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MINIREVIEW

Apoptosis, necrosis and cellular senescence: chaperone occupancy as a potential switch

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Summary

Chaperone function plays a key role in repairing proteotoxic damage and in the maintenance of cell survival. Here we compare the regulatory role of molecular chaperones (heat shock proteins, stress proteins) in cellular senescence, apoptosis and necrosis. We also review the current data on chaperone level and function in aging cells, and list some possible therapeutic interventions. Finally, we postulate a hypothesis, that increasing chaperone occupancy might be an important event which forces cells out of the normal cell cycle towards senescence. In the case of severe stress, this may lead to apoptosis or, following lethal stress, to cell necrosis.
Key words: apoptosis; chaperones; heat shock proteins; necrosis; senescence; stress proteins.

Introduction: molecular chaperones

Chaperones are ubiquitous, highly conserved proteins, and are key elements of the maintenance of the conformational homeostasis of proteins in our cells (Hartl, 1996; Bukau & Horwich, 1998). Classes of major chaperones are listed in Table 1. Chaperones either assist in the folding of newly synthesized or damaged proteins in an ATP-dependent, active process, or work in an ATP-independent, passive mode, sequestering damaged proteins for future refolding or proteasome-mediated degradation. Chaperones participate in signalling, protein traffic and many more cellular functions, and therefore they are vital for our cells during their whole lifetime. However, the demand increases after environmental stress, leading to proteotoxic damage. In stressed cells ATP levels drop significantly, and thus several chaperones may become ATP-independent 'holders' of damaged proteins, preventing their fatal aggregation. After the cell has been recovered, and the ATP level is increased, chaperones, which have a rather low affinity for ATP, regain their ATP-dependent mode, and are converted to 'folders' helping in the refolding, transport and/or ATP-dependent degradation of sequestered, damaged proteins.

Various targets of molecular chaperones include (1) newly synthesized proteins, (2) 'constantly damaged' (mutant) proteins and (3) newly damaged proteins. Chaperones also bind to numerous other proteins, such as other chaperones, co-chaperones, constituents of the cytoskeleton, etc. These targets and other chaperone-associated proteins might easily compete with each other (Csermely, 2001a,b). Chaperones may neutralize the conformational consequences of several mutations, and therefore 'buffer' their potential phenotypical changes and make them phenotypically silent (Rutherford & Lindquist, 1998; Roberts & Feder, 1999; Fares *et al*., 2002; Queitsch *et al*., 2002). An increased amount of damaged proteins may cause the phenotype of these mutations to re-appear by a competition for the chaperone-buffer. As we will discuss later, this phenomenon may occur in aging organisms and in cultured cells (Csermely, 2001b). Chaperone occupancy emerges as an integrator of cellular, organismal and populational responses.

Senescence, apoptosis and necrosis

Several cell types, such as human diploid fibroblasts, endothelial cells, T lymphocytes, epidermal keratinocytes, adrenocortical cells, smooth muscle cells, glial cells, lens epithelial cells and human pancreatic β-cells, exhibit only a limited number of replications in cell culture. Morphological and functional properties change until the cell reaches a non-dividing – senescent – state (Hayflick & Moorhead, 1961; Hayflick, 1965; Smith & Pereira-Smith, 1996). Continued proliferation of human cell cultures beyond the 'Hayflick limit' (e.g. by inactivation of the p53 and retinoblastoma growth inhibitory pathways) results in critical telomere erosion culminating in a period of massive cell death termed 'cellular crisis' (Wright & Shay, 1992). It was thought that the replicative capacity of senescent cells decreases with the age of the donor. However, later studies, including that of Cristofalo *et al*. (1998), found no significant correlation between the proliferative potential of various cell lines and the age of donors (Rubin, 2002).

However, cellular senescence is not a general phenomenon: certain rodent stem cells, human astrocytes, rodent glial cells and rodent oligodendrocyte precursor cells seem to have an indefinite lifespan in cell culture, while maintaining a normal phenotype (Rubin, 2002). Ramirez *et al*. (2001) reported that inadequate cell growth conditions may also lead to premature

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Table 1 Major classes of molecular chaperones

Most important eukaryotic representatives[a]	Recent reviews
Hsp25[b], Hsp27, crystallins, small heat shock proteins	Arrigo (2001); VanMontfort *et al*. (2002)
Hsp60, chaperonins	Bukau & Horwich (1998); Hartl (1996); Thirumalai & Lorimer (2001)
Hsp70, Hsc70, Grp78	Bukau & Horwich (1998); Hartl (1996); Mayer *et al*. (2002)
Hsp90, Grp94	Csermely *et al*. (1998); Pearl & Prodromou (2002); Pratt & Toft (2002); Richter & Buchner (2001); Young *et al*. (2001)
Hsp104	Porankiewicz *et al*. (1999)

[a]Neither the co-chaperones (chaperones which help the function of other chaperones listed), nor the so-called folding catalysts, the peptidyl-prolyl isomerases (immunophilins) and protein disulphide isomerases were included in this table, albeit almost all of these proteins also possess a 'traditional' chaperone activity in their own right. Several chaperones of the endoplasmic reticulum (e.g. calreticulin, calnexin, etc.), which do not belong to any of the major chaperone families, as well as some heat shock proteins (e.g. ubiquitin), which do not possess chaperone activity were also not mentioned.
[b]The abbreviations 'Hsp' and 'Grp' refer to heat shock proteins, and glucose-regulated proteins, chaperones induced by heat shock or glucose deprivation, respectively. Numbers refer to their molecular weight in kDa.

growth arrest. These findings emphasized the importance of the selection process of primary cells during biopsy and initial culturing, and raised the possibility that the 'stress history' of primary cells both in the donor organism and during biopsy/culture may also significantly influence the replicative lifespan of cultured primary cells. Premature senescence can be induced by a large number of stressors, e.g. increased oxygen, hydrogen peroxide, *tert*-butylhydroperoxide, γ-radiation, UV light, DNA strand breaks and pro-inflammatory cytokines (e.g. TNF-α or IL-1; Toussaint *et al.*, 2002), if the overall stress level is moderate. As an additional example, UV-B or oxidative stress induced replicative senescence of normal and telomerase-immortalized human foreskin fibroblasts (DeMagalhaes *et al*., 2002). Severe stress, however, causes cell death.

Cells typically die either by apoptosis or necrosis. These two forms of cell death are probably much closer to each other than previously thought (Proskuryakov *et al*., 2002). Both necrosis (where the cell membrane is ruptured, and the released cell content causes a massive inflammatory response) and apoptosis (where the cell content remains 'well-packed' in the apoptotic bodies, and inflammation does not occur) can be (1) caused by the same pathophysiological exposures, (2) prevented by anti-apoptotic mechanisms and (3) transformed from one form to the other by chemical interventions.

Senescent cells can stay viable at this state for several years with regular renewal of the medium (Bree *et al*., 2002). Senescent fibroblasts are resistant to programmed cell death (Wang, 1995), are unable to undergo p53-dependent apoptosis, and are shifted to necrosis upon DNA damage (Seluanov *et al*., 2001). However, apoptosis-resistance is not a general feature of senescent cells, which may also be apoptosis prone depending on the cell type and apoptotic stimuli, e.g. porcine pulmonary artery endothelial cells show an enhanced apoptosis when cultured for a prolonged period of time (Zhang *et al*., 2002). Senescent fibroblasts promote carcinogenesis of neighbouring cells by secreting tumorigenic factors (Krtolica *et al*., 2001). Therefore, the accumulation of senescent cells may contribute to the age-dependent dramatic increase of cancer incidences. Before elucidating the role of chaperones in cellular senescence, here we give a brief survey of the most important aspects of their multiple roles in apoptotic and necrotic processes.

Role of chaperones in apoptosis and necrosis

In agreement with the cytoprotective role of molecular chaperones it has been shown that they generally inhibit apoptosis (Samali & Orrenius, 1998). As an example, overexpression of Hsp70 in mice led to leukaemia due to a massive decrease of T-cell apoptosis in the thymus (Seo *et al*., 1996). Hsp70 and many other heat shock proteins can overcome both caspase-dependent and caspase-independent apoptotic stimuli and confer immortality in various human cell types (Nylandsted *et al*., 2000; Verbeke *et al*., 2001b). Hsp70 inhibits stress kinases, inducing a block in Bid activation and in all the consecutive steps of cytochrome c release, caspase-3 activation and poly ADP-ribose polymerase cleavage in human cell lines (Gabai *et al*., 1998, 2002). As a general anti-apoptotic effect, small heat shock proteins and Hsp70 protect against oxidative stimuli and thus block an important initiation factor of apoptotic processes (Su *et al*., 1999; Arrigo, 2001). Besides this, small Hsp-s also prevent cytochrome c release in Jurkat cells (Samali *et al*., 2001) and the activation of caspases in U937 cells (Garrido *et al*., 1999). As an additional mechanism, both Hsp70 and Hsp90 block the formation and activation of the Apaf-1 complex and, consequently, the activation of caspase-9 (Bree *et al*., 2002). Hsp90 is also a chaperone for PDK1 and Akt, the downstream members of the PI3K–PDK–Akt anti-apoptotic pathway (Sato *et al*., 2000; Fujita *et al*., 2002), and helps in the overexpression of the anti-apoptotic protein, Bcl-2 (Dias *et al*., 2002) in various human cell lines.

On the other hand, there are examples for the positive involvement of stress proteins in apoptotic signalling (Punyiczki & Fésüs, 1998). Hsp90 and its 75-kDa homologue Hsp75 participate in the signalling of tumour necrosis factor in human and rat cells (TNF-α; Song *et al.*, 1995; Galea-Lauri *et al*., 1996). The capacity of stress proteins may be exhausted due to a robust stress response resulting in protein misfolding and aggregation. Chaperone overload initiates either cell cycle arrest or apoptosis by two mechanisms: by proteasomal inhibition (which blocks cyclin degradation; Bence *et al*., 2001), and by induction of the JNK-dependent pathway suppressing the JNK-inhibitor, Hsp70 (Gabai *et al*., 1998, 2002) in human cell lines. Hsp90 inhibition also results in exaggerated apoptosis of TNFα-treated human cells (Lewis *et al*., 2000). Cytoplasmic translocation of mitochondrial Hsp60 is one of the pro-apoptotic signals, which (together with that of cytochrome c) promotes the activation of cytoplasmic caspases, when mitochondrial integrity becomes compromised in Jurkat T lymphocytes (Samali *et al*., 1999; Xanthoudakis *et al*.,

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1999). Several peptidyl-prolyl *cis-trans* isomerase cyclophilins, which are also apoptosis-activated nucleases, are potential direct elements in the apoptotic machinery in HeLa cells (Montague *et al*., 1997). One of the major endoplasmic reticulum chaperones, calreticulin, promotes both Ca-dependent apoptosis and necrosis pathways in several *C. elegans,* rat and human model systems (Nakamura *et al*., 2000; Xu *et al*., 2001; Kageyama *et al*., 2002). However, calreticulin protects neuroblastoma and renal epithelial cells from apoptosis upon cell differentiation as well as iodoacetamide treatment, respectively (Liu *et al*., 1997; Johnson *et al*., 1998).

In contrast to the necrosis-promoting effect of calreticulin in *C. elegans* (Xu *et al*., 2001), heat shock proteins generally protect cells against necrosis. In the absence of Hsp27 and Hsp70 the lethal stress increases ceramide generation, which induces cell necrosis in human fibroblasts (Verbeke *et al*., 2001b). In addition, Hsp70 helps to convert the ATP-depletion-induced cell necrosis to apoptosis (Vayssier & Polla, 1998).

During cell necrosis, cells release various chaperones, such as calreticulin, Hsp10, Hsp70, Hsp90, etc., which serve as a 'danger signal' (Basu *et al*., 2000). Extracellular chaperones provoke inflammation and induce the necrosis of nearby endangered human cells (Proskuryakov *et al*., 2002).

Involvement of chaperone function in cellular senescence

Several chaperones have a direct effect on cellular senescence (Table 2). Overexpression of Hsp27 in bovine arterial endothelial cells leads to an accelerated growth and senescence (Piotrowicz *et al*., 1995). Bag-1, a co-chaperone of Hsp70, slows down cell proliferation by impaired activation of the necessary Raf kinase, when Hsp70 levels are high enough, i.e. after stress (Song *et al*., 2001). When a senescence-promoting factor, mot-1, was isolated from cytoplasmic extracts of senescing (mortal) fibroblasts, it turned out to be a member of the Hsp70 chaperone family. Anti-mortalin antibodies rescued cells from senescence and induced cell proliferation. In contrast, overexpression of mot-2, another member of the mortalin family, in human fibroblasts permitted their escape from senescence (Wadhwa *et al*., 2002).

The previous examples may lead to the conclusion that chaperones promote cellular senescence. However, chaperone induction *per se* may counteract senescence, since repeated mild heat shock (a kind of hormesis) has been reported to delay fibroblast aging (Rattan, 1998), though it does not seem to extend replicative lifespan. Hsp90 is required for the correct function of telomerase, a major enzyme involved in determining the lifespan of cells (Holt *et al*., 1999). Up-regulation of telomerase activity in transformed prostate epithelial cells is completely due to an increased assembly by up-regulated chaperones (Harvey *et al*., 2002). It is reasonable to assume that the proliferative potential of telomerase-positive stem cells may correlate with their available chaperone capacity. Besides telomerase activity, protection of shortened/altered telomeres (Karlseder *et al*., 2002) might be another important element of chaperone-mediated senescence-delay. Interestingly, in contrast to the senescence-promoting effect of extra copies of Hsp27, when a mutant, non-phosphorylated form of Hsp27 was expressed in bovine arterial endothelial cells, cellular senescence was hindered (Piotrowicz *et al*., 1995). Hsp90 also chaperones cyclin-dependent kinases, and when Hsp90 level is diminished, even a mild heat shock induces cell cycle arrest (Nakai & Ishikawa, 2001).

Little is known about the action of chaperones in non-dividing, senescent cells. Apolipoprotein J (clusterin), an extracellular chaperone, has been described as a biomarker of senescence, and is implicated in the prevention/delay of apoptosis (Petropoulou *et al*., 2001; Trougakos & Gonos, 2002). As another example, impaired Hsp90 function leads to the activation of HSF-1, restoration of the heat shock response and slower chronological aging of non-dividing *Saccharomyces cerevisiae* (Harris *et al*., 2001). On the other hand, impaired Hsp70 induction (and probably function) of senescent cells leads to their reduced thermotolerance due to a loss of an Hsp70-mediated inhibitory control of stress kinase signalling (Volloch *et al*., 1998). Increased chaperone occupancy may significantly contribute to decreased p53 stability in senescent cells, causing an enhanced cell necrosis (Seluanov *et al*., 2001). Exhausted chaperones may thus lead to a reversal of the general apoptosis resistance of senescent cells.

Changes of chaperone function in cellular senescence

Like cells from aged animals (Sõti & Csermely, 2002) senescent cells tend to have increased chaperone levels: as one of the

Table 2 Role of molecular chaperones in cellular senescence

Chaperone	Change	References
Increased senescence		
Hsp27	increased senescence of endothelial cells decreased cell proliferation by decreased	Piotrowicz *et al*. (1995)
Bag-1	Raf activation after stress	Song *et al*. (2001)
cytoplasmic mortalin-1 (a Hsp70 homologue)	senescent fibroblast phenotype	Wadhwa *et al*. (2002)
Decreased senescence		
non-phosphorylatable Hsp27	senescence inhibition of endothelial cells	Piotrowicz *et al*. (1995)
mortalin-2 (a Hsp70 homologue)	malignant transformation of NIH 3T3 fibroblasts	Wadhwa *et al*. (2002)
Hsp90 (Hsp70, p23, Hsp40, Hop)	telomerase activation: extended proliferative lifespan	Holt *et al*. (1999); Harvey *et al*. (2002)

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examples, chondrocytes have an elevated Hsp27 level (Pfeuty & Gueride, 2000). Senescing fibroblasts possess higher Hsp70 as well as lower Hsp90 and Hsp27 levels. Repeated mild heat shock partially prevented the decrease in Hsp27 level, while making the changes in Hsp70 and Hsp90 levels more pronounced (Verbeke *et al*., 2001a).

Senescing fibroblasts are defective in the induction of several chaperones, such as Hsp70 and Hsp90 as well as the collagen-specific Hsp47 (Liu *et al*., 1989; Miyaishi *et al*., 1995; Bonelli *et al*., 1999). The exact mechanism of the defective Hsp-activation is not known. As with aged animals (Sõti & Csermely, 2002), a decreased activation and binding of heat shock factor-1 (HSF-1) to the respective DNA-element, HSE was reported in senescing cells (Choi *et al*., 1990). Lu *et al*. (2000) found a decreased trimerization of HSF-1 after hypo-osmotic shock in senescent fibroblasts. However, Bonelli *et al*. (1999) identified mRNA processing right before RNA translocation from the nucleus to the cytoplasm as the defective step of Hsp70 synthesis in senescent human fibroblasts. As another mechanism, the increased amount of damaged proteins in senescent cells may occupy Hsp70 and Hsp90, which may cause the release of HSF-1 from the Hsp70Hsp90HSF-1 complex (Morimoto, 1998). This would explain both the increase in the basal levels of major chaperones (an adaptive response) and also the defect in their further induction (a partially non-responsive state).

Possible therapeutic applications

Induction of the senescent state may be an important step in preventing malignant transformation. However, the elimination of senescent cells might be the ultimate goal in various forms of cancer, since senescent cells themselves play an important role in the initiation of malignant transformation (Wang, 1995; Krtolica *et al*., 2001). Low doses of Hsp90-antagonists inhibit telomerase without affecting the growth rate of tumour cells. Based on this finding it was postulated that inhibition of Hsp90 can provoke senescence by the inhibition of telomerase (Harvey *et al*., 2002). Hence, chaperone inhibition is a promising tool to decrease cytoprotection and to initiate apoptosis or necrosis of senescent cells. There are several drug candidates which inhibit chaperone function (Table 3). All these drug candidates may be used to induce senescence and, later, to eliminate senescent cells in patients.

On the other hand, either improving the function of post-replicative cells may be useful in several other disease states, such as in neurodegenerative diseases (Alzheimer's, Parkinson's, etc.). A class of drugs known as chaperone co-inducers have a well-established cytoprotective role (Vígh *et al*., 1997), and may help to preserve the functional reserve capacity of differentiated/senescent cells.

The protein homeostasis hypothesis of senescence

Senescence runs in parallel with an accumulation of damage at the molecular–cellular level. The attenuation of molecular chaperone inducibility and the simultaneous accumulation of damaged proteins raises the possibility that preservation of protein homeostasis is a major determinant in the occurrence and duration of cellular senescence. This concept leads to our 'protein homeostasis hypothesis of senescence' (Fig. 1), a key element of which is the balance between the amount of damaged (denatured) proteins and the available capacity of molecular chaperones to refold/repair them. When the balance is:

1 in favour of available chaperones (compensated stress): proliferative signals are transduced and cells may be temporarily arrested, but finally they divide normally again;

2 in favour of denatured proteins (stress, senescence): proliferative signals stop, cells become irreversibly arrested and enter into a prolonged senescent phase;

3 strongly in favour of denatured proteins (lethal stress): chaperones become unavailable, stress kinases are activated and proteasome inhibition leads to apoptosis;

4 very strongly in favour of denatured proteins (supralethal stress): complete loss of chaperone availability, ceramide accumulation and ATP depletion leads to necrosis.

Two intriguing consequences arise from the above hypothesis:

A When denatured proteins are just slightly, but permanently higher than the level of available chaperones (case 2, remitting in senescent state), silent mutations may be exposed, contributing to polygenic diseases, such as atherosclerosis, diabetes, cancer, etc. (Csermely, 2001b).

B In agreement with the assumption of Toussaint *et al*. (2002), the above hypothesis raises the possibility that many forms of cellular senescence are induced by the effects of prolonged stress on primary cells, when transferring and maintaining them

Table 3 Drug candidates influencing molecular chaperones

Drug candidate	Major effect	Company and web-site	References
Geldanamycin analogues	Hsp90 inhibition	Conforma Inc. (www.conforma.com)	Neckers (2002)
Geldanamycin-testosterone	Specific Hsp90 inhibition in tumours	Kosan Bioscience (www.kosan.com)	Harvey *et al*. (2002)
Radicicol	Hsp90 inhibition	Kyowa Hakko Kogyo Ltd. (www.kyowa.co.jp)	Soga *et al*. (1998)
Purine-scaffold Hsp90 binders, PU3	Hsp90 inhibition		Chiosis *et al*. (2002)
?	Hsp90 inhibition	RiboTargets Co. (www.ribotargets.com)	
Deoxyspergualine	Hsp70 inhibition	Nippon Kayaku Co. (www.nipponkayaku.co.jp)	
Arimoclomol, Iroxanadine	Chaperone co-induction	Biorex R & D Co. (www.biorex.hu)	Vígh *et al*. (1997)

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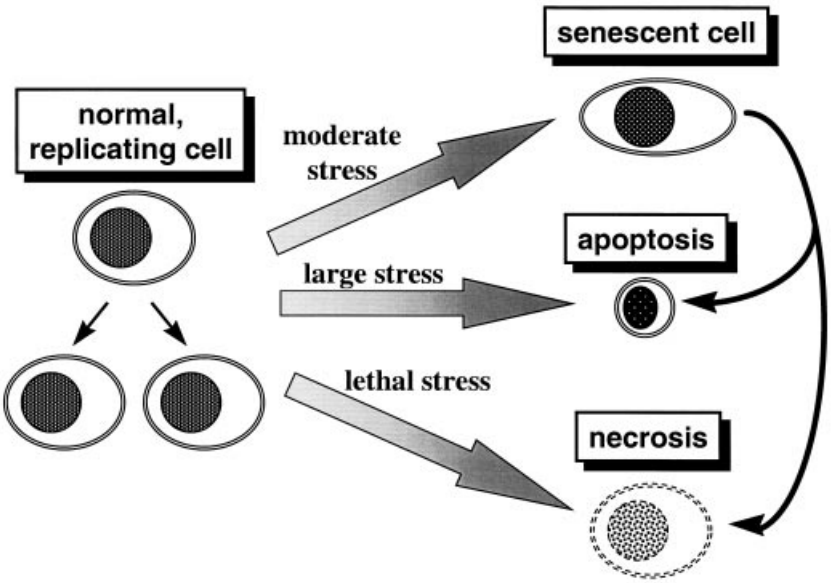

Fig. 1 Hypothetical relationships between chaperone occupancy (chaperone overload), cell proliferation, cellular senescence, apoptosis and necrosis. If there is no chaperone overload, i.e. the amount of damaged proteins does not exceed the amount of available chaperone capacity for a longer time period, cells proliferate normally. Chaperone overload may arise from a constant and large elevation in the amount of damaged proteins and/or a prolonged inefficiency of the cell to produce enough heat shock proteins and other chaperones to repair damaged proteins or a sudden discrepancy between protein damage and chaperone induction (such as a proteotoxic insult combined with the impaired chaperone induction). A modest chaperone overload makes the cells very sensitive to the senescent state. If chaperone overload becomes robust, cells become very sensitive to apoptosis. In the case of a complete, extreme chaperone overload, the possibility of cell necrosis is highly increased.

in culture. In agreement with the above hypothesis, in certain rodent stem cells, human astrocytes, rodent glial cells and rodent oligodendrocyte precursor cells, the use of appropriate culture media allows the cells to escape senescence while remaining untransformed (Rubin, 2002). In light of the above findings (which still have to be extended to more senescence-prone human cells), it is an exciting question to ask whether and how senescent cells exist *in vivo*. Our hypothesis would imply that a low level of persistent environmental stress is an important factor in inducing replicative senescence in living organisms by exhausting their capacity for chaperone-induction.

As a general conclusion, chaperones may not only constitute the most ancient defence mechanism of our cells, but also behave as direct sensors of their functional competence. Various levels of chaperone overload may make an important contribution to the signals directing the cell to senescence, apoptosis or necrosis.

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