Mechanisms of ageing

ALEXANDER BÜRKLE

ARTICLE

Abstract

Recent experimental work from a variety of biological systems, ranging from yeast to human beings, lends increasing support to the view that stochastic damage inflicted to biological macromolecules is the driving force for the ageing process. The damage is derived from small reactive molecules, most prominently reactive oxygen intermediates (ROI), that arise during normal cellular metabolism and are associated with important if not essential cellular functions. The major classes of macromolecules at risk are proteins, lipids and DNA, but damage to DNA (both nuclear and mitochondrial) may entail particularly severe consequences. Cellular dysfunction resulting from macromolecular damage can be detected as a variety of expressions, such as genomic instability, inappropriate cell differentiation events or cell death. While for post-mitotic cell types replacement of the dead cell by another cell of the same lineage is not possible, mitotic cell types may initially replace dead cells via cell proliferation. But exhaustion of the self-renewal capacity of the respective lineage, by either replication-associated or damage-associated telomere shortening, will ultimately also lead to loss of parenchymal cell mass and functional impairment of tissues, the latter being a typical feature of ageing of tissues and organs. It has been demonstrated in various experimental systems that the rate ageing of can be retarded by lowering the production of endogenous ROI or by improving cellular anti-oxidative defences. Whether augmentation of cellular DNA repair capacity will have the same effect remains to be seen.

Ageing can been defined as the time-dependent general decline of physiological functions of an organism, which is associated with a progressively increasing risk of morbidity and mortality.[1] The field of biogeronotology is currently in the process of developing into an important and competitive discipline of basic biomedical research, but the mechanisms underlying ageing are still far from being understood.

Table 1 presents a selection of the currently preferred biological systems in ageing research. The major results from work performed in all these diverse systems is summarised in a highly simplified scheme as given in Fig. 1, which is by no means intended to be comprehensive. The driving force for the ageing process seems to be 'damage' (i.e. stochastic chemical change) inflicted to biological macromolecules, which interferes with their function. Importantly, most damage is derived from small reactive molecules that arise during normal cellular metabolism and are associated with important if not essential cellular functions such as oxygen transport and respiration, phagocyte activity, or detoxification of xenobiotics. Reactive oxygen intermediates (ROI; comprising singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals) feature most prominently among these endogenous damaging agents and can create a state of 'oxidative stress'.[33] In addition, nitric oxide and its metabolite peroxinitrite, as well as endogenously formed alkylating agents and the aldehyde products of lipid peroxidation, all have the capacity to damage macromolecules. The major classes of macromolecules at risk are proteins, lipids and DNA, but damage to DNA may be particularly harmful, since in contrast to most other macromolecules there is little if any turnover of DNA to dilute the damage. In addition, since all genetic information of the cells resides in DNA and most genes are present at a low copy numbers, any errors in the coding function of DNA can 'amplify' to the level of proteins and their respective functions. It should be noted that mitochondria carry their own genome, and mitochondrial DNA is particularly vulnerable to oxidative damage to the close proximity to the respiratory chain, the major site of ROI formation,[34] and is subject to a very high mutation rate.[35]

The cellular dysfunction resulting from macromolecular damage can be detected as a variety of expressions, such as genomic instability, inappropriate cell differentiation events or cell death. Genomic instability is a term collectively describing alterations in the genome, such as point mutations in DNA, amplifications and deletions of DNA sequences, gene rearrangements, and structural or numerical chromosomal aberrations. Genomic instability is recognised as a driving force for the process of carcinogenesis, and indeed for most cancers the single most important risk factor is age.[36] Damage-driven cell differentiation events, on the other hand, can lead to secondary consequences, resulting from

A. Bürkle ✉
Department of Gerontology
University of Newcastle upon Tyne
IAH, Wolfson Research Centre
Newcastle General Hospital
Westgate Road
Newcastle upon Tyne
NE4 6BE, UK
Tel: +44 (0)191 256 3324
Fax: +44 (0)191 219 5074
e-mail:
Alexander.Buerkle@ncl.ac.uk

Table 1. *Preferred biological systems in current ageing research*

Species	Available biological material and typical experimental setting
Saccharomyces cerevisiae (yeast)	• Transformation experiments (gene knock-out/overexpression)[2,3]
Podospora anserina (filamentous fungus)	• Long-lived laboratory strains available[4] • Genes identified which confer life span extension if inactivated[4]
Caenorhabditis elegans (nematode)	• Mutants isolated with extended life span[5] • Transgenic worms with extended life span[5] • Caloric restriction to extend life span[6] • Drugs which mimic Mn-superoxide dismutase to extend life span[7]
Drosophila melanogaster	• Transgenic flies with extended life span[8] • Low-molecular-weight antioxidants in diet to extend life span[9]
Rodents	• Mutants with premature ageing (e.g. Klotho[10]) • Transgenic mice to study accumulation of mutations[11] • Gene knock-out mice to create symptoms resembling normal human ageing[12] or to extend life span[13] • Tissue stem cells and ageing[14] • Caloric restriction to extend life span[15,16] • Cell culture work (including transfection experiments) on cellular stress responses[17]
Primates	• Caloric restriction[18]
Humans	• Progeroid syndromes (e.g. Werner syndrome,[19,20] Progeria Hutchinson Gilford,[21] DNA repair deficiency syndromes[22]) • Comparisons between long-lived people (centenarians) and controls[23,24] • Cell culture work (including transfection experiments) on replicative ageing and cellular stress responses[25–32]

It should be noted that in all the systems listed above comparative studies between young and old individuals are being conducted concerning gene expression or at the level of organ/cellular function.

alterations in the metabolism of the differentiated cell (e.g. altered pattern of secretion of inflammatory mediators or extracellular matrix components). Finally, non-functional or dysfunctional cells can be eliminated by apoptosis (programmed cell death). The immediate consequences will differ according to cell type: For post-mitotic cells (e.g. neurones, muscle cells), replacement of the dead cell by another cell of the same lineage may not be possible. Instead, neighbouring cells may take over the function of the lost one to some extent, while the filling of the 'empty space' will rather occur by proliferating connective tissue, equivalent to scar formation. For mitotic cell types (e.g. stem cell systems, fibroblasts), the lost cell may initially be replaced via cell proliferation, thus preventing any immediate structural or functional impairment of the tissue. However, the proliferative self-renewal capacity of the respective lineage declines and eventually becomes exhausted, be it through reaching replicative senescence[25] (the 'Hayflick limit') due to the replication-associated loss of telomeric repeat units at the end of chromosomes[26] or due to accelerated telomere loss resulting from the accumulation of DNA damage in telomeres.[29] Then the same situation arises as outlined above for post-mitotic cells, and the loss of parenchymal cell mass as well as functional impairment will become manifest, the latter being a typical feature of ageing in many tissues and organs.

It is likely that cells may accumulate damage or genomic instabilities slowly over time without any significant phenotypic effect. Only when a critical threshold of cumulative damage (or of primary consequences of damage) is reached will the cells cease to function properly. Such functional deficit will first become manifest if the organism is undergoing some physical stress (such as trauma, vigorous physical activity, electrolyte and nutrient imbalances, infection), thus challenging the spare capacities of organ function which are lost with ageing.

Based on the above reasoning, the following may be deduced:

(1) *A low rate of endogenous ROI formation will keep the risk of DNA damage low.* In fact, a long-lived mutant of the filamentous fungus *Podospora anserina* displays reduced ROI production in mitochondria.[4] Likewise the rate of mitochondrial ROI formation has been shown to be much lower in long-lived versus short-lived vertebrate species.[37,38] Interestingly, caloric restriction (i.e. undernutrition coupled with a full supply of vitamins, minerals and other essential dietary components) in rodents seems to decrease ROI formation,[16] and this may be a crucial mechanism for its life-span extension and anti-cancer effects.

(2) *Efficient anti-oxidative defences will prevent at least some ROI from damaging DNA.* To some extent, cells are protected against the damaging effects of ROI by means of non-enzymatic and enzymatic antioxidant activities through which oxidants are detoxified before they can damage cellular macromolecules.[33] In a number of experimental systems involving genetic or pharmacological interventions it could be demonstated that an increased cellular anti-oxidative capacity retards the ageing process and extends life span.[7–9]

(3) *DNA repair mechanisms will antagonise the accumulation of DNA damage.* Once DNA damage has already been inflicted, at least some may be removed by DNA repair activities. The pivotal importance of efficient DNA repair systems in the protection against physical or chemical carcinogenesis has long been demonstrated in a number of experimental systems[22] and positive

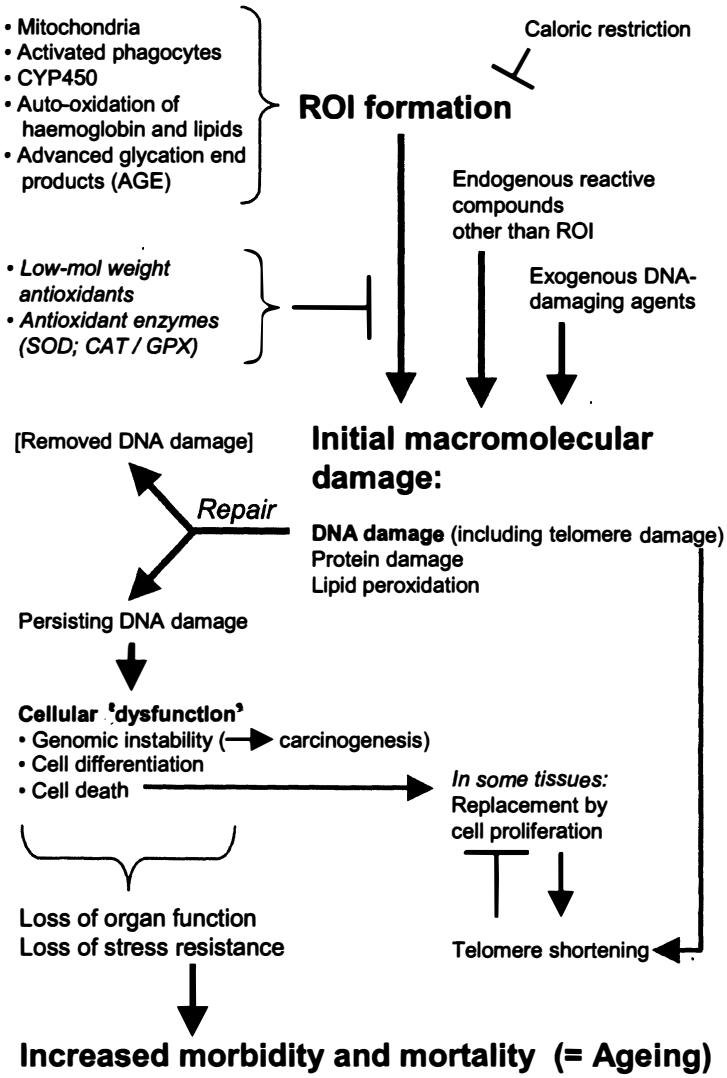

Fig. 1. *Macromolecular damage and the ageing process. CAT, catalase; CYP450, cytochrome P450; GPX, glutathione peroxidase; ROI, reactive oxygen intermediates; SOD, superoxide dismustase. For details see text.*

correlations have been described between the activity of DNA nucleotide-excision repair and life span of mammalian species.[39,40] Whether augmentation of cellular DNA repair capacity will have the same effect remains to be seen.

Our own work has been focused on an enzymatic activity, poly(ADP-ribosyl)ation,[41,42] which is triggered by DNA strand breaks and is associated with DNA base-excision repair[43] (i.e. a pathway that preferentially deals with oxidative DNA damage). In a comparative study we established a positive correlation between the cellular capacity to form poly(ADP-ribose) and life span in mammalian species,[44] and we have begun to unravel the underlying mechanism at the molecular level.[45] In addition, we showed an association between high cellular poly(ADP-ribosyl)ation capacity and longevity in humans.[24] Furthermore, we performed cell culture transfection experiments to further elucidate the biological role of poly(ADP-ribosyl)ation. Our recent data revealed that poly(ADP-ribosyl)ation acts as a negative regulator of genomic instability.[46] Viewed together with the fact that cells from long-lived species[44] or individuals[24] possess high poly(ADP-ribosyl)ation capacity, the picture emerges that poly(ADP-ribosyl)ation may actually be a key factor responsible for tuning the rate of genomic instability events, 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.[47]

Ageing-associated diseases and disabilities can affect any organ in the body and place an already enormous yet rapidly growing burden on the social and economic systems in developed countries. It is obvious that detailed studies of the multiple components of cellular damage, protection and repair pathways and their networks of interaction[48] are required to fully understand the molecular basis of the ageing process. Such understanding is indispensable if we are to develop novel modalities of prevention and treatment of ageing-associated pathologies.

Our own work cited here was supported by grants from the Deutsche Forschungsgemeinschaft (Bu 698/2-1, -2, -3, and -4) and from the EU Commission (Concerted Action Programme on 'Molecular gerontology: the identification of links between ageing and the onset of age-related diseases [MOLGERON]': BMH1 CT94 1710).

References

1. Bürkle A. Maintaining the stability of the genome. In: Rattan SIS, Toussaint O, editors. Molecular gerontology: Research status and strategies. New York: Plenum Press, 1996:25–36.
2. Jazwinski SM. Molecular mechanisms of yeast longevity. Trends Microbiol 1999;7:247–52.
3. Guarente L. Sir2 links chromatin silencing, metabolism, and aging. Genes Dev 2000;14:1021–6.
4. Osiewacz HD, Borghouts C. Mitochondrial oxidative stress and aging in the filamentous fungus *Podospora anserina*. Ann N Y Acad Sci 2000;908:31–9.
5. Murakami S, Tedesco PM, Cypser JR, Johnson TE. Molecular genetic mechanisms of life span manipulation in *Caenorhabditis elegans*. Ann N Y Acad Sci 2000;908:40–9.
6. Lakowski B, Hekimi S. The genetics of caloric restriction in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 1998;95:13091–6.
7. Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, *et al*. Extension of life-span with superoxide dismutase/catalase mimetics. Science 2000;289:1567–9.
8. Orr WC, Sohal RS. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science 1994;263:1128–30.
9. Brack C, Bechter-Thuring E, Labuhn M. *N*-Acetylcysteine slows down ageing and increases the life span of *Drosophila melanogaster*. Cell Mol Life Sci 1997;53:960–6.
10. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, *et al*. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 1997;390:45–50.
11. Vijg J, Dolle ME, Martus HJ, Boerrigter ME. Transgenic mouse models for studying mutations *in vivo*: applications in aging research. Mech Ageing Dev 1997;99:257–71.
12. Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, *et al*. Longevity, stress response and cancer in ageing telomerase-deficient mice. Cell 1999;96:701–12.
13. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, *et al*. The $p66^{shc}$ adaptor protein controls oxidative stress response and life span on mammals. Nature 1999;402:309–13.
14. Martin K, Potten CS, Kirkwood TB. Age-related changes in irradiation-induced apoptosis and expression of p21 and p53 in crypt stem cells of murine intestine. Ann N Y Acad Sci 2000;908:315–8.
15. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science 1996;273:59–63.
16. Merry BJ. Calorie restriction and age-related oxidative stress. Ann N Y Acad Sci 2000;908:180–98.
17. Gonos ES, Derventzi A, Kveiborg M, Agiostratidou G, Kassem M, Clark BF, *et al*. Cloning and identification of genes that associate with mammalian replicative senescence. Exp Cell Res 1998;240:66–74.
18. Lane MA, Ingram DK, Roth GS. Nutritional modulation of aging in nonhuman primates. J Nutr Health Aging 1999;3:69–76.
19. Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, *et al*. Positional cloning of the Werner's syndrome gene. Science 1996;272:258–62.
20. Nehlin JO, Skovgaard GL, Bohr VA. The Werner syndrome: a model for the study of human aging. Ann N Y Acad Sci 2000;908:167–79.
21. Mukherjee AB, Costello C. Aneuploidy analysis in fibroblasts of human premature aging syndromes by FISH during *in vitro* cellular aging. Mech Ageing Dev 1998;103:209–22.
22. de Boer J, Hoeijmakers JH. Cancer from the outside, aging from the inside: mouse models to study the consequences of defective nucleotide excision repair. Biochimie 1999;81:127–37.
23. Yashin AI, De Benedicts G, Vaupel JW, Tan Q, Andreev KF, Iachine IA, *et al*. Genes and longevity: lessons from studies of centenarians. J Gerontol A Biol Sci Med Sci 2000;55:B319–28.
24. Muiras ML, Müller M, Schächter F, Bürkle A. Increased poly(ADP-ribose) polymerase activity in lymphoblastoid cell lines from centenarians. J Mol Med 1998;76:346–54.
25. Hayflick L. The cell biology of aging. J Invest Dermatol 1979;73:8–14.
26. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345:458–60.
27. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, *et al*. Extension of life-span by introduction of telomerase into normal human cells. Science 1998;279:349–52.
28. Campisi J. Cancer, ageing and cellular senescence. In Vivo 2000;14:183–8.
29. von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. Ann N Y Acad Sci 2000;908:99–110.
30. Kletsas D, Pratsinis H, Zervolea I, Handris P, Sevaslidou E, Ottaviani E, *et al*. D. Fibroblast responses to exogenous and autocrine growth factors relevant to tissue repair: the effect of aging. Ann N Y Acad Sci 2000;908:155–66.
31. Toussaint O, Dumont P, Dierick JF, Pascal T, Frippiat C, Chainiaux F, *et al*. Stress-reduced premature senescence: essence of life, evolution, stress, and aging. Ann N Y Acad Sci 2000;98:85–98.
32. Atamna H, Paler-Martinez A, Ames BN. *N*-t-Butyl hydroxylamine, a hydrolysis product of alpha-phenyl-*N*-t-butyl nitrone, is more potent in delaying senescence in human lung fibroblasts. J Biol Chem 2000;275:6741–8.
33. Martin GM, Austad SN, Johnson TE. Genetic analysis of ageing: role of oxidative damage and environmental stresses. Nature Genet 1996;13:25–34.
34. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci USA 1994;91:10771–8.
35. Kopsidas G, Kovalenko SA, Heffernan DR, Yarovaya N, Kramarova L, Stojanovski D, *et al*. Tissue mitochondrial DNA changes: a stochastic system. Ann N Y Acad Sci 2000;908:226–43.
36. Riggs JE. Carcinogenesis, genetic instability and genomic entrophy: insight derived from malignant brain tumor age specific mortality rate dynamics. J Theor Biol 1994;170:331–8.
37. Ku HH, Brunk UT, Sohal RS. Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species. Free Radic Biol Med 1993;15:621–7.
38. Barja G, Cadenas S, Rojas C, Perez-Campo R, Lopez-Torres M. Low mitochondrial free radical production per unit O_2 consumption can explain the simultaneous presence of high longevity and high aerobic metabolic rate in birds. Free Radic Res 1994;21:317–27.
39. Hart RW, Setlow RB. Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammalian species. Proc Natl Acad Sci USA 1974;71:2169–73.
40. Cortopassi GA, Wang E. There is substantial agreement among interspecies estimates of DNA repair activity. Mech Ageing Dev 1996;91:211–8.
41. De Murcia G, Shall S. Poly(ADP-ribosylation) reactions: from DNA damage and stress signalling to cell death. Oxford: Oxford University Press, 2000.
42. Bürkle A. Poly(ADP-ribosyl)ation: a posttranslational protein modification linked with genome protection and mammalian longevity. Biogerontology 2000;1:41–6.

43. Molinete M, Vermeulen W, Bürkle A, Ménissier-de Murcia J, Küpper J-H, Hoeijmakers JHJ, *et al*. The poly(ADP-ribose) polymerase DNA-binding domain blocks alkylation-induced DNA repair synthesis in living cells. EMBO J 1993;12:2109–17.

44. Grube K, Bürkle A. Poly(ADP-ribose) polymerase activity in mononuclear leukocytes of 13 mammalian species correlates with species-specifics life span. Proc Natl Acad Sci USA 1992;89:11759–63.

45. Beneke S, Alvarez-Gonzalez R, Bürkle A. Comparative kinetic characterisation of baculovirus-expressed human and rat poly(ADP-ribose) polymerase-1. Exp Gerontol 2000;35:989–1002

46. Meyer R, Müller M, Beneke S, Küpper JH, Bürkle A. Negative regulation of alkylation-induced sister-chromatid exchanges by poly(ADP-ribose) polymerase-1 activity. Int J Cancer 2000;88:351–5.

47. Bürkle A. Poly(ADP-ribosyl)ation, genomic instability, and longevity. Ann N Y Acad Sci 2000;908:126–32.

48. Kirkwood TB. Molecular gerontology: bridging the simple and the complex. Ann N Y Acad Sci 2000;908:14–20.