Nuclear PARPs and genome integrity

Kameron Azarm and Susan Smith

Department of Pathology, Kimmel Center for Biology and Medicine at the Skirball Institute, New York University School of Medicine, New York, New York 10016, USA

Effective maintenance and stability of our genomes is essential for normal cell division, tissue homeostasis, and cellular and organismal fitness. The processes of chromosome replication and segregation require continual surveillance to insure fidelity. Accurate and efficient repair of DNA damage preserves genome integrity, which if lost can lead to multiple diseases, including cancer. Poly (ADP-ribose) a dynamic and reversible posttranslational modification and the enzymes that catalyze it (PARP1, PARP2, tankyrase 1, and tankyrase 2) function to maintain genome stability through diverse mechanisms. Here we review the role of these enzymes and the modification in genome repair, replication, and resolution in human cells.

Background: PARP1 and PARP2 and tankyrase 1 and tankyrase 2

Enzymes that catalyze PARylation

ADP-ribosylation (ADPr) is a posttranslational modification that regulates multiple cellular processes (Gupte et al. 2017). ADPr is catalyzed by the ADP-ribosyltransferase (ART) superfamily of enzymes (Palazzo et al. 2017). The best-studied family of this group is the poly(ADP-ribose) polymerases (PARPs). The PARP family is comprised of 17 members that share an ART diphtheria toxin-like (ARTD) domain and, hence, is also referred to as the ARTD family (Amé et al. 2004; Hottiger et al. 2010). PARPs transfer a single or multiple ADP-ribose unit(s) from nicotinamide adenine dinucleotide (NAD^+) to protein acceptors. PARP family members that perform poly(ADP-ribosyl)ation (PARylation) include PARP1 and PARP2, which synthesize long branched chains of up to 200 units, and tankyrase 1 and 2, which synthesize shorter unbranched chains of up to 20 units (de Murcia and Menissier de Murcia 1994; D'Amours et al. 1999; Rippmann et al. 2002; Vyas et al. 2014). The remaining members of the PARP family are not as well studied, but most catalyze mono(ADP-ribosyl)ation (MARylation) and have a range of functions throughout the cell (Lüscher et al. 2018). Here we focus on the four family members that catalyze PARylation (PARP1 and PARP2 and tankyrase 1 and tankyrase 2) and how they function in the nucleus to promote genome stability. We first compare and contrast PARP versus tankyrase and discuss the role of the poly(ADP-ribose) (PAR) modification. We provide an overview of PARPs in DNA repair, highlighting recent studies on their role during DNA replication and in phase separation. Finally, we focus on the role of tankyrases in telomere maintenance and chromosome resolution.

Compare and contrast: PARP1 and PARP2 versus tankyrase 1 and tankyrase 2

Structure, mode of action, and function

Structure PARP1, the founding member of the PARP family, is the most well studied and known for its role in DNA damage repair (DDR) (Martin-Hernandez et al. 2017; Ray Chaudhuri and Nussenzweig 2017). The primary structure of PARP1 comprises an N-terminal three-zinc-finger domain (Zn1, Zn2, and Zn3), an internal BRCA C terminus (BRCT) domain followed by a Trp–Gly–Arg (WGR) domain, and a C-terminal catalytic domain comprised of a helical domain (HD) in close association with the ART domain (Fig. 1A; Langelier et al. 2012, 2018a). One of the earliest events in DNA repair is recruitment of PARP1 to DNA. PARP1 binds to a range of DNA structures like cruciforms (Brázda et al. 2011) and DNA damage structures, including nicked and gapped single-strand breaks (SSBs) and overhang and blunt-ended double-strand breaks (DSBs) (Beck et al. 2014b). In the absence of genotoxic stress PARP1 is catalytically inactive. The HD regulates catalytic activity by blocking NAD^+ binding. Upon detection of DNA damage, the Zn fingers and WGR domain organize around the DNA break, inducing an allosteric destabilization of the HD that enables NAD^+ to access the catalytic active site (Dawicki-McKenna et al. 2015; Langelier et al. 2018b). PARP2 and the closely related PARP3 [a mono(ADP-ribosyl) transferase] lack the Zn fingers and BRCT domain of PARP1, but share

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Corresponding author: susan.smith@med.nyu.edu
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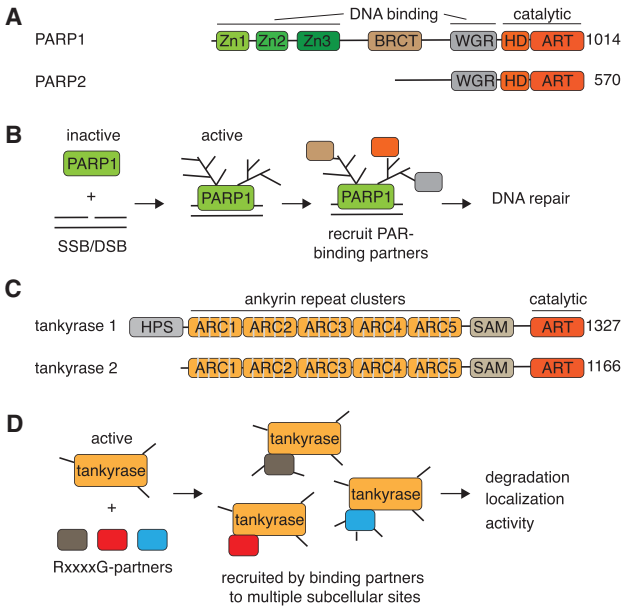

Figure 1. Primary structure and mode of action for PARP1 and PARP2 and tankyrase 1 and tankyrase 2. (*A*) Schematic representation of PARP1 and PARP2. (Zinc fingers) Zn1, Zn2, and Zn3; (BRCT) BRCA C terminus; (WGR) Trp–Gly–Arg domain; (HD) helical domain; (ARDT) ADP-ribosyltransferase. (*B*) PARP1 or PARP2 is regulated through its catalytic activity. Inactive PARP1 binds to breaks and is activated to undergo autoPARylation. PAR serves to recruit DNA repair proteins. (*C*) Schematic representation of tankyrase 1 and tankyrase 2. (HPS) His–Pro–Ser domain; (SAM) sterile α module; (ART) ADP-ribosyltransferase. (*D*) Tankyrase 1 or tankyrase 2 is regulated through its selection of binding partners. Constitutively active tankyrase binds to RxxxxG-containing proteins and localizes throughout the cell for multiple functions. Tankyrase may (or may not) PARylate its partner.

a common WGR domain that interacts with DNA (albeit with different substrate specificities) and regulates their catalytic activity in response to DNA damage (Langelier et al. 2014; Grundy et al. 2016; Obaji et al. 2016, 2018; Riccio et al. 2016a).

Binding to breaks stimulates rapid and extensive synthesis of PAR onto PARP1 itself (autoPARylation) (Satoh and Lindahl 1992). The PAR then may prevent immediate inappropriate processing of the damage by blocking access to nucleases (Caron et al. 2019) while simultaneously acting as a recruitment platform for nucleases (Wang et al. 2019) and other proteins that bind PAR to repair the damage (Fig. 1B). Excessive PARylation can induce release of PARP1 from the DNA. The turnover of the PAR chains is also regulated rapidly through the glycohydrolase PARG (Lin et al. 1997; Slade et al. 2011; O'Sullivan et al. 2019).

Tankyrase 1 and Tankyrase 2 are closely related proteins with diverse cellular functions ranging from chromosome resolution to Wnt/β-catenin signaling (Hsiao and Smith 2008; Haikarainen et al. 2014). They have a similar primary structure comprised of a C-terminal catalytic PARP domain, a SAM (sterile α module) domain, an ankyrin repeat domain, and an N-terminal HPS (His, Pro, and Ser) domain of unknown function, unique to tankyrase 1 (Fig. 1C; Smith et al. 1998). The SAM domain of tankyrases can promote homo- and hetero-oligomerization of tankyrase 1 and tankyrase 2; polymerization is required for full catalytic activity (De Rycker and Price 2004; Mariotti et al. 2016; Riccio et al. 2016b; Fan et al. 2018). A distinguishing feature of tankyrases is their ability to interact (through the ankyrin repeat domain) with a broad range of binding partners (Fig. 1D). The ankyrin domain is organized into five ankyrin repeat clusters (ARCs), which serve as a basic unit for recognizing an eight amino acid segment (with a strict requirement for Arg at position 1 and Gly at position 6 [RxxxxG]) in its binding partners (Sbodio and Chi 2002; Seimiya and Smith 2002; De Rycker et al. 2003; Guettler et al. 2011; Eisemann et al. 2016). Four of the five ARCs (1, 2, 4, and 5) are each capable of recognizing a peptide motif. Proteomic and in silico screens have identified hundreds of potential tankyrase interacting proteins (Guettler et al. 2011; Bhardwaj et al. 2017; Li et al. 2017). Over 40 human tankyrase-binding partners have been validated by coimmunoprecipitation; they localize throughout the cell and most contain a consensus peptide that (where it has been tested) binds to both tankyrase 1 and tankyrase 2 (Fig. 2). This review will focus on those partners that reside in the nucleus and have been shown to influence genome stability.

Mode of action Despite the fact that tankyrases and PARP1 and PARP2 share the ability to catalyze synthesis of ADP-ribose polymers, tankyrases differ from PARP1 and PARP2 in multiple ways. Tankyrases do not bind directly to DNA and they are not induced by DNA breaks to promote rapid synthesis of long PAR chains (Cook et al. 2002). The tankyrase 1 and tankyrase 2 catalytic ART domains lack the regulatory HD found in PARPs 1 and 2, rendering them accessible to NAD^+ binding. Analysis of the PARylation status in cells shows tankyrase 1 to be constitutively autoPARylated with relatively short PAR chains (Smith and de Lange 2000; Cook et al. 2002; Chang et al. 2005). So far, there does not appear to be a mechanism for dramatic induction of tankyrase PARP activity along the lines of the massive stimulation of PARP1 by DNA damage. Studies suggest that SAM domain-mediated polymerization can regulate tankyrase catalytic activity (Mariotti et al. 2016; Riccio et al. 2016b; Fan et al. 2018), although how this would be regulated in vivo to stimulate or induce tankyrase-mediated PARylation remains to be determined. Another distinguishing feature between PARP1 and PARP2 and tankyrases is that while PARP1 and PARP2 are predominantly nuclear, tankyrases (in addition to their nuclear localization) are distributed throughout the cell at many sites, consistent with their broad array of binding partners (Fig. 2). Thus, while the regulatory mechanism for PARP1 and PARP2 occurs through repression and activation of their catalytic ART domains, the regulatory mechanism for tankyrases occurs through its ankyrin repeat selection of RxxxxG-containing binding partners. Tankyrase-binding partners can recruit tankyrases to different subcellular sites (Hsiao and

A

PROTEIN	LOCATION	BINDING SITE	REFERENCE
TRF1	N	13-RGCADG	Smith et al, 1998
IRAP	PM, G	96-RQSPDG	Chi and Lodish, 2000
Grb14	PM, E	51-LPLPDG[b]	Lyons et al, 2001
TAB182	N, Cs	1508-RPQPDG	Seimiya and Smith, 2001
NUMA	N, SP	1743-RTQPDG	Sbodio and Chi, 2002
Mcl-1	N, C, M	78-RPPPIG	Bae et al, 2003
FBP17	PM, Cs, L	577-RTQPDG	Fuchs et al, 2002
Axin	N, C	26-RPPVPG	Huang, et al, 2009
FANCD2	N	1165-RVWPSG	Lyakhovich et al, 2011
RNF146	N, C	332-RSVAGG[a]	Zhang et al, 2011
BLZF1	N, G	18-RGAGDG	Zhang et al, 2011
CASC3	N, C	413-RQSGDG	Zhang et al, 2011
3BP2	N, C	415-RSPPDG	Levot et al, 2011
Disc1	N, Ce, M	223-RGEAEG	Guettler et al, 2011
Striatin	PM, Ce	302-RSAGDG	Guettler et al, 2011
Fat4	PM	4827-RNPADG[a]	Guettler et al, 2011
RAD54	N	691-RPPPDG	Guettler et al, 2011
BCR	C	116-RPDGEG	Guettler et al, 2011
MERIT40	N, C	28-RSNPEG[a]	Guettler et al, 2011
GMD	C	12-RGSGDG	Bisht et al, 2012
CPAP	Ce	1298-REYPDG	Kim et al, 2014
Miki	SP, G	------	Ozaki et al, 2012
PTEN	N, C, ER	15-RYQEDG	Li et al, 2015
AMOT	PM, Cs	77-RQEPQG[c]	Wang et al, 2015
MDC1	N	948-RGEPEG[a]	Nagy et al, 2016
PrxII	C	110-RLSEDYG[b]	Kang et al, 2017
ABRO1	N, C	333-RPQAVG	Tripathi et al, 2017
USP25	C, ER	1049-RTPADG	Xu et al, 2017
SSSCA1	N.D.	------	Li et al, 2017
GSK3β	N, C, PM	------	Li et al, 2017
PEX14	P	350-RRGGDG[a]	Li et al, 2017
ATG9A	E, A, P	233-RLPGLG	Li et al, 2017
HectD1	C	1107-RNLPYG	Bhardwaj et al, 2017
NKD2	C	16-RESPEG[c]	Bhardwaj et al, 2017
Notch2	N, PM	1726-RREPVG[c]	Bhardwaj et al, 2017
VAMP8	PM, E, L	33-RILARG	Bhardwaj et al, 2017
Dicer	N, C	656-RELPDG	Bhardwaj et al, 2017
CAP-D3	N	519-RSEPSG[a]	Daniloski et al, 2019
LKB1	N, C, M	86-RRIPNG[a]	Li et al, 2019

B

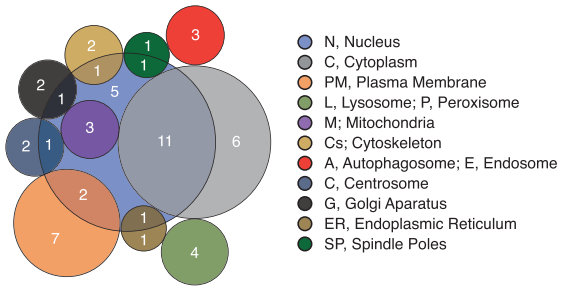

Figure 2. List of validated tankyrase-binding partners and their localizations throughout the cell. (*A*) A chronological list of human tankyrase-binding partners that have been validated by protein coimmunoprecipitation. For each protein, the list includes its subcellular location(s), its "RxxxxG" consensus tankyrase-binding site ("——" if not found in the amino acid sequence), and a reference for the publication identifying the interaction. Protein location(s) were curated through a literature search and the UniProt protein database. (N.D.) Not determined. ([a]) The protein contains additional binding motifs in its sequence. ([b]) The binding site is noncanonical. ([c]) The binding site is conserved among protein family members. (*B*) Schematic representation of the localization of tankyrase binding proteins (listed in *A*) to the indicated organelle(s). Circle size is based on the number of tankyrase-binding proteins localized to the organelle(s). The overlap between an organelle(s) circle with the "nucleus" circle represents the number proteins found in both.

Smith 2008). Once tankyrase binds a partner, it may or may not PARylate it (Smith et al. 1998; Bisht et al. 2012; Eisemann et al. 2019). Subsequently, tankyrase-mediated PARylation can influence the stability, localization, or function of the target.

Consequences of deletion The effect of knockout of these PARPs in a whole organism, such as mice, indicates a redundant and essential role for each class. Mice lacking PARP1 or PARP2 are viable, but exhibit sensitivity to genotoxic agents, consistent with a role for each protein in DNA repair (de Murcia et al. 1997; Wang et al. 1997; Masutani et al. 2000; Forsyth et al. 2002). Double deletion of PARP1 and PARP2 leads to early embryonic lethality at Day 8.0 in mice, indicating redundancy between the two proteins and suggesting a role in early development (Menissier de Murcia et al. 2003). The requirements for PARP1 and PARP2 at the stage during gastrulation when cellular proliferation increases dramatically, suggests a fundamental role for PARP in DNA replication (see below).

Mice lacking tankyrase 2 are viable and fertile, but have a short stature phenotype (Chiang et al. 2006; Hsiao et al. 2006). Mice deficient in tankyrase 1 appear to develop normally and have no defects in body size (Chiang et al. 2008), but suffer from a metabolic disorder (Yeh et al. 2009; Zhong et al. 2016). The double knockout is embryonic lethal, indicating functional redundancy and suggesting a role in development (Chiang et al. 2008). The requirement for tankyrases in development may be due to their interactions with signaling proteins such as Axin and Notch that play essential roles in development (Huang et al. 2009; Bhardwaj et al. 2017).

In contrast to the effect of deletion on mouse development, double deletion of PARP1 and PARP2 or tankyrase 1 and tankyrase 2 is not lethal in human cells grown in culture. Double-knockout *PARP1/PARP2* cell lines were generated in normal human (hTERT RPE-1) and cancer (U2OS) cells and double-knockout *TNKS1/TNKS2* cell lines were generated in cancer (HEK293T) cells (Bhardwaj et al. 2017; Hanzlikova et al. 2017; Ronson et al. 2018). The observation that these cell lines are viable indicate that (at least in the absence of exogenous genotoxic stress or other deficiencies) some human cell lines can survive without PARP1 and PARP2 or without tankyrase 1 and tankyrase 2.

PAR: a docking site for interacting proteins

The amino acid target sites of PARylation

Significant progress has been made on the proteome-wide determination of PARylated amino acids in human cells. Proteomic analyses have identified a diverse group of amino acids including Asp, Glu, Lys, Arg, and Cys (Daniels et al. 2015; Martello et al. 2016). PARP1 was previously found to be automodified on Asp, Glu, and Lys. However, recent analysis revealed Ser as the major target (Leidecker et al. 2016; Bilan et al. 2017) and further that the histone PARylation factor (HPF1) associates with PARP1 and PARP2 to target specifically Ser-ADPr (Gibbs-Seymour et al. 2016; Bonfiglio et al. 2017). Accurate analysis of ADPr site localization is inherently challenging. Recent advances in proteomic techniques will likely lead to

revisions of previous analyses and open up future discovery. Indeed, very recent work has expanded the ADP-ribosylome, identifying Tyr as an ADPr acceptor site (Leslie Pedrioli et al. 2018) and revealing crosstalk between Ser-ADPr and Ser phosphorylation (Larsen et al. 2018). Much less is known about tankyrase target sites. However, recent analysis for tankyrase 1 in vitro shows Glu and Asp as primary targets and also that HPF1 does not alter tankyrase 1 substrate specificity to target Ser for modification (Eisemann et al. 2019).

PAR-binding modules

The PAR modification can serve as a docking site for PAR-binding proteins (Teloni and Altmeyer 2016; Wei and Yu 2016). Proteins can bind to PAR through a range of interacting modules including: PAR-binding motifs (PBMs), short sequences (~20 amino acids) with a loosely defined consensus (Pleschke et al. 2000; Gagné et al. 2008); macrodomains, larger (~130- to 190-amino-acid) globular domains that bind to ADP-ribose monomer or the terminal ADP-ribose moiety in a PAR chain (Karras et al. 2005; Feijs et al. 2013); PAR-binding zinc fingers (PBZ), short modules (~30 amino acids) that bind to ADP-ribose monomers or to the ADP-ribose–ADP-ribose junctions of PAR chains (Ahel et al. 2008; Eustermann et al. 2010; Li et al. 2010; Oberoi et al. 2010); and WWE domains, comprised of conserved tryptophan (W) and glutamate (E) residues that bind to the iso-ADP-ribose moiety, the internal unit of the PAR polymer (Wang et al. 2012). Additional domains that can recognize PAR include: the forkhead-associated (FHA) domains of aprataxin (APTX) and polynucleotidekinase-3 phosphatase (PNKP), which interact with iso-ADP-ribose (like WWE domains) and the BRCA1 C-terminal (BRCT) domains of DNA ligase IV, XRCC1, and NBS1, which recognize the ADP-ribosyl moiety within PAR (Li and Yu 2013; Li et al. 2013; Breslin et al. 2015).

In addition to those described above, a number of protein motifs that bind to RNA and DNA have been found to also recognize PAR, thereby broadening the potential influence of PAR in nuclear functions. These motifs include the RNA recognition motif (RRM) found in hnRNP proteins (Gagné et al. 2008; Ji and Tulin 2009); serine/arginine repeats (SR) found in splicing factors (Malanga et al. 2008); the OB-fold found in the single-stranded binding proteins SSB1 and BRCA2 (Zhang et al. 2014, 2015a); the N terminus of the PilT protein (PIN) domain found in EXO1, GEN1, and SMG5 (Zhang et al. 2015b); and regions rich in arginines and glycines (RG/RGG), also termed glycine arginine-rich (GAR) domains, found in proteins involved in DNA damage signaling, transcription, and RNA processing (Thandapani et al. 2013).

PAR-binding modules in chromatin remodeling and DNA repair

Proteins containing PAR-binding modules can be recruited in a PAR-dependent manner by PARP1 to sites of DNA breaks to modify chromatin structure and facilitate DNA repair. For example, the chromatin remodeler amplified in liver cancer 1(ALC1), also known as chromodomain–helicase–DNA-binding protein 1-like (CHD1L), contains a PAR-binding macrodomain that is activated in a PAR-dependent manner to enable nucleosome remodeling and DNA repair (Ahel et al. 2009; Gottschalk et al. 2009, 2012). Two DNA damage repair proteins, checkpoint with forkhead and ring finger domains (CHFR) and aprataxin polynucleotide kinase-like factor (APLF) contain, respectively, single and tandem PBZ motifs (Ahel et al. 2008). APFL is recruited to sites of DNA damage through interactions with ADP-ribose through its PAR-binding PBZ motif to promote DNA repair by nonhomologous end joining (NHEJ) (Rulten et al. 2008, 2011). CHFR is required for the antephase checkpoint, dependent on its PBZ domain and PAR synthesis (Ahel et al. 2008). The chromatin remodeler CHD2 is recruited to DSBs by PAR binding through an uncharacterized motif, where it promotes chromatin decondensation to facilitate DNA repair by NHEJ (Luijsterburg et al. 2016). X-ray repair cross-complementing protein 1 (XRCC1) is recruited in a PARP1 dependent manner through a PBM, to act as a scaffold in assembly and activation of the DNA base excision repair (BER) machinery (Masson et al. 1998; Okano et al. 2003).

PAR-binding modules in protein degradation

Most examples of PAR-binding module recruitment that have been described thus far relate to PARP1. This may be due to the greater abundance of PARP1-induced PAR chains in cells compared with tankyrase-induced PAR chains. However, one PAR interactor, the E3 ligase RN146, associates specifically with tankyrase 1 and tankyrase 2. RNF146 interacts with PARylated substrates through its internal WWE domain (Callow et al. 2011; Kang et al. 2011; Zhang et al. 2011; Zhou et al. 2011; Wang et al. 2012). The PAR ligand allosterically activates the E3 ligase through a conformational change in the N-terminal RING domain to promote K48-ubiquitination of its targets (DaRosa et al. 2015). RNF146 can also bind to the ARC domains in tankyrase 1 or tankyrase 2 directly through motifs in its C terminus, thereby favoring PARylated tankyrases and their bound PARylated targets for ubiquitination and degradation (DaRosa et al. 2015, 2018). In this way, tankyrases regulate the cellular levels of several disease-related cytoplasmic proteins including Axin, 3BP2, PTEN, and the angiomotins, which are key regulators of the Wnt/β-catenin, SRC, AKT, and Hippo signaling pathways, respectively (Huang et al. 2009; Levaot et al. 2011; Li et al. 2015; Wang et al. 2015). Whether this mechanism is used to target degradation of tankyrase targets in the nucleus to influence genome integrity has not been determined.

Chromosome repair

PARP1 and PARP2 are central components in the single-strand break (SSB) repair pathway. They are also activated at double-strand breaks (DSBs) and at stalled replication

forks, where they promote homologous recombination (HR) and NHEJ. In contrast, tankyrase 1 and tankyrase 2 do not have a canonical role in DNA repair, although several recent studies suggest an indirect role through their binding partners. Finally, recent studies show that PARP1-dependent PAR can facilitate DNA repair through phase separation and transient compartmentalization of DNA damage sites.

PARP1 and PARP2 in DNA repair

Single-strand break repair SSBs can arise directly by oxidative damage to the sugar backbone, indirectly during DNA BER, or as a result of the abortive activity of topoisomerase 1 (Ray Chaudhuri and Nussenzweig 2017). Spontaneous single-strand breaks are rapidly detected and bound by PARP1. DNA-binding activates PARP1 to undergo autoPARylation (Satoh and Lindahl 1992). PAR recruits XRCC1, a core factor in SSBR that serves as a scaffold for SSB proteins (Caldecott et al. 1994). XRCC1 has an internal BRCT domain that binds directly to PARylated PARP1 or PARP2, thereby directing its recruitment to activated PARP1 or PARP2 (Caldecott 2019). XRCC1 then acts as a scaffold for protein-binding partners necessary to process damaged termini, including PNKP, APTX, and tyrosyl-DNA phosphodiesterase 1 (TDP1). XRCC1 recruits Pol β through its N-terminal domain to replace the single missing nucleotide at the SSBs and LIG3 through a C-terminal BRCT domain to ligate the nick (Ray Chaudhuri and Nussenzweig 2017; Caldecott 2019).

Double-strand break repair DSBs are produced following exposure to DNA-damaging agents such as γ-irradiation. DSBs are repaired by either HR or NHEJ (Martin-Hernandez et al. 2017; Ray Chaudhuri and Nussenzweig 2017). PARP1 detects and binds DS breaks, activating its autoPARylation. PARP1 may promote recruitment of the MRE11 nuclease (through its putative PAR-binding domain), which could contribute to DNA end processing and channel the pathway choice to HR (Haince et al. 2008). PARP1 also contributes to early recruitment of BRCA1 (through its PAR-binding domain), which subsequently loads RAD51 onto DNA, an essential step for HR (Li and Yu 2013). In G1, when the sister chromatid is not available for HR, NHEJ is the preferred mechanism for DSBR. DSBs are bound by KU70–KU80 dimers, which recruit DNA-PK catalytic subunit (DNA-PKcs). PARP1 binds and PARylates DNA-PKcs, stimulating its kinase activity, and may additionally help to recruit the chromatin remodeler CHD2 to promote LIG4-dependent classical NHEJ (cNHEJ) (Ruscetti et al. 1998; Spagnolo et al. 2012; Luijsterburg et al. 2016). Alternative NHEJ (aNHEJ) facilitates ligation independently of KU and LIG4, instead relying on MRE11 (Truong et al. 2013). PARP1 can compete with KU for access to DNA ends. In the absence of KU, PARP1 may promote recruitment of MRE11 to process the ends and channel repair to aNHEJ (Haince et al. 2008; Cheng et al. 2011). In a highly mutagenic process, the resected ends are then joined through sequence microhomology, and the gaps are filled in by POLQ and ligated by LIG3 (Ceccaldi et al. 2015; Mateos-Gomez et al. 2015).

Stalled replication forks An obstruction in the DNA template (such as an SSB in the leading strand template) can lead to a stalled fork, which can either undergo fork reversal where the nascent strands anneal in a "chicken foot" structure or fork collapse due to replication run-off of the leading strand (Cortez 2015). Either scenario results in a one-ended DSB that can be detected by PARP1 or PARP2 (Ray Chaudhuri and Nussenzweig 2017; Hanzlikova and Caldecott 2019). PARP-binding suppresses KU binding to prevent NHEJ, which can be toxic at one-ended DSBs (Hochegger et al. 2006; Sugimura et al. 2008). At the reversed fork PARP1 also inhibits the RECQ1 helicase to prevent premature fork reset and restart (Popuri et al. 2012; Berti et al. 2013). PARP activity can then promote the HR pathway. PARP1 or PARP2 physically recruits MRE11 (potentially through PAR binding) to promote DNA end-resection creating a 3′ single-stranded tail for BRCA2-assisted RAD51 loading (Haince et al. 2008; Li and Yu 2013). RAD51 facilitates DNA stand exchange/template switching to promote reannealing of the two strands of the reversed or collapsed fork with their complimentary sisters for DNA replication restart (Pasero and Vindigni 2017). In the case of reversed forks, this can also be achieved by RECQ1 once the lesion is repaired and PARP1 is released.

MARylating PARPs in DNA repair

Several PARP family members that catalyze mono(ADP-ribosylation) also function in DNA repair and genome integrity. PARP3, which is structurally related to PARP1 and PARP2, contributes to the cellular response to DSBs (Boehler et al. 2011; Rulten et al. 2011). PARP3 cooperates with KU to drive pathway choice to cNHEJ (Beck et al. 2014a) and facilitates association of APFL to damaged DNA, which promotes retention of XRCC4/DNA ligase IV to accelerate ligation during NHEJ (Rulten et al. 2011; Fenton et al. 2013). Two other MARylating PARPS, PARP10 and PARP14, which are structurally and functionally related to each other, play distinct roles in alleviating replication stress. PARP10 recognizes ubiquitinated PCNA, which is required to recruit translesion DNA polymerases for restart of stalled replication forks (Nicolae et al. 2014; Shahrour et al. 2016; Zafar and Eoff 2017). PARP10 and its interaction with PCNA is required for efficient translesion synthesis and resistance to replication fork stalling (Nicolae et al. 2014). PARP14 interacts with RAD51 to promote HR for relief of replication stress (Nicolae et al. 2015).

Tankyrase 1 and tankyrase 2 in DNA repair

Unlike PARP1 and PARP2, tankyrase 1 and tankyrase 2 do not bind directly to DNA and there is little evidence to suggest a direct role for tankyrases in DNA damage-induced repair. Depletion of tankyrase 1 does lead to an

increase in DNA damage foci, but this may be indirect due to defects in telomere and rDNA resolution (see below) (Hsiao and Smith 2009; Daniloski et al. 2019). However, several recent studies suggest that tankyrases may play a role in DNA repair, indirectly through its binding partners: mediator of DNA damage checkpoint protein 1 (MDC1), MERIT40, or TRF1. MDC1 is essential for spreading of the DDR signaling on chromatin surrounding DSBs and plays a role in DNA repair (Jungmichel and Stucki 2010). Tankyrase 1 and tankyrase 2 bind to MDC1 through tankyrase binding sites 948-RGEPEG and 1993-RRLLEG (Nagy et al. 2016). Tankyrase 1 or tankyrase 2 (overexpressed in the nucleus) localizes to sites of DNA damage (laser stripes or I-SCEI-induced DS breaks)-dependent on MDC1 and promotes DSB repair by HR (Nagy et al. 2016).

MERIT40, a component of the BRCA1-A and BRISC complexes was shown to bind to tankyrase 1 or tankyrase 2 through tankyrase binding sites 28-RSNPEG and 48-RSEGEG (Guettler et al. 2011). MERIT40 recruits tankyrase 1 (overexpressed in the nucleus) to γH2AX foci in X-ray irradiated cells, and the tankyrase-MERIT40 interaction is necessary for viability, suggesting a role for tankyrase in regulation of the DDR (Okamoto et al. 2018). It should be noted that in the above two examples tankyrase is artificially localized to the nucleus through a nuclear localization signal (NLS). This may amplify tankyrase function in the nucleus, since normally only a small fraction of endogenous tankyrase is nuclear.

Lastly, tankyrase 1 has been implicated in DNA repair through its interaction with the telomere-binding protein TRF1, which binds to tankyrase 1 and tankyrase 2 through a 13-RGCADG tankyrase-binding site (Smith et al. 1998). Tankyrase 1 can be recruited to sites of induced telomere oxidative damage where it PARylates TRF1 and leads to recruitment of XRCC1. Tankyrase 1 inhibition or expression of a TRF1 tankyrase-binding site mutant sensitizes cells to induced telomere oxidative damage, suggesting a role for tankyrase 1 in facilitating SSBR at damaged telomeres through PARylation (Yang et al. 2017).

PAR-induced liquid demixing in DNA repair

Liquid–liquid demixing or phase separation can dynamically organize soluble intracellular components into confined compartments. Phase separation can be initiated by intrinsically disordered proteins (IDPs) that contain low-complexity domains (LCDs): unstructured repetitive sequences that can phase separate into liquid droplets (Chong and Forman-Kay 2016; Banani et al. 2017). Some LCD-containing RNA-binding proteins can self-assemble into structures, and in some cases assembly can be nucleated by RNA itself (Chong et al. 2018). One example is FUS, an RNA-binding protein containing an N-terminal LCD (comprised of a prion-like domain; PLD) and multiple RGG domains (Thandapani et al. 2013). Studies showed that RNA binding could nucleate formation of higher order FUS structures, dependent on the PLD and RGG domains (Schwartz et al. 2013). PAR polymers (like RNA) could serve as seeding platforms to recruit proteins that multimerize through their LCDs to form cellular compartments (Leung 2014). Indeed, FUS binds directly to PAR in vitro through its RGG domains and is recruited to sites of DNA damage, dependent on PARP1 and PARylation activity (Mastrocola et al. 2013; Rulten et al. 2014).

Analysis of FUS compartments in vivo reveals all the hallmarks of liquid droplets: They undergo rapid internal rearrangement, are spherical, and two droplets can fuse and relax into one sphere (Patel et al. 2015). Further analysis shows that FUS, as well as two other members of the TET family of proteins, EWS and TAF15, are recruited to laser micro-irradiated sites of DNA damage in a PAR-dependent manner with the RGG domains serving as PAR sensors (Altmeyer et al. 2015). The N-terminal PLDs assemble into spherical structures in cells by liquid demixing. Live-cell microscopy shows that PAR seeds the liquid demixing at sites of DNA damage and further that the activity is enhanced by depletion of PARG and inhibited by PARP inhibitors (PARPi). The purified TET proteins or a model protein comprised of a PLD and RGG form aggregates in vitro that are consistently larger in the presence of PAR, demonstrating the intrinsic ability of PAR to nucleate IDP-aggregation.

Recently, this model was elaborated in vitro at the single molecule level using atomic force microscopy to dissect the steps (Singatulina et al. 2019). FUS is recruited to PAR synthesized by PARP1 on damaged DNA, and FUS triggers formation of large compartments in which damaged DNA is enriched. Consistent with the in vivo studies described above, both the PLD and RGG motifs are required for formation of the compartments. These compartments can reversibly dissociate by hydrolysis of PAR by PARG. Together these data indicate that PAR-mediated recruitment of FUS can facilitate DNA repair through transient phase-separated compartmentalization of DNA damage sites. Whether tankyrase-dependent PAR can promote liquid demixing remains to be determined.

Chromosome replication

As described above, PARP1 and PARP2 are activated by SSBs, DSBs, and stalled replication forks. PARP1 and PARP2 bind to the damaged DNA structure, become catalytically active, and PARylate themselves (and other proteins) to promote DNA repair. However, what about during a normal S phase in proliferating cells in the absence of exogenous damage? Is there a role for PARP1 during DNA replication? Historically it has been difficult to identify sites of PAR in unperturbed cells, although several studies hint at a role for PARP1 and PAR in DNA replication. Recent studies suggest a role for PARP1 in normal proliferating cells during S phase in Okazaki fragment processing (Hanzlikova et al. 2018) and regulation of replication fork speed (Maya-Mendoza et al. 2018). To date a role has not been described for tankyrase 1 and tankyrase 2 in replication fork progression; however, they do play a role in replication of chromosome ends by telomerase.

PARP1 and Okazaki fragment processing

During DNA replication PARP1 can bind directly to nicks of the lagging strands. Early studies showed increased PARP activity upon initiation of DNA replication and in newly replicated chromatin (Lehmann et al. 1974; Anachkova et al. 1989). Depletion of PARG, the enzyme that cleaves PAR, using siRNA led to slowing of replication fork progression and to accumulation of defective replication intermediates (Ray Chaudhuri et al. 2015). PARG interacts directly with PCNA and colocalizes to PCNA-containing replication foci (Mosavi et al. 2004; Kaufmann et al. 2017). Recent advances have led to new, cell-active in vitro chemical probes, which offer potent and selective inhibition of PARG in the cellular context (James et al. 2016). Armed with this new class of PARG inhibitor, Caldecott and colleagues set out to detect endogenous PAR by treating cells briefly (15–60 min) with inhibitor (Hanzlikova et al. 2018). PAR was detected specifically in S phase of the cell cycle and at sites of DNA replication (based on proximity to PCNA), dependent on PARP1 activity. The S phase PAR was not due to DNA damage or replication stress, rather it resulted from unligated Okazaki fragments. Okazaki fragments are first processed by FEN1, and then ligated together by LIG1. Treatment of FEN1-inhibited or LIG1-depleted cells with PARG inhibitor led to a >10-fold increase in S-phase PCNA-associated PAR. Moreover, treatment with emetine, an inhibitor of DNA replication that prevents formation of Okazaki fragments, completely blocked the appearance of S phase PAR. The authors postulated that a subfraction of the 30 to 50 million Okazaki fragments that are synthesized during replication of the human genome might escape canonical processing by FEN1 and LIG1, and instead, through PAR synthesis recruit the SSBR machinery to complete ligation (Hanzlikova et al. 2018; Hanzlikova and Caldecott 2019). Indeed, it has been demonstrated that the SSBR scaffold protein XRCC1 is recruited to these sites of incomplete replication, dependent on PARP1 and PARP2 (Breslin et al. 2015). Together these data indicate the PARP-dependent SSBR machinery as a "backup" pathway for processing unligated Okazaki fragments.

PARP1 and replication fork speed

Another way in which PARP1 may impact chromosome replication is through regulation of replication fork speed. A recent study identified a new regulatory network involving PARylation and p21 as suppressors of DNA replication fork speed (Maya-Mendoza et al. 2018). Treatment of cells with PARP inhibitors for 24 h resulted in a 60% increase in fork speed measured by DNA fiber analysis (from 1.0 to 1.6 kb/min), in contrast to the accepted model that PARP inhibitors induce fork stalling (Bryant et al. 2005). The increased fork speed was accompanied by a DDR in cells. Depletion of LIG1 and FEN1 led to accelerated fork speed (1.2 and 1.3 kb/min, respectively), but it was less than that induced by PARPi and did not induce a DDR. Indeed, when PARPi was titrated down in concentration or duration to induce acceleration <40%, a DDR was not induced, suggesting that increases >40% lead to a DDR. Knockdown of PARP1 protein led to increased fork speed (1.2 kb/min), but it was less than the effect of PARPi. The PARP knockdown cells did not show reduced PAR levels at the fork, indicating that other PARPs may be involved in fork-speed control. PARPi treatment of PARP1 knockdown cells did not further accelerate fork speed or a DDR, indicating a requirement for PARP1 protein.

To gain insight to control of the pathway, a connection between replication fork speed and PARP1, PARylation, and p53–p21 was explored. p53 activates p21 and is itself PARylated, and PARP1 binds p21 and controls its expression (el-Deiry et al. 1993; Waga et al. 1994; Frouin et al. 2003; Madison and Lundblad 2010; Lee et al. 2012). p21 levels were found to increase in PARP1 knockdown cells, but not in PARPi-treated cells. Depletion of p21 did not affect PARylation levels, but did lead to increased fork speed (1.7 kb/min) that was additive with PARPi (2.3 kb/min). A fork speed regulatory network was proposed with p21 and PARP1/PARylation as interacting arms that are connected through PARylation-mediated regulation of p53 activity, as well as PARP1-mediated inhibition of p21 expression. Reduction of p21 or PARylation would lead to an imbalance, resulting in increased fork speed. Thus, at steady state during normal DNA replication, the network would limit fork speed to ensure genome stability (Maya-Mendoza et al. 2018; Quinet and Vindigni 2018).

Tankyrase 1 and tankyrase 2 and telomere length maintenance

Human telomeres are comprised of TTAGGG repeats and shelterin, a six-subunit complex that includes the double-stranded telomere DNA-binding proteins TRF1 and TRF2 (de Lange 2005). Telomeres rely on shelterin and shelterin-binding proteins to mediate the specialized mechanisms required for their replication (Gilson and Géli 2007; Stewart et al. 2012) and protection (Palm and de Lange 2008; de Lange 2018). In normal human cells after multiple rounds of cell division, shortened telomeres are unable to recruit sufficient shelterin to protect chromosome ends, resulting in a persistent DNA damage response that signals replicative senescence (d'Adda di Fagagna et al. 2003; Takai et al. 2003). Shortening can be counteracted by telomerase a reverse transcriptase (Greider and Blackburn 1985, 1987; Lingner et al. 1997) that is repressed in the human soma (Wright et al. 1996), but is up-regulated in most human cancers (Kim et al. 1994) and is required for tumor cell growth (Hahn et al. 1999). Telomere length is regulated by TRF1; overexpression leads to telomere shortening and depletion to telomere lengthening by telomerase (van Steensel and de Lange 1997).

Tankyrase 1 was initially identified as a binding partner for TRF1 (Smith et al. 1998). Tankyrase PARylates TRF1, and this modification releases TRF1 from DNA, much in the way PARyation of PARP1 releases it from DNA breaks. Tankyrase is recruited to telomeres by TRF1, dependent on its tankyrase-binding site (Hsiao and Smith 2008). Overexpression of tankyrase 1 or tankyrase 2 in

the nucleus evicts TRF1 from telomeres, resulting in its degradation and allowing telomerase-mediated recruitment and telomere lengthening (Smith and de Lange 2000; Cook et al. 2002; Chang et al. 2003). In these experiments (as described above for tankyrase in DNA repair) artificial expression of tankyrase in the nucleus may amplify its nuclear function. CRISPR-generated knockout of tankyrase 1 or tankyrase 2 in human HEK293T cancer cells has no effect on telomere length, but the double knockout induces telomere shortening, which can be rescued by reintroduction of tankyrase 1 or tankyrase 2 (Bhardwaj et al. 2017). Thus, tankyrase is required to maintain telomere length, but either tankyrase 1 or tankyrase 2 is sufficient.

Chromosome resolution

Resolution of sister chromatids in mitosis is essential for proper distribution of genetic material to the daughter cells. Sister chromatids are held together from the time of their replication in S phase until their separation at mitosis by protein complexes termed cohesins (Nasmyth and Haering 2009; Nishiyama 2019). In human cells, cohesins are removed in two stages: first in prophase from chromosome arms, and second in metaphase from centromeres (Waizenegger et al. 2000; Peters and Nishiyama 2012). Human repetitive chromosomal regions (telomeres and ribosomal DNA) rely on additional specialized tankyrase-dependent mechanisms for their resolution. Here we focus on the role of tankyrase in chromosome resolution and genome integrity. The mitotic functions of tankyrase and PARPs were recently reviewed elsewhere (Slade 2019).

Telomere resolution requires tankyrase 1 and tankyrase 2

Cohesion between telomeres is normally resolved at the same time as chromosome arms in G2/M (Ofir et al. 2002; Yalon et al. 2004). However, when cells are treated with tankyrase siRNA chromosome arms and centromeres resolve, but telomeres remain cohered in mitosis (Fig. 3; Dynek and Smith 2004). Cells undergo a prolonged anaphase, but ultimately exit mitosis; although some cell types (like HeLa) exhibit a prolonged mitotic arrest (Dynek and Smith 2004; Kim and Smith 2014). Cells also exhibit an increase in DNA damage foci and in sister telomere fusions (Hsiao and Smith 2009). Rescue of persistent cohesion requires a PARP-active tankyrase 1 (Bisht et al. 2013b). The persistent cohesion phenotype is recapitulated in CRISPR-generated HEK293T *TNKS1* knockout cell lines (Bhardwaj et al. 2017). Surprisingly, *TNKS2* knockout cells show the same phenotype as *TNKS1* knockout, and the double-knockout cells show an even greater level of persistent cohesion than the single knockouts. Thus, in contrast to telomere length maintenance where either tankyrase 1 or tankyrase 2 is sufficient, resolution of telomere cohesion requires both tankyrase 1 and tankyrase 2 (Bhardwaj et al. 2017). CRISPR-generated mutation of the tankyrase-binding site in TRF1 (from 13-

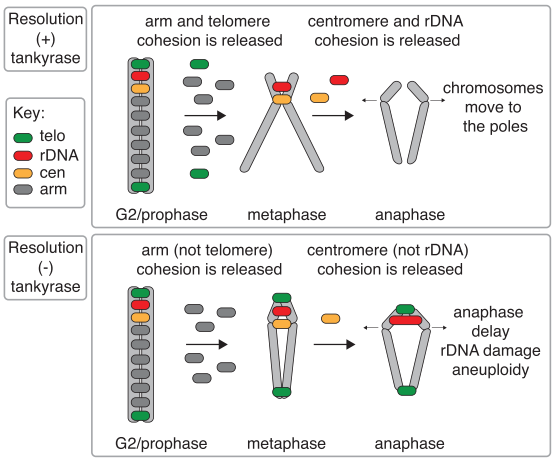

Figure 3. Tankyrase is required for resolution of telomeres and rDNA. (*Top* panel) In control cells, arm and telomere cohesion is released in G2/prophase, followed by resolution of centromere and rDNA at the metaphase to anaphase transition. (*Bottom* panel) In the absence of tankyrase 1, arm cohesion is released in G2/prophase, followed by release of centromere cohesion at the metaphase to anaphase transition. Resolution of telomeres and rDNA is delayed until anaphase leading to anaphase delay, rDNA damage, and aneuploidy.

RGCADG to RGDADP) leads to persistent telomere cohesion, indicating that TRF1-mediated recruitment of tankyrase is required for resolution (Azarm et al. 2020).

Telomere resolution requires RNF8-mediated stabilization of tankyrase 1 Tankyrase 1 is recruited to telomeres in late S/G2 phase, aligned with the timing of sister telomere resolution (Bisht et al. 2012, 2013a). What regulates tankyrase to act specifically at telomeres within this window? The answer lies in cell cycle-regulated ubiquitination and deubiquitination of tankyrase 1. Tankyrase is normally turned over rapidly due to RNF146-mediated K48-linked ubiquitination and degradation by the proteasome (Callow et al. 2011; Zhang et al. 2011). However, in late S/G2, the damage-responsive E3 ubiquitin ligase RNF8 promotes K63-linked ubiquitination on tankyrase 1 (Tripathi and Smith 2017). The damage activating RNF8 originates from the endogenous ATM-mediated signaling at newly replicated telomeres (Verdun et al. 2005). The K63-linked ubiquitin promotes transient stabilization of tankyrase 1 to permit timely resolution of cohesion in late S/G2. RNF8 depletion (like tankyrase 1 depletion) leads to persistent telomere cohesion and sister telomere fusions (Hsiao and Smith 2009; Tripathi and Smith 2017).

Following resolution of cohesion, upon nuclear envelope breakdown, tankyrase 1 is exposed to the cytoplasmic K63-deubiquitinating enzyme complex BRISC (Cooper et al. 2010; Feng et al. 2010; Hu et al. 2011). Tankyrase 1 binds to a 333-RPQAVG tankyrase binding site in the BRISC scaffold subunit ABRO1 (Tripathi and Smith 2017). ABRO1 depletion leads to increased K63-ubiquitinated tankyrase 1, premature resolution of telomere cohesion, and sister telomere fragility/loss, demonstrating that

limiting tankyrase 1 stabilization and activity to the S/G2 window of the cell cycle is vital for telomere integrity. These phenotypes can be rescued by introduction of wild-type ABRO1, but not a tankyrase binding site mutant (from 333-RPQAVG to RPQAVR). This study suggests that K63-ubiquitination can shunt tankyrase 1 out of the RNF146-mediated K48-ubiquitination degradation cycle into a transiently stabilized state to function in resolution of telomere cohesion (Tripathi and Smith 2017).

Telomere resolution in aging cells As human cells age and their telomeres shorten they exhibit a natural block in resolution of telomere cohesion. In presenescent cells aged in culture and early passage cells from aged individuals telomeres are cohered in mitosis, whereas other chromosomal regions are resolved (Ofir et al. 2002; Yalon et al. 2004). Introduction of telomerase into presenescent cells rescues the persistent telomere cohesion, suggesting that it is coordinated with telomere shortening (Yalon et al. 2004). Tankyrase levels are not reduced in aging cells, yet overexpression of PARP-active tankyrase 1 rescues the persistent telomere cohesion, hinting at a mechanism that prevents endogenous tankyrase from acting at telomeres in these aged cells (Kim and Smith 2014). Indeed, a recent study shows that shortened telomeres recruit insufficient TRF1 and as a consequence inadequate tankyrase 1 to resolve sister telomere cohesion (Azarm et al. 2020). Overexpression of wild-type TRF1, but not a tankyrase-binding site mutant (from 13-RGCADG to RGDAAA), promotes recruitment of tankyrase 1 to aging cell telomeres and forces resolution of cohesion. Surprisingly, persistent cohesion is beneficial to aging cells; forcing resolution results in deleterious phenotypes: excessive interchromosomal subtelomere recombination, DNA damage, and premature activation of checkpoint-mediated senescence (Azarm et al. 2020). The limited recruitment of tankyrase at shortened telomeres promotes persistent telomere cohesion, protecting chromosome ends from engaging in damage-inducing subtelomere recombination that signals premature cell cycle arrest. Thus, in aging cells the gradual loss of telomere repeats and the accompanying limited recruitment of tankyrase 1 ensures an integrated and measured onset of replicative senescence.

Telomere resolution in ALT cancer cells Persistent telomere cohesion in mitosis has also been observed to occur naturally in cancer cells that use alternative lengthening of telomeres (ALT), a recombination-based mechanism of telomere maintenance (Bryan et al. 1997; Ramamoorthy and Smith 2015; Sobinoff and Pickett 2017). As with aging cells, tankyrase 1 levels are not reduced in ALT cells, yet overexpression of PARP-active tankyrase 1 can rescue the persistent telomere cohesion (Ramamoorthy and Smith 2015). ALT cell telomeres range from exceptionally long to critically short (Henson et al. 2002). Could the population of shortened telomeres with insufficient TRF1 contribute to persistent telomere cohesion as in aging cells? Indeed, introduction of telomerase into ALT cells rescues the persistent cohesion, linking the phenotype to shortened telomeres (Azarm et al. 2020). Overexpression of wild-type TRF1, but not a tankyrase-binding-site mutant, recruits tankyrase 1 to telomeres, forces resolution of cohesion, and leads to similar phenotypes in ALT as in aging cells: subtelomere recombination, DNA damage, and (in checkpoint-proficient U2OS ALT cells) senescence (Azarm et al. 2020).

A noncanonical role for the PAR-binding macroH2A1.1 In both aging normal cells and ALT cancer cells, reduced TRF1 at shortened telomeres limits tankyrase 1 recruitment (Ramamoorthy and Smith 2015; Azarm et al. 2020). There appears to be an additional mechanism (at least in ALT cells) that acts directly through tankyrase 1. A common feature of ALT cancer cell lines is loss of the SWI/SNF-like ATPase ATRX (Heaphy et al. 2011; Lovejoy et al. 2012). Along with its roles in chromatin remodeling and histone deposition, ATRX sequesters the soluble pool of macroH2A (Ratnakumar et al. 2012), a histone variant comprised of an N-terminal H2A-like domain and a C-terminal PAR-binding macrodomain (Gamble and Kraus 2010; Cantariño et al. 2013). MacroH2A exists as three isoforms (macroH2A1.1, macroH2A1.2, and macroH2A2), but only one macroH2A1.1 (by virtue of an alternative splice) binds PAR (Kustatscher et al. 2005; Timinszky et al. 2009). Loss of ATRX in ALT cells frees the soluble pool of macroH2A1.1 to bind and sequester PARylated tankyrase 1 away from telomeres (Ramamoorthy and Smith 2015). Overexpression of ATRX (or depletion of macroH2A1.1) forces resolution of telomere cohesion, phenocopying TRF1 or tankyrase 1 overexpression. Whether tankyrase 1 is regulated through a similar mechanism in aging cells remains to be determined, but the possibility is supported by studies showing that macroH2A1.1 is highly expressed in cells undergoing senescence (Sporn et al. 2009; Chen et al. 2015).

Ribosomal DNA resolution

To accommodate the high demand for ribosomal RNA, the genes encoding the ribosomal RNA (rDNA) exist in many copies (Gonzalez and Sylvester 1995). In humans the rDNA is in repetitive clusters on the short arms of the five acrocentric human chromosomes adjacent to the telomeres (Henderson et al. 1972; McStay 2016). While it was known that the bulk of the human genome resolves by prophase, except for the centromeres, which resolve at the metaphase-to-anaphase transition (Waizenegger et al. 2000; Nagasaka et al. 2016), the timing of rDNA resolution was unknown. Two recent studies show that rDNA is not resolved until late in anaphase after the rest of the genome (Daniloski et al. 2019; Potapova et al. 2019) and also that tankyrase 1 is required. In tankyrase 1-depleted cells the rDNA loci of sister chromatids remain connected and stretch across the segregating DNA mass in anaphase (Fig. 3; Daniloski et al. 2019). The stretching results from the inaction of condensin II. Two condensin complexes (condensin I and condensin II) with complementing activities exist in

human cells (Ono et al. 2003, 2004; Hirota et al. 2004). They each have the common SMC2 and four subunits plus three additional related, but unique, subunits. An in-silico search for tankyrase-binding sites across all human condensin subunits revealed them in only one, CAP-D3 of condensin II. Depletion of CAP-D3 (but not its condensin I counterpart, CAP-D2) led to the same rDNA stretching as tankyrase 1 depletion. Tankyrase 1 binds to CAP-D3 through its 519-RSEPSG tankyrase-binding site, and wild-type CAP-D3 (but not a tankyrase-binding site mutant, RSEPSA) rescues the rDNA stretching and facilitates topoisomerase IIα-mediated segregation of rDNA.

Analysis of the fate of the rDNA in tankyrase 1-depleted cells reveals dramatic consequences for genome integrity: rDNA-specific damage in mitosis, rDNA-containing micronuclei, and nondisjunction of rDNA-containing acrocentric chromosomes (Daniloski et al. 2019). How does defective rDNA resolution lead to damage? The rDNA clusters, like subtelomeres, are "hot spots" for recombination (Killen et al. 2009; Stults et al. 2009; Salim and Gerton 2019). Defective rDNA resolution may lead to aberrant recombination that induces damage, similar to the subtelomere recombination-driven damage in replicative senescence described above.

Telomere and rDNA resolution—a connection through tankyrase 1

Two types of human repetitive sequences (telomeres and the rDNA) require tankyrase 1 for their resolution in mitosis. Surprisingly, despite the common role for tankyrase, resolution of telomeres and rDNA occurs at different stages in mitosis: prophase and anaphase, respectively (Fig. 3). Is their resolution connected? Tankyrase 1 is recruited to telomeres through TRF1, but what directs it to the rDNA? Considering the timing of resolution (telomeres in G2/prophase, and rDNA in anaphase), perhaps tankyrase is initially recruited to telomeres (including those of acrocentric chromosomes) through TRF1, and then relocalizes to the adjacent rDNA locus. The studies described above show timely rDNA resolution occurs in normal cells and in telomerase-positive cancer cells. It remains to be determined whether the process is defective (like resolution of telomere cohesion) in aging and ALT cancer cells. Loss of ATRX in ALT cancer cells has been connected to a reduction in rDNA copy number (Udugama et al. 2018). As described above, loss of ATRX in ALT cells sequesters tankyrase away from telomeres, preventing resolution of telomere cohesion (Ramamoorthy and Smith 2015). Perhaps it also keeps tankyrase 1 away from rDNA, preventing rDNA resolution. The observed loss of rDNA in ALT could be a consequence of defective rDNA resolution.

Perspectives

In this review, we discussed the role of PARP1, PARP2, tankyrase 1, and tankyrase 2 in chromosome repair, replication, and resolution in human cells. Though these four proteins share the ability to PARylate themselves and their binding partners, they differ in their contributions to genome integrity. PARP1 and PARP2 auto-PARylation provides a harbor at sites of DNA damage, disrupted replication forks, or unprocessed Okazaki fragments, for PAR-binding proteins to dock and resolve these aberrations. The harboring of proteins to preserve genome stability is taken further through evidence of compartmentalization through PAR-mediated liquid-liquid demixing at sites of DNA damage. Auto-PARylation of tankyrases, on the other hand, does not appear to motivate function. Rather, it is the ability to interact with an array of diverse binding partners at specific locations and times, as evidenced by the example of tankyrase 1 localization to telomeres through its binding partner TRF1 in G2/M to promote timely resolution of sister telomere cohesion. Together, the combined activities of these four PARPs contribute significantly to genome maintenance and stability. Against the backdrop of expanding strategies for PARP inhibitors in the clinic, future research will continue to identify new targets and provide new insights.

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