PARP-1: A Regulator of Genomic Stability Linked with Mammalian Longevity

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1. Introduction

Ageing of organisms is commonly defined as the time-dependent general decline of physiological functions accompanied by a progressive increase in the risk of morbidity and mortality.[1] It appears that the major driving force of the ageing process is damage inflicted on cellular macromolecules, which interferes with their function. Such damage is mostly derived from low molecular weight reactive compounds that arise within the body during normal metabolism and are linked with important cellular functions such as oxygen transport, respiration, phagocyte activity or detoxification reactions. Most prominent among such endogenous damaging agents are reactive oxygen species (ROS), which can lead to a state termed oxidative stress, if produced in excessive amounts.[2] It is the damage to DNA that may be particularly hazardous, since unlike other macromolecules DNA is subject to little if any turnover, which could dilute the damage. DNA damage and some of its irreversible consequences that are collectively referred to as "genomic instability" (e.g. chromosomal aberrations, sister chromatid exchange [SCE] as well as DNA translocations, deletions, amplifications and other kinds of mutations) would rapidly accumulate and disturb DNA replication, gene expression and ultimately cellular and tissue homeostasis if there were no effective cellular defence and repair systems in place. A central prediction of the "disposable soma theory"[3] is that longevity has evolved by allocation of increasing proportions of available bioenergy to somatic maintenance and repair pathways, thereby making cellular components, cells and organisms more resistant to stress (including genotoxic stress) and allowing for better functional preservation (including integrity and stability of the genome) over time.

2. Poly(ADP-ribosyl)ation

Our research interest has been focused on poly(ADP-ribosyl)ation, a posttranslational modification of various nuclear proteins (Figure 1), representing an immediate cellular response to DNA damage induced by ionizing radiation, alkylating agents and oxidants.[4–14] Poly(ADP-ribosyl)ation is catalysed mostly by the 113-kDa enzyme poly(ADP-ribose) polymerase-1 (PARP-1) with NAD^+ serving as substrate. Recently, several additional polypeptides catalysing poly(ADP-ribosyl)ation have been identified. These new members of the "PARP family" seem to account for about 10% of cellular poly(ADP-ribose) formation stimulated by DNA breaks. PARP-1 is constitutively expressed, at a level depending on the type of tissue or cell. However, it is the contact with DNA single- or double-strand breaks, mediated by two zinc fingers located in the amino-terminal DNA-binding domain (DBD) of the enzyme, that causes activation of the catalytic centre residing within the carboxy-terminal NAD^+-binding domain. The crystal structure of the latter domain has been determined, revealing a striking homology with catalytic domains of bacterial toxins that act as mono-ADP-ribosyl transferases and allowing the authors to propose a detailed reaction mechanism of PARP-1 at the molecular level.[4] In living cells PARP-1 itself is the major target protein ("acceptor") for covalent modification with poly(ADP-ribose). This automodification is thought to occur mostly on a specific domain located between the DBD and the NAD^+-binding domain. Several additional acceptor proteins have been identified in living cells, such as histones and topoisomerases, and in vitro many more proteins can undergo poly(ADP-ribosyl)ation. The existence of poly(ADP-ribose) in cells is transient and tightly linked with the existence of DNA strand breaks, since the half-life of poly(ADP-ribose) is very short under conditions of DNA breakage, due to rapid degradation by poly(ADP-ribose) glycohydrolase and other catabolic enzymes.

While the molecular functions of PARP-1 and/or poly(ADP-ribose) have not been fully elucidated yet, a fairly large number of hypotheses have been proposed,[4, 7] many of which are based on work on subcellular systems only and therefore are of uncertain relevance for the in vivo situation. Functions of PARP-1 and/or poly(ADP-ribose) have been proposed (i) in the signalling of DNA damage induced by alkylating agents, oxidants and ionizing radiation and in the recruiting of enzymes involved in DNA base excision repair; (ii) in the regulation of genomic stability in cells under genotoxic stress (see Section 4); (iii) in DNA replication; (iv) as a co-activator of transcription; and (v) in

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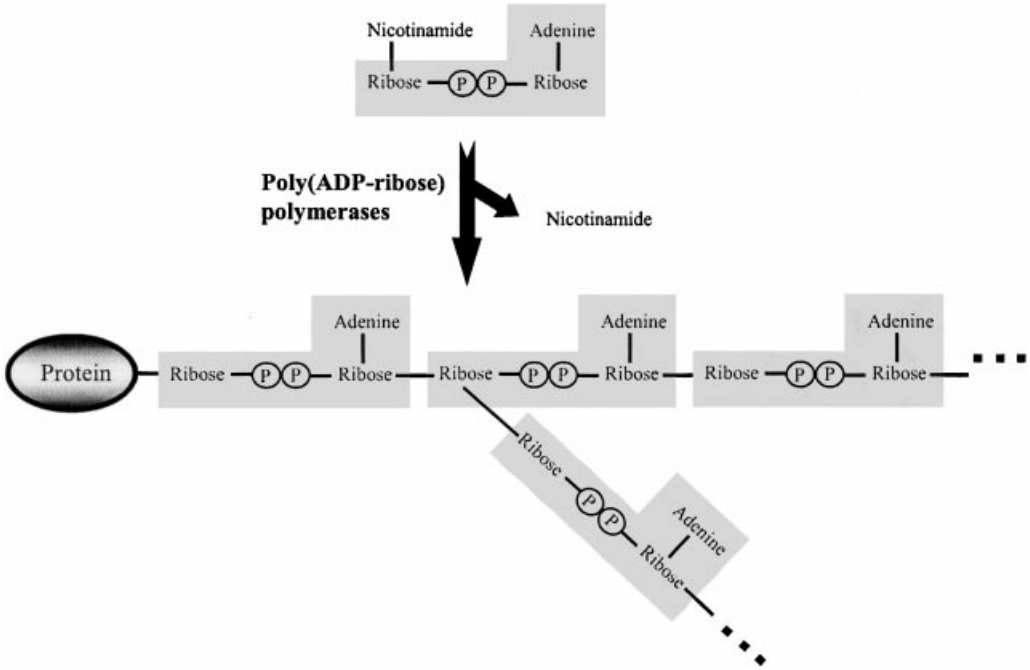

Figure 1. *Schematic representation of poly(ADP-ribose) synthesis. Poly(ADP-ribose) polymerases cleave the glycosidic bond between nicotinamide and ribose in NAD^+ and transfer the resulting ADP-ribosyl moiety to glutamate residues of acceptor proteins ("initiation reaction") or to other ADP-ribosyl moieties already transferred ("elongation reaction"). Repeated reaction cycles then lead to the formation of poly(ADP-ribose) chains, with branching events occurring at regular intervals.*

energy metabolism, with repercussions on apoptotic and necrotic cell death. Some of the proposed scenarios are not mutually exclusive.

A vast number of studies have been performed at the cellular and in vivo level using various strategies to abrogate poly(ADP-ribosyl)ation. These strategies include use of competitive low molecular weight PARP inhibitors, expression of a dominant negative PARP-1 version, *PARP-1* antisense RNA expression, or *PARP-1* gene disruption in the mouse germ line. Such studies have firmly established two contrasting functions of PARP-1, their respective relevance depending on the intensity of DNA damage inflicted and the cellular proliferation or differentiation status: (i) In proliferating normal and malignant cells exposed to low-level DNA damage, poly(ADP-ribosyl)ation significantly contributes to cellular recovery from cytotoxicity. This effect has been linked mechanistically with an involvement of PARP-1 in DNA base excision repair. Furthermore, there is clear evidence that poly(ADP-ribosyl)ation counteracts the induction of genomic instability by DNA damage as assessed by several biological markers, such as chromosomal aberrations, SCE, gene amplification, or mutagenesis. (ii) In contrast to such "cytoprotective" functions, PARP-1 overactivity can lead to cell suicide due to severe and irreversible depletion of NAD^+ and consequently of ATP pools. Whether this overactivity is due to acute, unusually strong activation or to significantly prolonged activation at rather normal activity levels, or whether it represents a "relative" overactivation due to an unusually low cellular regeneration capacity for NAD^+ is unknown as yet. Further, whether the ensuing cell death occurs as necrosis under all circumstances or may include apoptosis as well remains to be established. Suicidal PARP-1 overactivation has been observed in several nonproliferative cell types in vivo and in culture, including (i) pancreatic islet cells exposed to ROS, nitric oxide or streptozotocin; (ii) neurones after regional ischaemia-reperfusion damage of the brain (known to induce release of ROS and nitric oxide); and (iii) dopaminergic neurones exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; a drug known to induce production of ROS selectively in the dopaminergic neurones of the substantia nigra, thereby leading to selective neuronal death and Parkinson's syndrome). Cell death in post-ischaemic heart and skeletal muscle and renal tubular cells also appears to result from excessive PARP-1 activity. PARP-1 is instrumental in the above-mentioned pathological conditions not only as an effector of cell death by energy depletion, but also as a co-transactivator with nuclear factor-κB (NF-κB), a crucial transcription factor in inflammatory processes and circulatory shock, mediating induction of inducible nitric oxide synthase (iNOS) expression and enhanced formation of nitric oxide (NO) and consequently peroxynitrite, thus providing another source of DNA strand breakage.

There has been considerable interest in the role of PARP-1 and poly(ADP-ribosyl)ation specifically in apoptotic cell death. Apoptosis is clearly associated with dramatic changes concerning the poly(ADP-ribosyl)ation system.[15] There is a well-documented proteolytic cleavage of PARP-1 into a 85-kDa and a 25-kDa fragment by activated caspase-3 during the execution phase of apoptosis, which is one of the most common biochemical markers of apoptosis. This cleavage should abrogate the responsiveness of PARP-1 to DNA strand breaks. Given the extremely large number of endogenous DNA strand breaks being formed during apoptosis, any ensuing PARP-1-mediated NAD^+/ATP depletion could represent a serious obstacle to completion of the apoptotic programme known to require substantial amounts of bioenergy. Therefore, PARP-1 cleavage

could help to safeguard sufficient bioenergy for successful completion of apoptosis. Furthermore, inactivation of PARP-1 as a "survival factor" may prevent futile cycles of repair, thus contributing to an irreversible commitment to cell death. On the other hand, it should be noted that there is evidence from studies in several different cell systems for a substantial accumulation of poly(ADP-ribose) (i.e. the product of PARP activity) in apoptotic cells. In conclusion, no clear picture has emerged so far as to whether there are, in the various phases of apoptosis, any causative roles to play either for intact PARP-1 protein, for its cleavage products, for poly(ADP-ribose) synthesis, or for the associated consumption of NAD^+ (or their presumed prevention by PARP-1 cleavage, respectively).

3. Relationship between poly(ADP-ribosyl)ation and mammalian life span

We first addressed the question of a possible relationship between poly(ADP-ribosyl)ation and longevity several years ago, measuring the poly(ADP-ribosyl)ation capacity (i.e. maximally inducible PARP activity) of permeabilised mononuclear leukocytes from 13 mammalian species with life spans ranging from 4 to 120 years.[16] We detected a positive correlation of poly(ADP-ribosyl)ation capacity with life span, the difference between the shortest lived (rat) and longest lived species (human) in our study being fivefold. To our surprise, the correlation was not due to any systematic differences in the PARP-1 expression levels, suggesting some qualitative difference of PARP-1 in cells from different species. In order to see whether this was related to variation in the primary structure of PARP-1, we then overexpressed PARP-1 cDNA from rat[17] and man[18] in the baculovirus system, purified the recombinant proteins and performed a comparative enzymological study. In analyses of the automodification reaction, human PARP-1 consistently displayed up to twofold higher activity than the rat counterpart.[19] Therefore, it appears that some (as yet unknown) differences at the level of primary structure of the enzyme have a significant impact on specific enzyme activity but, at the present state of knowledge, cannot fully account for the longevity-related differences in cellular poly(ADP-ribosyl)ation capacity. We currently speculate that accessory factors, known to interact with PARP-1 protein and to modulate its level of DNA-break-induced activity, may also have important roles to play in this context.

In an independent line of research, we have shown that lymphoblastoid cell lines established from peripheral blood samples from a French population of centenarians possessed significantly higher poly(ADP-ribosyl)ation capacity than cell lines from controls.[20] Intriguingly, specific enzyme activity was a more powerful parameter to discriminate between centenarian and control samples than total activity. Assuming that this could be a direct consequence of genetic polymorphisms in the PARP-1 gene, we have recently sequenced the complete PARP-1 open reading frame from 18 subjects and identified a total of four new polymorphisms, one of which causes a codon change. We then studied a much larger number of human samples (324 centenarian and 324 controls) by allele-specific PCR for the frequency of these new polymorphisms, but our data did not reveal an association of any of the alleles with longevity,[21] indicating that genetic factors other than variation in the primary structure of PARP-1 polypeptide should underlie the observed increase in poly(ADP-ribosyl)ation capacity in cells from centenarians.

4. Poly(ADP-ribosyl)ation and the maintenance of genomic stability

Over the past decade we have developed molecular genetic approaches to study the biological role of PARP-1 in cells: We could show in transient transfection assays that overexpression of the PARP-1 DBD leads to transdominant inhibition of poly(ADP-ribosyl)ation by competition for DNA strand breaks.[18] To analyze in detail the biological consequences, we established stable transfectants that overexpress the PARP-1 DBD under the control of the hormone-inducible mouse mammary tumour virus promoter. We could show that overexpression leads to a dramatic sensitization of the cells to the cytotoxic effects of γ radiation or alkylating agents (i.e. agents known to strongly stimulate PARP-1 activity under normal conditions) while leaving normal cell growth undisturbed.[22] PARP-1 DBD overexpression also potentiated the induction of gene amplification[23] and of mutations[24] by alkylating agents. In line with results from other groups that used other strategies to block cellular poly(ADP-ribosyl)ation, our data therefore indicated that PARP-1 activity contributes to cell survival and maintenance of genomic stability under conditions of genotoxic stress.

In a complementary approach, we overexpressed full-length, wild-type human PARP-1 in hamster cells and demonstrated that this intervention leads to above-normal levels of poly(ADP-ribose) in living cells.[25, 26] Studying cell survival under genotoxic stress, we detected a slight but significant sensitization of the cells to γ radiation, which, viewed together with our above-mentioned results on transdominant inhibition of poly(ADP-ribosyl)ation, indicated that the natural level of cellular poly-(ADP-ribose) is optimized for cell survival. Importantly, however, SCE induction by the alkylating agent MNNG was strongly suppressed under conditions of PARP-1 overexpression.[26] If this result is viewed together with a substantial body of data from the literature showing that abrogation of PARP(-1) activity leads to upregulation of carcinogen-induced SCE, PARP-1 emerges as an important regulator of alkylation-induced SCE formation, imposing a control that is strictly negative and commensurate with enzyme activity level.

5. Summary and outlook

After more than 30 years of research into poly(ADP-ribosyl)ation, many ideas and scenarios that have been proposed concerning its biological and molecular function are still highly controversial. Nevertheless, there is a general agreement that abrogating PARP(-1) activity under conditions of mild DNA damage leads to significant potentiation of genomic instability. This has led to considering PARP-1—like a number of other proteins interacting with damaged DNA—a "guardian of the genome".[27] By keeping the incidence of genomic instability events low, PARP-1 would

be expected to be a retarding factor both in carcinogenesis and in ageing (Figure 2). However, based on abrogation experiments alone, it cannot be resolved whether this function is

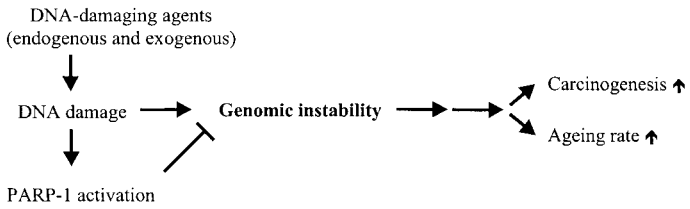

Figure 2. *Working hypothesis on the role of PARP-1 in controlling the rate of carcinogenesis and ageing through its function as a negative regulator of genomic instability. A tilted "T" sign denotes inhibition. For details see text.*

"constitutive" or regulatory. Based on our recent PARP-1 overexpression experiments[26] in conjunction with our comparative work showing a link between high cellular poly(ADP-ribosyl)ation capacity and long life span,[16, 19, 20] the picture is emerging that PARP-1 actually behaves as a regulatory factor, responsible for *tuning* the rate of genomic instability events, which are provoked by the constant attack by endogenous and exogenous DNA-damaging agents, to a level that is just appropriate for the longevity potential of a given organism or species (Figure 2). It will be exciting to see if this scenario holds true in vivo.

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