 1; MRN, Mre11–Rad50–Nbs1; PARP1, poly(ADP-ribose) polymerase 1; PCNA, proliferating cell nuclear antigen; PNKP, polynucleotide kinase 3′-phosphatase; TDP1, tyrosyl-DNA phosphodiesterase 1; XPF, xeroderma pigmentosum, complementation group F (also known as ERCC4); XRCC1, X-ray repair cross-complementing protein 1.

SSBR and the cell cycle

The SSBR processes described above most probably operate throughout the genome and throughout the cell cycle to rapidly detect and remove the majority of chromosomal SSBs, and are therefore denoted 'global SSBR'. However, it is possible that cellular SSBR capacity and mechanism is regulated to some extent depending on cell-cycle status. For example, it has been suggested that XRCC1 expression is regulated by FOXM1 and E2F1 — transcription factors that regulate a variety of genes required for DNA replication and proliferation[110,115]. XRCC1 has also been reported to be downregulated in terminally differentiated muscle cells, although how applicable this observation is to other non-proliferating cells remains to be determined[116]. There is also mounting evidence that S-phase cells possess one or more additional SSBR processes for repairing SSBs, possibly once they are encountered by the DNA replication machinery[93,117–120] (FIG. 4). The molecular detail of this hypothetical process (which we have previously denoted replication-coupled SSBR, RC-SSBR)[41,121] is unclear, but is probably similar to the long-patch subpathway of global SSBR described in FIG. 2 because many of the enzymes involved in the long-patch repair process (for example, Pol δ/ε, PCNA, LIG1 and FEN1) are core components of the replication machinery. RC-SSBR is likely to operate in conjunction with HR because SSBs that are encountered by a replication fork can be converted into DSBs[8,9] (FIG. 4b). Consequently, RC-SSBR and HR might function together as an effective back-up mechanism that ensures that SSBs that escape global repair do not irrevocably block DNA replication. RC-SSBR might also involve some global SSBR factors, such as XRCC1 and possibly components of the global SSBR end-processing machinery. However, a key feature of this process is likely to be the availability of structure-specific nucleases that can remove a variety of damaged termini (FIG. 4). As proposed previously[122,123] and discussed below, this feature of RC-SSBR and HR might in part account for the lack of genetic instability and cancer in human genetic diseases such as ataxia-oculomotor apraxia 1 (AOA1) and SCAN1 (see below) in which global SSBR proteins are mutated. Thus, whereas global SSBR might be the primary determinant of genetic integrity and survival in non-cycling cells in response to SSBs, RC-SSBR (in conjunction with HR) might be the major determinant of these end-points in proliferating cells.

SSBR and hereditary genetic disease

Ataxia-oculomotor apraxia 1. AOA1 is an autosomal recessive spinocerebellar ataxia syndrome that resembles Friedreich ataxia and ataxia-telangiectasia (AT) neurologically, but which lacks non-neurological features such as immunodeficiency and telangiectasia[124]. Initial studies also suggested that AOA1 lacks the overt cellular and chromosomal sensitivity to ionizing radiation that accompanies AT[125]. Although subsequent studies have revealed considerable clinical variation within AOA1, characteristic features of AOA1 seem to be variable onset (1–16 years), cerebellar atrophy and ataxia (uncoordinated movement and gait), late axonal
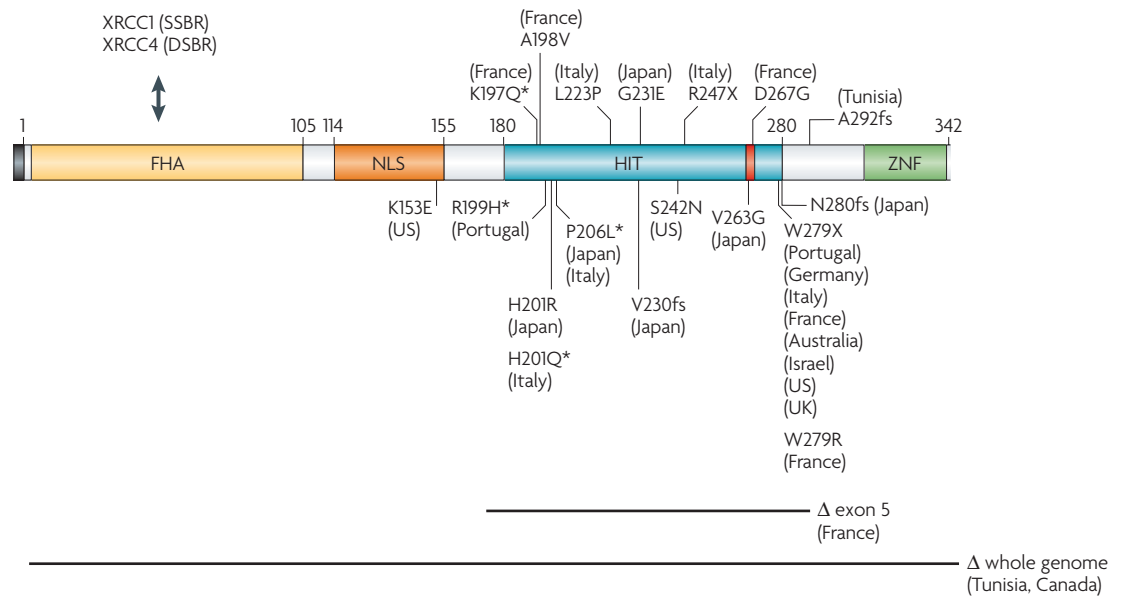
Figure 5 | **Aprataxin and ataxia-oculomotor apraxia 1 (AOA1) mutations.** Known domains are indicated, along with the location of established direct interactions with X-ray repair cross-complementing protein 1 (XRCC1) and XRCC4. The red box denotes the position of the catalytic histidine triad (HIT). The black box denotes the additional 14 amino acids that are present at the amino-terminal in the longest (356 aa) alternative splice variant. Asterisks denote mutations that are associated with particularly late-onset and/or mild clinical presentation. Parentheses denote the countries in which each mutation has been reported. Δ denotes deletion, followed by its location. fs, denotes frameshift: the nucleotide mutations associated with the frameshifts are; V230fs, 689insT; N280fs, 840delT; A292fs, splice-site mutation 875-1G>A (IVS7-1 G>A). Citations for mutations are omitted for brevity. DSBR, double-strand break repair; FHA, forkhead associated; ZNF, zinc finger; NLS, nuclear localization signal; SSBR, single-strand break repair.

peripheral neuropathy, and oculomotor apraxia (limited eye movement on command). In addition, a number of other features appear in AOA1, including cognitive impairment, hypercholesterolaemia, hypoalbuminaemia and involuntary movements (choreoathetosis and dystonia). In a large study covering approximately half of the Portuguese population, AOA1 accounted for ~21% (22 patients in 11 families) of 107 individuals with recessive spinocerebellar ataxia, a frequency second only to Friedreich ataxia (38%)[126].

Five of the families included in the study described above were subsequently linked to a locus designated as *AOA1* (REF. 127), along with five Japanese patients with early-onset cerebellar ataxia with hypoalbuminaemia (EOCA-HA). A recent study of 14 French, Italian and Algerian patients with AOA1 from nine families supports the likelihood that, although cerebellar atrophy, ataxia, and sensorimotor axonal neuropathy are common to most patients with AOA1, the presence and severity of other features is more variable (~85% in the case of oculomotor apraxia)[128]. Variation in clinical impact and/or severity of AOA1 is further suggested by reported clinical overlap with other neurological diseases and conditions, such as multiple system atrophy (MSA) and ataxia with coenzyme Q10 (coQ10) deficiency[129–131]. Intriguingly, Baba *et al.* reported that two patients with clinical features resembling the cerebellar subtype of MSA (MSA-C) harboured novel heterozygous mutations in APTX (FIG. 5; mutations K153E and S242N). AOA1 accounts for 5–10% of all autosomal recessive

cerebellar ataxias and has a variable age of onset (typically 1–18 years), with a mean of ~5 years[127,128]. Unlike AT cells, AOA1 cells are only mildly sensitive, if at all, to ionizing radiation and other genotoxins, and exhibit normal cell-cycle checkpoint control and chromosome stability following ionizing radiation[74]. Consistent with these observations, elevated cancer incidence has not been reported in patients with AOA1.

In late 2001, the gene that is mutated in AOA1 was identified and designated *APTX*[132,133]. The protein product of *APTX*, aprataxin, was initially characterized as a polypeptide of 342 residues. However, the longest form of this polypeptide might include an extra 14 amino acids at the amino terminus, owing to alternative splicing at the 5′ end of the gene[134]. The prevalence and function of the extra sequence is unclear, but as it is located immediately upstream of a putative mitochondrial targeting sequence (K.W.C., unpublished observations) it might influence the sub-cellular localization of APTX.

APTX contains a central histidine triad (HIT) domain and is a member of the HIT-domain superfamily of nucleotide hydrolases and/or transferases. The amino terminus of APTX exhibits homology to PNKP[133], and like PNKP encodes a divergent FHA domain[122]. Two splice variants of APTX were originally reported, one of which encoded a protein lacking the FHA domain. The FHA domains of both APTX and PNKP facilitate constitutive interactions with CK2-phosphorylated XRCC1 (REFS 73–77,122,135). It is noteworthy that a third member of this divergent FHA-domain family has
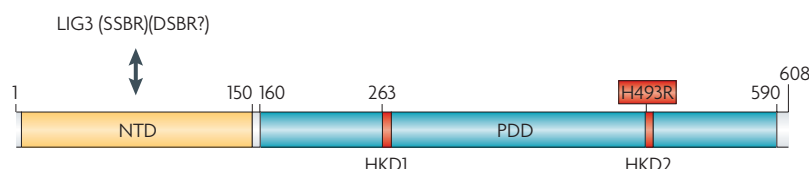
Figure 6 | **Tyrosyl-DNA phosphodiesterase 1 (TDP1) and spinocerebellar ataxia with axonal neuropathy 1 (SCAN1) mutation.** Known domains are indicated, along with the location of a direct interaction with ligase 3 (LIG3). The red boxes denote the position of the two HKD catalytic active sites. The H493R mutation in SCAN1 is indicated in red. NTD, N-terminal domain; PDD, phosphodiesterase domain.

been identified (APLF, also known as PALF and Xip1) and shown to bind CK2-phosphorylated XRCC1 (REFS 107,108,136). In fact, all three FHA-domain proteins are also sequestered into the DSB repair (DSBR) machinery through FHA domain-mediated interaction with CK2-phosphorylated XRCC4 (REFS 74,108,137). It is thus highly likely that APTX, PNKP, and APLF have roles both in SSBR and DSBR. APTX also associates with PARP1 and tumour suppressor protein p53, and with the nucleolar proteins nucleolin, nucleophosmin and upstream-binding factor 1 (UBF1)[75,138]. The association and partial co-localization of APTX with nucleolar proteins is also mediated by the FHA domain, although whether these associations are direct or indirect remains to be determined. Nevertheless, the association of APTX with nucleolar proteins might indicate that SSBR and/or DSBR is particularly important at sites of high transcriptional activity, perhaps to prevent SSBs from blocking gene expression.

Most of the mutations identified in AOA1 so far are located within the HIT domain or just upstream of the C-terminal ZNF motif, consistent with a crucial requirement for the HIT domain for normal neurological function (FIG. 5). Many of these mutations greatly reduce the stability and/or cellular level of APTX and might therefore be functional null alleles[75,139,140]. Some mutations seem to be associated with later disease onset and/or milder clinical features[133,141–143], which in some cases seem to have less effect on APTX stability and/or activity[139].

On the basis of sequence comparisons and substrate specificity, APTX seems to be a discrete branch of the HIT-domain superfamily[144]. Indeed, although APTX can hydrolyse substrates typical of either the fragile histidine triad (FHIT) or the histidine triad nucleotide-binding protein (HINT) branch of HIT-domain proteins, releasing AMP from diadenosine tetraphosphate or AMP-lysine, respectively, its catalytic activity is low ($Kcat < 0.03$ s^{-1})[139,144]. APTX has also been reported to process 3′-phosphate and 3′-phosphoglycolate termini, raising the possibility that it is an end-processing factor. However, the activity of APTX on such substrates is also low ($Kcat \sim 0.0003$–0.003 s^{-1}). A more likely physiological substrate for APTX are 5′-AMP termini[71,72]. DNA strand breaks in which the 5′-terminus is linked to AMP are normal intermediates of DNA ligation, but if they arise before 3′-DNA end processing has occurred ligation is inhibited. APTX can remove AMP from the

5′-terminus of DNA breaks at such 'abortive' DNA-ligation events, and is thus a DNA deadenylase that can 'proofread' the DNA ligase reaction (see REF. 145 for a recent review). In addition to the HIT domain, the C-terminal ZNF is important for APTX activity on 5′-AMP, most probably to increase the affinity and/or specificity of APTX for 5′-AMP substrates[72]. The ability to process 5′-AMP termini is an elegant activity, although it remains to be determined whether this type of terminus arises *in vivo*.

Spinocerebellar ataxia with axonal neuropathy 1 (SCAN1). Takashima *et al.* identified an autosomal recessive ataxia that they denoted SCAN1 (REF. 146). Like AOA1, patients with SCAN1 lack chromosomal instability and cancer predisposition but exhibit cerebellar atrophy and peripheral neuropathy. Mild hypercholesterolaemia and hypoalbuminaemia are similarly present. SCAN1 has a later age of onset (~15 years) than AOA1 and does not involve cognitive decline, on the basis of the limited number of patients available to date. SCAN1 is associated with a mutation in TDP1, an end-processing factor that repairs TOP1–SSBs. The level of TOP1 strand breaks is induced exogenously by treatment with camptothecin, which increases the half-life of TOP1 cleavage complexes, and endogenously by 'trapping' of cleavage complexes by other DNA lesions[147–152]. The second observation might explain why TOP1-dependent strand breaks are also induced by H_2O_2 and ionizing radiation[153–156]. It is worth noting, however, that although TOP1-linked termini are likely to be the primary physiological substrate of TDP1, this protein might also process other types of 3′-termini, and possibly 5′-termini, particularly at DSBs[157–160].

To date, SCAN1 is restricted to nine patients from a single Saudi Arabian family, three of which have been examined in detail[146]. Affected individuals possess a common homozygous mutation (H493R) within the second HKD motif of the TDP1 active site (FIG. 6). The ability of TDP1 to remove TOP1 peptide and create 3′-phosphate termini is reduced ~25-fold in experiments using recombinant mutated TDP1 and ~100-fold in experiments using SCAN1-cell extracts[7,161]. The greater defect in SCAN1 extracts is likely to reflect the 2–3-fold reduction in TDP1 protein levels in these cells, owing presumably to instability of the mutant protein. Intriguingly, the mutant TDP1 protein can partially process TOP1-linked termini, removing TOP1 while remaining trapped covalently on the 3′-terminus[161,162]. The extent to which mutant TDP1 converts TOP1-breaks into TDP1-breaks is concentration dependent, and the relative proportion of unprocessed (and therefore TOP1-associated) versus partially processed (and therefore TDP1-associated) termini that accumulate in SCAN1 cells is unclear. Consequently, the relative contribution of these termini to the neurological defects in SCAN1 is unknown. It is worth noting, however, that in *Tdp1*$^{-/-}$ mice, which can accumulate TOP1-linked but not TDP1-linked termini, there is an age-dependent decrease in cerebellar size, consistent with an effect of TOP1-linked termini on neurological function[163].

SSBs and/or DSBs: causes of SCAN1 and AOA1?

Both SCAN1 lymphoblastoid cells and *Tdp1*^{−/−} primary mouse post-mitotic neurons display significantly reduced global rates of chromosomal SSBR[7,156,163]. This is true not only following exposure to camptothecin and ionizing radiation, but also following treatment with H_2O_2. Because H_2O_2 is a physiologically relevant oxidizing agent, these observations provide compelling support for the idea that SCAN1 neurons might possess higher steady-state levels of this lesion. By contrast, neither non-replicating SCAN1 lymphoblastoid cells nor *Tdp1*^{−/−} primary mouse post-mitotic neurons display a measurable defect in DSBR following treatment with camptothecin, ionizing radiation, or H_2O_2[7,156,163]. This might indicate that the frequency of TDP1 DSB substrates is too rare to detect by existing cell-based assays, or that the role of TDP1 is highly redundant during DSBR in non-cycling cells. The situation is more complex in AOA1, however. APTX constitutively interacts with XRCC1 and XRCC4, and AOA1 cells exhibit mild to moderate hypersensitivity to genotoxins, consistent with a role for APTX in both SSBR and DSBR[74,75]. However, AOA1 cells lack a measurable defect in DSBR, and the evidence for a defect in SSBR is conflicting[75,82,164]. Thus, although it is likely that APTX is involved in the repair of both SSBs and DSBs, the relationship between this disease and either or both of these lesions remains to be established.

SSBs and cancer

One of the most striking features of SCAN1 and AOA1 is the absence of increased genetic instability and cancer. At first glance this seems surprising, given that unrepaired SSBs can result in potentially clastogenic DSBs during DNA replication. As we have proposed previously[7,122,165] and discussed above, this might in part reflect that proliferating cells possess alternative end-processing factors (for example, the structure-specific nucleases ERCC1–XPF, FEN1 and the MRN (Mre11–Rad50–Nbs1) complex) that efficiently and accurately remove DNA strand breaks during DNA replication (FIG. 4). Consistent with this idea, lymphoblastoid cells from patients with SCAN1 have a 3-fold increased frequency of 'spontaneous' sister-chromatid exchange, a hallmark of HR[7]. In short, it is likely that the roles of TDP1 and APTX are redundant in proliferating cells, at physiological levels of SSBs at least. Genetic instability in SCAN1 and AOA1 might be further limited by committing to apoptosis those few cells in which a strand break avoids repair and/or is involved in a genetic rearrangement. These arguments do not exclude an effect of SSBR on cancer frequency in other circumstances, however. Mutations in proteins with a role in RC-SSBR, or with a more extensive involvement in global SSBR such that the steady-state levels of SSBs is elevated above that which HR can tolerate, might have an impact on genetic instability. Although many such mutations are likely to be embryonic lethal because they are incompatible with rapid cell proliferation during embryonic development, hypomorphic or somatic mutations in such genes could affect cancer incidence. For example, the possible correlation between cancer incidence and a number of XRCC1 polymorphisms is the focus of intense epidemiological investigation, and mutations in Pol β have been identified in ~30% of human tumours, some of which can affect polymerase fidelity and/or activity during SSBR[166].

SSBs and neurodegeneration

The existence of dedicated pathways for dealing with unrepaired SSBs during S phase might explain why SCAN1 and AOA1 are not associated with measurably increased genetic instability and cancer. However, this cannot explain why the impact of these diseases on post-mitotic cells is largely restricted to the nervous system. One possibility is that neurons might be more dependent on TDP1 and APTX for DNA end processing than are other post-mitotic cells, owing to a more limited availability of alternative end-processing factors. Another factor we have previously postulated is the high level of oxidative stress encountered by the nervous system, which consumes ~20% of inhaled oxygen and possesses low levels of antioxidant enzymes[122,165,167]. In addition, there is a high transcriptional demand in post-mitotic neurons, which might further increase the dependency of these cells on SSBR. Finally, the limited regenerative capacity of neurons, compared with other non-cycling cell types that are more readily replaced by precursors, might render this tissue particularly sensitive to cell dysfunction or loss. Further analyses of mouse models of these diseases will hopefully shed light on these crucial issues.

Conclusions and future questions

SSBs are the most common lesions arising in cells, and chromosomal SSBR is a rapid and efficient process. Most breaks are removed within minutes by a global pathway that is accelerated by the SSB sensor protein PARP1 and the molecular scaffold protein XRCC1. Two of the proteins that are implicated in DNA end-processing during global SSBR, TDP1 and APTX, are mutated in the hereditary genetic diseases SCAN1 and AOA1, respectively, implicating unrepaired SSBs in progressive neurological dysfunction. Whereas post-mitotic cells seem to be dependent on global SSBR for genetic integrity, proliferating cells might possess an additional SSBR pathway that operates at replication forks in conjunction with HR. This might allow proliferating cells to tolerate SSBs that escape global SSBR, at physiological levels of damage at least, perhaps explaining why SCAN1 and AOA1 are not associated with elevated genetic instability and cancer. Many questions remain, however. For example, do unrepaired DSBs contribute to these diseases, or are they strictly SSBR-defective syndromes? What is the nature of the damaged DNA termini that lead to neuronal dysfunction, and can we detect their accumulation *in vivo*? How do unrepaired SSBs lead to neuronal dysfunction, and why are these diseases selectively associated with defects in specific regions of the nervous system? Last, but not least, are defects in SSBR associated with other neurological disorders in which oxidative stress is an etiological factor (for example, Parkinson disease and Alzheimer disease), and do SSBs contribute to neurological decline during normal human ageing?

Clastogenic
Capable of causing chromosome damage and/or rearrangements.

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DATABASES
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LIG1 | LIG3 | LIG4
OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
ataxia-oculomotor apraxia 1 | ataxia-telangiectasia | Friedreich ataxia | spinocerebellar ataxia with axonal neuropathy 1
UniProtKB: http://ca.expasy.org/sprot
APEX1 | APTX | FEN1 | PARG | PARP1 | PNKP | TDP1 | TOP1 | XRCC1

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