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Minireview

Transcription factors in cellular senescence and death

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The main manifestation of cell senescence is their limited ability to proliferate. It is believed that this process is genetically controlled. The end of the proliferation period of cells growing *in vitro* is associated with protooncogene c-*fos* repression and diminished activity of transcription factor AP-1, a dimer composed of Fos and Jun proteins. On the other hand, induction of c-*fos* and other protooncogenes like c-*jun* and c-*myc*, whose protein products act as transcription factors, is observed in cells undergoing apoptosis (programmed death). This would suggest that the proliferative and apoptotic cell programs utilize the same early pathway to regulate the transcription of late-expressed genes responsible for the different biological endpoints, i.e. cell proliferation and cell death. The open question is whether or not senescent cells that cannot proliferate and do not express c-*fos* protooncogene undergo apoptosis.

Cellular senescence as a genetic phenomenon

A fundamental feature of some normal cells of higher eucaryotes is their limited ability to proliferate. In tissue culture at the end of proliferative period, which for normal human fibroblasts is about 50 population doublings, the cells stop dividing, continue to metabolize for a few months, and then die [1]. This process, referred to as replicative senescence, is common to other cell types grown *in vitro* such as lymphocytes, hepatocytes, chondriocytes and keratinocytes [2]. It is believed that senescence *in vitro* reflects the aging process which normally occurs *in vivo*. There is a direct correlation between proliferative capability and maximum lifespan in different species, and an inverse correlation between proliferative capability and donor age [3]. Immortalized and neoplastic cells, in contrast to normal ones, can proliferate in culture indefinitely without senescing.

Two main theories have been proposed to account for cellular senescence. According to the first, cellular senescence is a random process caused by progressive accumulation of damage which in turn is the consequence of errors in synthesis of proteins and other macromolecules. According to the second theory, cellular senescence is an active, genetically controlled phenomenon, similar to cell differentiation [4]. Although there are good reasons to believe that aging is a multifactorial process influenced by genetic and environmental factors (especially oxygen radicals), experimental data are in favour of the genetic hypothesis. It has been shown that the senescent phenotype is dominant over immortal phenotype [5] and that the fusion of different tumour cells results in four complementation groups of hybrids with a limited lifespan [6]. These data suggest that at least four genes are responsible for the immortal phenotype, and that immortalization can be achieved by changes in one of these four genes, which can complement each other to give a finite phenotype. The question is why and how the cells that have finished their proliferation die. Do they become senescent and

die because they cannot proliferate any more, or is the end of proliferation capacity the last step of a senescence program?

Cell death

Two common forms of cell death, necrosis and apoptosis, have been described. Necrosis is cell death caused by sudden injury such as ischemia, hyperthermia, physical or chemical trauma. The plasma membrane seems to be the major site of damage. The cells swell and rupture; their contents are spilled into surrounding tissue space provoking an inflammatory response. Apoptosis (programmed death), which has been described as the opposite to necrosis (accidental death), can be initiated by a number of external signals such as glucocorticoids, irradiation or withdrawal of growth factors. Apoptosis is characterized by several morphological and biochemical events, such as cellular shrinkage, chromatin condensation and marginalisation, DNA breaks and fragmentation, and formation of apoptotic bodies [7, 8, 9]. In several systems apoptosis has been shown to require both DNA and protein synthesis suggesting that this form of cell death is an active process [7]. Indeed, in different cell types undergoing apoptosis there is a requirement for new gene expression for both the morphological changes and death itself to occur. Its very interesting that some of these genes are expressed also in cells stimulated to proliferate [9].

Transcription factors in apoptosis

The regulation of proliferation as well as many other complex biological processes occurs at many different levels; one of the most fundamental processes is regulation of RNA transcription, the first step in the expression of a gene. The frequency of initiation of mRNA synthesis depends on proteins called transcription factors, that interact with specific elements in gene promoters and enhancers [10]. It is believed that all protooncogenes whose protein products reside in the nucleus belong to transcription factors [11]. We assume that some transcription factors that regulate proliferative events play a role in the induction of apoptosis as well. Indeed, genes encoding proteins forming transcription factors such as protooncogenes c-*myc*, c-*fos*, c-*jun* have been proved to be activated in cells undergoing apoptosis. Buttyan *et al.* [12] demonstrated c-*fos* and c-*myc* induction in rat prostate undergoing apoptosis following androgen deprivation caused by castration. Similarly, androgen withdrawal increased the c-*fos* expression in Shiongi mouse mammary carcinoma [13]. Increase of c-*myc* RNA levels has been observed during apoptosis of estrogen-dependent MCF-7 cancer cells following estrogen ablation [14]. Expression of c-*fos* and c-*jun* protooncogenes was rapidly induced after growth factor deprivation in IL-6 and IL-2 dependent mouse lymphoid cells [15]. Antisense oligonucleotides directed against c-*fos* and c-*jun* mRNA consistently reduced the expression of these genes in treated cells and increased the survival of growth factor deprived lymphoid cells [15]. Grassilli *et al.* [16] showed that an early accumulation of c-*fos*, c-*jun* and c-*myc* mRNA occurred in rat thymocytes undergoing apoptosis after glucocorticoid treatment, as well as in thymocytes stimulated to proliferate with concanavalin A. Protooncogenes c-*fos* and c-*jun* encode proteins which form a dimeric transcription factor named AP-1 [17]. Dimer formation is necessary for DNA binding. Fos proteins cannot dimerize with each other and thus cannot independently bind to DNA; only Jun-Jun and Jun-Fos can form dimers. Since heterodimers are more stable than homodimers, this accounts for their much greater DNA-binding affinity. Members of the Jun family can dimerize with each other and with every member of the Fos family, giving 18 different combinations [11]. AP-1 is characterized by its ability to alter gene expression in response to growth factors, cytokines, tumor promoters, carcinogens and increased expression of various oncogenes [17]. Recently, we have shown for the first time that the AP-1 DNA binding activity increased in rat thymocytes stimulated either to proliferate or to undergo apoptosis [18].

The c-*myc* protooncogene is related to apoptosis in two ways: either it is expressed in cells undergoing apoptosis (see above), or its modulation affects the process. Fanidi *et al.* [19] observed an increased rate of apoptotic death in fibroblasts that were transformed with the c-*myc* protooncogene. Chinese hamster ovary cells transfected with c-*myc* under the control of heat shock promoter underwent cell death following heat shock [20]. Moreover, the antisense oligonucleotide corresponding to c-

myc blocked the death of T cell hybridomas [21]. Recently, it has been observed that c-*myc* expression induces apoptosis in rat fibroblasts deprived of growth factors [22]. The c-*myc* protooncogene is usually implicated in cell transformation, differentiation and cell-cycle progression [23]. The c-Myc protein, in a heterodimeric complex with factor Max, binds DNA specific sequences and act as a transcription factor [23]. It is interesting to speculate, that both proliferation and programmed cell death, two apparently opposite processes, are driven by the same c-*myc* protooncogene. It is possible that successful proliferation in normal cells requires c-*myc* and a second survival signal, such as that provided by growth factor and receptor interaction. If such a second signal is absent, the cells are not allowed to progress through the cell cycle and c-*myc* induces apoptosis. In this two signal model, c-*myc* can provide the first signal, driving cells either to apoptosis or to progression, and certain growth factors may provide a second signal, to inhibit apoptosis and allow c-Myc to drive cells into the cell cycle. Thus, c-Myc can promote cell death either by activating genes required for the induction of apoptosis, such as p53, or by repressing genes such as *bcl-2*, whose products suppress the apoptotic process. The wild type of p53 is a tumor suppressor gene which not only arrests cell growth but also induces apoptosis in several cell systems [24 - 26]. p53 Acts as a transcriptional factor, upregulating the expression of some genes and repressing many others [27]. Indeed, Reisman *et al.* [28] have shown recently that the c-Myc/Max heterodimers *in vitro* translated bind directly to the p53 promoter. One can assume that there exists a subtle but very complicated regulation at the level of transcription, driving cells to proliferation or to death. Figure 1 shows a model for the regulation of cellular proliferation, senescence and death by some protooncogenes and tumor suppressor genes encoding for transcriptional regulator.

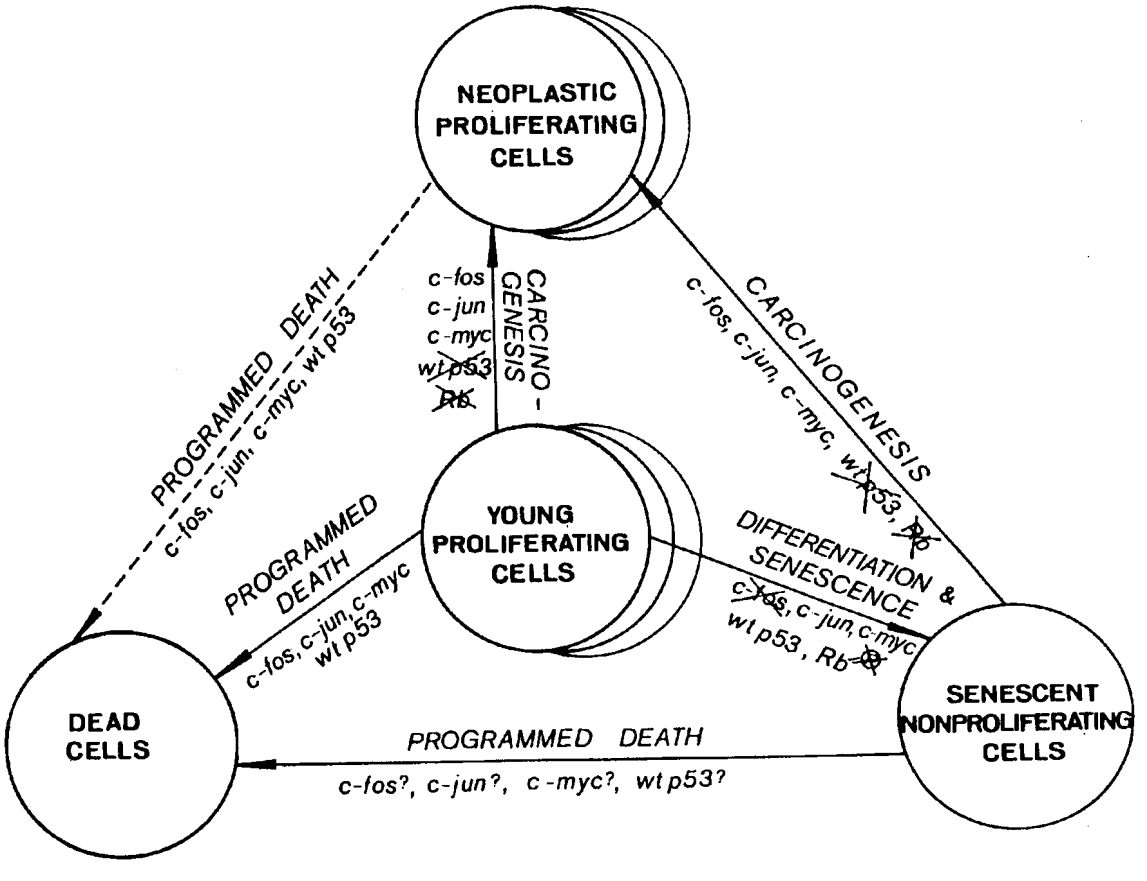

Fig. 1. A model for the regulation of cellular proliferation, senescence and death by some protooncogenes and tumor suppressor genes coding for transcriptional regulators.
The genes which are marked by crossing over are suppressed during the respective processes. P crossed over denotes lack of phosphorylation of the Rb protein.

Recently, several death-associated mRNA's have been isolated from glucocorticoid-treated thymocytes [29]. One of them, RP-8, is probably a transcriptional factor. Its expression is increased in apoptotic cells in a variety of tissues including thymus and brain [30].

Transcription factors in cellular senescence

The regulation of cell proliferation in eucaryotes involves two control points: a point in G1 phase that commits the cell to entry into S phase, and a point in G2 phase that regulates enter into mitosis. Expression of several genes is required for the cell to progress through the G1-S transition and enter DNA synthesis [31]. Senescent human fibroblasts, upon stimulation by serum, cannot enter the S phase, but express many cell cycle-dependent genes activated in young cells including c-*myc* and c-*jun* [31, 32]. However, serum-stimulated senescent fibroblasts fail to express the c-*fos* protooncogene [32]. We have shown that, like human fibroblasts stimulated to proliferate, mouse splenocytes derived from old animals and induced to growth with concanavalin A had decreased expression of c-*fos* protooncogene [33]. In addition, we observed that there was no detectable AP-1 DNA binding activity in splenocytes derived from old mice, but levels of other transcription factors, AP-2 and AP-3, were unchanged [33]. Diminished AP-1 DNA binding activity was also observed in lymphocytes derived from individuals with Down syndrome [34]. People with Down syndrome show some signs of premature aging; a major manifestation includes alteration of the immune system similar to that in an aged person. The decreased AP-1 DNA binding activity was correlated with a diminished capability of the lymphocytes to proliferate. Riabowol *et al.* [35] demonstrated directly that AP-1 activity is required for human cells to proliferate in response to serum. They also found that activity of the AP-1 complex was reduced in old human fibroblasts prior to their entering a fully senescent state. Moreover, the composition of the AP-1 complex was changed, so that old cells produced predominantly Jun-Jun homodimers instead of Fos-Jun heterodimers [35, 36]. Our preliminary data suggest as well the possibility of formation of AP-1 composed from Jun-Jun proteins in lymphocytes derived from Down syndrome patients. The AP-1 complex has much lower affinity to DNA than the heterodimer Fos-Jun [37]. These data suggest that changes in AP-1 activity may contribute to the inability of senescent cells to proliferate in response to mitogens. It has been shown recently, however, that induction of c-*fos* expression and AP-1 activity in senescent human fibroblasts by microinjection of oncogenic c-*Ha-ras* protein was not sufficient for DNA synthesis [38]. Similarly, Philips *et al.* [39] showed that populations of senescent human fibroblasts that had completed their full proliferative life span did not respond with DNA synthesis to the transfections of exogenous c-*fos* protooncogene [39]. These results suggest that the senescence cannot be solely attributed to the absence of c-*fos* expression and that the proliferative block in senescent cells involves multiple molecular mechanisms. Another potential mechanism underlying senescence involves the regulation of the retinoblastoma susceptibility gene product Rb. Rb protein is a tumor suppressor gene that functions in a growth-inhibitory manner in normal cells. Traversion of the G1/S cell cycle boundary, allowing DNA synthesis to occur, is dependent upon the hyperphosphorylation of the Rb protein, which in senescent cells does not occur [40]. Rb is not a transcription factor by itself, but it acts in concert with c-Myc [41] or E2F proteins [42] which are able to bind to DNA. Although so far no one has been able to describe the exact mechanism of the cell proliferation block in senescent cells, the role of transcription factors in the process is unquestionable.

Death of senescent cells

The inhibition of c-*fos* expression in senescent cells, which have a diminished capability to divide, has been proved in many laboratories [32 - 36]. The induction of this protooncogene in cells undergoing apoptosis has been shown in many systems [12 - 16]. Does this mean that senescent cells as unable for c-*fos* induction by mitogens cannot die by apoptosis? This possibility can not be excluded, especially in that the capability for undergoing apoptosis seems to be a feature of the cells able to proliferate. On the other hand, even in senescent cells, c-*fos* can be induced by oncogenic proteins [38]. Perhaps the signal for apoptosis in senescent cells is dependent on c-*fos* as well. Unfortunately, the data concerning the death of senescent cells are

very scarce. To our best knowledge only Bayreuther *et al.* [43] showed that senescent fibroblasts are able to express c-*fos* and that they are dying by apoptosis [43]. Alternatively, it can be supposed that the mechanism of induction of apoptosis is different in proliferating and non-proliferating, terminally differentiated cells. In the latter, the main role in driving cells to apoptosis could be played by the c-*myc* protooncogene, which is known to be expressed in senescent fibroblasts. One can imagine that, in senescent cells induction of c-*myc* is able only to drive cells to die, not to proliferate. Wild type p53 also seems to be a good candidate for a death inducer in senescent cells. Analysis of p53 in haemopoietic cells showed elevated levels of p53 in the normal population of nonproliferating mature lymphoid granulocytic and monocytic cells compared to their proliferating precursors [44]. Additionally, wild type p53 functions only in a growth-inhibitory manner, never inducing the proliferative response.

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