Poly(ADP-ribose) polymerases in aging – friend or foe?

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Running title: PARPs in aging

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Abstract
Poly(ADP-ribose) polymerases were originally described as DNA repair enzymes. PARP-1, PARP-2 and PARP-3 can be activated by DNA damage and the resulting activation of these enzymes facilitate DNA repair, a prerequisite of successful aging. Through more fit DNA repair systems PARP activation helps to maintain genomic integrity, however, in parallel these enzymes limit metabolic fitness and make the organism more prone for metabolic diseases. In addition, several other pathways (e.g. proteostasis, nutrient sensing, stem cell proliferation or cellular communication), all contributing to aging, were shown to be PARP mediated. In this review we aim to summarize our current knowledge on the role of PARPs in aging.

Keywords: PARPs, mitochondria, DNA repair, aging, healthspan, energy stress
Ageing, ageing-related pathologies

With increases in lifespan, Western countries face an ever-growing number of aging people and growing number of ageing-related diseases. Aging is a natural process that is characterized by genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, stem cell exhaustion, cellular senescence and altered intercellular communication [1]. On the course of ageing the prevalence of certain diseases increase as compared to the young(er) or mixed-age population such as metabolic diseases (type II diabetes, obesity), certain malignancies, macular degeneration, osteoporosis, hearing loss - just to list a few. It is evident that identifying molecular targets that improve healthspan or the quality of life during ageing has obvious advantages for the society.

Poly(ADP-ribose) polymerases (PARPs) were originally described as DNA repair proteins and in the late ‘90s Muiras and Bürkle [2] have shown that in centenarians in comparison to the average population have higher PARP activity in leukocytes suggesting that higher PARP activity is associated with successful aging. In the past years our understating of the relationship between PARP activity and aging has greatly improved and in this review we try to provide a comprehensive picture of the role of PARPs in ageing.

The enzymology of poly(ADP-ribosyl)ation

Poly(ADP-ribose) polymerases (PARPs) form a family of 17 in humans that share the same catalytic domain. PARPs are multidomain proteins contain additional domains – among others – domains for protein-protein interactions (e.g. 3rd zinc finger in PARP-1, BRCT domain, ankyrin repeats, etc., macrodomains for binding or cleavage of ADP-ribose, DNA/RNA binding domains (zinc finger or SAP domain) and nuclear or nucleolar localization signals [3, 4].

Poly(ADP-ribosyl)ation (PARylation) is an evolutionarily conserved biochemical process. Activated PARP-1 binds and cleaves NAD+ to ADP-ribose and nicotinamide (NAM) then binds ADP-ribose to glutamate, aspartate side chains [3]. To the first ADP-ribose moiety the enzyme can subsequently join new ADP-ribose units forming large, branched polymers counting up to 200 individual ADP-ribose residues [3]. To date PARP-1, PARP-2 and tankyrases (TNK) are known to perform PARylation, the rest of the family performs oligo- or mono-ADP-ribosylation (inefficient or no elongation), can be inactive (mutation of catalytic amino acids) or its activity had not been characterized [5]. It must be noted that single ADPR units can be further elongated by PARPs capable of performing PARylation [6] 85-90% of the basal and DNA break-triggered PARP activity is covered by PARP-1, PARP-2 is responsible for the remaining 10-15%, while the activity conferred by the rest of the family is negligible as compared to the total activity [5]. PARP-1, PARP-2 and PARP-3 are activated by DNA strand breaks, however
different posttranslational modification may also modulate the activity of PARPs (for review see [7, 8]).

PAR molecules can be joined onto PARP-1 or PARP-2 (autoPARylation) or onto other acceptor molecules (transPARylation). AutoPARylation inhibits PARP-1 activation and therefore inhibit PARP-1 overactivation, while removing PAR reactivates PARP-1 [5]. The PAR polymer is rapidly degraded in cells to ADPR. There are several enzymes capable of degrading PAR, such as poly(ADP-ribose) glycohydrolase (PARG) ADP-ribosyl-acceptor hydrolase 3 (ARH3), ADP-ribosyl lyase and macromodain-containing proteins [8]. Poly(ADP-ribose) glycohydrolase (PARG) removes PAR reverting and reactivating PARP-1. ADPR can be further fragmented to AMP by Nucleoside Diphosphate Linked to X (NUDIX) pyrophosphatases; increases in cellular AMP has pivotal role in mitochondrial deterioration inflicted by PARP activation [8].

PARPs are involved in a plethora of biological processes, hereby – due to space limitations - we refer the reader to reviews [4, 9].

Primary events of aging

Genomic instability and telomere attrition

Accumulating genetic damage during aging is unavoidable as genomic material is continuously challenged by exogenous (physical, chemical and biological) and endogenous (replication errors, hydrolytic reactions and oxidative damage) factors [10]. In order to maintain genetic integrity, a vast repertoire of DNA repair mechanisms stand ready to prevent DNA damage and accumulation of these lesions over time. It seems more likely that genomic instability during aging is rather a consequence of declined or impaired repair functions [10]. Should repair mechanisms fail, cells undergo apoptosis or suffer senescence as a tumor suppression mechanism.

Many DNA repair mechanisms, counteracting the above-detailed changes, are PARP-dependent processes. PARP-1, PARP-2 and PARP-3 were shown to be activated by DNA strand breaks [11]. PARPs have role in nucleotide excision repair to attack bulky adducts, base excision repair processes to counteract base modifications and in the repair of single and double strand breaks (both non-homologous end joining and homologous repair) and to maintain genomic integrity [11]. The deletion or pharmacological inhibition of PARP-1, PARP-2 or PARP-3 does not lead to spontaneous genomic instability [11, 12], however, under genotoxic stress the loss of PARPs does lead to genomic instability [11]. In line with these observations, the simultaneous deletion of PARP-1 and PARP-2 or PARPs and other DNA repair enzymes lead to embryonic lethality that is characterized by the accumulation of DNA damage [11].
There are specific regions of the genome that seem to be more prone to DNA damage. Telomeres, affected especially by age related shortening, are among these regions [13]. In somatic human cells, telomeres shorten with every round of replication and cells are triggered into replicative senescence once telomeres shorten to a critical length [13]. Telomeres can be rebuilt by the enzyme telomerase that has cofactors, such as telomeric repeat binding factor 1 and 2 (TRF1 and TRF2, respectively) [13].

PARP-1, TNK1 and TNK2 are involved in telomere maintenance through different mechanisms. TNKs interact with TRF1 (but not TRF2) and both are localized together near the physical ends of metaphase chromosomes, implying TNKs as a components of the human telomeric complex [14]. TNKs have intrinsic PARP activity towards at least two substrates (TRF1 and themselves) [14] resulting in decreased binding of TRF1 to telomeres and induces proteasome-mediated degradation of TRF1, hence TRF1 is a negative regulator of telomere length [15]. In addition to TNKs, PARP-1 is also involved in telomere maintenance, wherein PARP-1 is a negative regulator of TRF2 [16]. Indeed, the absence of PARP-1 did lead to dramatically shortened telomeres in PARP-1−/− mice and cells [17, 18] and in such cells chromosomes are prone to end-to-end fusions [19].

Muiras and Bürkle [2, 20-22] has linked better DNA repair capabilities upon higher total PARP activity with prolonged lifespan. Indeed, in centenarians, who actually aged successfully, higher PARP-1 activity was detected as compared the average of population [2]. Indeed, a subsequent study by Mangerich and co-workers [23] showed that the overexpression of an extra copy of PARP-1 in mice reduced the incidence of several malignant diseases clearly validating the original observations of Muiras [2].

Epigenetic alterations and changes in gene expression

Epigenetics has been defined as the inheritance of changes in gene function without any change in the DNA nucleotide sequence. A variety of epigenetic alterations including posttranslational histone modifications and chromatin remodeling could be associated to influence aging [24]. PARP-1, PARP-2 and TNK1 were shown to modulate chromatin structure, to mediate both condensation and decondensation events and hence play indispensable role in establishing facultative and constitutive heterochromatin (Barr body, centrosome, pericentrosome, telomere, inactivated sex chromosome during meiosis, inactive rRNA genes, etc.) [25]. Moreover, PARP-1 impacts on epigenetic marks by PARylating histones and chromatin remodeling enzymes such as KDM5B, ISWI, ALC1, DNMT-1, CTCF etc. (for review see [26]) or can rearrange nucleosome and linker histone binding through which PARP-1 influences transcription [26]. PARP-1 binds to 90% of the Pol II-transcribed genes and mediates around 3.5% of all transcribed RNAs [26]. Besides PARP-1, PARP-2, -7, -10, -14
regulates gene expression [4]. It is not yet fully confirmed by experimental data, however it is likely that the modulation of epigenetic factors by PARPs could impact on aging.

**Loss of proteostasis**

Proteostasis means the maintenance of protein turnover and functional proteins in all cellular compartments. Different forms of stress have an impact on the cell proteome which uses a vast number of protein stabilization and refolding mechanisms (via heat-shock proteins) to maintain the functionality of affected proteins. If proteins can’t be rescued, the incorrectly folded structures are targeted for lysosomal or proteasomal degradation to avoid their accumulation and aggregation [1]. Dysfunctional proteostasis is associated with aging and age related diseases like Alzheimer’s or Parkinson’s disease [1].

Overloading the protein translation machinery leads to the production of misfolded proteins that declutches a signaling mechanism called ER stress that slows down protein synthesis to enable cells to cope with misfolded, dysfunctional proteins. PARP-1 has regulatory role in translation [27], moreover, PARP-1 has a role in ER-stress signaling [28].

Upon oxidative stress the 20S proteasome in the nucleus is rapidly activated in a PARP-1 and PARylation-dependent fashion [29-32] that is closely linked to reduced rate of aging, increased proliferative capacity and tumor growth [33, 34]. In contrast to that, genetic or pharmacological inhibition of PARP-1 reduce proteolytic activity and induce senescence [35-38].

Proteostasis is not limited to the removal of damaged proteins, but to the preservation of the balance between the protein content or protein composition between cellular organelles such as the balance between mitochondrial and nuclear proteins [39]. The regulatory circuit balancing between mitochondrial and nuclear protein pools is comprised of two arches. Energy stress sensor pathways (e.g. SIRT1, AMPK, etc.) help in translating shortcomings in cellular energetics into nuclear transcription programs that then upregulate mitochondrial energy production. The other arch is called retrograde signaling translating mitochondrial dysfunction (e.g. decreased expression or function of complex I) into nuclear transcriptional programs that can restore mitochondrial oxidation [39]. The activation of PARP-1 had been shown to influence mitonuclear protein balance through modulating SIRT1 activity [40].

**Mitochondrial dysfunction**

Mitochondrial oxidation capacity deteriorates on the course of aging [1]. The negative effect of PARP-1 on mitochondrial activity was described in 1998 by Virág and co-workers [41]. PARP-1 overactivation blunts mitochondrial activity through several parallel pathways (for comprehensive review see [8, 42]): 1) inhibition of mitotropic transcription factors (e.g. SIRT1) [5, 43], 2) induction of transcription factors that suppress mitochondrial activity (e.g. HIFs) [44,
45], 3) inhibiting mitochondrial membrane and membrane-associated enzymes (e.g. ANT, the hexokinase-ANT complex) by cytosolic PAR polymers [8, 46, 47], 4) impairs mitophagy and nuclear-mitochondrial protein balance [40, 48].

In contrast, the deletion or pharmacological inhibition of PARP-1 or PARP-2 enhances mitochondrial activity through inducing SIRT1 activity by enhancing cellular NAD⁺ levels or by inducing SIRT1 expression, respectively [5, 40]. Enhanced SIRT1 activity leads to the deacetylation of mitotropic transcription factors, such as FOXO1 or PGC-1α that induce transcription programs that support mitochondrial oxidative metabolism [5, 40]. PARPs interact with energy sensor (e.g. AMPK, NRFs, SIRT3 – for review see [8]), however, the involvement of these pathways in PARP-mediated changes in mitochondrial activity is poorly characterized.

The balance between SIRT1 and PARP-1 has central role in setting the rate of mitochondrial oxidative metabolism. Both enzyme use a common substrate, NAD⁺ and can limit each other’s activity [5]. Several elegant studies from Braidy and co-workers (e.g. [49, 50]) have shown that the activity of PARP-1 enhances upon aging that reduces cellular NAD⁺ levels and consequently SIRT1 activity that blunts mitochondrial oxidation. Importantly, mitochondrial biogenesis – most probably in the skeletal muscle – upon the deletion of PARP-1 or PARP-2 protect against type II. diabetes that is a classical aging-related metabolic disease [12]. In line with these findings the overexpression of PARP-1 in mice leads to type II. diabetes [23]. There are other PARPs with metabolic properties [12] suggesting that these enzymes may also participate in the metabolic rearrangements in aging.

**Deregulated nutrient sensing**

Nutrient availability defines the balance between anabolism and catabolism, moreover it is involved in the regulation of key aging-related processes, such as autophagy, cell divisions, hormonal signaling, diurnal rhythms, calorie restriction, etc. The systems that are involved in nutrient sensing are often referred as energy (stress) sensor pathways [51] and involve – among others – the AMPK-mTOR system, nuclear receptors or hormonal signaling. These sensor systems can be pharmacologically modulated to mimic dietary changes that modulate lifespan and healthspan (e.g. calorie restriction) [51].

The deletion of PARP-1 in mice led to perturbed diurnal rhythm and increased food uptake and negative energy balance [12] suggesting dysfunction of the central (hypothalamic) circadian regulation. Although direct molecular proofs are missing for the defects of central regulation, hepatic (i.e. peripheral) circadian oscillations are perturbed upon the pharmacological inhibition or the deletion of PARP-1 due to the absence of the PARylation of CLOCK protein and the consequent dysregulation of the CLOCK-BMAL/PER-CRY circuitry [52].
PARP activity responds to nutrient availability, in skeletal muscle autoPARylation and protein level of PARP-1 increased upon feeding mice with hypercaloric high fat diet, while fasting sharply reduced PARP-1 activity [12]. As an extension to that, several nutrient sensor pathways were shown to be mediated by PARP-1. PARP-1 influences the phophatidil-inositol-3 kinase (PI3K)-Akt and glucagon-like peptide (GLP)-1 pathway through which PARP-1 mediates insulin (and probably growth factor) signaling [53-56]. There are numerous examples where PARPs interact with nuclear receptors and hence mediate sensing intra or extracellular lipid levels [8]. The impact of the interactions between PARP-1 and energy sensors (AMPK, mTORC1, NRFs, HIFs, SIRTs - see also the previous chapter and [4, 8]) on nutrient sensing had not been assessed. Taken together, the current data suggest that PARP-1 influences both central and peripheral sensing of lipids, glucose and amino acids. Little is known about PARPs other than PARP-1, primarily due to the lack of dedicated in vivo studies, however, PARP-2 is known to interplay with beta cell function and certain nuclear receptors [57], while tankyrases impact on beta cell function and feeding [58].

**Integrative events of ageing**

*Stem cell exhaustion*

With the progress of aging it is very common for different tissues to have an impaired regenerative ability due to compromised stem cell renewal capacity [1]. Importantly, PARP-1 and PARP-7 define in stem cells whether cells undergo terminal differentiation or renewal [59]. PARP-2 activity had been also linked with sustaining hematopoietic stem cell (HSC) production [60, 61] or thymopoiesis [62]. The application of PARP inhibitors, mostly through inhibiting PARP-1, influence the differentiation of bone marrow-derived cells, neurons, spermatocytes, skeletal muscle, osteoblasts and adipocytes [4]. Although it is very likely that PARP activation may alter the renewing capacity of stem cells, yet, direct evidences are missing.

*Cellular senescence*

A hallmark of cellular senescence is the slowdown or complete stop of cell cycle [1]. PARylation levels change on the course of the cell cycle [4], being the highest in G2 and S phase, while the lowest in G1 that is probably linked to DNA repair. The degradation of PAR or TNK1 silencing leads to a pre-anaphase block, PARP-1 is required for primase activity and replication-coupled repair and its absence leads to G2/M arrest when genotoxic stress is present. PARP-6 is necessary to leave the S-phase, while PARP-3 regulates the G1 to S phase progression. Taken together, PARPs are required for sustaining the progression of cell cycle.
Compromised intercellular communication

There are several modalities of communication between cells of an organism, the hormones of the endocrine system, cytokines or chemokines, etc. Although our knowledge is limited on the endocrine functions of PARPs, it is clear that PARPs are involved in the function of pancreatic beta cells [12] or different peptide hormones, among them several adipokines (Fig. 1. and [63, 64]). The involvement of PARP-1 in the age related changes in insulin secretion and the consequent appearance of type II. diabetes was suggested by Mangerich et al. [23], pointing towards the possibility of other PARP-mediated, age-related endocrine changes.

Ageing-related changes in the inflammatory system involve inflammaging. In inflammaging the normal function of the immune system is compromised: on the one hand, low grade inflammation commences in tissues (e.g. white adipose tissue) that on the long run deteriorates the function of these tissues, while on the other, the elimination of pathogens is limited. PARP-1 and PARP-2 control the expression of a plethora of chemokines, cytokines, inducible nitric oxide synthase (iNOS) and polyunsaturated fatty acids and exert pro-inflammatory roles in Th1 and Th2-mediated pathologies [65, 66]. It is tempting to speculate that ageing-related enhancement of PARP activity may feed-forward the low grade inflammation that accompanies inflammaging. Furthermore, PARP-1 and PARP-2 plays role in free radical-induced cell death that often accompany inflammation (also called a bystander effect) [4, 42] adding another modality to the pro-aging role for these enzymes. It must be noted that there are anti-inflammatory PARPs as well (e.g. tankyrases), however the role of these PARPs in aging is completely unknown. Finally, PARP-1 regulates endothelial NOS function and the production of oxygen-centered reactive species (e.g. mitochondrial uncoupling) through which it impacts on the function of gaseotransmitters [67].

Conclusions

Since the first pioneering observation, where higher PARP-1 expression was found in centenarians [2] that was linked to improved DNA damage sensing and more efficient repair, a way more complex picture was obtained on the role of PARPs in aging. PARP-1 and, to a smaller extent, PARP-2 are key factors in aging-related diseases (neurodegenerative diseases, metabolic diseases, etc.) [4], finally the competing nature of the anti-aging action of PARP-1 and PARP-2 (maintaining DNA integrity) and the pro-aging actions (increased susceptibility to metabolic diseases) was discovered [23] (see graphical abstract). In other words, PARP activation is a double-edged sword in aging, while enhanced PARP activity ensures DNA integrity, PARP activation at the same time makes the organism more susceptible to metabolic diseases [23]. Recent advances uncovered novel, PARP-mediated pathways (loss of proteostasis, changes in nutrient sensing, stem cell exhaustion or the
deregulation of intercellular communication) that may contribute to aging, warranting further research in these directions. It is important to underline that PARP activation was detected in several aging-related diseases: neurodegenerative diseases such as Alzheimer’s disease, type II diabetes and other metabolic diseases, ocular degenerative diseases [4] that clearly underlines the role of PARPs in the definition of healthspan, however, according to our current knowledge, the action of PARPs on lifespan is more limited.

There are several questions that remain still open. The studies that define the impact of PARPs on lifespan are missing similarly to the studies aiming to understand the role of minor PARP isoforms in aging. Finally, the applicability of the administration of NAD+ or NAD+ precursors and other NAD+-dependent enzymes (e.g. sirtuins, such as SIRT6) still needs to be better elucidated.

Competing interests
The authors declare no conflict of interest.

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References


**Legends to Figures**

**Figure 1.** PARP-2 modulates the expression of adipokines.

The expression of the above adipokines were determined in the white adipose tissue of PARP-2^{+/+} and PARP-2^{-/-} male mice (n=6/7, 6 months of age). Error bars represent SEM.