Trends in Genetics

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Review

Perspectives on PARPs in S Phase

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Accurate copying of DNA during S phase is essential for genome stability and cell viability. During genome duplication, the progression of the DNA replication machinery is challenged by limitations in nucleotide supply and physical barriers in the DNA template that include naturally occurring DNA lesions and secondary structures that are difficult to replicate. To ensure correct and complete replication of the genome, cells have evolved several mechanisms that protect DNA replication forks and thus maintain genome integrity and stability during S phase. One class of enzymes that have recently emerged as important in this process, and therefore as promising targets in anticancer therapy, are the poly (ADP-ribose) polymerases (PARPs). We review here the roles of these enzymes during DNA replication as well as their impact on genome stability and cellular viability in normal and cancer cells.

Highlights

PARP1 and PARP2 are essential for embryonic viability, and chemical inhibition of these enzymes in the clinic selectively kills homologous recombination-defective cancer cells (e.g., those harbouring mutations in BRCA1 or BRCA2).

PARP1 and PARP2 are activated by potentially pathogenic nucleic acid structures such as DNA breaks, DNA single-strand gaps, and stalled or broken DNA replication forks.

PARP activity signals the presence of these structures by modifying themselves and other proteins with poly (ADP-ribose), thereby promoting their repair.

Unligated Okazaki fragment DNA replication intermediates are primary inducers of PARP activity in normal proliferating cells.

Unligated Okazaki fragments are candidate drivers of genome instability and/or cell death in developing embryos and in homologous recombination-defective cancer cells in which PARP activity is absent or inhibited.

The PARP Family: Key Contributors to Cell Proliferation

The ADP-ribosyltransferases are a superfamily of 17 enzymes that modify themselves and other proteins with **mono(ADP-ribose)** (see Glossary) or **poly(ADP-ribose)** using NAD^+ as an enzyme cofactor [1]. Proteins can be mono- or poly-ADP-ribosylated on a variety of residues, but the most common sites are Ser, Glu, and Asp [2,3]. Protein ribosylation is a highly dynamic post-translational modification that is rapidly removed from proteins by several glycohydrolases, the primary and most active being poly(ADP-ribose) glycohydrolase (PARG). The importance of protein poly-ADP-ribosylation is indicated by the embryonic lethality observed in mice if the genes (*Parp1 and Parp2*) encoding the two major poly(ADP-ribose) polymerases (PARPs) are both deleted [4]. Although the precise cause of this lethality is unknown, it is most likely associated with a requirement for Parp1 and Parp2 during the rapid cell proliferation that occurs in the epiblast during gastrulation [5], the stage of embryonic development at which lethality is observed. Consistent with this idea, a role for PARP1 during normal S phase was suggested more than 30 years ago by the observation that PARP activity is associated with DNA replication complexes and is enhanced in S phase cells in newly replicated chromatin [6,7].

In contrast to mouse embryonic development, loss of both PARP1 and PARP2 activity is not lethal in human cell lines cultured *in vitro*, at least in the absence of exogenous genotoxic stress [8,9]. However, deletion or inhibition of both of these enzymes results in hypersensitivity and/or **synthetic lethality** in cells lacking efficient **homologous recombination (HR)-mediated repair**, an error-free repair process that is crucial for the maintenance of genetic integrity during DNA replication [9–11]. The activity of PARP1 and/or PARP2 thus appears to play an important role in protecting DNA replication from one or more DNA structures which, in the absence of efficient HR, result in aberrant genome duplication and cell death. The source and identity of the DNA structures that arise during normal S phase and are detected by PARP1 and/or PARP2 therefore has major implications for cell viability, embryonic development, and cancer, and are the focus of this review.

PARP Activity and DNA Replication Fork Metabolism

PARP Activity at the DNA Replication Fork

Perhaps surprisingly, PARP1 does not appear to be enriched at DNA replication forks, possibly because it is abundant throughout chromatin [12–15]. By contrast, the poly(ADP-ribose)

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catabolic enzyme PARG is enriched at DNA replication forks via a direct interaction with PCNA, suggesting that the local level of poly(ADP-ribose) is tightly controlled during DNA replication by polymer degradation [14,16,17]. Consistent with this idea, short incubations with PARG inhibitor reveal that the primary detectable sites of PARP activity in normal proliferating cells are located close to or at sites of DNA replication, and are associated with PARP1 activation at unligated **Okazaki fragment** intermediates of DNA replication [18]. In contrast to short periods of PARG inhibition, loss of PARG activity for extended periods (>12 h) results in extensive DNA replication fork damage, confirming that tight control of poly(ADP-ribosylation) is important during DNA replication [19]. This could reflect an impact of prolonged PARG loss on the metabolism of stalled or broken DNA replication forks, and/or an impact on other PARP-dependent processes such as **DNA single-strand break (SSB)** repair or Okazaki fragment maturation [18,20].

The physiologically relevant target(s) of PARP activity at DNA replication forks are currently unknown. Mass spectrometric analyses of proteins recovered using poly(ADP-ribose) binding reagents identify hundreds of ribosylated proteins in unperturbed cells, and >1000 in cells treated with genotoxins [21–23]. Perhaps surprisingly, relatively few components of the replisome are ribosylated, with RFC, PCNA, RPA, and FEN1 being notable exceptions. Instead, PARP1 itself and chromatin proteins such as histones are the primary targets of PARP activity.

Intriguingly, in contrast to PARP1 and PARP2, both PARP10 and PARP14 have been reported to interact with PCNA. Although these enzymes are implicated in the response to DNA replication stress, definitive evidence that they are active at DNA replication forks is currently lacking, perhaps because they are mono-ADP-ribosyltransferases and mono(ADP-ribose) is difficult to detect [24–26].

PARP Activity and DNA Replication Fork Progression

The rate of DNA replication fork progression is actively slowed following treatment of cells with genotoxins via mechanisms involving PARP1 and HR-mediated repair [27–29]. This is not simply an impact of the established role of PARP1 in SSB repair, reflecting a delay incurred by the latter process ahead of the DNA replication fork, because depletion or deletion of other SSB repair proteins under the same experimental conditions does not affect fork progression [27,30]. Instead, as discussed below, this phenomenon reflects a direct role for PARP in regulating replication fork reversal and/or repair [28,30,31]. In contrast to the situation in cells treated with genotoxin, an impact of PARP activity on the rate of fork progression in unperturbed cells is not normally detected [27,30,31], although such an impact was recently reported under conditions of prolonged PARP inhibition [32]. Regulation of DNA replication fork reversal and/or repair by PARP enzymes is thus most likely relatively rare during normal S phase.

PARP Activity and 'Toxic' NHEJ at DNA Replication Forks

Lesions or obstructions in the DNA template such as SSBs can result in DNA replication fork stalling (Figure 1A), and the subsequent formation of a reversed fork structure known as the 'chicken foot' in which the nascent DNA strands are annealed (Figure 1B). Alternatively, at some types of lesion or obstruction, stalled forks might collapse or break (Figure 1C), for example by replication run-off at an unrepaired SSB [33–37] or by topoisomerase or nucleolytic cleavage of the stalled fork [38–40]. A feature common to both of these scenarios is the presence of a **one-ended double-strand break (DSB)** – a structure that can trigger PARP activity [41–43]. PARP activity can regulate the metabolism of one-ended DSBs several ways, depending in part on whether the DSB is associated with a reversed replication fork or a broken replication fork, as discussed below.

Glossary

DNA single-strand breaks (SSBs): breaks or gaps in one strand of a DNA duplex. SSBs can be induced by the abortive activity of topoisomerases, by direct ionisation of DNA (e.g., by ionising radiation such as X-rays), or by attack of DNA by reactive oxygen species, and are also obligate intermediates of DNA excision repair pathways. SSBs are typically 1–3 (depending on their source) orders of magnitude more common than DSBs. Unligated Okazaki fragments are a special form of SSB in which Okazaki fragment processing and ligation has failed.

DNA strand exchange/template switching: the process by which the exchange of DNA strands between adjacent sister chromatids is achieved. RAD51 recombinase assembles on the 3′ single-stranded tail formed during DNA end-resection in a filament-like structure and promotes the capture of the identical single strand in the adjacent sister chromatid. This can switch the strands that are employed as DNA replication templates, thereby reforming the fork and enabling bypass of any lesion or blockage persisting in the original template strand.

DSB end-resection: degradation of the 5′-end of a DSB during HR-mediated repair by a nuclease activity such as MRE11, to create a 3′-single-stranded tail on which filaments of RAD51 recombinase can assemble.

Homologous recombination (HR)-mediated repair: a DSB or gap repair process that uses an intact sister chromatid as a sequence template to repair a damaged sister chromatid during S–G2 phase of the cell cycle. This pathway is relatively slow but is largely error-free.

Mono(ADP-ribose): the monomeric product of mono-ADP-ribosyltransferase activity, and of PARG activity on poly (ADP-ribose), in which proteins are modified with an ADP-ribose monomer.

Nonhomologous end-joining (NHEJ): a DSB repair process in which two-ended DSBs can be directly joined together. This pathway can operate throughout the cell cycle and is relatively fast, but is error-prone because it does not employ a sister chromatid as a sequence template at the break.

Okazaki fragments: a normal intermediate of discontinuous DNA replication. These structures are several hundred nucleotides in length, and must

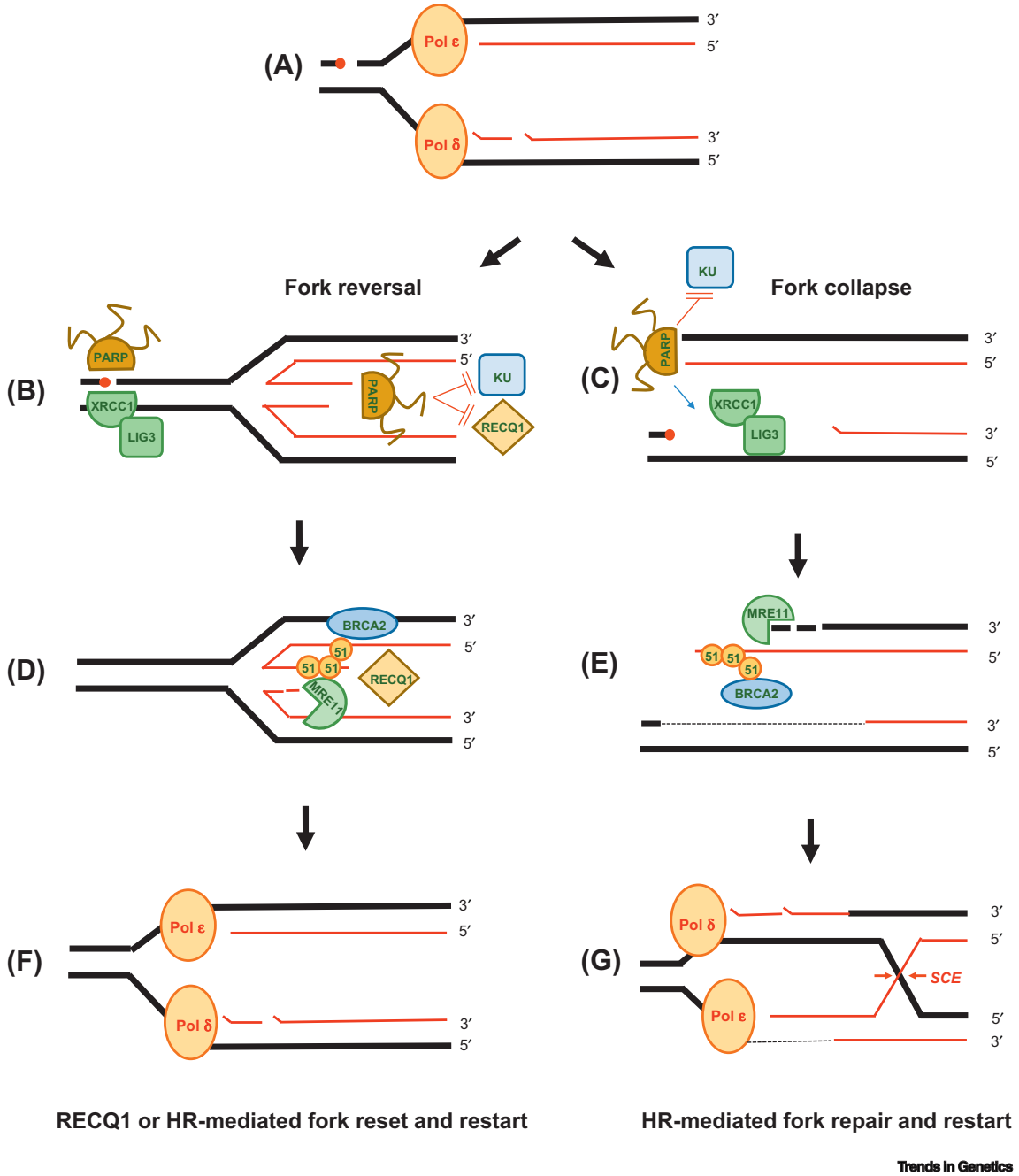

Figure 1. Poly(ADP-Ribose) Polymerase (PARP) Activity at Stalled DNA Replication Forks. A DNA replication fork stalls at a single-strand break (SSB) in the leading-strand template (A). Red lines denote nascent DNA and the red circle denotes a damaged 3′-terminus at the SSB. The replication fork reverses creating a chicken foot structure (B), or alternatively runs into the SSB and collapses the fork (C), in both cases creating a one-ended double-strand break (DSB) that is detected by PARP1 and/or PARP2. PARP activity (brown wavy lines) also promotes XRCC1 recruitment and repair of the SSB that is located either ahead of the reversed fork (in B) or on the adjacent sister chromatid of the collapsed fork (in C). In addition, PARP activity suppresses KU-dependent toxic end-joining at the one-ended DSBs, and prevents the premature reset and restart of the reversed fork by inhibiting RECQ1. Finally, PARP activity can promote MRE11 recruitment, DSB end-resection, and BRCA2-assisted RAD51-loading (‘51’ circles) at the reversed (D) or collapsed (E) fork, enabling homologous recombination (HR)-mediated fork reset and restart (F,G). At reversed forks (D), fork reset and restart can also be achieved by RECQ1 helicase activity. Abbreviation: SCE, sister-chromatid exchange.

One likely role for PARP1 and/or PARP2 at both types of one-ended DSB is to suppress the 'toxic' binding of proteins such as Ku and 53BP1 which are involved in **nonhomologous end-joining (NHEJ)** [27,44–46] (Figure 1B,C). This role appears to be important to increase the access of one-ended DSBs by proteins involved in HR-mediated repair, and is particularly important for cells in which HR capacity is reduced. NHEJ is a DSB repair pathway in which **two-ended DSBs** are ligated together without employing an undamaged sister chromatid as a template for repair [47]. Although this is a legitimate, albeit potentially error-prone, approach for repairing two-ended DSBs such as those induced by genotoxins, NHEJ activity at one-ended DSBs can lead to inappropriate ligation with another DSB at a distal region of the genome, thereby resulting in a chromosome translocation and/or cell death [48,49]. Interestingly, the inability to block toxic NHEJ at one-ended DSBs may account for the lack of HR-mediated fork slowing in PARP-defective cells, either because NHEJ is a rapid process that can repair broken forks more quickly than HR-dependent processes (albeit at the expense of genome instability and/or cell viability) or because it can promote PCNA loading and replication-restart downstream of the blocked fork [27,50].

PARP Activity and the Restart and/or Repair of DNA Replication Forks

PARP activity is implicated in promoting HR at one-ended DSBs not only by suppressing toxic NHEJ, as described above, but also by regulating the recruitment and activity of MRE11 nuclease [51]. The recruitment of MRE11 by PARP1 or PARP2 at stalled or broken replication fork structures is mediated by physical interaction, possibly by an ability of MRE11 to bind to poly (ADP-ribose) [52]. MRE11 facilitates **DSB end-resection** (Figure 1D,E), the process by which an extended 3′-single-stranded tail can be generated and on which RAD51 recombinase can load and facilitate **DNA strand exchange/template switching** [53]. DNA strand exchange/template switching results in (re)annealing of the two strands of the reversed or broken DNA replication fork with their complementary strands in the intact sister chromatid, thereby resetting or repairing reversed and broken forks in readiness for DNA replication restart (Figure 1F,G). It should be noted that, although the use of DSB end-resection and RAD51 loading is normally an effective way to protect and/or repair one-ended DSBs, it can have pathological consequences if RAD51 functionality is attenuated (e.g., as in *BRCA2*-mutated cells), because MRE11 recruitment can lead to excessive fork degradation and cell death [54–56]. It is also important to note that the roles described above for PARP activity in promoting HR at stalled or broken DNA replication forks were observed in cells that were treated with genotoxins. However, the net impact of losing PARP activity in otherwise unperturbed cells is increased HR [57–60]. This apparent paradox is most likely explained by the overriding role played by PARP activity in promoting SSB repair (see below) which, by suppressing the number of SSBs persisting in S phase, reduces the number of HR events.

At reversed DNA replication forks, PARP1 can also regulate the longevity of the regressed fork structures by inhibiting RECQ1 (Figure 1B), a helicase that can 'reset' reversed forks independently of RAD51-mediated HR by 'pushing' them back to their original position [30,31]. By negatively regulating fork reset in this way, PARP activity may influence the decision regarding whether the lesion ahead of the fork is repaired before fork restart (e.g., as shown at a SSB in Figure 1B) or is bypassed in an error-free manner. It is noteworthy that PARP activity is particularly appropriate at DNA replication forks that are stalled or collapsed at a SSB. This is because, in addition to the roles discussed above in regulating the repair and restart of the DNA replication fork, this activity can recruit **XRCC1 protein complexes** [8]. These complexes can rapidly repair the SSB either ahead of the stalled fork (as in Figure 1B) or following collapse of the fork, after which the SSB is present on the adjacent sister chromatid (as in Figure 1C).

be ligated together to generate new intact DNA 'lagging' strands.

Okazaki fragment processing: the process by which most Okazaki fragments are ligated during DNA replication. This typically involves displacement of the 5′-end of a downstream Okazaki fragment by Pol δ-mediated extension of the 3′-end of the upstream fragment, followed by cleavage of the displaced single-strand flap by FEN1 and ligation of the nick by LIG1.

One-ended DSBs: DSBs in DNA that do not have an adjacent double-stranded end to ligate to. These structures arise primarily at replication forks that have broken, and require HR-mediated repair for their error-free removal and for reformation of an intact replication fork.

Poly(ADP-ribose): the polymeric product of poly-ADP-ribosyltransferase/poly(ADP-ribose) polymerase activity, composed of repeating units of ADP-ribose. These molecules can be short oligomers or long polymers of several hundred units.

Synthetic lethality: cell death induced by a combination of two genetic or chemical states, each of which has little or no toxic impact when applied separately. It refers here to cell death induced by the loss and/or chemical inhibition of two DNA repair pathways of overlapping function, of which the loss of either alone is not toxic.

Two-ended DSBs: DNA double-stranded ends that do have an adjacent double-stranded end to ligate to. These structures arise via the cutting of both strands of a DNA duplex in close proximity, such that the chromosome is severed. Stochastic (random) two-ended DSBs can be induced by the abortive activity of topoisomerases, by direct ionisation of DNA (e.g., by ionising radiation such as X-rays), or by attack of DNA by reactive oxygen species.

XRCC1 protein complexes: XRCC1 is a cigar-shaped scaffold protein with binding sites for the enzymes that repair most types of SSBs, including Pol β, PNKP, APTX, and LIG3. It is recruited to SSBs by a binding site for poly(ADP-ribose). Mutation of XRCC1 or several of its partners results in hereditary neurological disease.

PARP Activity and Two-Ended DNA DSBs

Two-ended DSBs can arise during S phase by the encounter of a SSB with two DNA replication forks, approaching from opposite directions. In addition, they can arise directly from DNA attack by genotoxic treatments such as ionising radiation or by endogenous reactive oxidative species or abortive topoisomerase activity. If an intact sister chromatid is present at the site of the DSB, as is the case in G2 phase of the cell cycle or in S phase at replicated regions of the genome, then HR-mediated repair is a viable and potentially error-free mechanism for their repair. Several studies have suggested a role for PARP activity in HR-mediated repair of genotoxin- or nuclease-induced two-ended DSBs, by promoting the rapid recruitment of BRCA1, BRCA2, or Timeless [61–64] or by suppressing 53BP1-dependent NHEJ [65]. By contrast, other studies in which two-ended DSBs were induced at specific sites of the genome by nucleases or during gene targeting have failed to detect a role for PARP activity in stimulating HR [59,66], or have detected an negative regulatory role mediated by the suppression of BRCA1 function [67]. Consequently, it is currently unclear whether or not PARP enzymes play a role during HR-mediated repair of two-ended DSBs.

In addition to HR, two-ended DSBs can also be repaired by NHEJ [68–70]. PARP3 can detect DSBs and promote NHEJ by recruiting the histone chaperone APLF, by stabilising NHEJ protein complexes, and by suppressing DNA end resection and HR [71–74]. However, PARP3 contributes very little to the total level of poly(ADP-ribose) in normal cells because this enzyme is primarily a mono-ADP-ribosyltransferase [71,75,76]. PARP1 has also been implicated in NHEJ, via interactions of this protein and/or poly(ADP-ribose) with DNA-PK, MRE11, and NBS1 [52,77,78]. However, convincing evidence that PARP1 promotes NHEJ has not yet been reported. By contrast, there is convincing evidence that PARP1 can promote an 'alternative' pathway of NHEJ that operates if the classical (KU-dependent) pathway is absent, possibly by recruiting the error-prone DNA polymerase Pol θ and/or LIG3 [79–81]. Nevertheless, because classical NHEJ is normally very active, it is unlikely that this alternative pathway contributes significantly to PARP activity during normal cell proliferation.

PARP Activity, DNA SSBs, and Okazaki Fragments

As discussed above, SSBs are a major threat to DNA replication because they can result in DNA replication fork blockage or collapse. SSBs are potent activators of PARP activity, which in turn enables recruitment of the scaffold protein XRCC1 and its associated SSB repair proteins. SSBs can arise stochastically by the disintegration of oxidised deoxyribose or indirectly as obligate intermediates of excision repair pathways or abortive topoisomerase activity [82]. Surprisingly, however, the predominant source of SSBs that induce PARP activity in normal proliferating cells, as measured by the detection of poly(ADP-ribose), are not stochastic SSBs but instead are unligated Okazaki fragment intermediates of DNA replication [18]. Indeed, these structures appear to account for the majority, if not all, of the detectable poly(ADP-ribose) in normal proliferating cells. Although other sources of PARP activity such as stochastic DNA breaks and aberrant DNA replication fork structures almost certainly arise during normal S phase, they are either relatively rare or are too transient to contribute to the level of detectable PARP activity.

It is currently unclear to what extent **Okazaki fragments** escape the canonical pathway for **Okazaki fragment processing** in normal cells. Although this pathway is highly efficient, it may not be sufficient to process all of the 30–50 million Okazaki fragments generated during each human S phase. For example, it has been estimated that 15–30% of human DNA polymerase Pol δ molecules dissociate from the extending nascent lagging strand before the downstream Okazaki fragment is encountered [83]. If only 1 in 1000 Okazaki fragments escapes canonical processing this would equate to 30 000–50 000 SSBs each S phase. In addition, unligated

Okazaki fragments may reflect not only stochastic errors by the lagging-strand DNA replication machinery but also may arise as a result of lesions and/or unusual DNA secondary structures in the nascent lagging strand or lagging-strand template.

It seems likely that one role of PARP activation at unligated Okazaki fragments is to facilitate the repair of these fragments (Figure 2). This idea may explain the reported association of PARP1 and XRCC1 with lagging-strand DNA polymerases [84–87] and also the ability of the XRCC1 protein partner LIG3 to maintain the viability of proliferating chicken DT40 cells lacking LIG1 [88]. A role for PARP1 in the repair of a subset of Okazaki fragments might also explain the observation, more than 30 years ago, that PARP inhibition results in the accumulation of a subfraction of nascent lagging-strand intermediates that are ~10 kb in size [89,90]. Assuming an average Okazaki fragment size of several hundred nucleotides, this would be equivalent to ~1 in every 20–50 Okazaki fragments requiring PARP-mediated repair.

PARP Activity and S Phase Disease Pathology

PARP Activity and Genome Instability

As discussed above, although several nucleic acid structures can trigger PARP activity during S phase, unligated Okazaki fragments appear to predominate. As such, it seems likely that these canonical DNA replication intermediates are a major driver of genome instability, particularly in cells in which HR is reduced or absent. This is because any unligated Okazaki fragments that escape both canonical and PARP-dependent processing (Figure 3A) can be converted into single-strand gaps and/or DSBs behind the DNA replication fork (Figure 3B), or alternatively can result in DNA replication fork reversal or collapse in the subsequent S phase (Figure 3C). These structures are excellent substrates for repair by HR in S/G2 phase of the same or subsequent cell cycle (Figure 3D), as indicated by the increased frequency of HR observed in bacterial and eukaryotic cells in which canonical Okazaki fragment processing is perturbed [91–93]. If HR

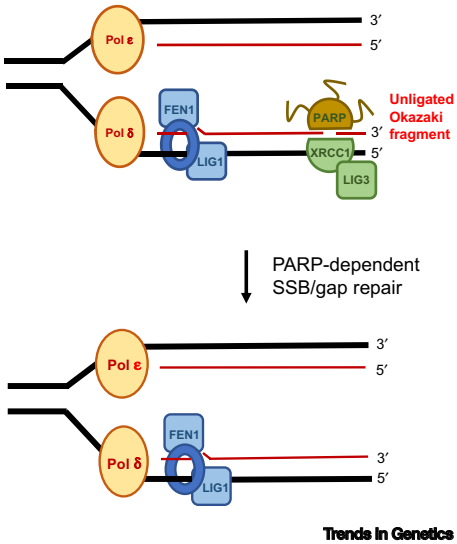

Figure 2. Poly(ADP-Ribose) Polymerase (PARP) Activity at an Unligated Okazaki Fragment. An unligated Okazaki fragment that has escaped processing by the canonical Okazaki processing machinery involving PCNA, FEN1, and LIG1 is detected by PARP1 and/or PARP2, either behind the DNA replication fork (as shown here) or at some distance once the fork has departed. PARP1 and/or PARP2 automodify themselves and/or other nearby proteins with poly(ADP-ribose) (brown wavy lines) resulting in recruitment of the XRCC1 single-strand break (SSB) repair complexes and/or other enzymes necessary for repair of the single-strand gap, flap, or nick. The red lines denote nascent DNA.

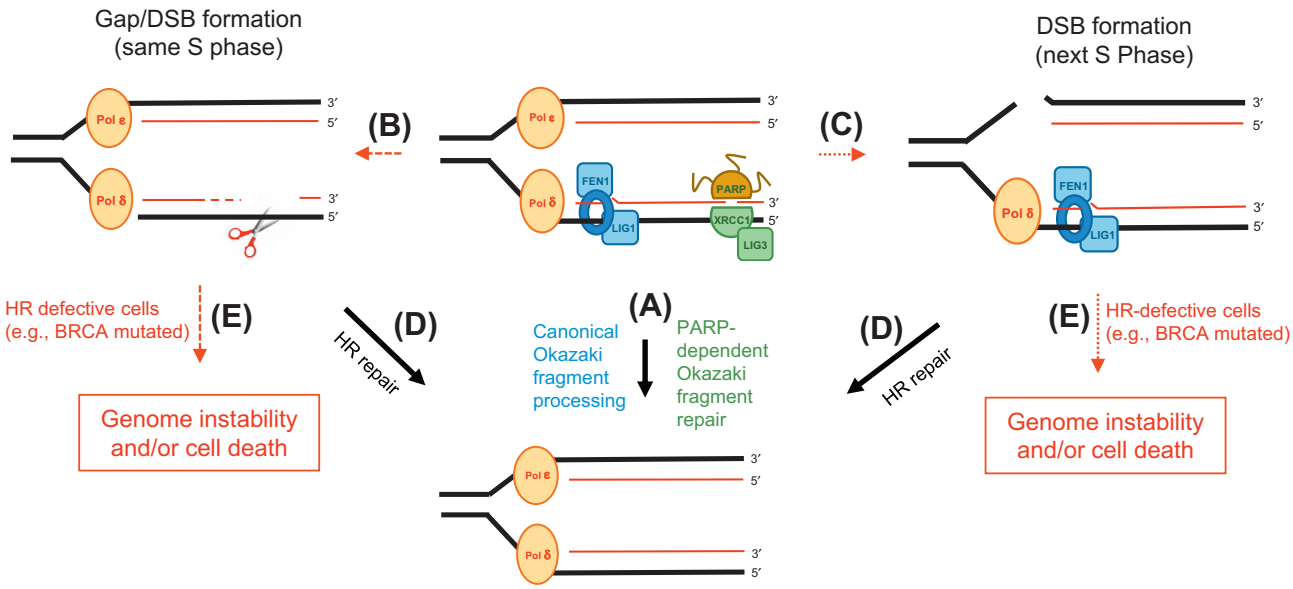

Figure 3. A Model for the Impact of Unligated Okazaki Fragments on Poly(ADP-Ribose) Polymerase (PARP) Activity and Genome Instability in Homologous Recombination (HR)-Defective Cells. (A) An Okazaki fragment is processed by canonical Okazaki fragment processing involving FEN1 and LIG1 (blue) or, if this fails, by PARP1-dependent single-strand break (SSB) repair (green). However, if both canonical and PARP-dependent processing fail (e.g., by chance or in the presence of a PARP inhibitor), the unligated Okazaki fragment is converted into an extended single-strand gap and/or double-strand break (DSB) in the same S phase (e.g., by a nuclease activity such as Mre11) (B), or is converted into a reversed or collapsed (as shown here) replication fork in the next S phase (C). In both cases the single-strand gap, DSB, or aberrant replication fork structure are repaired by HR-mediated repair (D). However, if HR-mediated repair is absent (e.g., in BRCA-mutated cancer cells), the aberrant structures arising from the unligated Okazaki fragments can drive genome instability and/or cell death (E).

is inefficient, however, such as in *BRCA1 or BRCA2*-mutated cancer cells, the DNA single-strand gaps, DSBs, and aberrant DNA replication fork structures that can arise from unligated Okazaki fragments are potential sources of chromosome degradation, rearrangement, and mitotic catastrophe [56,94,95] (Figure 3E). Indeed, in *Escherichia coli* it is known that unligated Okazaki fragments that escape HR can lead to DSB formation behind the DNA replication fork, resulting ultimately in excessive chromosome degradation and cell death [93,96].

PARP Activity and Embryonic Lethality

The observation that unligated Okazaki fragments are the major source of PARP activity in proliferating cells also implicates these structures as a major contributor to the embryonic lethality observed in mice in which *Parp1* and *Parp2* are deleted [4]. This idea is consistent with the overlapping role of Parp1 and/or Parp2 in the recruitment of XRCC1 protein complexes during SSB repair [8] because an overlapping role for these proteins is also the defining feature of their requirement during embryonic development. It is also consistent with the observation that *Xrcc1* deletion triggers embryonic lethality at a similar stage of development [97]. It is not yet clear why HR-mediated repair cannot compensate for the loss of SSB repair efficiency in developing embryos lacking Parp1 and Parp2, as would be expected from the model presented in Figure 3, but one possibility is that HR capacity is overwhelmed by the number of unligated Okazaki fragments that arise during the rapid cycles of cell proliferation.

PARP Activity and Cancer Therapy

Small-molecule inhibitors of PARP1 and PARP2 result in hypersensitivity and/or synthetic lethality in cells lacking efficient HR-mediated repair [10,11]. This finding is now exploited clinically to selectively kill cancers in which the HR regulatory proteins BRCA1 or BRCA2 are mutated [98,99]. The mechanism of cell killing is believed to reflect the 'trapping' of PARP on its nucleic acid substrate/s, rather than loss of PARP function *per se*, resulting in protein-bound DNA lesions that require HR-mediated repair for their removal. This is supported by a correlation between the clinical efficacy of different PARP inhibitors and their ability to trap the enzyme on DNA breaks [100]. However, it should be noted that this observation does not by itself exclude a loss-of-function model because PARP trapping may simply slow SSB repair to a greater extent than PARP loss if other DNA repair enzymes are unable to access the break. The strongest evidence that trapping rather than loss-of-function underlies the cytotoxicity of PARP inhibitors is that loss of PARP1 results in resistance to PARP inhibitors [101]. However, this has been convincingly demonstrated only in wild-type cells, which are killed at much higher concentrations of PARP inhibitors than are HR-defective cells. Indeed, when normal and BRCA2-defective cells were compared side by side, PARP1 loss was a resistance mechanism only in the former [102].

Once again, the discovery that unligated Okazaki fragments are the major source of detectable PARP activity in S phase implicates these as the structures on which PARP is trapped, and thus the structures that induce synthetic lethality in HR-defective cancer cells. Similarly to the scenario described in Figure 3 above, unligated Okazaki fragments on which PARP is trapped may be converted into DNA single-strand gaps, DSBs, and/or collapsed DNA replication forks in the same or subsequent S phase. However, because the trapped Okazaki fragments cannot be ligated by PARP-dependent repair, the elevated number of aberrant DNA structures arising from these unligated Okazaki fragments may drive not only genome rearrangements but also cell death in HR-defective cancer cells (Figure 3E). Consistent with this idea, the loss or inhibition of FEN1 activity also induces synthetic lethality in BRCA2-defective cells [103].

The above concepts also raise possible new avenues for anticancer therapy. For example, perhaps the use of PARP inhibitors can be extended beyond HR-defective tumours to include cancers in which Okazaki fragment processing is perturbed, such as those in which FEN1, LIG1, or Pol δ are mutated. The observations that FEN1-deficient cells are hypersensitive to PARP inhibitor [92,100], and that PARP-deficient cells are hypersensitive to FEN1 inhibitor [18], are consistent with this idea. Finally, perhaps even the judicious use of combinations of inhibitors that target both PARP enzymes and FEN1 might have therapeutic value, especially for HR-defective cancers.

Concluding Remarks and Future Perspectives

This review has highlighted several nucleic acid structures that can activate PARP enzymes during S phase, including stochastic DNA breaks and aberrant DNA replication fork structures. However, most of the studies that have addressed the activity and role of PARP enzymes at such structures have done so in cells treated with exogenous genotoxins. The extent to which these studies reflect the physiological role/s of PARP activity during normal S phase are thus unclear (see Outstanding Questions). Of those endogenous nucleic acid source/s of PARP activity that have been detected, unligated Okazaki fragments appear to be the most prevalent. Understanding how these and other endogenous sources of PARP activity arise and are repaired will be important not only for understanding the diseases associated with loss of PARP activity during cell proliferation but also for the future clinical exploitation of inhibitors of ADP-ribose metabolism.

Outstanding Questions

Unligated Okazaki fragments appear to be the major source of PARP activity in normal proliferating cells. Are these the nucleic acid structures that underpin the embryonic lethality in Parp1/Parp2 defective embryos, and/or synthetic lethality induced by PARP inhibitor in HR-defective cancers?

To what extent do the roles of PARPs in the repair and/or processing of stochastic SSBs, DSBs, and stalled/broken replication forks contribute to the critical role(s) of PARPs in normal cells?

Why do some Okazaki fragments escape the canonical Okazaki fragment processing and ligation machinery? What is the nature of these structures – are they nicks, gaps, flaps, or some other type of single-stranded interruption?

Could PARP inhibitors be applied to selectively kill tumours other than those lacking efficient HR, such as cancer cells in which deregulated DNA replication perturbs canonical Okazaki fragment processing?

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