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Aging Research Worldwide

Ageing research in Greece

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Abstract

Ageing research in Greece is well established. Research groups located in universities, research institutes or public hospitals are studying various and complementary aspects of ageing. These research activities include (a) functional analysis of Clusterin/Apolipoprotein J, studies in healthy centenarians and work on protein degradation and the role of proteasome during senescence at the National Hellenic Research Foundation; (b) regulation of cell proliferation and tissue formation, a nationwide study of determinants and markers of successful ageing in Greek centenarians and studies of histone gene expression and acetylation at the National Center for Scientific Research, 'Demokritos'; (c) work on amyloid precursor protein and Presenilin 1 at the University of Athens; (d) oxidative stress-induced DNA damage and the role of oncogenes in senescence at the University of Ioannina; (e) studies in the connective tissue at the University of Patras; (f) proteomic studies at the Biomedical Sciences Research Center 'Alexander Fleming'; (g) work on *Caenorhabditis elegans* at the Foundation for Research and Technology; (h) the role of ultraviolet radiation in skin ageing at 'Andreas Sygros' Hospital; (i) follow-up studies in healthy elderly at the Athens Home for the Aged; and (j) socio-cultural aspects of ageing at the National School of Public Health. These research activities are well recognized by the international scientific community as it is evident by the group's very good publication records as well as by their direct funding from both European Union and USA. This article summarizes these research activities and discuss future directions and efforts towards the further development of the ageing field in Greece.

Keywords: Ageing; Proteomics; Clusterin; Centenarians; Histone; Presenilin-1; APP

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1. National Hellenic Research Foundation

1.1. Functional analysis of Clusterin/Apolipoprotein J involvement in the development of the ageing and cancer phenotypes

Various studies have revealed that cellular senescence relies on a genetic background. We have cloned several genes that associate with mammalian replicative senescence by taking advantage of conditionally immortalized rat embryonic fibroblast cell lines which undergo senescence upon SV40 T Ag inactivation (Gonos et al., 1996; Powell et al., 1999). One of the cloned genes encodes for Clusterin/Apolipoprotein J (ApoJ; Gonos et al., 1998). Subsequent studies have shown that the gene is overexpressed in human and rat embryonic fibroblasts as well as in human trabecular osteoblasts undergoing ageing in vitro (Gonos et al., 1998; Dumont et al., 2000). Interestingly, partial or full transformation by *ras* oncogenes (Barradas et al., 2002) of immortal fibroblasts induced ApoJ down-regulation. We then pursued our studies and analyzed ApoJ function in normal human diploid fibroblasts (HDFs) upon the development of the senescence phenotype. We found that in vitro ageing of HDFs is accompanied by a significant up-regulation of the ApoJ mRNA and protein levels, but with no altered biogenesis, or intracellular distribution of the two ApoJ protein forms (cytoplasmic and secreted) detected. To analyze the relationship between senescence and ApoJ protein accumulation, we stably overexpressed the ApoJ gene in primary and immortal HDFs and showed no effect at normal cellular lifespan (Petropoulou et al., 2001; Dumont et al., 2002). Thus ApoJ is a novel senescence biomarker that is not directly related to the cell-cycle progression checkpoints or to the induction of the senescence phenotype, but its induction is probably a result of stress accumulation during ageing.

To conclude about ApoJ function we undertook a detailed analysis of ApoJ gene regulation and function in human osteosarcoma cells (OS) upon apoptosis and/or growth arrest induction by chemotherapeutic drugs (Trougakos et al., submitted for publication). Our analysis showed that exposure of various OS cell lines to adriamycin (ADR), resulted in increased ApoJ mRNA and protein levels. The observed up-regulation of ApoJ gene is ADR dose-dependent and is abolished by blocking the protein kinase C (PKC) activity. Interestingly, chemoresistant OS cells expressed elevated levels of the ApoJ gene. Moreover, stable ApoJ overexpression in OS cells resulted in significantly higher resistance rates to the cell-death or growth arrest induced by ADR, providing evidence that ApoJ functions in OS cells as a cytoprotective molecule. Further, we analyzed ApoJ biogenesis in normally proliferating OS cells and identified two novel ApoJ protein forms being localized in the nucleus and plasma membrane with yet unknown function. In addition, we showed that the ApoJ protein forms physically interact in the cytoplasm and plasma membrane with the DNA-PK components, a feature that directly involves ApoJ in the process of DNA repair. In summary, our on-going studies on ApoJ function have revealed unanticipated results regarding its function in normal and cancer human cells and raised the hypothesis that the gene probably exerts distinct functions in normal and transformed human cells (Trougakos and Gonos, 2002). This work is supported by two European Union grants, a Biomed-2 ‘Genage’ grant and a QLRT ‘Functionage’ grant.

1.2. Model systems for studying cellular ageing: healthy centenarians

Lifespan is a multifactorial quantitative trait, which is affected by both genetic and environmental factors. Healthy centenarians represent the best example of successful ageing, because they have escaped the major age-associated diseases and most of them are in good mental and physical condition (Gonos, 2000). Over the past years we have developed banks of cells (skin fibroblasts and peripheral blood monocytes), RNA and DNA from dozens of donors of various ages including healthy centenarians, which has allowed us to envisage on the genetic and environmental factors that are related to ageing.

Skin fibroblasts cultures derived from biopsies of healthy centenarians and from adult donors of different ages were established and further studied. Determination of cultures’ growth kinetics, as well as examination of various molecular and cellular markers (e.g. β-gal staining, gene expression levels, telomeres’ length, etc.) has revealed that centenarian derived cultures follow the typical Hayflick limit, as they enter senescence after 20–35 CPDs (Mondello et

al., 1999). However, analysis of several proteasome subunits RNA and protein expression levels, determination of two proteolytic activities and identification of oxidized proteins in these samples revealed that centenarians cultures differ from elderly donors derived cultures as they have a functional proteasome (Chondrogianni et al., 2000). Finally, the relevant subsets of differentially expressed genes among these samples were identified by using a combination of differential screening methods (Simoes and Gonos, 2002). For some of the cloned genes, a similar expression pattern was observed in all centenarians samples so far examined, though a genetical trait has still to be determined, as well as if any of these genes will have an effect on the extent of cellular lifespan. This work is supported by two European Union grants, a Biomed-2 'Genage grant' and a QLRT 'Cellage' grant.

1.3. Protein degradation during ageing: the role of proteasome

During ageing the cellular homeostatic machinery becomes progressively impaired (Petropoulou et al., 2000). The proteasome represents the major proteolytic mechanism among the ones that are considered to play an important role in cellular maintenance (Chondrogianni et al., 2002). We have shown an impaired proteasomal function in HDF that senesce in vitro. The three major proteolytic activities were found decreased in late passage cells as compared to early passage cells and, moreover, senescent cells exhibited high levels of oxidized proteins. The different complexes of the proteasome, 20S, 26S and PA28:20S, were also analyzed by gel filtration column separation. Initial results show that there is not a difference in the ratio of those complexes between early and late passage cells. However, it was shown a decrease in the amount of several proteasomal subunits to relate to a decreased level of those complexes observed in late passage cells. The importance of the function of proteasome in senescence is also revealed by inhibition experiments since treatment of early passage cells with the specific proteasome inhibitor MG132, elicits a senescent-like phenotype. Young proliferating cells that were treated with MG132 exhibit a phenotype similar to that seen at normal senescent cells as revealed by β-gal staining, DNA synthesis rate, immunoblot analysis of several senescence-related proteins and levels of the chymotrypsin-like activity. Interestingly, this activity could not be restored in the cells that were treated with the inhibitor, even after a long recovery period.

Since the proteasome appears to play a major role during ageing, we have also attempted a restoration of the proteasomal function by transfection experiments. Two constitutively expressed catalytic subunits (X and Y) were overexpressed in WI38/SV40 T Ag cells. The three specific proteasomal activities were found increased in clones that were overexpressing either of those two subunits. Furthermore, it was found that cells that were overexpressing one of these subunits were also overexpressing the other subunit suggesting the existence of an auto-regulatory pathway. Two-dimensional electrophoresis experiments are in progress, in order to reveal differences between the proteasomal composition of cells that overexpress those subunits and control cells. Taken together, our results demonstrate that as the process of ageing progresses the function of the proteasome is impaired, leading to chain-reaction phenomena such as the accumulation of impaired proteins and the abnormal degradation of normal cellular proteins that, in turn, may lead to several pathogenic situations. This work is supported by a European Union QLRT 'Protage' grant.

2. National Centre for Scientific Research, 'Demokritos'

2.1. Regulation of cell proliferation and tissue formation during ageing

Our aim is to study the regulation of tissue homeostasis during development and ageing, and especially the role of growth factors in this process. A prominent demonstration of homeostasis, which is severely compromised during ageing, is tissue repair. Accordingly, we have studied in vitro several important parameters of the repair process, e.g. cell proliferation and extracellular matrix (ECM) formation, in fibroblasts derived from healthy young and aged individuals. Our data have shown no age-related decline—at the cellular level—in any of these aspects that could possibly account for impairments of the tissue repair in the elderly, suggesting the importance of systemic factors

in the regulation of the healing process (Kletsas et al., 1998, 2000). This is further supported by more recent findings, indicating that systemic stressful agents, such as elevated corticosteroid levels in the blood, alter the cell's responses even in vitro (manuscript submitted).

Concerning the regulation of cellular proliferation, our data indicate that while there is no major change in the proliferative ability of fibroblasts during ageing in vivo, there is a qualitative alteration during the fetal-to-adult transition in the cells' response to exogenous growth factors, especially to TGF-β, as well as to autocrine growth factors (Pratsinis et al., 1997, 2000a; Kletsas et al., 2000). Finally, we are also studying structural and functional features of in vitro senescent human fibroblasts and conditionally immortalized smooth muscle cells (Hsieh et al., 2000; Pratsinis et al., 2000b). In particular, we are investigating ageing-related changes in aspects of cell proliferation, such as growth factor receptors, their signaling pathways, secondary messengers and cell-cycle markers (Psarras et al., 1994; Kletsas et al., 1995, Papazafiri and Kletsas, submitted for publication), in addition to the expression of structural nuclear proteins that are connected with cell cycle progression, such as lamins and LAPs. The above aim at the delineation of the mechanisms underlying both the ageing process and cell proliferation.

2.2. A nationwide study of Greek centenarians: determinants and markers of successful ageing

In this project, we address the cohort of the 'oldest old' and namely the centenarians. To this end, we conducted a pilot research at the national level aiming at the localization and identification of Greek centenarians and collection of information concerning their state of health and lifestyle, as well as environmental and socio-economic parameters, in face-to-face interviews according to a structured questionnaire. The profiles of approximately 450 centenarians have been inserted in a database, which is currently under completion. Interestingly, some of them show a satisfactory level of good health and—most important—considerable autonomy. Accordingly, our next objective is to study this group also in terms of clinical parameters, biochemical and genetic key markers and lifestyle determinants in a multidisciplinary approach, in order to acquire insight into the basic mechanisms of successful ageing.

2.3. Ageing and histones: histone gene expression and acetylation

During the ageing process numerous biochemical pathways are altered leading to G1 arrest and inhibition of entrance into the S phase. A cascade of genetically programmed events take place leading to this post-mitotic senescent state, which, in recent years, have been intensely studied. These events bare many similarities to events leading to the differentiated state. Least studied, are the changes in the constitution of chromatin which must inevitably occur for the progression of senescence-related events. Prerequisite to chromatin remodeling and the initiation of transcription are changes in the histone variant constitution and changes in their post-translation modifications. These changes are well documented during the process of terminal differentiation. Using the in vitro model ageing cell system of HDF and a HDF cell system where ageing is induced artificially by the histone deacetylase inhibitors, sodium butyrate and trichostatin A, as well as long-term T-lymphocyte cell cultures (Sourlingas et al., 1999) our work has focused on changes in histone variant gene expression which may occur as a function of the increasing age of the culture and in post-mitotic senescent cell populations. Of special interest are the results obtained with the well known, as a differentiation-associated histone variant, the H1 linker histone variant, H1o. In mitotically active HDF cell populations, though cell cycle related changes occur in H1o synthesis rates, no differences in these rates were found as a function of increasing CPDs (Tsapali et al., 2000a). However, specific changes in H1o mRNA levels were observed as a function of the phases of the cell cycle and increasing age of the culture (Tsapali et al., 2000b). When post-mitotic senescent HDF cultures were studied, increased levels of both H1o synthesis rates and intensely increased H1o mRNA levels were observed (Tsapali et al., 2001). The behavior of this linker histone variant in the in vitro HDF model system is similar to that found in numerous terminally differentiating cell systems studied.

Moreover, within the framework of our participation in a European Thematic Network on Immunology and Ageing in Europe (ImAginE), we are continuing

our histone ageing research work with T-lymphocytes. Using histone deacetylase inhibitors in peripheral blood lymphocytes, we observed the concomitant induction of apoptosis, histone acetylation and H1o gene expression (Sourlingas et al., 2001). In fact when the effect of the highly specific histone deacetylase inhibitor, trichostatin A on the level of induced H1o expression, H4 acetylation and S phase total histone synthesis inhibition of peripheral blood lymphocytes from young and old donors was analyzed, differential responsiveness/sensitivity and/or differential expression of histone deacetylases with increasing age was observed (Sourlingas et al., 2002). It is known that H1o is tightly related to the more compact structures of chromatin and that histone acetylation is a prerequisite to the initiation of transcription. These results may be of pivotal importance during immunosenescence and senescence in general in which chromatin remodeling may be an important event and may prove of special benefit to the further elucidation of chromatin structural changes and programmed gene expression during ageing.

3. University of Athens

3.1. Molecular and cell biology of the amyloid precursor protein and Presenilin 1

Since 1991, our research has focused on the biochemistry, molecular and cell biology of the Alzheimer's amyloid precursor protein (APP) and Presenilin 1 (PS1). Under the mentorship of Dr Robakis (Mount Sinai School of Medicine, New York), we have investigated the role of signal transduction pathways and G-proteins on the processing and secretion of APP in addition to its trafficking and post-translational modifications (Efthimiopoulos et al., 1994, 1996a; Refolo et al., 1995; Wu et al., 1997; Pangalos et al., 1995, 1996; Hook et al., 1999). We have also examined the effects of receptor agonists and depolarization on the regulated secretion of APP in primary chromaffin cells (Efthimiopoulos et al., 1996b), the mechanism of amyloid beta peptide (Aβ) induced neurotoxicity (Fagarasan and Efthimiopoulos, 1996; Pappolla et al., 1996) and the inhibition of Aβ production by peptidyl-aldehydes (Pereira et al., 1998). During the last 3 years, we have been studying the subcellular localization of PS1 in human and murine tissues and its interaction with the cadherin/catenin adhesion system. More specifically, in mouse brain, we detected PS1 immunoreactivity mainly in neuronal cell bodies and dendrites (Elder et al., 1996). Axons showed marked PS1 staining in portions of brain stem and spinal cord (Elder et al., 1996). In subcellular fractionation experiments, we found that PS1 proteolytic fragments are enriched in clathrin coated vesicles suggesting that PS1 may be expressed at the cell surface (Efthimiopoulos et al., 1998). Indeed, Georgakopoulos et al., 1999 showed that PS1 is expressed at the cell surface of MDCK cells where it colocalizes and forms complexes with the cadherin/catenin adhesion system. Furthermore, we have shown that the interaction of wild type PS1, but not of ΔE9 PS1 mutant, with the cadherin/catenin adhesion system results in its stabilization and increased cell–cell aggregation (Baki et al., 2001).

We are also studying the effects of familial Alzheimer's disease-linked PS1 mutations on the intracellular trafficking and on the expression at the cell surface of APP, E-cadherin, and Notch 1. This line of research is based on the hypothesis that changes in the intracellular trafficking of these proteins and their expression at the cell surface may affect their proteolysis and function thus leading in neuronal dysfunction and the development of Alzheimer's disease neuropathology. In addition, we are investigating to identify the intracellular compartment where PS1 interacts with APP and Notch 1 and the PS1-dependent γ-secretase proteolytic activity takes place. This work is supported by a grant from the American Health Assistant Foundation and a grant from the European Union.

4. University of Ioannina

4.1. Oxidative stress-induced DNA damage and ageing

Oxidative stress is a major challenge to all living cells and oxidative damage to cell constituents represents a strong candidate for a primary mechanism of ageing. DNA is especially vulnerable to oxidative damage and is regarded as a key target for a variety of oxidizing agents. Accumulation of unrepaired

oxidative DNA lesions is likely to play a causative role in ageing. However, our knowledge about the molecular mechanisms regarding oxidant-induced DNA damage as well as DNA repair is limited.

Research efforts in our laboratory have concentrated mainly towards understanding the molecular mechanisms of action of agents that are generated in vivo, such as hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl). By using the 'single cell gel electrophoresis assay' which detects single strand breaks and alkali labile sites in nuclear DNA, we observed a fast (within a few minutes) and calcium-dependent induction of DNA damage, when Jurkat cells in culture were exposed either to H_2O_2 or $ONOO^-$ (Panagiotidis et al., 1999; Doulias et al., 2001). The dependence of calcium as well as other observations indicate the possibility that an enzymatic process is taking place. Following these observations, the role of redox-active iron and copper ions has been reinvestigated (Barbouti et al., 2001; Galaris et al., 2002). By using the same technology, we have also studied the role of a variety of substances that are able to protect cells from H_2O_2-induced DNA damage (Tselepis et al., 2001).

The role of heme iron in relation to interactions of peroxides with heme-containing proteins has also been investigated by using H_2O_2 and myoglobin as prototypes of peroxides and heme proteins, respectively (Galaris and Korantzopoulos, 1997; Galaris et al., 1998). Finally, we are presently investigating the relation of the fast effects on DNA with oxidant-induced apoptosis (manuscript submitted) as well as ways of protecting cells from these effects. In this regard, apart from using a variety of substances, transfections of cells with plasmids containing ageing-related genes have been performed in order to evaluate their involvement in oxidant-induced DNA damage.

4.2. Oncogenes in cellular senescence

Cultured normal human fibroblasts very rarely, if ever, spontaneously produce immortal clones and their immortalization and transformation by chemical carcinogens, mutagens or activated oncogenes is a very rare event. Several possible mechanisms have been suggested to explain the manner in which diploid cells senesce and by which immortal cells evade senescence (Rosenberger et al., 1991). Among the factors essential for cell immortality and for initiating neoplastic changes is the activation of oncogenes.

Comparison of HDFs (MRC-5) with their SV40-immortalized and transformed counterparts (MRC-5V1) revealed multiple molecular changes mainly in the expression of growth factor and receptor genes and ECM components, characterized by the absence of fibronectin, collagen α2(I) and decorin in MRC-5V1, gene products that are often overexpressed in senescent cells (Kolettas et al., 1997). Downregulation of decorin, a TGFβ-1 and collagen-binding ECM proteoglycan, was found to be related to the induction of anchorage-independent growth caused by the *v-src* gene product in MRC-5 without affecting their lifespan (Kolettas and Rosenberger, 1998). Constitutive expression of *v-fos*, the cellular homologue of which has been implicated in cellular senescence, failed to rescue MRC-5 or a conditionally immortalized rat cell line from growth arrest (Kolettas et al., 2001).

Comparison of the growth and transformation properties of embryonic human (MRC-5) and rat (REF) fibroblasts carrying *v-Ha-ras* and *v-myc* oncogenes acting alone or in combination showed that the two cell types behaved differently in a number of ways. Acting together, the two viral oncogenes markedly reduced the growth rate, the plating efficiency and the saturation density of the human but not the rodent cells. MRC-5 co-expressing viral *myc* + *ras* died by apoptosis and never formed confluent monolayers. Further, in contrast to REFs, they converted only a small proportion of human fibroblasts to anchorage-independent or focus forming cells and this correlated with changes in the expression of decorin and lumican, gene products with transformation suppressor function. Although co-expression of viral *myc* + *ras* in REFs promoted immortalization and tumorigenic growth, it yielded no clones of MRC-5 capable of unlimited growth and failed to induce tumors in nude mice. We are currently investigating the molecular basis underlying these changes in the two cell types.

5. University of Patras

5.1. Studies in the connective tissue

During the last two decades we have been engaged with research on various aspects of connective tissue

biochemistry. The activities of our group have been well recognized by the international scientific community as we were entrusted to organize the XVII meeting of the Federation of European Societies of Connective Tissue (FECTS) in Patras, in July 2000.

One line of research is the alteration of proteoglycans/glycosaminoglycans with ageing in cartilage, brain and blood vessels. We have shown that aged cartilage contains reduced amounts of proteoglycans and total glycosaminoglycans, which may be attributed to decreased biosynthetic rates. Whereas the size of the proteoglycans and that of the chondroitin sulfate is reduced with ageing, that of keratan sulfate increases in size as well as amount (Theocharis and Tsiganos, 1985; Theocharis et al., 1985). On the other hand, the keratan sulfate content of sheep brain decreases with ageing and becomes undetectable in very old animals (Papageorgakopoulou et al., in preparation). Using a methodology developed in our laboratory we have found that autoantibodies against aggrecan, the main cartilage proteoglycans are increased with ageing in sera of healthy subjects (Vynios et al., in preparation).

Furthermore, we have studied several factors contributing to the development of abdominal aortic aneurism (AAA). At the level of GAGs, it was found that AAA is associated with a statistically significant decrease of hyaluronan and heparan sulfate with a significant increase in the content of chondroitin sulfate as compared to normal aorta. On the other hand, the amount of dermatan sulfate remained almost constant. An important finding was the altered distribution of structures sulfated at C-4 and C-6 of the repeating units of chondroitin and dermatan sulfates (Theocharis et al., 1999). This differential pattern was attributed to the decreased protection of LDL oxidation observed in the process of atherosclerosis. At the level of PGs, we found that differential expression of versican splice variants V0 and V1 as well as the different localization of decorin, which is localized at the sites rich in collagen, are the worth noticing events closely associated to the process of atherolsclerosis and the development of AAA (Theocharis et al., 2001). Cell populations (SMCs and macrophages) play also crucial roles. SMCs are significantly decreased, whereas the number of macrophages is increased in discrete sites of the tissue. These results suggested that the content, fine chemical structure and localization of GAGs/PGs are among the factors contributing to organization, properties and dilataration of aneurysmal aorta.

6. Biomedical Sciences Research Center ‘Alexander Fleming’

6.1. A proteomic approach to ageing, senescence and growth regulation

The laboratory, apart from carrying out research on signal transduction pathways, provides a core facility for the analysis and characterization of proteins and their interactions with other biomolecules. A strong emphasis has been given on the acquisition of state-of-the-art specialized instruments such as an ion-trap mass spectrometer, a nanospray source and a surface plasmon resonance biosensor. Funding for this equipment has come from a major competitive grant from the European Union Second Operational Program for Research and Technology.

One of the current projects in our laboratory involves a proteomic approach to ageing and cellular senescence (in collaboration with the laboratories of Dr E.S. Gonos and Dr D. Kletsas). We apply 2D electrophoresis and protein sequencing by mass spectroscopy to the discovery of proteins whose expression is altered during cellular senescence and to compare expression patterns between fibroblasts from donors of different ages. The involvement of specific signal transducing networks in these processes is also being investigated. These approaches allow the mapping of protein distribution profiles in normal and diseased tissues and cells and the generation of information on the relative abundance and post-translational modifications of proteins. We want to consolidate the position of our laboratory as the leading proteomics facility in Greece and expand further its capabilities by developing methodologies for a more efficient characterization of post-translational modifications and by increasing the throughput of samples, both at the level of 2D gels and mass spectrometry processing with the aim to be able to accommodate clinical samples.

7. Foundation for Research and Technology

7.1. Molecular aspects of ageing: the Caenorhabditis elegans connection

Elucidation of the basic molecular mechanisms underlying the progressive decline in cellular function that accompanies ageing will have an immediate impact on the design of novel interventions that could reduce or delay age-related deterioration in humans. Protein synthesis and degradation are the two essential, interlinked cellular processes responsible for maintaining a functional protein content in every cell. Damage to cellular macromolecules such as proteins has been postulated to be a major contributor to the ageing of diverse organisms. Damage can be limited by maintaining high antioxidant defences and by clearing/repairing damage efficiently. Protein turnover is one of the main routes by which functional proteins are maintained and damaged proteins are removed.

We exploit the experimental strengths of the *C. elegans* model system in an effort to identify the specific biochemical steps underlying alterations of protein turnover during ageing and under caloric restriction (Tavernarakis and Driscoll, 2002). Our testable working hypothesis is that the delicate balance between detrimental protein modification and protein turnover, which exists early in life, is tipped in favor of deleterious protein modification during late stages of life. The rate at which a protein pool is refreshed at any given point in time is determined by the rate of protein synthesis and protein degradation at that particular point. Protein turnover cannot keep pace with the ever-increasing accumulation of damaged proteins during ageing. Increased protein turnover might consequently be one of the major causes of lifespan extension under caloric restriction or in long-lived mutants, by facilitating the maintenance of a fresher pool of proteins with less accumulated damage. The specific goals of our work are (1) to characterize the age-related changes in protein turnover and to correlate these changes with the accumulation of aberrant proteins, in wild type and long-lived *C. elegans* strains; (2) to experimentally determine whether directed modulation of protein synthesis rates affects lifespan and/or other biomarkers of *C. elegans* ageing; and (3) to investigate the effects of dietary restriction on protein turnover and ageing in *C. elegans*.

We have shown that experimentally increasing protein turnover can extend lifespan in *C. elegans*, while a decrease in protein turnover has the opposite effect on longevity. We have generated nematode strains with increased protein turnover rates that have a lengthened lifespan and also strains with decreased protein synthesis rates that have a shortened lifespan. Moreover, we have shown that long-lived nematode mutants show a significant increase in protein synthesis rates (Tavernarakis and Driscoll, 2001, 2002; Xu et al., 2001). Our data provide the first causal molecular evidence linking lowered protein synthesis/degradation rates with senescent decline.

8. 'Andreas Sygros' Hospital

8.1. Investigating the role of ultraviolet radiation in skin ageing

The ageing process in human skin is strongly influenced by the effect of various environmental factors, the most prominent one being chronic exposure to ultraviolet (UV) radiation. Photoageing refers to a series of characteristic macro- and microscopic changes of the skin that are caused by chronic sun exposure and are distinctly different from those observed in sun-protected, chronologically aged skin. Although the clinical and histological characteristics of photoaged skin have long been recognized, recent research has eluded the role of several molecular pathways in the pathophysiology of photoageing, such as the generation of reactive oxygen species, the altered expression of several transcription factors (AP-1, NF-kB), and the induction of matrix metalloproteinases and mitochondrial DNA mutations.

Our Unit is focused in three main areas of research. First we are conducting clinical studies to evaluate the efficacy of antioxidants in preventing or reversing the clinical changes of photoageing. These are long-term human volunteer studies in which topical preparations of antioxidants (vitamin C and E, flavonoids), used on a daily basis, are assessed in view of their capacity to reduce wrinkles and remove pigmented alterations of aged skin.

The second area of focus pertains to the experimental reproduction of the histologic changes of photoageing. By using artificial light sources emitting UVA (320–400 nm) or UVB radiation (290–320 nm) we perform repetitive exposures of small areas of human skin in vivo to suberythemal doses of UV radiation and observe histologically the progressive changes in the structure of collagen and elastin fibers. In addition, we use sunscreens of different degrees of protection (SPF) prior to UV exposure in order to assess their efficacy in preventing these structural alterations. In collaboration with the A. Fleming Hospital we also use specific immunohistochemical assays to assess apoptosis and p53 mutations in chronically UV-exposed skin, in an effort to determine whether these molecular events may contribute to the photoageing phenotype, as they do in skin photocarcinogenesis (Stratigos and Antoniou, 2000).

The third area of our current research work involves the chronic effects of photochemotherapy (PUVA therapy), a widely used method employing artificial UV radiation for the treatment of psoriasis, eczema, and other skin disorders. Despite its beneficial effect in these skin conditions, chronic administration of PUVA can result in photoageing and the development of skin malignancies. In our cohort of psoriasis patients who have received photochemotherapy on a long-term basis (total cumulative UVA exposure dose > 1000 J/cm^2), we are currently investigating the structural changes induced in collagen and elastin in comparison to those seen in sun-protected as well as chronically sun-exposed skin. This project is done in collaboration with the 'Department of Pathologic Anatomy of the General State Hospital'. In the context of this study, we are also examining the expression of specific matrix metalloproteinases in order to assess any potential differences in the activity of these degrading enzymes between PUVA-treated and chronically sun-exposed skin.

9. Athens Home for the Aged

9.1. Long follow-up studies in healthy elderly

For the past 25 years, we have studied the major factors reducing renal and respiratory function during ageing. The project started with examinations of clinically healthy subjects 70–75 years old which were followed at the 'Athens Home for the Aged' with complete annual clinical and laboratory check-ups for 10 years. The presence of urinary tract infection, even asymptomatic bacteriuria and elevated blood pressure was found. Male sex and age as such are factors reducing the GFR, renal blood flow and proximal tubular activity during the whole life of the individual. In addition, important factors of reduction include age as such, acute and chronic obstructive lung disease (COLD) especially if this infection appears at an early age. Modifiable factors also include smoking which, however, acts through the development of COLD and not by direct reduction of the respiratory volumes. Increase in weight or frank obesity have also a negative impact on the level of these functions (Staszewska-Pistoni et al., 1995; Dontas et al., 1996; Giamarellou et al., 1998).

Moreover, to the above program we have studied as part of the Seven Counties study, the prevalence and incidence of coronary heart disease in complete population samples in defined rural areas of the islands of Crete and Corfu. The inhabitants of these islands have a special position among the remaining six countries (very low coronary heart disease prevalence and incidence, low overall mortality rate and a long life expectancy) which are due to the diet, the medium–large habitual physical activity, exercise and lack of stress. In particular, the diet includes small daily amounts of protein calories, large amounts of total fat composed largely of olive oil and an abundance of fruits and vegetables. The Greek islands diet is the prototype of the Mediterranean diet which is presently promoted world-wide as a longevity promoting diet (Kafatos et al., 1997; Dontas et al., 1998, 1999). The collaborators on these large topics are continuing their studies for more than 30 years on the survivors of these programs.

10. National School of Public Health

10.1. Socio-cultural aspects of ageing

Our research interests are centered on the socio-cultural aspects of ageing and 'third', even fourth age (Agrafiotis, 2002). The questions explored, concern the status of third age and more concretely

how different discourses approach the limits of the age groups? Which mechanisms in our societies lead to the definitions of third age? What are the consequences of these definitions on socio-cultural level? What is the future of third age groups? The main findings of this analysis suggest that third age is a group not only with a 'past but also with a future': the group has limits, of course, on the level of functional participation in the production system and on the level of symbolic power (because of the 'obsolete' character of their experience) but they gain on the level of free time and (for a great number) on economic resources.

Another area of investigation is the analytical approach of the lifestyles of the aged in relation to new technologies. In other terms, the objective of this inquiry is to investigate to which degree the 'issue of ageing' can be transformed to a field of innovations—technological and socio-cultural. The result of this work indicated that technologies of communication, of continuous or distance learning and transports could be candidates for the exploration of new ideas.

Ageing has obtained a dominant place in multiple fields of collective life. A lot of factors have contributed to this evolution. The question is, what is the impact and the socio-cultural conditions of this phenomenon? What kind of 'problematique' of socio-cultural nature is it possible to conceive? What kind of discourses and scientific analyses are used to construct this interrogation? To what extent the economico-demographic argument is capable to face the multidimensionality of ageing? Is it possible to propose an interdisciplinary approach in order to study ageing in late modernity? The main idea here is that 'ageing' creates the setting in which research and technology and institutional arrangements have more powerful influences for the ageing than biological–physiological factors. That is to say a socio-cultural determinism tends to be more crucial than the biological mechanism of ageing. In this situation 'life', 'death', 'normal duration of life', 'quality of life' have to be re-defined; but such a redefinition is also related to a global evolution of modern societies. In this perspective ageing could be a catalyst for socio-cultural changes. Finally, one aspect of third age studied was sexuality. The question seeks to explore how Greek society perceives sexual life of the aged persons. A preliminary study has showed that traditional stereotypes of no-sexual life and the no-right to sexual life are still quite strong (Agrafiotis, 1997).

11. Concluding remarks

Ageing research in Greece is well established. As it is presented in this article many groups located in universities, research institutes or public hospitals are studying various and complementary aspects of ageing. Their research activities are well recognized by the international scientific community. This is evident by the seven European Union (four to Dr Gonos group at the National Hellenic Research Foundation, one to Dr Sekeri-Pataryas group at Demokritos, one to Dr Panayotou group at the Fleming Institute and one to Dr Efthimiopoulos group at the University of Athens) and an American Health Assistant Foundation (to Dr Efthimiopoulos group) grants that have been awarded to these research groups since 1998. In addition and during the same period, the groups have published 47 articles in various aspects of ageing in peered reviewed journals. Equally important is the fact that Greece has hosted the XVII FECTS meeting (Patras, 2000) and the Second EuroConference on 'Biological Ageing' (Spetses, 2002) thanks to efforts of colleagues at the University of Patras (Prof. C.P. Tsiganos) and at the National Hellenic Research Foundation (Dr E.S. Gonos), respectively. Despite the international recognition, at domestic level, a substantial and continuous financial support by the Hellenic State is required, if Greece wishes to be part of the leading countries in this field of research. The very first step of such an effort could be the establishment and the funding of a 'Hellenic Network of Ageing Research'. Such a network will not only bring closer the existing research groups (up to now—January 2002—there is only one joint PENED grant between Drs Gonos, Kletsas and Panayotou groups supported by the Hellenic General Secretariat of Research and Technology), but it will also provide the platform for Greece to play a major scientific role in Ageing Research in Europe. It is inevitable that, sooner or later, Greece will confront such a challenge.

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