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Foreword

The reviews and research reports in this issue of *Biological Signals* deal with changes in transcription factors in mammalian cells that have undergone increasing numbers of population doublings. It has been known for some time that as mammalian fibroblasts age, whether in the intact organism or in cell culture, they exhibit a progressive loss of proliferative potential (senescence) accompanied by diverse changes in gene expression. To what extent this is relevant to, or explains, aging of the mature multicellular mammalian organism is an intriguing question. That it does have some bearing is attested to by the fact that a number of genetic diseases that result in premature aging (e.g. progeria, Werner's syndrome) yield fibroblasts with a reduced capacity, relative to normal controls, for in vitro proliferation.

Although gene expression is controlled at various levels, most of the focus of recent research has been at the transcriptional level since this is considered to represent the most critical phase of gene expression. The identification of transcription factors involved in the regulation of 'age-dependent' genes and clarification of their mode of regulation and mechanisms of action will help elucidate the universal cellular decline of biological aging and an important obverse manifestation, the rare escape of cells from senescence leading to immortalization and oncogenesis. As the cells become senescent they cease dividing and enter a 'crisis' stage, where they may remain for a considerable period of time under appropriate conditions. Rodent fibroblasts at this stage will, if passaged appropriately, frequently undergo a process of spontaneous immortalization. Human and avian cells, in contrast, rarely if ever immortalize spontaneously.

Meyyappan et al. have summarized part of what is known about how expression of various genes, including those encoding transcription factors, changes with cell age; expression of individual genes assessed at the mRNA or protein (including activity and phosphorylation status) level may increase, decrease or remain constant. They have also discussed in detail their discovery that the serum response factor is unable to bind to the serum response element in the c-*fos* promoter, and other promoters, because it is hyperphosphorylated in senescent human diploid fibroblasts. This observation appears to account for the reduction in c-*fos* expression in senescent cells and it suggests that substantial changes in signalling mechanisms mediated by protein phosphorylation may occur with replicative aging.

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Wynford-Thomas et al. have presented a perceptive analysis of the role of p53, a transcription factor, as a tumor suppressor and regulator of the proliferative life span. The essence of their thesis is that p53, which is seen as a 'guardian of the genome' by virtue of its ability to induce growth arrest in response to DNA damage, is activated by telomer erosion (the shortening of the telomers with increasing population doublings/replication cycles) and inducing growth arrest. The current paradigm is that DNA damage causes modification of P53 (phosphorylation?), and that this in turn leads to increased transcription of various genes, including $p21^{WAF1,CIP1,SDI1}$, which is itself an inhibitor of the kinase activity of certain cyclin/cyclin-dependent kinases necessary for progression through G1/S. Telomers shorter than a critical length are perceived as DNA damage, resulting in a cessation of DNA replication and cell proliferation. This is hypothesized to occur at what has been called, in human diploid fibroblasts, the M1 checkpoint; escape from this growth arrest state (senescence) can occur by loss of p53 function. After limited further proliferation the cell reaches the M2 checkpoint and enters a crisis period, a form of cellular homeostasis that ends in the death of a cell. Escape from M2, and permanent immortality, is thought to require loss of Rb function and a restoration of functional telomers. Wynford-Thomas et al. also develop both direct and indirect (anticipatory) models for: 'What drives selection for p53 mutation during clonal progression [in a tumor]'. Finally, they have dicussed the extent to which these conclusions drawn from studies on fibroblasts are relevant to epithelial cells, from which most human cancers arise.

Dimri et al. discuss the causes of the reduction in activity of three transcription factors (AP-1, Id, and E2F) that occur in human diploid fibroblasts with increasing replicative age in culture. They also review what is known about the role of QM (also known as Jif for Jun-interacting factor). The decline in mitogen-inducible (but not DNA damage-inducible) AP-1 activity appears to result primarily from the reduced transcription of c-*fos*, as discussed above. QM/JIF, whose expression changes little with replicative age, can suppress cell proliferation when Jun levels fall below a threshold. It is of interest that c-*fos* remains serum-inducible in senescent Werner syndrome cells, in contrast to its loss of inducibility in normal cells. The helix-loop-helix Id proteins negatively regulate basic HLH transcription factors and are essential for a proliferative response to mitogens. Recent data suggest that Id-1, which is not expressed in senescent cells, may be capable of suppressing the activity of a growth inhibitory bHLH protein. E2F-1, which stimulates transcription of various genes that are necessary for the proliferative response and is inhibited by pRB (phosphorylated retinoblastoma protein), is not expressed in senescent cells for unknown reasons. E2F-4 and E2F-5 are also expressed at lower levels in senescent cells.

Good and Chen present a detailed study of proteins able to bind to a sequence from the human TK promoter that contains an inverted CCAAT box. There is a clear decrease in the binding activity of CBP/tk (CCAAT-binding protein for thymidine kinase gene) activity with increasing in vitro age of IMR-90 cells. The question whether NF-YA or NF-YB were the proteins responsible for CBP/tk activity was investigated. NF-YA and NF-YB protein levels were reduced in senescent cells, relative to young cells, but the absence of a quantitative relationship between the physical abundance of the protein and CBP/tk binding activity suggested that either another (related?) protein was responsible for the CCAAT-binding activity or that the DNA-binding activity of the NF-Y proteins was regulated independently of the protein level.

The decline with age in the transcription of the gene encoding the androgen receptor (AR), a ligand-activated transcription factor, is the focus of the contribution by Supakar and Roy. They have identified several transcription factors (ADF, Sp1, and SRF) that enhance transcription of the AR gene and exhibit less activity in the liver of old rats as compared to young rats. Concomitantly, there is an increase in the activity of both AP3 binding activity and NFB, a negative regulator of the AR promoter. Interestingly, the activity of AP-1 and a number of other transcription factors (CREB, NF1/CTF, C/EBP, HNF-1, and HNF-4) did not decrease with the age of the rat liver.

While recent studies on cellular aging have focused primarily on determining the nature and cause of the loss of dividing potential, it is possible that at least part of the aging phenotype seen in vivo occurs due to an age-related functional decline of cells rather than as a consequence of the loss of DNA synthesis. In this context, age-dependent decline in induction of the heat shock transcriptional response and changes in the regulation and function of the heat shock transcription factor are the focus of the article by Lee et al. The authors reviewed the evidence of an attenuated response to stress in a number of mammalian aging model systems, and presented their recent findings on age-dependent changes in the regulation and function of the heat shock transcription factor, HSF1. The authors suggested that functional decline in the heat shock transcriptional response, and other cellular defense mechanisms, are likely to contribute to the increased morbidity and mortality of the aging human.

As guest editors of this special issue on transcription factors and cell aging, we want to stress that the articles are meant to provide a snap shot of an evolving and ongoing discovery process aimed at understanding the molecular basis of aging. Because of the very limited space, we cannot possibly cover many of the important recent findings in this and other related fields, including: 1. the role of DNA oxidative damage and excision repair in cell aging [1, 3, 6, 7]; 2. telomere length and cell aging [2]; 3. possible role of DNA helicase – an enzyme that unwinds the DNA double helix and is necessary for DNA repair, replication, or expression of the genetic material – in premature aging [5, 9]. Some of these topics are covered in recent reviews on patterns of aging [4, 8].

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