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REVIEW

Protein Poly(ADP-ribosyl)ation System: Changes in Development and Aging as well as due to Restriction of Cell Proliferation

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Abstract—It is well known that the number of dividing cells in an organism decreases with age. The average rate of cell division in tissues and organs of a mature organism sharply decreases, which is probably a trigger for accumulation of damage leading to disturbance of genome integrity. This can be a cause for the development of many age-related diseases and appearance of phenotypic and physiological signs of aging. In this connection, the protein poly(ADP-ribosyl)ation system, which is activated in response to appearance of various DNA damage, attracts great interest. This review summarizes and analyzes data on changes in the poly(ADP-ribosyl)ation system during development and aging *in vivo* and *in vitro*, and due to restriction of cell proliferation. Special attention is given to methodological aspects of determination of activity of poly(ADP-ribose) polymerases (PARPs). Analysis of relevant publications and our own data has led us to the conclusion that PARP activity upon the addition of free DNA ends (in this review referred to as stimulated PARP activity) is steadily decreasing with age. However, the dynamics of PARP activity measured without additional activation of the enzyme (in this review referred to as unstimulated activity) does not have such a clear trend: in many studies, the presented differences are statistically non-significant, although it is well known that the number of unrepaired DNA lesions steadily increases with aging. Apparently, the cell has additional regulatory systems that limit its own capability of reacting to DNA damage. Special attention is given to the influence of the cell proliferative status on PARP activity. We have systematized and analyzed data on changes in PARP activity during development and aging of an organism, as well as data on differences in the dynamics of this activity in the presence/absence of additional stimulation and on cellular processes that are associated with activation of these enzymes. Moreover, data obtained in different models of cellular aging are compared.

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In our previous review [1], we discussed the role of poly(ADP-ribosyl)ation system in the maintenance of genome stability (repair of DNA damage, prevention of chromosome breaks, etc.), as well as the interaction of poly(ADP-ribose) polymerase-1 (PARP-1) with specific proteins of progeria. In particular, based on numerous data indicating an important role of PARPs in an organism's reaction to DNA damage when the organism is exposed to damaging factors, we supposed that PARP-1 could prevent tumorigenesis. PARP is especially interesting for investigations because its "positive" functioning as an enzyme involved in the DNA repair is observed under "normal" conditions, i.e. in the absence of pathologies. Inactivation and knockout of PARP-1 lead to a decrease in genetic stability and increased disposition for tumorigenesis. In the case of development of acute or chronic pathologies, such as inflammation, ischemia, etc., the role of PARP becomes "negative", and its activation aggravates the course of disease. PARP inhibitors are used in such cases as pharmaceuticals reducing the duration and severity of the pathological process. This "dualism" seems promising for discriminating the development of age-related diseases and "successful" aging. Moreover, the data were summarized and analyzed on the role of protein poly(ADP-ribosyl)ation in determination of lifespan (correlation between maximal lifespan and PARP activity) and on the influence on lifespan of the expres-

Abbreviations: CPD, cell population doubling; DIV, days *in vitro*; PAR, poly(ADP-ribose); PARP, poly(ADP-ribose) polymerase; PARP-1, poly(ADP-ribose) polymerase 1; PBMC, peripheral blood mononuclear cells; SA, stimulated activity; UnSA, unstimulated activity.

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sion of the gene encoding the PARP-1 protein, as well as of single-nucleotide polymorphisms in the relevant gene [1].

The present review considers age-related changes in PARP activity and the influence on it of cell proliferative status, methodologies of its determination, and different approaches to activation of the enzymes by biologically active substances (DNA-damaging agents and mitogens) *in vivo* and *in vitro*.

AGE-RELATED CHANGES IN THE PROTEIN POLY(ADP-RIBOSYL)ATION SYSTEM *in vivo*

On the boundary of the 1990s, the first works appeared about the connection of PARP with aging. In these studies, the role of these transferases was studied in age-related changes of the eye lens [2, 3]. Then many reports were published on age-related changes in activity and expression of PARP-1 [4-12].

Before analyzing these works, we mention some methodological problems concerning the choice of approaches for determination of PARP activity in investigations of different authors. In particular, in publications about studies on cultures of an organism's tissues and cells, there are significant differences in use of such term as "the PARP activity", and there is no standard generally accepted procedure for its measurement. Moreover, there are many specific methodical problems associated with gerontological studies on enzymes. Kanungo [13] listed the following difficulties associated with comparison of age-related changes in the enzyme activity: using different methods for its measurement and expression of parameters in different units (per gram wet weight, per gram dry weight, per mg protein, or per mg DNA) – and all these "denominators" can change with age, as well as the enzyme activity; activities of the enzymes are investigated under conditions that are far from physiological; existence of circadian and other rhythms of activity; differences in heterogeneity of animal cohorts studied among strains and laboratories. Moreover, there is no general opinion of what age animals can be considered old [13]. The proliferative status of studied tissue (presence and proportion of proliferating cells) is an additional factor influencing PARP activity (see section "Influence of cell proliferative status on activity of poly(ADP-ribose) polymerases" for detail).

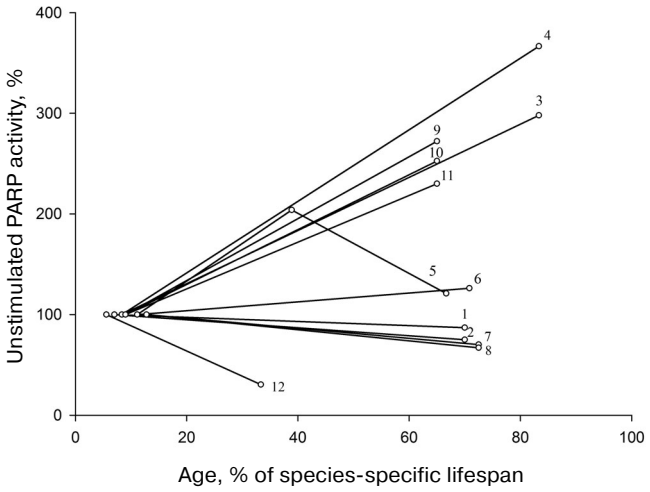
Age-related changes in PARP activity *in vivo*. Careful consideration of methodical aspects of procedures on determination the PARP activity (or of what it is designated by the term) revealed that all methods used in gerontological studies led to one of two parameters: (i) "stimulated activity of PARP" (SA PARP), which represents the rate of poly(ADP-ribosyl)ation determined under external stimulation of PARP by DNA breaks induced in various ways. In this case, on PARP saturation with substrate and excess DNA breaks this parameter reaches maximal values limited by the amount of PARP; (ii) "unstimulated activity of PARP" (UnSA PARP), which represents the rate of poly(ADP-ribosyl)ation determined without additional stimulation of PARP by DNA breaks. With excess PARP, it is limited by the number of DNA breaks.

It should be noted that virtually all the literature data about PARP activity are results not of longitudinal, but of cross-sectional studies; therefore, although we follow the tradition established in the literature and use the term "age-dependent changes in PARP activity", it is necessary to emphasize that it would be more correct to speak about age-related differences.

Since PARP-1 is a protein containing "zinc fingers", decrease in its enzymatic activity could be associated with age-related deficit of zinc ions caused by inadequate diet and/or disturbance of intestinal absorption in elderly people [14]. In fact, in a work by Kunzmann et al. [15] a correlation (although a weak one) was found between SA PARP and zinc concentration in human peripheral blood mononuclear cells (PBMC) ($r^2 = 0.1779$, $p < 0.05$) [15]. Moreover, an age-related decrease in PARP activity in a population of Italians ($r^2 = 0.3965$, $p < 0.05$), but not of Poles or Greeks, was revealed. However, no significant age-related changes were found in the combined group. Grube and Burkle determined SA PARP in human and rat PBMC over a wide age range: from 0 to 100% of the maximal lifespan in rats (BN/BiRj strain) and from 0 to 85% of maximal lifespan in humans [6]. In both cases, an inverse correlation was found between age and PARP activity: the correlation coefficients were –0.54 ($p < 0.001$) and –0.34 ($p < 0.005$) for humans and rats, respectively. Decrease in maximal PARP activity during the life in humans and rats was 59 and 39%, respectively. The most significant differences were found on comparison of PARP activity in PBMC of young (about 30 years old) and old (about 80 years old) people. Zaremba et al. found great dispersion of individual values of SA PARP in PBMC, as well as gender differences, including those probably caused by differences in hormonal regulation [16].

Many more studies have compared UnSA PARP in preparations from tissues of Wistar rats of different age (see figure). As mentioned above, this parameter characterizes the number of DNA breaks in specimens taken for analysis.

In Mandel's laboratory, where PARP was first discovered, age-related changes in UnSA PARP (termed simply as "PARP activity") in nuclei of bovine lens epithelial cells were also studied [2, 3]. These studies are especially interesting because the lens is one of the best-known objects for modeling aging and age-related diseases [17]. The researchers showed that UnSA PARP in old bulls (113 months) was six times higher than in young animals (4.5 months) [2]. The number of DNA breaks in



Age-related changes in unstimulated PARP activity in Wistar rats. PARP activity in young animals is taken as 100%. The data are from the following works: 1) Ushakova et al. [10], spleen; 2) Ushakova et al. [10], brain; 3) Messripour et al. [8], brain, neurons; 4) Messripour et al. [8], brain, astrocytes; 5) Strosznajder et al. [9], brain, cortex; 6) Strosznajder et al. [11], brain, cortex; 7) Mishra et al. [7], brain; 8) Mishra et al. [7], liver; 9) Braidy et al. [12], liver; 10) Braidy et al. [12], kidneys; 11) Braidy et al. [12], heart; 12) Quesada et al. [5], prostate

the adult animals (54 months) was only slightly higher than in the young bulls (24 months) (the difference is 12.9%, $p < 0.05$). At the same time in the old animals (94 months), the number of DNA breaks was 2.3-fold higher ($p < 0.02$) than in the young ones (24 months), and this was expected because of the direct interrelation of these parameters. In another study performed in the same laboratory and with the same approaches, UnSA PARP was found to significantly increase with age in neurons and astrocytes of Wistar rat brain [8]. It was found that UnSA PARP in neurons and astrocytes of old rats (30 months) was, respectively, 3.5- and 3.9-fold higher than in young animals (3 months). The level of DNA damage in the old rats was also significantly higher: 2.7-fold in neurons and 1.3-fold in astrocytes.

Strosznajder et al. compared UnSA PARP in nuclei isolated from different parts of the brain of young (4 months), adult (14 months), and old (24-27 months) Wistar rats [9]. Significant age-related changes in UnSA PARP were detected only in the hippocampus: in the adult rats, it was two times higher than in the young animals, and in old rats it was 2.1 times lower than in the adult rats ($p < 0.02$). In brain cortex and cerebellum, there were no significant differences in UnSA PARP in the young and old rats [9]. In another work, the same group of researchers did not find differences of UnSA PARP in brain of young and old rats [18].

Summarizing the data on UnSA PARP shown in the figure, we note that the graphs are of two groups: a group characterized by increase in UnSA PARP with age (according to the initial hypothesis, because the number of DNA breaks in tissues accumulates with age), and a group without such increase. We also note that the second group mainly includes tissues with low proliferative index, whereas in the upper part there are tissues that mainly have high index. However, these differences in UnSA PARP between tissues with low and high proliferative index described in different works are lower than differences between tissues described within the same work. This is likely due to methodology (specimen preparation, conditions of analyzing, choice of age of control and experimental animals).

Age-related dynamics of UnSA PARP can be explained based on results of a work by Strosznajder [9] in which UnSA PARP was compared not in two, but in three age groups: young, adult, and old animals. The figure shows that it increases in adult animals and decreases in old animals to the level characteristic for young animals. Since the number of DNA breaks does not decrease with age, it can be supposed that the ability of cells to react to DNA damage becomes worse.

Moreover, it was shown that in nuclear fraction from old rat hippocampus, SA PARP did not increase in response to strong oxidative/genotoxic stress induced by $FeCl_2$ and ascorbate, whereas in young rats this parameter significantly increased [11]. DNA damage caused by the alkylating agent *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine also caused strong, 2.6-fold, increase ($p < 0.05$) in level of poly(ADP-ribosyl)ation of proteins in hippocampus of young but not of old rats [11]. Moreover, it was shown that in hippocampus the amyloid peptide and N-methyl-D-aspartate caused (apparently indirectly) DNA damage that in turn led to nearly 80% increase in PARP activity in brain cortex and hippocampus of young (4 months) rats. However, these preparations did not influence this parameter in old (24-27 months) animals [9].

Malanga et al. reported similar results: they showed that in cerebellum of old rats (20-27 months) the ability to activate PARP in response to enzymatic cleavage of DNA by DNase I (SA PARP) was lower than in young (2 months) animals [19]. However, UnSA PARP in forebrain and cerebellum was almost the same in young and old rats [19].

In a work of Gaziev et al., UnSA PARP (termed "constitutive activity of PARP") and maximal SA PARP (synthesis of poly(ADP-ribose) (PAR) stimulated by gamma-irradiation (10 Gy)) were studied in nuclear fraction of brain and spleen from young (2 months) and old (29 months) rats [10]. Irradiation of the animals with dose 10 Gy was sufficient for full activation of PARP (i.e. they showed that it was the correct and maximal SA PARP; this is very seldom done in studies on SA PARP). The UnSA PARP in nuclei from brain and spleen cells was, respectively, 13 and 25% lower in old rats than in young rats [10]. Maximal SA PARP in brain and spleen

nuclei from young rats was about two times higher than in nuclei from old rats.

Thus, literature data on decrease in efficiency of repair of DNA damage induced by gamma-irradiation in old animals can be attributed to decrease in ability of cells for poly(ADP-ribosyl)ation of nuclear proteins [10].

In some other studies, age-dependent decrease in UnSA PARP was reported (SA PARP was not investigated). Thus, Mishra and Das reported that UnSA PARP in brain and liver nuclei of old rats (110-115 weeks) was ~30% lower than in young rats (20 weeks, $p < 0.001$). Age-related decrease was also observed in degree of poly(ADP-ribosyl)ation of both histone and non-histone proteins [7].

Age-related changes associated with the system of chromatin poly(ADP-ribosyl)ation were also detected by Schroder et al. [4]; they reported that UnSA PARP (termed "PARP activity") in chromatin fraction from oviduct of old (3-3.5 years) quail *Coturnix coturnix* females was two times lower than in young birds (7-10 months). However, activity of DNA topoisomerase II increased with age. They supposed that PARP could play an important role in age-related changes of topoisomerase II activity through its posttranslational modification [4].

In this connection, results reported by Thakur and Prasad are also interesting: they studied poly(ADP-ribosyl)ation of nonhistone proteins in nuclei of liver from adult (14 weeks) and old (113 weeks) Wistar rats [20]. It turned out that UnSA PARP in combined fraction of nonhistone proteins was the same in both groups, although spectra of PAR-modified proteins were different. Perhaps not only level and activity of PARP family proteins can change with age, but also direction of their action (which determines the spectrum of PAR-modified proteins, length and branching of PAR chains, etc.).

Thus, studies of UnSA PARP in tissues from animals of different age gave contradictory results (see figure). In some works, it was reported that UnSA PARP in nuclear fraction increased the age, which could be associated with accumulation of unrepaired DNA damage [12]. At the same time decreased ability of cells from old animals to synthesize PAR in response to induction of such damage (SA PARP) can be explained by decrease in amount of active enzyme capable of interacting with newly produced DNA breaks. However, in some works the result was opposite – UnSA PARP decreased with age (figure).

Braidy et al. [12] compared levels of PAR, UnSA PARP, and intracellular NAD^+ in liver, heart, kidneys, and lungs of female Wistar rats of ages from 3 to 24 months, i.e. embracing all age groups (young, adult, and old animals). UnSA PARP was shown to significantly increase with age in all organs: 1.1-1.2-fold ($p < 0.01$) by 12 months old and 2-2.2-fold ($p < 0.01$) by 24 months old as compared to age of 3 months. Moreover, it was reported that PAR accumulated with age, intracellular level of NAD^+ was significantly decreased, and amount of DNA damage increased in all studied tissues. They supposed that high intracellular content of NAD^+ could be an important biochemical factor positively affecting lifespan [12].

The same group of researchers investigated UnSA PARP in human skin samples from non-sun exposed areas of the bodies of people of different age. The level of DNA damage was reported to have good correlation with age in both men ($p = 0.029$, $r = 0.490$) and women ($p = 0.003$, $r = 0.600$). At the same time UnSA PARP significantly increased with age in men only and if the samples were taken from 0 to 77 years old individuals ($p < 0.0001$, $n = 27$, $r = 0.768$). On considering men only during post-puberty and women at the age of 36-76 years, significant changes in UnSA PARP were not found [21]. It can be supposed that the presence/absence of age-related correlations in this case strongly depends on intensity of the organism's growth and, respectively, on the proliferative status of cells (about this see below in detail). Obviously, growth is very active during the postnatal-prepubescent period, whereas during postpubertal period, especially during late post-puberty, there is no active growth. Thus, we conclude that the available data are insufficient for explaining decrease in SA PARP with age. From the considered data, it follows that observed decrease in this parameter with age, along with increase in the amount of DNA damage, can lower the ability of cells to react to DNA lesions and, as a result, cause different disturbances in the genome. Therefore, it is reasonable to suppose that growth of the number of DNA breaks during aging will be associated with increase in level of poly(ADP-ribosyl)ated proteins and that consistently increasing fraction of PARP will be inactivated at any moment, especially if we consider age-related decrease in activity of poly(ADP-ribose) glycohydrolase as reported by Bizec et al. [2]. However, other pathways for regulation of PARP activity may exist, e.g. due to formation of complexes with other proteins or other posttranslational modifications of proteins (acetylation, phosphorylation, mono(ADP-ribosyl)ation, including modifications of PARPs themselves).

Age-related changes in level of PARP-1 expression. Messripour et al. reported that in brain of old (30 months) rats the content of PARP-1 protein was nearly 3.5-fold higher than in young (3 months) rats [8]. In another study on PARP-1 immunoreactivity in young (4 months) and old (27 months) rats, no differences were found in PARP-1 expression in hippocampus and cerebellum, whereas in brain cortex and especially in striatum of old rats the content of PARP-1 was lowered [1]. However, in a work by Malanga et al., no significant difference was found in the amount of PARP-1 protein in cerebellum of young (2 months) and old (20-27 months) rats [19].

Age-related changes in PARP-1 content were found in humans by O'Valle et al. [22]. They showed immunohistochemically that PARP-1 level in kidneys destined for transplantation increased with the donor's age ($r = 0.408$, $p = 0.006$) and positively correlated with the period of

functional activity recovery (effective diuresis) in recipients ($r = 0.386$, $p < 0.01$). At the same time the donor's age varied in narrow limits – from 53 to 64.8 years (on average, 58.9 years). Thus, these data do not allow us to firmly conclude that there is distinct age-related dynamics of PARP-1 expression.

INFLUENCE OF CELL PROLIFERATIVE STATUS ON ACTIVITY OF POLY(ADP-RIBOSE) POLYMERASES

Proliferative status and cell aging. It is known that many parameters used in investigations of aging can be in good correlation with the organism's age but be quite unrelated with increase in death probability with age [23, 24], which is necessarily included in classical determination of aging of living organisms (see for example [25, 26]). Therefore, it is recommended to perform gerontological studies either in longitudinal experiments or in experiments using so-called "gist"/essential models [27, 28]. These imply model systems that are based not only on correlations identified in gerontological studies (for example, the well-known Hayflick model), but on specific mechanisms of aging postulated by the authors. One of such models is the "stationary phase aging" model that was proposed already 30-40 years ago [29-31]. It is based on the concept that the fraction of proliferating cells decreases in an organism with age, which promotes accumulation of macromolecular damage and subsequent disorders in functioning of organs and tissues leading to increase in death probability.

Similar destructive changes accumulate also in the cell culture if its proliferation is restricted. This has been called "stationary phase aging". It was supposed that cell culture growth and monolayer formation could be compared to growth and development of the whole organism [32-34]. In the framework of this concept, it was proposed to use stationary cell cultures for studies on age-related changes that occur in cells of an aging organism [24, 26, 28, 31, 35].

It is supposed [27, 31, 35] that treatment with a geroprotector (from the standpoint of aging deceleration) would be optimal on accumulation in cells of DNA damage initiated by preventing dilution of such lesions at the level of the whole cell population (in the body or in the cell culture) due to slowing or complete cessation of appearance of new cells. If we use a geroprotector in the stage of implementation of already accumulated DNA damage resulting in all other "age-related" changes and diseases, the efficiency of such treatment will be much lower because it changes the rate of aging only for a short time. Thus, differences in lifespan (including species-specific ones), most likely, will be determined only by differences in the programs responsible for reliability of systems (cells, organs, tissues) of a given organism [23].

Influence of cell proliferative status on PARP activity. There are many hypotheses [35, 36] about triggering destructive ontogenetic changes leading to increase in death probability with age (and just this is aging). As possible causes of such increase, there are the action of free radicals, accumulation of somatic mutations, the genetic program, etc. [25]. According to the viewpoint of most modern molecular gerontologists, aging of a living organism (which seems to be determined by destructive changes in cells) is based on accumulation of DNA damage, because DNA is a matrix for renovation of virtually all elements of the cell [37]. In many organisms studied, aging is associated with genetic instability [38]. It is very likely that DNA damage caused by exogenous and endogenous agents that are always attacking the genome of living organisms (e.g. free oxygen radicals, reducing sugars, other physiological metabolites of cells, environmental carcinogens or radiation) play an important role in triggering the aging process.

This viewpoint is confirmed by positive correlation between lifespan of mammals and level of DNA repair: DNA repair more efficiently counteracts accumulation of damage in long-living species and, thus, in such species genome integrity and stability can be maintained more efficiently during the whole lifespan [39]. This can be a factor that determines the appearance of tumors in long-living species later than in species with short lifespan [38]. The intensity of PAR metabolism was compared in proliferating and resting cells isolated from mouse mammary gland tumor [40], and it was shown that both UnSA PARP (termed as "baseline activity" in the paper) and SA PARP did not correlate either with survival or with repair of DNA breaks. It is interesting that under activation by DNase I, SA PARP in one cell line studied was two times higher in proliferating cells than in resting ones, whereas in another line its values were nearly equal. In another series of experiments, under radiation with 50 Gy dose, SA in one cell line was two times higher in proliferating cells than in resting ones, whereas in another cell line the difference was 3.5-fold. Results of analysis of UnSA PARP in resting and actively dividing cell cultures were not so uniform. In one line, this parameter in resting cells was 2.5-fold lower than in proliferating ones, whereas in another line, the picture was opposite – this parameter in resting cells was three times higher, although these two lines were initially received from the same tumor. This indicates once more difficulties in interpretation of data on UnSA PARP. Note that proliferating and resting cells were not different in rate of PAR cleavage. It was also shown that UnSA PARP was higher in proliferating cells of CV-1 line (kidney epithelium of African green monkey) before reaching confluent monolayer state than in these cells after it, but lower than in ERas-transformed cells whose proliferation cannot be stopped by contact inhibition [41]. It was concluded that the activation of PARP-1 and increase in DNA synthesis

are the most striking diagnostic parameters of cancer cells [41].

Salminen at al. also reported that immortalization of cells with SV40 virus (i.e. abolishment of the Hayflick limit and activation of proliferation) leads to increase in PARP expression in culture of human diploid fibroblasts [42].

Spina Purello et al. investigated influence of some mitogenically active growth factors on UnSA PARP in cultures of "young", "mature", and "aged" cells of rat astroglia obtained by cultivation for 30, 90, and 190 days (DIV, days *in vitro*), respectively. Treatment for 12 h of "young" cells with insulin-like growth factor I and basic fibroblast growth factor and treatment of "aged" cells with epidermal growth factor, insulin, or basic fibroblast growth factor significantly increased UnSA PARP [43]. However, none of the above-listed growth factors changed UnSA PARP on treatment of "mature" cells.

Tanigawa et al. studied the influence of protein ADP-ribosylation on DNA synthesis in nuclei from the livers of chick embryo and of adult hens [44]. They found that activation of PARP by addition of 5 mM NAD stimulated DNA synthesis in nuclei of the embryo, but suppressed it in nuclei of adult hens. Based on these results, it was supposed that in nuclei of embryo and adult hen, proteins differently influencing DNA replication could be ADP-ribosylated. Moreover, Porteous et al. found that UnSA PARP in nondifferentiated epithelial cells of the lower part of guinea pig small intestine crypta was nearly 10-fold higher than in differentiated maturing epithelial cells of upper crypta of villi [45]. Analyzing data on age-dependent changes in SA PARP and UnSA PARP with age, we note that these parameters are influenced not only by expression level of PARP family members, mainly of PARP-1 and PARP-2, but also by the character and degree of PARP modification and, possibly, by some other factors. Thus, on activation of PARP-1, the enzyme itself is pronouncedly poly(ADP-ribosyl)ated, which results in its inactivation and dissociation of DNA–PARP-1 complex.

Role of cell proliferative status in changes of the protein poly(ADP-ribosyl)ation system during organism development. It is known that PARP activity is much higher in active chromatin, i.e. in actively proliferating or differentiating cells [44, 46]. This is explained by easier availability of unpacked chromatin to various enzymes including PARPs. Poly(ADP-ribosyl)ation of nuclear proteins disturbs their association with DNA, which promotes DNA replication in S-phase [13]. Model systems are especially interesting that provide analysis of changes in PARP activity (functioning of the cellular poly(ADP-ribosyl)ation system) in germline cells and in relevant tissues and organs (testes, prostate, oviducts, etc.) in response to biologically active substances (hormones, mitogens, etc.).

Muller et al. reported that stimulation with estrogen of 35-day-old Japanese quail (*Coturnix japonica*) females increases the rate of cell proliferation and differentiation and sixfold increases oviduct weight, accompanied by increase in activity of DNA polymerase and UnSA PARP [47].

It has been established that in rat testes the major acceptor protein is the specific histone H1T [48]. Poly(ADP-ribosyl)ation of histone proteins was very low in isolated intact nuclei of testes of 8-day-old animals (in seminiferous tubules there were only spermatogonia), significantly increased by the age of 16 days (pachytene spermatocytes were formed), and by 32 days reached the state characteristic for adult rats (condensed spermatids were present) [48]. In the same laboratory, it was found that in testes of rats at age 28-130 days, the maximum SA PARP was reached by the age of 30 days in tetraploid spermatocytes and in haploid and diploid spermatids, and then it decreased [49]. At the same time the maximal amount of SA PARP was observed in tetraploid spermatocytes subjected to meiotic division, whereas activity of poly(ADP-ribose) glycohydrolase was the same in all studied germ cells. Moreover, expression maxima of PARP-1 and SA PARP in rat testes did not match, but corresponded to the age of 60 and 30 days, respectively [49]. The authors suggested that the protein poly(ADP-ribosyl)ation system is controlled by several regulatory mechanisms during spermatogenesis.

It was shown that in nuclear fraction of Sprague–Dawley rat brain, UnSA PARP fell steadily with fetal maturation [50]. The authors suggested that decrease in UnSA PARP is associated with slowing of cell proliferation, and its increase after birth is associated with activation of brain cell differentiation.

UnSA PARP in cardiomyocytes of 90-day-old rats was lower than in 5-day-old animals [51]. The observed decrease in UnSA PARP can be explained not by "aging" of cardiomyocytes (90-day-old rats can be considered as young animals), but by change in their proliferative status during postnatal development of the organism (see below for details).

Thus, we suppose that during the early period of development of an organism, when soon after birth growth rate decreases and, accordingly, the average mitotic activity of cells also decreases, SA PARP, which reflects the general ability of the cell for poly(ADP-ribosyl)ation, will only decrease. UnSA PARP also decreases, and we suppose that this reflects decrease in DNA availability for PARP because of its compactization, and lower intensity of cell metabolism in the already formed organism and decrease in transcription level. Then, already in resting cells, UnSA for some time will increase, but not due to functional realignments in DNA but due to age-related or pathological processes that increase the level of DNA damage. The accumulation of such damage will increase UnSA only to a certain time, because the poly(ADP-ribosyl)ation capacity decreases with age, as noted above.

THE POLY(ADP-RIBOSYL)ATION SYSTEM IN DIFFERENT MODELS OF CELL AGING

Concerning studies on cell cultures, in populations of intensively proliferating cells (including transformed cells) no accumulation of destructive damage is really observed. This can be explained as follows: if even in rapidly proliferating cells some damage to DNA structure appears because of the effect of free radicals, some chemical agents, or simply temperature-associated movement of molecules, they are either eliminated during replicative repair, or cells with life-incompatible damage simply die, and the population is constantly replenished with young undamaged cells [34]. Therefore, the average amount of DNA damage in the cell population (calculated per cell) does not change. We conclude that restriction of proliferation rate is a trigger for accumulation in the cell population of destructive lesions, including DNA damage. It seems that this situation is adequate to events that occur in an aging organism (decrease in number of proliferating cells and worsening of cell nutrition) [24].

It looks like the widely used Hayflick model [52] based on exhaustion of mitotic potential of a cell culture after approximately 50 passages (for human diploid fibroblasts) does not very accurately represent the situation in a whole organism. Thus, the fraction of dividing cells in an organism is not very high. Moreover, the organism dies not because of exhaustion of mitotic potential or decrease in cell proliferation rate, whereas according to Hayflick, the population of cultured cells is considered "aged" on its inability to double their number within a definite period (two weeks). Comparison of results obtained in models of replicative and "stationary phase aging" can significantly increase our knowledge about cell aging mechanisms. Thus, in the yeast *Saccharomyces cerevisiae*, the mean replicative lifespan is about 15 divisions [53], but already after termination of two-three cell cycles resistance of mother cells to some stresses, e.g. heat shock and salt stress, decreases [54]. This may be due to decreased ability of cells after four and more divisions of realigning their defense systems compared with newly produced mother cells with replicative "age" of two-three divisions [55].

However, yeast cells that were passed into stationary state by immobilization in a special system providing full-value nutrition realigned their phenotype and gene expression pattern, became more resistant to stress, and retained viability >95% for 17 days [56]. In the absence of such system (with usual "chronological aging" (see [57, 58]) the cells die, apparently not only because of stepwise accumulation of internal damage but also because of stress caused by acidification of the medium with products of metabolism; however, the second factor does not seem to be decisive in this phenomenon [58].

Changes in the protein poly(ADP-ribosyl)ation system during cell replicative aging in culture. We already mentioned that in "stationary phase aging" cells restriction of proliferation, as in an organism, does not cause cell death but only triggers accumulation of different lesions [24]. It seems that accumulation of unrepaired DNA damage which was shown to occur, first in nondividing cells, plays the fundamental role in aging [59]. The damage can be very different: DNA–protein cross-links, depurination, substitution of one base for another, demethylation, etc. [13, 60, 61]. DNA strand breaks are the most serious damage. DNA repair enzymes work in senescent cells less efficiently than in young cells, and this leads to disturbances in structure and, correspondingly, worsening of the functional state of DNA.

Dell'Orco and Anderson [62] studied changes in UnSA PARP and SA PARP during "Hayflick-type" aging in permeabilized human diploid fibroblasts obtained from fetal lung (IMR-91 strain) and from newborn foreskin (CF3 strain). In both cell strains, number of cells in late passages (60-80% of maximal number of cell population doublings (CPD)) decreased by 30-60% compared to the decrease in early passages (<60% of maximal number of CPD). SA PARP (stimulated by DNase I) did not depend on cell strain and number of CPD, and the authors concluded that "age-related" changes in PARP-1 protein level were absent [62]. However, this statement contradicts data of Salminen et al. that PARP-1 content dramatically decreased in late passages in a culture of human diploid fibroblasts [42]. To resolve this contradiction, additional studies must be performed with concurrent measurement of the two parameters. It is most likely that PARP activation with DNase I in the experiments of Dell'Orco and Anderson had some methodical errors. This is another argument in favor of using for PARP activation double-strand oligonucleotides as a source of DNA free ends.

Changes in protein poly(ADP-ribosyl)ation system in "stationary phase aging" of cultured cells. It was already mentioned that on slowing of proliferation, PARP activity (and, consequently, ability to react to DNA breaks) changes. These data are especially important because in case of both replicative aging and "stationary phase aging" the appearance of "senescent" phenotype is associated with restriction of proliferation. Populations of postmitotic or very slowly propagating cells inevitably appear during development, and just the presence of such populations in an organism triggers aging; therefore, aging can be considered a "byproduct" of the development program [27, 63]. In a report of Zaniolo et al. [64], SA PARP and expression of PARP-1 in nuclear fractions were compared before and somewhat after monolayer formation in primary cultures of rabbit cornea epithelial cells (RCEC), human cornea epithelial cells (HCEC), human derma keratinocytes (HDK), human umbilical vein endothelial cells (HUVEC), smooth muscle cells from human umbilical vein (HVSMC), and retinal pigmented epithelium from rabbit eye (RPE). All these cells were grown to submonolayer state (about 70% monolay-

er) or monolayer state (100% monolayer) and then were cultured for 2, 4, 5, 15 days (5% CO_2, 37°C). On long-term resting after reaching the monolayer state, SA PARP decreased 2-12-fold in all the cell cultures. Moreover, PARP-1 protein level significantly decreased after monolayer state was reached.

Similar results were obtained earlier by Salminen et al. [42] on cultures of human diploid fibroblasts. They observed significant decrease in PARP-1 protein level on cell transition to resting state in early passages. They showed that this decrease was not associated with increase in apoptosis or with activation of proteolytic enzymes cleaving PARP-1.

In the mentioned work of Spina Purello et al. [43], influence of serum deprivation for 36 h on UnSA PARP in primary culture of rat astrocytes during different growth stages, "young" (30-DIV), "mature" (90-DIV), and "aged" (190-DIV), was studied. As markers of cell "age", cytoskeleton proteins were used: vimentin present mainly in immature astrocytes and glial fibrillar acidic protein, which is the major protein of intermediate filaments in mature astrocytes. In "young" culture (30-DIV) mainly vimentin was expressed, whereas in 90-DIV and 190-DIV cultures glial fibrillar acidic protein was most strongly expressed. In "aged" cells (190-DIV), specific morphological changes were observed, which indicated degenerative processes in the culture. UnSA PARP was strongly increased in "aged" cells (190-DIV) compared to this parameter in 90-DIV cells, which clearly indicated accumulation of DNA damage with "age".

We studied changes in UnSA PARP and SA PARP at different stages of "stationary phase aging" in culture of transformed fibroblast-like strain B11dii-FAF28 of Chinese hamster cells, i.e. on arresting culture growth and further being in stationary phase during cultivation without changing the medium [65]. After cultivation of the cells for 9-10 days, the amounts of both SA and UnSA PARP decreased almost to zero. Thus, SA decreased 2.5-fold after 5 days of cultivation, i.e. after transition of the culture to stationary phase. By the "age" of 10 days, SA PARP decreased nearly 10-fold, and by 13 days it was virtually undetectable [65]. Number of living cells (unstained with trypan blue) lowered only to slightly less than 50%. Thus, similarly to *in vivo* experiments (see above), SA PARP was shown to decrease with age also in the "stationary phase aging" model.

Moreover, we showed that in the Chinese hamster cell culture the number of attached cells decreased, and among the cells maintained on the growth surface the percentage of dead cells increased, which was revealed by staining with trypan blue (from 3% on the 5th day to 50% after 10 days of cultivation). Moreover, the culture medium state changed: pH decreased and debris appeared. Such "exogenous" processes could play a noticeable role in aging, although their role must not be overestimated. We found earlier that with increase in "age" of the culture medium (0-24 days) during "stationary phase aging", its ability to stimulate proliferation of "young" cells decreased by 30-40%. This is not a full explanation of the "stationary phase aging" phenomenon, because even for the "oldest" medium (24 days) the labeling index (calculated as percent of cells with nuclei labeled on incubation in [^{3}H]thymidine-containing medium) was 65%, whereas for "young" medium (3 days) this index was 100% [66]. Based on these results, we have concluded that the "stationary phase aging" phenomenon is based only on internal factors.

Summarizing our results and literature data, we supposed that PARP activity and cell culture viability are closely related parameters. Decrease in cell culture viability with "age" is accompanied by activation of cell death processes, which can cause a decrease in PARP activity. And by contrast – a gradual increase in level of protein poly(ADP-ribosyl)ation caused by increase in level of DNA damage can decrease cell viability through regulation of activity of factors involved in transcription and through enhancement of signals triggering cell death.

As stated above, decrease in proliferation rate is accompanied by accumulation of defects in biological macromolecules including DNA. The idea of DNA damage as a major cause of age-dependent degradation of an organism underlies many concepts of aging, e.g. the free radical theory of Harman [67]. In this connection, many authors have noted that load on systems acting as "sensors" of DNA damage also increases with age, and for their correct functioning their number or activity must be increased [68, 69]. Undoubtedly, the poly(ADP-ribosyl)ation system is such a "sensor". The literature data suggest that accumulation of damage to macromolecules in a cell can be caused by decrease in activity of enzymes responsible for cleavage of PAR, which in turn leads to accumulation of poly(ADP-ribosyl)ated proteins including PARPs themselves. Thus, accumulation of PAR-modified proteins accompanies aging.

Thus, it seems that PARP activity correlates with both species-specific lifespan (this finding motivated active studies of PARPs) and age of animals, as well as with duration of cell cultivation during the "stationary phase aging" and replicative aging. However, we cannot yet conclude that there is any essential relation between poly(ADP-ribosyl)ation and mechanisms underlying age-related increase in death probability, although its involvement in mechanisms of cell death directly is now obvious. However, there are many data indicating that PARP activation aggravates the condition in very different pathologies (ischemic brain stroke, Alzheimer's disease, Parkinson's disease, diabetes mellitus, psoriasis, etc.) [70-72].

In total, during the early period of life, SA PARP decreases accompanied by slowing of proliferation and activation of cell differentiation. In the presence of mito-

gens, UnSA PARP (at least) increases. Thus, PARP activity in a cell both *in vivo* and *in vitro* seems to be regulated not only by external and internal DNA-damaging agents, but also by factors influencing proliferation, including intercellular interactions and action of mitogens. During aging of an organism, SA PARP steadily decreases. It is reasonable to suppose that accumulation of automodified PARPs can be a cause of SA PARP decrease with age. This worsens the ability of PARPs to react to newly arising DNA damage and leads to decrease in efficiency of repair of this damage. From this standpoint, it is very informative to determine in aging biological systems DNA breaks and PARP activity as parameters of a cell's ability to function normally. As to UnSA PARP, it decreases on restriction of proliferation – apparently because of chromatin compactization and decrease in fraction of DNA available for PARP, but later it grows to a certain level – due to accumulation of DNA damage during aging. This is accompanied by accumulation with age of poly(ADP-ribosyl)ated proteins (including PARPs themselves) with partially or complete impaired functioning because of this modification.

However, then the "cost" of reaction to DNA damage becomes "exorbitantly high" for cells of an aging organism (because this is associated with expenditure of great amounts of NAD and ATP). As a result, cells gradually lose their ability to synthesize PAR, possibly not only because of decrease in expression of the enzyme but also due to its inactivation through different posttranslational modifications and decrease in activity of enzymes cleaving this polymer. This is indirectly confirmed by the finding that SA PARP (i.e. total amount of the enzyme that can be activated) is steadily decreasing with age both *in vitro* and *in vivo*. Respectively, data on "age-related" changes in SA and UnSA PARP in the models of replicative aging and "stationary phase aging" suggest that similar changes in cells of an aging organism are determined, at least partially, by change in proliferative status of the cells and not, for example, by the genetic program of aging.

The similarity of changes in the poly(ADP-ribosyl)ation system during aging and on restriction of cell proliferation (both *in vivo* and in model systems) is another indication of viability of the concept asserting that restriction of proliferation is a major cause of accumulation in cells of age-related macromolecular changes leading further, through the chain of various events, to increase in probability of an organism's death, i.e. to aging [27, 73]. However, it must be taken into consideration that just the process of proliferation restriction itself, even unrelated with subsequent accumulation of macromolecular damage, can lead to changes in cell status (intensity of metabolism, etc.), which in turn can influence parameters of the poly(ADP-ribosyl)ation system.

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