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Signaling mechanisms involved in the response to genotoxic stress and regulating lifespan

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Abstract

Ageing is defined by the loss of functional reserve over time, leading to a decreased capacity to maintain homeostasis under stress and increased risk of morbidity and mortality. Ageing is extremely heterogeneous between individuals and even between tissues within an organism, making it challenging to identify the molecular basis of ageing. Much of our current understanding of ageing comes from genetic studies in model organisms seeking genes that either accelerate or decelerate the ageing process. These studies revealed not only causes of ageing, but also signaling mechanisms that both promote and protect against ageing. In all cases, the signaling pathways that influence lifespan are familiar mechanisms that regulate cellular metabolism, growth, proliferation, differentiation and survival. This review highlights the significant overlap in signaling mechanisms implicated in both the cellular response to genotoxic stress and regulation of organism lifespan.

Keywords

genotoxic stress; insulin-like growth factor; lifespan regulation

Introduction

There is abundant evidence that time-dependent accumulation of stochastic damage to cellular macromolecules is a driving force in ageing (Kirkwood, 2005). All cellular components are vulnerable to damage, but DNA is unique in that it is the only macromolecule repaired rather than degraded when damaged, demonstrating its pivotal role in maintaining cell function. The genome of every cell is damaged tens of thousands of times per day by reactive oxygen species produced during oxidative metabolism and due to the inherent chemical instability of nucleic acids. This is further exacerbated by environmental exposures and replication of the genome, which causes DNA double-strand breaks and telomere attrition. DNA damage can lead to mutations and chromosomal aberrations that cause cancer. It can also block essential cellular processes such as transcription and replication, triggering signaling events that culminate in programmed cell death and loss of functional cells (Mitchell et al., 2003). Based on the frequency and consequences of DNA damage, it is a likely culprit in the ageing process. In support of this, inherited defects in genome maintenance mechanisms cause progeroid or accelerated ageing symptoms (Hasty et al., 2003). Furthermore, DNA damage has recently

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been demonstrated to limit the ability of stem cells to restore homeostasis after stress (Rossi et al., 2007), a hallmark of ageing.

Cascades and Key Molecules

To maintain the integrity of the genome, a cell challenged with genotoxic stress must sense the damage and respond to it by stalling cell cycle progression until the damage is repaired. If the damage is catastrophic, then pro-apoptotic mechanisms are activated, resulting in cell death. At the organismal level, chronic genotoxic stress causes systemic dampening of growth signals (Niedernhofer et al., 2006), affording another layer of protection. Many of the key regulators of the signaling pathways affected by genotoxins when similarly affected via genetics or pharmaceuticals extend the lifespan of cells or organisms. This implies that DNA damage triggers a network of signaling mechanisms aimed at somatic preservation. Here we review how the cellular response to genotoxic stress integrates several key regulators of lifespan (Figure 1).

I. DNA damage response

The precise signaling mechanisms governing the response to genotoxic stress are best characterized for DNA double-strand breaks (DSBs). ATM is a PI3-like kinase that acts as a signal transducer in response to genotoxic stress, most notably DSBs (Shiloh, 2006). Once a DSB is detected by the MRE11-RAD50-NBS1 complex, ATM is activated and phosphorylates numerous effector proteins that promote cell cycle arrest and DNA repair. A key substrate of ATM is p53, a transcription factor that regulates the G1/S checkpoint and apoptosis. Phosphorylation of p53 leads to its stabilization and activation. ATM also phosphorylates MDM2, blocking its ability to ubiquitinate p53 and target it for degradation. ATM phosphorylates IKKγ, the regulatory subunit of the IκB kinase IKK, which activates the transcription factor NF-κB (see below). Like p53, NF-κB promotes apoptosis in response to genotoxic stress (Barkett and Gilmore, 1999). A third transcription factor, E2F1, is also a substrate for ATM (Shiloh, 2006). E2F1 induces expression of SIRT1, a histone deacetylase associated with lifespan regulation (Longo and Kennedy, 2006), which tempers the pro-apoptotic activity of p53 and NF-κB directly and activates other pro-survival pathways. E2F1 also regulates the expression of other important cell cycle regulatory and survival factors such as $p19^{ARF}$ in response to certain stress stimuli. ATM regulates translation in response to insulin and growth factors by phosphorylating 4E-BP1, causing it to release eIF-4E, which then binds the N-terminal cap of mRNA to initiate protein synthesis.

p53 and NF-κB are activated by other genotoxins, including ultraviolet light (UV) and reactive oxygen species (ROS), independently of ATM. Interestingly, hyperactivation of p53 promotes ageing (Maier et al., 2004) and hyperactivation of NF-κB is associated with ageing in mammals, leading to speculation that either of the transcription factors or the pathways that regulates them can drive ageing (Giardina and Hubbard, 2002).

II. IGF1

IGF-1 is a mitogenic peptide produced primarily by the liver in response to growth hormone (GH). Binding of IGF-1 to its receptor (IGF-1R) leads to phosphorylation of numerous downstream effectors that promote cell survival, proliferation and differentiation. IGF-1 protects cells against apoptosis in response to genotoxic stress. Seemingly in contradiction to this, *in vivo* chronic genotoxic stress causes a systemic reduction in circulating IGF-1 (Niedernhofer et al., 2006). This may be mediated by p53, which inhibits expression of IGF-1R and IGF-II while increasing the expression of IGFBP-3 (an IGF-1 binding protein that retains IGF-1 in the serum), all of which dampen IGF-1 signaling (Butt et al., 1999). GH-IGF-1 levels are also known to decline with age in mammals.

It is well-established that chronic suppression of GH-IGF-1 signaling extends the lifespan of worms, flies and mice (Kenyon, 2005). Suppression of GH-IGF-1 signaling is also the primary mechanism by which caloric restriction extends lifespan. This endocrine response appears to be a highly conserved mechanism aimed at conserving energy resources for maintenance and repair rather than utilizing them for proliferation, and growth and was likely evolved to cope with stress (nutritional or genotoxic). As a consequence of suppressed GH-IGF-1, oxidative metabolism is decreased, while anti-oxidant defenses and DNA repair are increased (Kenyon, 2005; Niedernhofer et al., 2006). Suppression of GH-IGF-1 signaling in response to genotoxic stress therefore affords protection, minimizing further damage, including DNA damage. However, in the long term, as with ageing, accumulation of DNA damage is inevitable. Thus suppression of this pro-survival signaling cascade driven by IGF-1 may ultimately contribute to the loss of tissue reserves or regenerative capacity.

III. mTOR

mTOR (mammalian Target of Rapamycin), like ATM, is a PI3-related kinase that phosphorylates Ser-Thr residues of proteins rather than lipids. It resides in one of two cytoplasmic complexes: mTORC1, comprised of mTOR, mLST8 (GβL) and Raptor, which senses the nutrient status of cells and promotes cell survival and proliferation in response, or mTORC2, containing mTOR, mLST8 and Rictor that is involved in actin organization. mTOR serves to integrate multiple signals to regulate growth including responses to growth factors, stress, nutrients, and energy. Stimulation of cells with mitogens such as IGF-1 results in activation of AKT/PKB. AKT then phosphorylates and inactivates the TSC1-TSC2 complex, an inhibitor of mTOR signaling. Depriving cells of nutrients, such as leucine, results in rapid dephosphorylation of mTOR targets S6K1 and 4E-BP1, inhibiting translation, whereas addition of nutrients results in rapid phosphorylation of these targets by mTOR through both TSC1-TSC2-dependent and independent pathways.

Genotoxic stress leads to inhibition of mTOR via a p53, AMPK and TSC1-TSC2-dependent mechanism. Similarly, expression of proteins involved in AKT-mTOR signaling pathways is significantly down-regulated in tissues of aged mice (L.J.N., submitted). Thus, like IGF-I, the pro-survival and proliferation signals of mTOR are dampened in response to genotoxic stress and with ageing. Also like IGF-1, suppression of mTOR signaling is associated with lifespan extension. Dominant negative forms of TOR or its downstream effector S6K1 or overexpression of the upstream inhibitors of TOR, TSC1 and TSC2, extend lifespan in *Drosophila* (Kapahi et al., 2004). Similarly, deletion of TOR1 and many of its downstream targets in yeast (Kaeberlein et al., 2005) or deletion of the downstream TOR target e1F4E in worms confers longevity (Syntichaki et al., 2007).

IV. NF-κB

NF-κB is a family of transcription factors that regulates cell proliferation and survival in response to stress and inflammatory cytokines. The NF-κB family includes five members: p65, c-Rel, Rel-B, p50, and p52. In the absence of stress, NF-κB proteins are retained in the cytoplasm through their association with IκB proteins. Cellular stress leads to phosphorylation of IκB and its subsequent polyubiquitination and degradation by the proteosome. This releases NF-κB, which then translocates to the nucleus and induces transcription. Phosphorylation of IκB is mediated by the IκB kinase (IKK) complex. IKK is made up of two catalytic subunits, IKKα and IKKβ and a regulatory subunit IKKγ. NF-κB is generally thought of as a survival factor, inhibiting apoptosis, driving cell proliferation and regulating differentiation. However, in response to genotoxic stress, activation of NF-κB promotes apoptosis (Barkett and Gilmore, 1999). NF-κB is activated by a variety of different types of genotoxic stress (Janssens and Tschopp, 2006). Furthermore, NF-κB is activated in numerous tissues of aged organisms (Giardina and Hubbard, 2002).

V. Sirtuin 1

SIRT1 is member of the sirtuin family of class III histone deacetylases implicated in regulation of lifespan (Longo and Kennedy, 2006). Several non-histone targets of this enzyme have been identified. SIRT1 deacetylates FOXO transcription factors and the DNA repair protein KU70 leading to their activation and expression of stress response genes and inhibition of BAD-mediated apoptosis, respectively. SIRT1 also deacetylates p53 and NF-κB, leading to their inactivation and inhibition of apoptosis. In addition, SIRT1 can regulate NF-κB through a deacetylase-independent mechanism through interaction with the transcriptional repressor, TLE1 (Ghosh et al., 2007).

Overexpression of orthologs of SIRT1 in yeast, worms or flies (Sir2) extends the lifespan of these organisms. Similarly, resveratrol, a SIRT1 agonist, extends the lifespan of worms and flies. Treating adult mice on a high fat diet with resveratrol induces endocrine changes associated with longevity, such as suppression of IGF-1 and insulin sensitivity, and extends lifespan (Baur et al., 2006). Resveratrol also dramatically inhibits mTOR activation in response to stress through a TSC2-dependent pathway, at least in part through SIRT1 activation (P.D.R, submitted). Therefore, in apparent contrast to other pro-survival and growth promoting signaling mechanisms (*e.g.*, IGF-1 and mTOR), SIRT1 hyperactivity appears to delay ageing, partly through suppression of IGF-1 and mTOR signaling. Consistent with this, SIRT1 expression is induced rather than suppressed by caloric restriction (Cohen et al., 2004) and SIRT1 orthologs are required for lifespan extension via caloric restriction in yeast, worms and flies (Haigis and Guarente, 2006).

There are, however, several observations that counter the notion that SIRT1 is a longevity factor. First, deletion, rather than overexpression of Sir2 increases resistance to oxidative DNA damage and significantly extends the chronological life span of yeast (the longevity of a non-dividing cell) (Fabrizio et al., 2005). Second, deletion of SIRT1 or inhibition with nicotinamide leads to resistance to oxidative stress and extends the replicative lifespan of primary fibroblasts (Longo and Kennedy, 2006). Furthermore, *Sirt1*$^{-/-}$ mice overexpress IGFBP-1, an inhibitor of IGF-1, and are insulin sensitive, dwarfed and have decreased fertility, mimicking caloric restriction. However, the effect of loss of SIRT1 on lifespan in mice has not yet been reported. An interesting clue as to how to resolve these conflicting protective and inhibitory functions of SIRT1 comes from the observation that deletion of Sir2 in yeast dramatically extends lifespan dramatically, but only under conditions of nutrient deprivation or when IGF-1 signaling is inhibited (Fabrizio et al., 2005). This suggests that Sir2/SIRT1 may be protective and extend longevity under non-stressful conditions, but may be detrimental under stressful conditions because it counters the protective signaling network mediated by the GH-IGF-1 axis (Figure 1). This raises the question: will SIRT1 inhibitors or agonists delay age-associated degenerative changes in humans given our complex and relatively stressful environment?

Therapeutic implications

Mutation or overexpression of a single gene that extends lifespan in lower eukaryotes invariably comes with a price in mammals (*e.g.*, dwarfism, infertility, glucose intolerance). That is because all longevity genes identified encode proteins that are key regulators of signaling pathways that govern cellular metabolism, growth, proliferation, differentiation and survival. However, longevity genes are also key regulators of how a cell responds to its environment. Thus developing rational strategies for preventing or delaying ageing-associated functional decline will necessitate understanding not only the complex signaling pathways, but also how signaling changes in response to various types and levels of common stress, including genotoxic stress.

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References

Barkett M, Gilmore TD. Control of apoptosis by Rel/NF-kappaB transcription factors. Oncogene 1999;18:6910–6924. [PubMed: 10602466]

Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006;444:337–342. [PubMed: 17086191]

Butt AJ, Firth SM, Baxter RC. The IGF axis and programmed cell death. Immunol Cell Biol 1999;77:256–262. [PubMed: 10361258]

Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de Cabo R, Sinclair DA. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 2004;305:390–392. [PubMed: 15205477]

Fabrizio P, Gattazzo C, Battistella L, Wei M, Cheng C, McGrew K, Longo VD. Sir2 blocks extreme life-span extension. Cell 2005;123:655–667. [PubMed: 16286010]

Ghosh HS, Spencer JV, Ng B, McBurney MW, Robbins PD. Sirt1 interacts with transducin-like enhancer of split-1 to inhibit NF-kappaB mediated transcription. Biochem J. 2007

Giardina C, Hubbard AK. Growing old with nuclear factor-kappaB. Cell Stress Chaperones 2002;7:207–212. [PubMed: 12380689]

Haigis MC, Guarente LP. Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction. Genes Dev 2006;20:2913–2921. [PubMed: 17079682]

Hasty P, Campisi J, Hoeijmakers J, van Steeg H, Vijg J. Aging and genome maintenance: lessons from the mouse? Science 2003;299:1355–1359. [PubMed: 12610296]

Janssens S, Tschopp J. Signals from within: the DNA-damage-induced NF-kappaB response. Cell Death Differ 2006;13:773–784. [PubMed: 16410802]

Kaeberlein M, Powers RW 3rd, Steffen KK, Westman EA, Hu D, Dang N, Kerr EO, Kirkland KT, Fields S, Kennedy BK. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science 2005;310:1193–1196. [PubMed: 16293764]

Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Curr Biol 2004;14:885–890. [PubMed: 15186745]

Kenyon C. The plasticity of aging: insights from long-lived mutants. Cell 2005;120:449–460. [PubMed: 15734678]

Kirkwood TB. Understanding the odd science of aging. Cell 2005;120:437–447. [PubMed: 15734677]

Longo VD, Kennedy BK. Sirtuins in aging and age-related disease. Cell 2006;126:257–268. [PubMed: 16873059]

Maier B, Gluba W, Bernier B, Turner T, Mohammad K, Guise T, Sutherland A, Thorner M, Scrable H. Modulation of mammalian life span by the short isoform of p53. Genes Dev 2004;18:306–319. [PubMed: 14871929]

Mitchell JR, Hoeijmakers JH, Niedernhofer LJ. Divide and conquer: nucleotide excision repair battles cancer and ageing. Curr Opin Cell Biol 2003;15:232–240. [PubMed: 12648680]

Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, Appeldoorn E, Odijk H, Oostendorp R, Ahmad A, van Leeuwen W, Theil AF, Vermeulen W, van der Horst GT, Meinecke P, Kleijer WJ, Vijg J, Jaspers NG, Hoeijmakers JH. A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature 2006;444:1038–1043. [PubMed: 17183314]

Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 2007;447:725–729. [PubMed: 17554309]

Shiloh Y. The ATM-mediated DNA-damage response: taking shape. Trends Biochem Sci 2006;31:402–410. [PubMed: 16774833]

Syntichaki P, Troulinaki K, Tavernarakis N. eIF4E function in somatic cells modulates ageing in Caenorhabditis elegans. Nature 2007;445:922–926. [PubMed: 17277769]

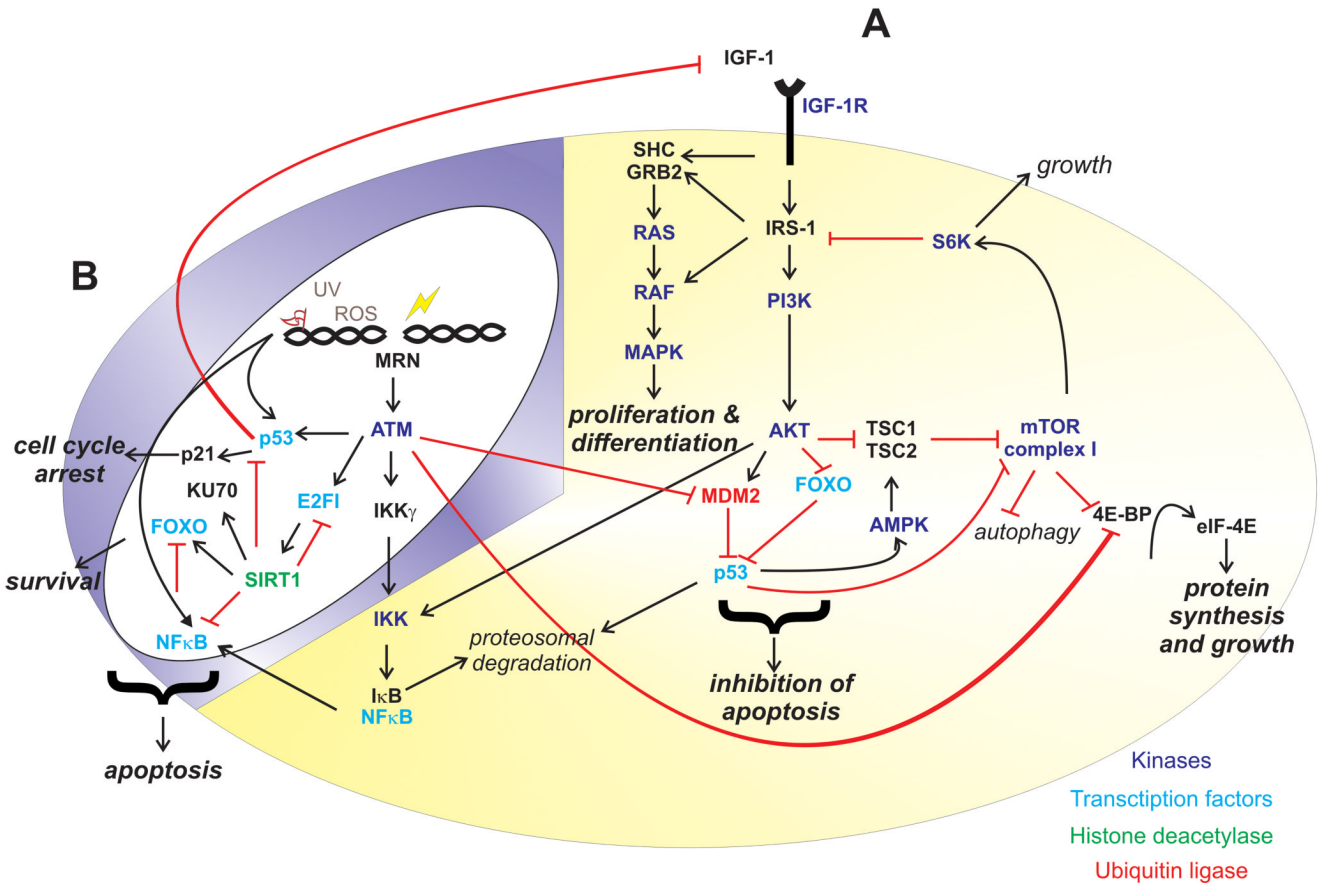

Figure 1.

Signaling mechanisms involved in lifespan regulation and the cellular response to genotoxic stress. **(A) Signals promoting growth and survival** (yellow portion of the cell). IGF-1 is secreted primarily by the liver in response to growth hormone and is the key mediator of its growth-promoting effects. IGF-1 binds its cell surface receptor IGF-1R, a tyrosine kinase that phosphorylates numerous substrates including SHC, which then recruits the adaptor protein GRB2 and activates the RAS-MAPK pathway, which stimulates cell proliferation and differentiation. A second key substrate is IRS-1, which leads to activation of the serine-threonine kinase AKT via PI3-kinase (PI3K). AKT promotes cell survival by directly phosphorylating and inhibiting pro-apoptotic factors including caspase-9, BAD and FOXO transcription factors. AKT also phosphorylates a subunit of IKK leading to activation of the transcription factor NF-κB, which drives expression of pro-survival genes. AKT inhibits p53-mediated apoptosis by phosphorylating and activating MDM2, an E3-ubiquitin ligase that polyubiquitinates p53, targeting it for proteosomal degradation. AKT also phosphorylates the TSC complex, allowing activation of mTOR. mTOR is a PI3K-like kinase that resides in multiprotein complexes. mTORC1 positively regulates growth by directly phosphorylating and inhibiting 4E-BP, causing it to release its binding partner eIF-4E, which then binds mRNA caps, the rate limiting step in protein translation. mTOR also negatively regulates autophagy and activates S6K, which promotes ribosomal maturation and growth. Decreased expression of the growth promoting factors IGF-1, IGF-1R, PI3K, AKT, TOR, e1F4E or S6K leads to lifespan extension in at least one model organism (yeast, worms, flies or mice).

(B) Pro-apoptotic signaling in response to genotoxic stress (blue portion of cell). DNA double strand breaks are sensed by the MRE11-RAD50-NBS1 (MRN) complex, which in turn activates the signal transducer ATM. ATM is a PI3K-like kinase with numerous protein targets.

ATM phosphorylation of the transcription factor p53 leads to its stabilization and activation, promoting cell cycle arrest and apoptosis. ATM also phosphorylates IKKγ, the regulatory subunit of IKK, causing its translocation to the cytoplasm, activating IKK, which phosphorylates IκB. Phosphorylation of IκB leads to its polyubiquitination and proteosomal degradation, releasing NF-κB transcription factor, which moves to the nucleus and in response to genotoxic stress increases expression of pro-apoptotic genes. A third substrate of ATM is the transcription factor E2F1, which among other things induces expression of the histone deacetylase SIRT1. SIRT1 deacetylates numerous non-histone substrates including KU70 and FOXO transcription factors, leading to their activation and inhibition of apoptosis. SIRT1 also deacetylates p53 and NF-κB, causing their inactivation and dampening of pro-apoptotic signals. Hyperactivity of the pro-apoptotic factor p53 accelerates aging and NF-κB is associated with old age. Deletion of SIRT1 extends lifespan in metazoans and appears to delay aging in mammals, consistent with it being primarily growth promoting.