# Stress-Induced Senescence: Molecular Pathways

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## Contents



**Abstract**

Senescence is an irreversible state of the cell, in which the cell becomes incapable of further cell division. The concept of stress-induced senescence indicates that the major causes of senescence are various types of stresses that act on cells. These stresses act via intracellular pathways, which may be multiple, to a final common state of irreversible cell division. In this state the cell is held in the nondividing state by the combination of cyclin-dependent kinase inhibitor (CDKI) activity, heterochromatin formation, gene expression changes, and other mechanisms. While the mediators of stress-induced senescence are multiple and complex, the p38 MAP kinase pathway has a prominent role in linking stresses to the permanently nondividing state. The means by which it may become activated by stresses and the means by which it acts to cause senescence are being unraveled. While most of the past studies on senescence have used cells in culture, the recent emergence of novel mouse models in which senescence can be studied has opened up exciting avenues toward mechanistic insights and possible therapeutic interventions.

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**Keywords**

Senescence • Stresses • Cyclin-dependent kinase inhibitors • p38 MAP kinase

## Introduction

The defining characteristic of cellular senescence is the inability of the senescent cell to undergo cell division in response to physiological stimuli that normally cause cell proliferation in that cell type. Cellular senescence is one possible end-point as a reaction of cells to various types of stress; it forms one possible outcome of the effects of severe stresses, in addition to various forms of cell death and in addition to full repair of the damage and complete recovery (Kuilman et al. 2010). During the development of the field, and continuing to the present day, senescence has been closely linked with telomere biology: senescence and telomere shortening, the originally identified cause of senescence, have often been considered as a single phenomenon. However, there are many examples of stresses that cause senescence that either have no connection with telomere biology, or at least a nonobvious connection. Additionally, telomere shortening is not invariably associated with senescence. Thus, senescence is now understood to be a general reaction of cells to a wide range of forms of cellular damage. This chapter reviews the basic biology of cellular senescence as it occurs in cell culture and in tissues in vivo, and gives an overview of some of the molecular mechanisms involved.

## Cellular Senescence as the End-Point of Cellular Response to Stresses

Almost any stress can potentially cause cells to enter senescence (Fig. 11.1). In fact, many vaguely-characterized phenomena in cultured cells, in which cells have been seen to enter a permanently nondividing state, can now be recognized as examples of cellular senescence. Depending on the nature of the stress, its intensity, and the cell type, the result may be cell death, cellular senescence, or repair and recovery. At least in some cell types, lower levels of damage cause senescence and higher levels cause cell death. Conversely, some cell types are less likely

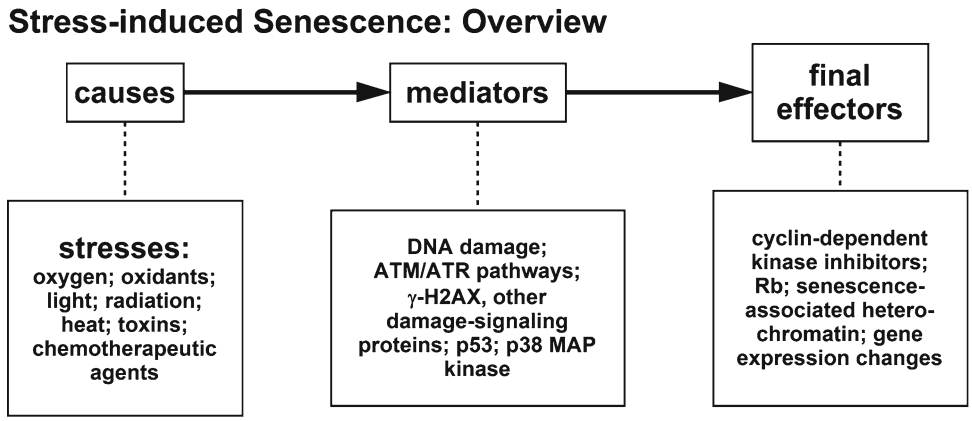


**Fig. 11.1** Causes, mediators and effectors of stress-induced senescence. A wide variety of stresses act as initiators of stress-induced senescence, which thereby acts as a common final state of these various forms of damage. These include, but are not limited to, those listed in the diagram. As discussed in this chapter, the mediators by which stresses activate pathways that lead to a state of irreversible cell division are not yet clear. Many act via initiation of DNA damage. These initial actions activate intracellular pathways, including the p53 and p38 MAP kinase pathways, that produce changes leading to an irreversible state of nondivision. The final status of the cells, involving elevated activity of cyclin-dependent kinase inhibitors and heterochromatin changes, are common to senescence that results from stresses, telomere dysfunction, or oncogene activation

to enter senescence under any circumstances and more likely to undergo apoptosis. Even cancer cells, despite multiple abnormalities, can still be forced into senescence under the action of chemotherapeutic drugs (Acosta and Gil 2012). While the defining characteristic of senescence is complete unresponsiveness to physiological stimuli that cause cell proliferation, reinitiation of cell division in senescent cells is possible under some experimental circumstances, other than exposure to appropriate mitogens (Beausejour et al. 2003). Senescence also involves numerous changes in gene expression, but the process is not a form of terminal differentiation as it is normally understood.

The discovery that senescence could be caused by events other than telomere shortening resulted initially from studies of the overexpression of oncogenic Ras in cultured human cells (Serrano et al. 1997). This was the first of many examples in which expression of the activated form of an oncogene was shown to cause senescence, a phenomenon now termed oncogene-induced senescence (Gorgoulis and Halazonetis 2010). Although the range of stresses that are capable of inducing senescence is large, many of them, but possibly not all, involve some type of DNA damage (Fig. 11.1). Activated oncogenes cause DNA damage via replication stress, including perturbation of replication origin firing, rereplication, and delayed fork progression (Ruzankina et al. 2008; Gorgoulis and Halazonetis 2010; Kuilman et al. 2010).

Frequently the term "stress-induced premature senescence" has been used to refer to senescence resulting from cellular events other than telomere dysfunction (e.g., Passos et al. 2010; de Jesus and Blasco 2012; Mirzayans et al. 2012). It would be more appropriate to refer to the process as "rapid" senescence rather than "premature" senescence. Here, the process is simply called stress-induced senescence. While senescence has many causes, the final senescent state always has common molecular features, whether it results from various stresses, telomere dysfunction, or activated oncogenes. However, it is not yet clear that it is valid to place all nontelomere-based mechanisms together into one class. There may be multiple distinctions among senescent cells that have reached that state via different routes. Features of senescence may also vary considerably dependent on the cell type.

## Senescence in Tissues In Vivo: Animal Models for Stress-Induced Senescence

As an outcome of the effects of stresses/damage to the cell, senescence is one example of an outcome that does not result in perfect repair; in other words, it does not result in the continued existence of a normal healthy cell, either as a cell with continued division potential or a postmitotic cell. One alternative outcome, recovery from the immediate effects of the damage, but with fixation of the damage in the form of a mutation, is evidently an undesirable outcome, because such a mutated cell may be a precursor to cancer. Both senescence and apoptosis, as outcomes of the effect of damage, have the result of removing the cell from potential contribution to future cell generations, thereby acting to prevent tumorigenesis (Kuilman et al. 2010). Nevertheless, apoptosis removes the cell completely, while senescence leaves it in place, unable to divide, but metabolically active and with the potential to disrupt tissue function (Tchkonia et al. 2010). Additionally, senescence of a stem cell is equivalent to a functional loss of the cell. Defects in stem cell proliferation could be of vital importance in determining the properties of old tissues (Kim and Sharpless 2006; Ruzankina et al. 2008; Waterstrat and Van Zant 2009).

Thus, as a terminal state of cells, distinct from apoptosis and differentiation, the selective value of senescence is much less understood than those other cellular processes. Potentially, future research on senescence may provide evidence that senescence actually has advantages over apoptosis as a way to prevent the potential harm done by stresses that damage cells.

Of course, the validity of this discussion is called into question unless senescence occurs in tissues in vivo as well as in cell culture. During the characterization of cellular senescence as a

cell culture phenomenon, its relevance to in vivo biology was often questioned. Over time, it has become clear that senescence occurs in tissues in vivo, and its importance to the general processes of tissue aging has been increasingly recognized. At the same time, the more recent development of animal models in which the mechanisms and significance of senescence can be studied has the potential to greatly advance the field (Tchkonia et al. 2010; Baker et al. 2011; Tilstra et al. 2012).

Tissue aging could result from damage to macromolecules (DNA, RNA, protein), or by the cellular reaction to such damage, but there is no consensus on this topic. Because damage can cause senescence, the senescent cell could be both a consequence of damage and an amplifier of its effects, by disrupting tissue function. Over the past few years, evidence has accumulated that at least some of the molecular changes that are observed in tissues during aging are consistent with the direct or indirect presence of senescent cells. Three important sets of observations are (1) an increase in $p16^{INK4A}$ mRNA in tissues in aging animals (Kim and Sharpless 2006); (2) increased numbers of nuclear DNA damage foci in tissues (Sedelnikova et al. 2004; Herbig et al. 2006); and (3) the probable involvement of intracellular pathways that are well established to be involved in inflammatory processes, including NF-κB (Tchkonia et al. 2010; Tilstra et al. 2012). DNA damage foci are sites of double strand breaks, together with characteristic proteins such as γ-H2AX and 53BP1 (Polo and Jackson 2011), and are at least one potential cause of the increase in p16, as explained further below (Fig. 11.2). Elevated p16 levels may have a variety of effects via altering the activity of Rb and E2F transcription factors, and, as a consequence, the expression of many types of genes (Kim and Sharpless 2006; Takahashi et al. 2006).

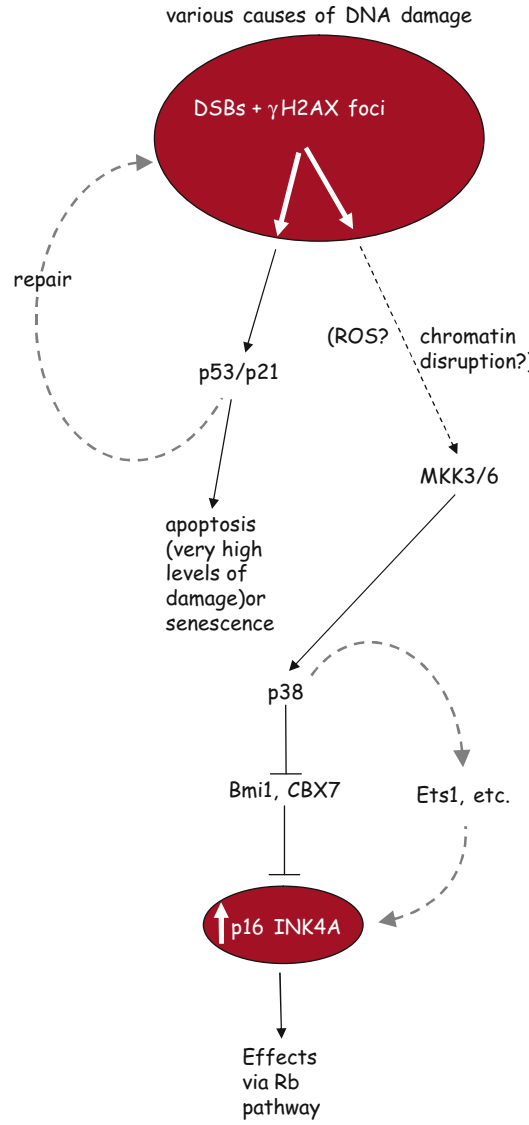


**Fig. 11.2** Hypothesized pathway from DNA damage to p16 in cultured cells and in tissues in animals. Potential indirect mediators from DNA damage to p38 are shown as reactive oxygen species (ROS) arising as an indirect consequence of DNA damage and disruption of chromatin via DNA damage that is an indirect cause of cellular stress. Further downstream the pathway from DNA damage to p16 may be via p38 MAPK and factors that regulate p16, including Bmi1 and Ets1

These are examples of an evolving variety of markers that may be employed to detect and measure senescent cells in tissues (de Jesus and Blasco 2012). As these observations have become more extensive it has been realized that some tissues are preferential sites of the accumulation of senescent cells. In particular, one of the more significant sites of senescent cell accumulation in aging is adipose tissue (Tchkonia et al. 2010). In that site, the secreted products of senescent cells

may have adverse consequences for the overall health, contributing to insulin resistance and type 2 diabetes (Tchkonia et al. 2010). In summary, while an age-related increase in the occurrence of senescent cells is an established phenomenon, its causes are likely multiple and its consequences a topic of active investigation, with few firm conclusions as yet.

## Possible Causes of DNA Damage in Tissues

If cells with DNA damage accumulate as a result of various stresses which the tissues have encountered, it may be further speculated that among the stresses to which cells are exposed in tissues are endogenously generated reactive oxygen species (ROS), widely thought to be one of the causes of aging and a universal hazard against which cells must be protected (Tchkonia et al. 2010; Shao et al. 2011; Tilstra et al. 2012). ROS generation has an established potential to induce DNA damage foci (Hornsby 2010). Specific examples include: ischemia/reperfusion injury, which is thought to be mediated by oxidative damage, increases nuclear γ-H2AX foci in the heart; treatment of animals with agents that act in part via ROS causes the formation of γ-H2AX foci; chemotherapeutic drugs and irradiation of experimental animals produce γ-H2AX foci in many tissues (Hornsby 2010; Rodier et al. 2011).

ROS are among several potential sources of endogenous and environmental damage in tissues, as well as the action of activated oncogenes (Fig. 11.1). Toxins from the environment and possibly even electromagnetic radiation could be of importance. The implications for stress-induced senescence generally are that, while cells in culture may be exposed to stresses that are not normally encountered by cells in living tissues, tissue cells are exposed to sufficient stresses to undergo stress-induced senescence after sufficiently long periods of exposure. Elucidating these mechanisms will be important for understanding both senescence and aging.

## The Critical Role of the Cyclin-Dependent Kinase Inhibitors p21 and p16 in Linking DNA Damage to Stress-Induced Senescence

Although stresses may potentially act via damage to any macromolecules, in many cases DNA damage is the initiating factor; or alternatively, stresses that initially affect some other cell component secondarily cause damage to DNA. A key molecular lesion known to induce senescence is the DNA double strand break (Ruzankina et al. 2008; Polo and Jackson 2011). The induction of senescence by a variety of chemotherapeutic drugs (e.g., bleomycin, adriamycin, mitomycin) provides the most direct evidence that senescence can begin with double strand break damage. Because of the well-understood molecular process by which these agents damage DNA, they form a good model for stress-induced senescence. The senescence-inducing action of short or dysfunctional telomeres is also thought to result from the action of the uncapped telomere as a double strand break (Polo and Jackson 2011; Cao et al. 2011). The response of the cell to such damage is the assembly of large protein complexes around the breaks which stabilize the structure and prepare for potential repair. γ-H2AX and 53BP1 are two proteins present in these foci and have often been used as markers for double strand breaks. They also have been used as indicators of senescence in conjunction with other cellular markers (Sedelnikova et al. 2004; Herbig et al. 2006; de Jesus and Blasco 2012).

DNA damage activates ATM and ATR kinases, thereby initiating a series of signaling events that culminates in the imposition of a cell cycle block via activation of p53 and the cyclin-dependent kinase inhibitor (CDKI) p21 (Ruzankina et al. 2008; de Lange 2010; Polo and Jackson 2011). Early work showed that p21 appeared likely to be at least partially responsible for the nondiving state of senescent cells. These findings led rapidly to studies of whether p21 might be involved in tissue aging in humans and animals. It soon emerged that p21 was generally not elevated in tissues of old animals, but

it did respond in tissues to various kinds of injury, such as ischemia/reperfusion (Hornsby 2010). One of the key issues for both cells in culture and tissues in vivo is the existence of DNA damage foci that represent the site of unrepaired/unrepairable damage, rather than a transient accumulation of proteins that actually results in appropriate repair of the damage (Polo and Jackson 2011). These persistent DNA damage foci (DNA-SCARS; "DNA segments with chromatin alterations reinforcing senescence") are characterized by association with PML nuclear bodies, lack of the DNA repair proteins RPS and RAD51, a lack of DNA synthesis, and accumulation of activated CHK2 and p53 (Rodier et al. 2011). DNA-SCARS were detected in mouse tissues following irradiation. While further study is needed, it appears that DNA-SCARS provide a persistent activation of the DNA repair pathway, which maintains the p53-dependent growth arrest and the gene expression changes characteristic of the senescent state (Rodier et al. 2011).

Following the early discovery of p21, more CDKIs were described, particularly $p16^{INK4A}$, one of two products of the CDKN2A gene (Kim and Sharpless 2006). How p16 is increased as a response to DNA damage is not as well-established as the mode of induction of the p53/p21 pathway, although mechanisms are beginning to be understood (Mirzayans et al. 2012). Agents that cause DNA damage elevate levels of p16; while the p53/p21 pathway is activated as an immediate response to DNA damage, cells that have been senescent for long periods no longer have high p21, but often have high levels of p16 (Takahashi et al. 2006; Mirzayans et al. 2012).

While DNA damage elevates the levels of p16, the molecular pathway is complex (Mirzayans et al. 2012). Some possibilities are indicated in Fig. 11.2 (Hornsby 2010). As most of this work on p16 in the context of senescence has been in cell culture, it is important to consider models in which p16 can be experimentally modulated in tissues in vivo in order to determine the pathophysiologically relevant mechanisms (Kim and Sharpless 2006; Baker et al. 2011) as well as in pathological conditions in human diseases (Lichterfeld et al. 2012).

## Telomere Biology in Relation to Stress-Induced Senescence

An area of continuing research activity concerns the role of telomere biology in senescence in aging tissues. While it is well established that telomere shortening in culture causes senescence (the classical "end-replication problem": Kuilman et al. 2010; de Lange 2010; Cao et al. 2011; de Jesus and Blasco 2012), it is much less certain that senescence in vivo is normally the result of shortened telomeres, or that cells that undergo telomere shortening in vivo actually enter a senescent state. Because human cells have short telomeres, and typically very little telomerase activity, telomere shortening leading to senescence is well established in human cells in culture and therefore may potentially occur in vivo (Herbig et al. 2006). However, it has been difficult to unambiguously link telomere shortening in human tissues in vivo to subsequent cellular senescence. Many cell types undergo progressive telomere shortening as a function of age in humans, but in most cases this has not been linked to a resultant increase in senescent cells (Hornsby 2001). One example in which such an association is stronger is provided by CD8 T cells during chronic HIV infection; the excessive proliferation characteristic of HIV disease is associated with telomere dysfunction and the induction of specific senescence features (Lichterfeld et al. 2012). While a complete dissociation of the phenomena of telomere shortening and senescence in human tissues is unlikely, the occasionally encountered opposite assertion—that replication-based telomere shortening is one of the key causes of aging—is equally improbable.

An early concept was that telomere shortening, caused by the "end-replication problem" in telomerase negative cells, was the main cause of senescence. It is now understood that this is one of many causes of senescence; nevertheless, telomere biology plays a prominent role in many forms of stress-induced senescence even when it does not result from progressive telomere shortening (Kuilman et al. 2010; de Lange 2010; de Jesus and Blasco 2012).

What is the evidence that senescent cells in tissues arise via a stress-induced mechanism versus telomere shortening and dysfunction? Although DNA damage is observed at telomeres in cells as well as at nontelomeric sites (Herbig et al. 2006) this could occur via a greater susceptibility of telomeres to DNA damage from causes such as oxidative stress (Passos et al. 2010; de Lange 2010; Hewitt et al. 2012; Fumagalli et al. 2012) rather than shortening as a result of excessive cell proliferation. Although there is little direct evidence, it is significant that dermal fibroblasts, cells in which senescent features are observed, generally have a low rate of cell division in situ; labeling of human skin shows epidermal cells in the cell cycle but typically almost none in the dermis. While there is a lack of direct experimental data, a working hypothesis is that the accumulation of senescent cells and accompanying biochemical changes, such as elevated levels of p16, are the result of stress-induced senescence rather than excessive cell proliferation. Nevertheless, damage may be particularly localized to telomeres, even if this is not related to the effects of continued cell division.

In the mouse, the much longer telomeres (in comparison to humans), combined with higher levels of telomerase in mouse tissues, would seem to make progressive telomere shortening an unlikely cause of senescence in this species. Nevertheless, more recent data show consistent telomere shortening and an increase in the percentage of short telomeres in aging mouse tissues (de Jesus and Blasco 2012). However, because mouse cells do not normally undergo telomere shortening in culture, and therefore do not typically exhibit telomere-based senescence in culture (Hornsby 2003), it seems unlikely that short telomeres in cells in tissues in vivo are the result of progressive, cell division-based telomere shortening. In contrast, telomere shortening does occur in mice as a function of age, even though telomere length varies with strain, and mice generally have much longer telomeres than humans (de Jesus and Blasco 2012). Mouse tissues show age-related senescence changes (Sedelnikova et al. 2004; Baker et al. 2011; Kosar et al. 2011; Tilstra et al. 2012).

Thus, the relationship between telomeres and senescence in tissues is more complex than originally envisioned. While telomerase (hTERT) maintains telomeres and so prevents senescence via telomere shortening, the enzyme has additional effects on senescence beyond its role in telomere maintenance. A variety of extratelomeric effects of hTERT have been reported; such actions include the prevention of apoptosis in neurons, prevention of oxidative damage in many cell types, enhancement of genomic stability by upregulating DNA repair capacity, upregulation of growth-promoting genes, and downregulating genes that promote apoptosis such as TRAIL (reviewed in Chung et al. 2005). In tumorigenicity studies, hTERT allowed Ras/SV40 TAg-expressing human fibroblasts to grow when implanted as a cell suspension under the skin in immunodeficient mice, while they do not need hTERT in the same location when implanted in collagen gel (Hornsby 2010). Moreover, $ALT^+$ cells (alternative lengthening of telomeres), which are telomerase-negative, need hTERT for growth when implanted under the skin, but not when implanted with better microenvironmental support, i.e., in collagen gel or by subrenal capsule transplantation; moreover, in an unusual animal model of cancer resistance, the naked mole-rat, hTERT overcame the resistance of the cells of this mammal to neoplastic transformation by Ras/SV40 TAg (Liang et al. 2010).

While most of these published studies have added a variety of phenomena to the extratelomeric effects of hTERT, recent data strongly indicate that TERT in both mouse and human tissues stimulates cell proliferation via Wnt signaling (Choi et al. 2008; Shkreli et al. 2012). Conditional telomerase induction causes proliferation of hair follicle stem cells in the mouse. The reverse transcriptase catalytic activity of TERT is not needed for the activation of proliferation of hair follicle stem cells by mTERT, thus clearly demonstrating that this cannot be an effect via classical telomerase activity. These experiments also defined the intracellular pathways that are the main targets of TERT; mTERT stimulation of stem cell proliferation was shown to depending on the activation of the Wnt pathway via β-catenin (Choi et al. 2008).

Evidence that HIV-associated nephropathy in both human and mouse kidneys is accompanied by increased expression of TERT and activation of Wnt signaling indicates that these mechanisms may not require ectopic hTERT expression and may reflect a normal link of TERT with cell division control (Shkreli et al. 2012).

## Chromatin Changes in Stress-Induced Senescence

Senescence is a permanent state of nondivision (in response to mitogens that would normally cause cell proliferation) but the reasons for its irreversibility have only partially been elucidated. As with telomere-based senescence and senescence caused by oncogenes, stress-induced senescence involves both CDKIs and changes in chromatin structure (Kuilman et al. 2010). Foci of senescence-associated heterochromatin (SAHF) were first described as a downstream consequence of high-intensity Ras signaling and telomere dysfunction (Serrano et al. 1997). More recently it has been suggested that the formation of SAHF follows the expression of p16 in senescent cells and is dispensable for senescence (Kosar et al. 2011; Jeanblanc et al. 2012).

These chromatin changes in senescent cells are also accompanied by a wide range of changes in gene expression, called the "senescence associated secretory phenotype," SASP, or the "senescence messaging secretome," SMS (Fumagalli and d'Adda di Fagagna 2009; Kuilman et al. 2010). These changes, resembling the acute inflammatory response of fibroblasts in tissues reacting to tissue injury, are very similar in cells that have reached senescence via telomere shortening and those that have undergone stress-induced senescence. In particular, the overexpression of matrix metalloproteinases (MMPs) was recognized in early studies. The SASP may have various adverse effects on tissues, and may be responsible for the deleterious consequences of the presence of senescent cells (Tchkonia et al. 2010). Experimental evidence for the tumor-promoting effects of senescent cells, via MMPs and similar factors, has been obtained in xenograft models in immunodeficient mice. Fibroblasts with senescence induced by bleomycin treatment (stress-induced senescence) behaved in a very similar manner to those made senescent by telomere shortening (Hornsby 2010).

## Role of Stress-Induced Kinase, p38 Map Kinase

During the investigation of intracellular pathways that can drive cells into senescence in culture, it became apparent that one common mechanism that may be widely involved is the activation of p38 mitogen-activated protein kinase (MAP kinase; MAPK14). Activation of p38 MAPK is a general reaction to many cellular stresses, including DNA damage and telomere dysfunction; however, this does not require critical shortening of telomeres, thus playing a role in stress-induced senescence (Passos et al. 2010; Gorgoulis and Halazonetis 2010; Shao et al. 2011; Jeanblanc et al. 2012). Agents that cause senescence, such as hydrogen peroxide, activate p38 (Hornsby 2010; Barascu et al. 2012). p38 is attractive as a candidate mediator of stress-induced senescence, because, as a stress-activated kinase, it is responsive to those forms of damage that cause stress-induced senescence, and it is in a pathway that may lead to elevated levels of p16 (Hornsby 2010; Passos et al. 2010; Kim et al. 2011; Kuilman et al. 2010; Shao et al. 2011; Mirzayans et al. 2012; Jeanblanc et al. 2012) (Fig. 11.2).

Some evidence for the role of p38 has come from the use of pharmacological inhibitors of this kinase. Two p38 kinase inhibitors developed by SmithKline Beecham in the early 1990s, SB203580 and SB202190, have been widely used and are highly selective. In some cell culture models, p38 inhibitors oppose senescence (Passos et al. 2010). One interesting example of this is provided by cells from patients with Werner syndrome, a syndrome with features of premature aging. Cultured cells from Werner patients stop dividing prematurely when telomeres are still relatively long when compared to normal fibroblasts. The lack of the Werner RecQ helicase may cause a greater level of replication stress in

cells. Growth of Werner cells in a p38 inhibitor prevents the early telomere-based senescence that is characteristic of this syndrome (reviewed in Hornsby 2010).

While there are other potential candidates for intermediate cellular pathways between stresses and effectors of the permanent senescent state, p38 provides an example of one that fulfills the criteria of appropriate action and appropriate downstream targets. Future experiments in cells in tissues may provide more definitive evidence for the key role of this pathway.

## Generation of ROS as a Consequence of DNA Damage

If p38 MAP kinase is a significant mediator of stress-induced senescence then it would be expected that it can be activated by DNA damage or other changes at the chromosomal level. A major potential mediator of the activation of p38 is ROS, generated as result of DNA damage. Although it is well-established that ROS can damage DNA, there is also evidence that DNA damage in itself increases the level of ROS in cells (Hornsby 2010; Mirzayans et al. 2012). Transfection of DNA fragments increases the levels of ROS; human fibroblasts have higher levels of ROS as they approach complete senescence, i.e., during a period when telomeres are shortening to the stage where they function as double strand breaks; inhibition of ROS damage by antioxidants slow the progression of cells to senescence, suggesting that although telomere dysfunction causes cessation of cell division, the indirect effect of telomere dysfunction elevated ROS production has an additional effect on cell proliferation. In some cases antioxidants can prevent both p53-mediated senescence and the activation of p38 MAP kinase (Jung et al. 2004; Barascu et al. 2012). These results are consistent with a model in which DNA damage, including telomere dysfunction, affects two pathways: first, ATM/p53/p21, resulting in a cell cycle block; and second, ROS/p38/p16, also resulting in a cell cycle block.

A key finding is that the generation of ROS downstream of DNA damage in turn acts to create more DNA damage and thereby forms a positive feedback loop (Passos et al. 2010). The essential feature of the loop is that long-term activation of p21 induces mitochondrial dysfunction and ROS generation, via the intermediacy of p38 activation. ROS replenish short-lived DNA damage foci and maintain an ongoing DNA damage response. This loop appears to be both necessary and sufficient for the stability of growth arrest in senescence (Passos et al. 2010). Moreover, DNA damage at telomeres is linked to mitochondrial dysfunction and ROS generation (Sahin and DePinho 2012).

Further evidence is provided by mice with disruption of the ATM gene. They exhibit higher ROS levels and defects in stem cell proliferation in these mice can be corrected by antioxidant administration (Ruzankina et al. 2008; Hornsby 2010). $ATM^{-/-}$ mice have continuously-present unrepaired double strand breaks and increased ROS generation. p38 MAP kinase is activated and pharmacological inhibition of p38 can normalize the defects in stem cell proliferation (Kim et al. 2011). A key mediator of the effects of ROS in ATM-deficient cells is laminin B1, which accumulates via activation of p38 (Barascu et al. 2012). Lamin B1 accumulation causes nuclear shape alterations in stress-induced senescence; lamin B1 overexpression is sufficient to induce nuclear shape alterations and senescence in wild-type cells, while normalizing lamin B1 levels in ATM-deficient cells reduces both nuclear shape alterations and senescence (Barascu et al. 2012).

Other research suggests more direct links between ROS generation and activation of p16/induction of senescence. A mouse model has been generated in which luciferase has been knocked into the INK4A locus, thus providing a real-time readout of the transcriptional activity of the INK4A locus (Ohtani et al. 2010). The authors confirmed a transcriptional increase in p16 as a function of age. They proposed that accumulation of DNA damage activates ROS production, which is linked to increases in $p16^{INK4A}$ mRNA via a block of expression of the

DNA methyltransferase DNMT1 and consequent derepression of the INK4A locus by alterations in the methylation status of the promoter (Ohtani et al. 2010).

Thus, one general model for stress-induced senescence is that DNA damage causes increased ROS, which act as a general stress in the cell, activating the p38 pathway and increasing levels of p16. ROS generation is well-established to activate p38 (Hornsby 2010; Barascu et al. 2012). The facts that antioxidants, which reduce the effects of ROS, can improve cell division and decrease p38 activation in the presence of DNA damage suggests that it may be possible to use antioxidants to intervene in the age-dependent generation of senescent cells in tissues; tests of this hypothesis will likely be a major focus for future research.

## Disruption of Chromatin Structure as a Potential Mediator of Stress-Induced Senescence

A second possible pathway for the activation of p38 by DNA damage is via disruption of chromatin structure. Large-scale changes in chromatin structure could provide a form of stress that is transmitted to p38 MAP kinase. Some tentative evidence is provided by the fact that increased histone acetylation, causing gross changes in chromatin structure, activates p38 . Histone deacetylase inhibitors increase γ-H2AX foci. However, DNA double strand breaks, if unrepairable or unrepaired, might lead to chromatin disruption or reorganization and act as a stress, activating p38 (Fig. 11.2) (Hornsby 2010).

## Summary

Stress-induced senescence is a very broad concept encompassing a variety of stresses and sources of damage to cells. These stresses act via intracellular pathways, which may be multiple, to a final common state of irreversible cell division. In this state the cell is held in the nondividing state by the combination of CDKI activity, heterochromatin formation, and gene expression changes. Here, a case is made that, although the mediators of stress-induced senescence may be multiple, the p38 MAP kinase pathway stands out as potentially the most important. However, both the means by which it may become activated by stresses and the means by which it acts to cause senescence are both as yet unclear. Future studies should focus on appropriate in vivo models in which stress-induced senescence can be studied under pathophysiologically relevant conditions.

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