MOLECULAR BIOLOGY OF AGEING

RESEARCH PROGRAMME OF THE EURAGE MOLECULAR BIOLOGY GROUP

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PREFACE

This document is a report written by a group of outstanding European molecular biologists who are involved in experimental ageing research. These experts were invited by EURAGE (a concerted action of ageing and diseases of the European Community) to prepare a research programme which could provide a concerted attack on the important molecular biological aspects of mammalian ageing under study within the EURAGE framework.

The report describes an integrated European research programme with the main overall objective to uncover the molecular events underlying age-related functional decline and the genetic factors predisposing to the disease processes occurring with a high incidence in the aged. In addition, the presented programme guarantees a sound molecular biological basis for many ongoing EURAGE research activities.

Rijswijk, March 1988
D.L. Knook
Project Leader EURAGE

I INTRODUCTION

At the present time we are in the midst of a revolution in the biological sciences. A series of extraordinary technical breakthroughs in molecular biology, among which the emergence of recombinant DNA technology is the most notable, has provided us with the tools for analyzing basic molecular processes in cells and to be able to mimic such processes in the laboratory with the aim of producing some most valuable natural compounds on an industrial scale. Some spectacular successes include mapping of disease genes and the transfer of cloned genes into animals. In particular, a crude map of the human genome has been made (see Danis-Heller, H. et al., A genetic linkage map of the human genome, Cell 51:319-337, 1987) and programmes for the sequencing of the complete human genome have been proposed (see Dulbecco, R., A turning point in cancer research: sequencing the human genome, Science, 231: 1055-1056, 1987).

It is now generally acknowledged that molecular biology has an important part to play in the unraveling of the mechanisms involved in the etiology and pathogenesis of age-related disorders. Indeed, it is conceivable that ageing itself is caused by alterations in biological macromolecules and hence predisposes to the ever increasing number of diseases that may find their origin in one or more molecular defects. Elucidation of the molecular mechanisms of ageing and the genes and gene products involved could lead to an understanding of the fundamental processes underlying age-related diseases and functional decline in humans.

At present, the EURAGE research programme is organized in 4 topic oriented research groups (TORGs): 1. Ageing of the liver; 2. Ageing of the immune system; 3. Ageing of the lens; and 4. Ageing of the brain and senile dementia. In many of the participating groups molecular biological techniques have become essential tools for attaining their research aims. However, in spite of the often high quality of molecular biological research already carried out within the

framework of EURAGE, there is at present no organized structure that actively promotes application of advanced molecular biological techniques and that can communicate molecular biological knowledge to physiologists, pathologists, clinicians. etc. (see Rattan, S.I.S., Ageing trends and the European effort: editorial, BioEssays 5:51-52, 1986).

The importance of organizing molecular biological expertise in view of its critical role at the interface between fundamental research on ageing and its clinical applications in the form of better diagnosis and treatment of diseases in the aged, has been recognized by the American National Institute on Ageing. This organization has recently announced the programme "Molecular Biological and Genetic Basis of Ageing".

It appears that the best guarantee for abundant and timely high quality information on the latest developments in the molecular biology of ageing is the establishment of a EURAGE research programme in this field. With this aim in mind a number of European molecular biologists (many of which were already participating in EURAGE research groups) met in Amsterdam on December 18, 1987. The main conclusion with absolute consensus was that a EURAGE research group on "Molecular Biology of Ageing" was highly desirable. Such a group should have a research programme finely tuned to specific European circumstances and demands. Although fundamental research was considered important, the general feeling was that the group should operate near to and in close association with the clinic. In addition, such a group should closely collaborate with comparable organizations in the United States and other parts of the world. Finally, the group should organize the transfer of molecular biological knowledge and expertise to all other EURAGE scientists.

In this publication the research programme and the future activities of the proposed EURAGE molecular biology group are presented.

II EVALUATION OF MODEL SYSTEMS FOR GENETIC ANALYSIS OF AGEING PROCESSES

Ethical aspects and some practical considerations, such as high complexity and long generation times, dictate the use of a variety of model systems in research directed towards the genetic analysis of human ageing processes. These models may be whole organisms such as rodents, insects, nematodes, or even fungi; they may be biopsy material taken to study a particular tissue (or tissues) in isolation from the body; or they may be cells grown in tissue culture, possibly in co-culture with other cell types.

The range of possible models in ageing research is exceptionally broad since most characteristics of a great variety of organisms can be found to undergo some alteration with age. Not all of these changes, however, reflect primary features of senescence and an efficient strategy of research will result only if great care is directed to the choice of model systems and their characteristics relevant to ageing.

Whole organisms

Ageing of whole organisms is most clearly defined by a life-history in which growth to reproductive maturity is followed by a phase when intrinsic mortality rises progressively with age, at a rate specific to the species, until eventually the risk of dying becomes so great that an effective limit is imposed on the lifespan. This life-history pattern, which may best be seen when a population is maintained in a protected environment away from common extrinsic hazards such as predation and starvation, is found in most mammals, birds and reptiles, and it is also common among fish, insects and some groups of invertebrates (1,2).

The shaping of the genetic determinants of ageing processes during evolution is likely to have been closely linked to the life-history pattern of the species in question and, within a given life-history plan, to the relative importance of the various processes

needed to maintain life. Species for which genetic analyses of ageing processes are most likely, therefore, to yield results relevant to human ageing are species with comparable life-histories to our own, and with broadly similar physiology.

In this respect, rodents are an especially useful model in view of their relatively short lifespan and low maintenance costs. However, there can be advantage in working with simpler organisms when the object is either to test specific hypotheses or genetic factors likely to apply across a wide range of species, or to conduct genetic studies, such as selection experiments, for which a short lifespan is essential (3). Such organisms include the filamentous fungus Podospora anserina, of which all wild strains become senescent and die after prolonged vegetative propagation; the nematode Caenorhabditis elegans, which has a well-characterised developmental process; and the fruit-fly Drosophila melanogaster, which is easy to culture and for which a great deal of genetic information is available already.

Analysis of life-histories has an important practical role to play in guiding the choice of model organisms and in interrelating research studies carried out using different species models. Further, while making inter-species comparisons it is important to develop reliable biological parameters of age, for example percent lifespan completed or time units before and after the reproductive period.

Choice of research materials

The actual research material within a given species model may be the organism itself, an organ, biopsy material, or a cell culture. Studies on whole organisms are relevant in experiments using artificial selection to test general theories on the form of genetic control over ageing processes, and in comparative studies. The possibility to create transgenic animals containing genes for more efficient repair and maintenance systems raises new scope for studies on whole organisms in conjunction with techniques of molecular genetics.

The use of organs and biopsy material gives opportunity for study of tissues in isolation from the donor individual. Such materials provide an important level of research intermediate between the organism and the cell. These materials may be valuable in studies on the genetic basis of age-related changes in tissue structure and in cell-cell interactions.

The use of cell cultures in ageing research is well established (4), following the discovery that populations of diploid human fibroblast can replicate only a finite number of times in vitro (5). A related observation that many carcinogenic agents will "immortalise" normal, limited lifespan cultures into cell lines which grow indefinitely suggests an association between, on the one hand, finite and infinite growth in vitro and on the other hand, ageing and cancer in vivo. Although there is some supporting evidence for the relationship of finite cell growth in culture to senescence in vivo, this interpretation is not universally held and the suggestion that normal cells cease to proliferate simply because they enter a post-mitotic state of terminal differentiation still finds support. Further basic studies will be needed to establish firmly the relevance of cell culture models.

Methods are also available to reconstruct in vitro some of the tissues, for example the skin epidermis, as an intermediary between pure cell culture and the tissue in vivo. These are useful not only as models for research but also for clinical use, such as grafting for burn victims and some cosmetic purposes.

III MOLECULAR BASIS OF HUMAN AGEING PROCESSES

From a medical point of view, the relevance of studying human ageing at the molecular level lies in the fact that the incidence of certain (often fatal) diseases increases greatly as we grow old. Although this has often been explained in terms of the long incubation time these diseases need to become manifest (for example, cancer), the possibility of a more fundamental role of ageing in their etiology should not be neglected. Indeed, the existence of human syndromes of accelerated ageing (e.g. Hutchinson-Gilford's, Werner's, Down's), featuring a much earlier onset of age-related pathologies than in normal individuals, indicate an association with biological rather than with chronological age.

The sole fact that many age-related diseases seem to find their cause in a defect at the molecular level warrants an aggressive approach towards unraveling the molecular mechanisms of ageing itself. What is needed is to assess the role of genetic factors as determinants of lifespan and to analyze the molecular aspects of cellular information in relation to age, from the DNA via information transfer to the protein-based cellular phenotype.

Hereditary factors in human longevity

Several lines of evidence indicate that genetic factors contribute to the ageing process. In favour of this view are data regarding different maximum lifespans of different species. In humans, apart from syndromes of accelerated ageing on a genetic basis, as described above, data are scanty. However, there are findings which suggest that longevity runs in families and that identical twins age much more in the same way and at the same rate than non-identical twins. Although it is not clear which genetic factors are responsible, an as yet unestablished involvement of the major histocompatibility complex (MHC) has been suggested by studies on rodents by Walford and co-workers several years ago (6) and recently by data on humans (7).

Since molecular biological techniques have now made many genes

available for study, emphasis should be placed on which genes are worthwhile to be studied and in which populations. A ranking order of importance of particular genes for the ageing process is likely to exist. Several genes involved in the regulation of the neuro-endocrine and immune system have been cloned and are interesting to study in view of their interconnective roles. Genes involved in protective mechanisms (e.g. SOD, cytochrome P-450, etc.) are also prime candidates for studies on their heterogeneity in human populations and the preferential occurrence of genetic variants in nonagenarians and centenarians. Another strategy which can be used is a random search for particular DNA sequences shared by very old people. These studies should be placed within the framework of the emerging knowledge of the DNA sequence organization of the human genome and the increasing number of sophisticated techniques capable of detecting variability among individual genomes.

However, these studies are difficult to perform on humans owing to the great genetic heterogeneity of the human population and to interfering cultural factors. It can be predicted that the search for genetic biomarkers of longevity will be successful only if a sufficient number of very old people is studied. In fact, the genetic factors involved might be different among different European populations but still could be involved in the control of the same biological processes. To overcome these problems a collaborative effort is needed in order to collect suitable material, for example blood specimens, from centenarians, longevity families, very old mono-zygotic twins and people affected by rare progeroid syndromes in the different EC countries. The establishments of cell banks and other data regarding biological parameters of European centenarians and the sharing of data and DNA probes will provide the basis for the collaborative effort which is needed in this field.

Genetic instability

The possibility that DNA alterations are a (or probably the) central cause of ageing was proposed decades ago by several authors

(for a review, see 8). The initial concept of an accumulation of random mutations in structural genes is now been considered unlikely in view of the rather low (10^{-6} to 10^{-5} per cell per locus) mutation frequencies demonstrated in the in vivo situation over one generation (9,10). Instead, major attention is now focused on the possible role of DNA rearrangements in the etiology of ageing and age-related diseases such as cancer (11,12). At the microscopic level there is convincing evidence that in mammals the incidence of chromosomal aberrations increases with age (13,14). It is not inconceivable that at the molecular level this reflects multiple DNA rearrangement events, which might be caused by overreplication-recombination (11) or meiosis-like chromosome exchanges (15). The basic cause of such events could be increased "genomic stress" due to the continuous exposure of ageing individuals to naturally present DNA damaging agents, such as oxygen radicals, body heat, glucose etc. (16,17).

For lower organisms there is strong evidence that DNA rearrangements are associated with senescence. For the filamentous fungus, Podospora anserina it has been demonstrated that during senescence discrete mitochondrial DNA sequences are excised and amplified. This process results in the accumulation of additional circular extrachromosomal DNA (18). Interestingly, in somatic cells from Drosophila and even in mammalian cell lines, such as Hela, extrachromosomal cccDNA has been reported to be present, perhaps as a consequence of excision events in the genomic DNA. There is evidence that in human fibroblasts an accumulation of this type of DNA occurs with replicative age in culture (19).

To get insight into the relevance of genetic rearrangement events for human ageing and the age-related increase in cancer incidence it is important to investigate whether DNA rearrangements have a causal relationship with senescence. At present the most suitable model systems are lower organisms like Podospora, which lend themselves well to detailed genetic analysis (see Chapter II). Furthermore, it is necessary to investigate the age-related frequency and characteristics of DNA rearrangements in mammalian cells in

vitro and in vivo. The recent emergence of highly advanced recombinant DNA techniques allows one to address this problem (20).

Finally, the genes governing the rate and characteristics of genomic instabilities in humans and other mammals should be identified and cloned. The recent successful cloning of genes belonging to the complex of enzymatic systems collectively termed DNA repair, is of major importance in this respect (21). For testing the function of such genes and their role in determining longevity the use of transgenic animals is of crucial importance.

Gene expression

The relevance of alterations in the pattern of gene expression for the deteriorative aspects of ageing is a crucial problem. In general it has proved to be difficult to distinguish between deteriorative and adaptive changes during ageing. From a practical point of view qualitative and quantitative changes in gene expression during ageing could provide biomarkers for a great number of ageing phenomena including the rate of ageing itself. Research in this field should be conducted along the following lines.

Differential hybridization. Using preparations of poly $(A)^+$ RNA (messenger RNA) from the same tissues or organs of young and old animals/humans or from early and late passage fibroblasts, age-specific cDNA clones can be selectively isolated by differential hybridization. This may lead to the identification of genes with altered expression during ageing.

Long-lived mRNAs. The factors determining the stability of long lived mRNAs during ageing (lens crystallins, myosine, collagen) should be studied in post-mitotic tissues and in G° mitotic tissues.

Growth factors. The control of gene expression by growth factors should be studied in relation to ageing. This is of basic importance in order to obtain information about the hierarchy of the genetic programme regulating cell proliferation. It should include the following points:

- establishment of the expression pattern of oncogenes during ageing;
- the preparation of transgenic mice with inducible growth factor genes that can be triggered in all the tissues;
- the construction of cDNA libraries of differential expression between mitotic and post-mitotic cells;
- elucidation of the signals triggering division and differentiation with respect to their transduction to the nuclei during ageing and during ontogeny.

Splicing. In view of its important role in gene regulation the splicing process and alterations therein should be studied in relation to ageing.

Regeneration. Gene expression patterns should be studied during tissue regeneration.

Epigenetic mechanisms

Epigenetic modulations can be found at all levels of genetic information transfer, such as DNA replication, transcription and translation. At the level of DNA, methylation of certain cytosine residues in the genomic sequences appear to be crucial for the regulation of gene expression. The role of DNA methylation during ageing and immortalization is beginning to be understood and such changes are termed epimutations (22). Intensive studies are required in order to understand the nature and mechanisms of progressive failing to maintain the extent and pattern of DNA methylation during ageing. Similarly, various epigenetic processes involved in RNA splicing, processing and transport may be crucial in maintaining the stability and accuracy of genetic information transfer that might be altered during ageing. These include polyadenylation, capping, methylation and others. Further studies need to be undertaken in this - as yet the least explored - area in gerontology for a better understanding of the molecular mechanisms of ageing.

Several epigenetic modifications involved in the regulation of protein structure and function might be of critical value in relation

to ageing. This is because proteins are involved at all levels of biological organisation and functioning including the transfer of genetic information, maintenance and repair, and inter- and intracellular interactions. A large body of evidence suggests that age-related changes in the stability and activities of numerous enzymes may be primarily due to post-translational modifications, such as phosphorylation, acetylation, sulphation, deamidation, glycosylation, methylation and others. Many of these changes have been observed both in the houskeeping proteins and in the proteins involved in genetic information transfer (23).

Accuracy and activities of various components of the protein synthetic machinery, such as the initiation factors and elongation factors are subject to change due to post-translational modifications. Studies are under way on characterizing age-, cell cycle-, transformation- and stress-related changes in post-translational methylation of protein elongation factors (24). Similar studies on epigenetic changes in other proteins are essential for understanding why and how the structure, conformation, function and stability of proteins change during ageing. These studies will help to develop new approaches towards understanding and intervening in the ageing processes and age-related diseases (25).

Molecular regulation of integrative mechanisms

The importance of the immune and neuroendocrine system in the ageing process has been recognized for many years. However, it is becoming clear that immune-, neuro- and endocrine-system form a deeply interconnected network responsible for the integrative mechanisms at the organismic level. Many immune functions appear to be modulated by the nervous and the endocrine systems through specific receptors on lymphocytes (and on other cells of the immune system) for otherwise classical neuromediators and hormones. Conversely, receptors for molecules which mediate immune responses (monokines, lymphokines etc.) are present on the surface of cells belonging to the other above cited systems (26, 27). In recent years

many of these mediators and growth factors, often shared by the three systems, have been purified and their genes cloned.

Other molecules of great biological interest for development and likely for the ageing process, such as cell adhesion molecules, are also shared by, for example, the immune and the nervous system (28).

These observations open new horizons which can be assessed at the molecular level by studying the expression of such genes and of their mutant forms in different tissues and organs from aged animals and humans. Such an approach offers, for the first time, the advantage of studying the ageing process at the molecular level within the general hypothesis that all the three major integrative systems are involved in the ageing process in an interconnected way. Moreover, this approach can be particularly interesting to explain the precocious age-related involution of organs such as the thymus that, according to recent studies (29), has to be considered a part of a broader neuroendocrine circuit.

IV MOLECULAR ANALYSIS OF AGE-RELATED DISEASES

In the 21st century, in Europe women on the average will live into their nineties and men into the late eighties. This is an enormous increase since 1900 when average life expectancy at birth was only around 45 years. People are now living longer at all ages and infant mortality is substantially reduced. This has resulted in the known rectangularization of the survival curves. Since no one wants longer life if it is accompanied by disease, the most important component for the Europeans in their 70's, 80's and 90's is the quality of their health. To meet the challenge of increasing numbers of older people in our countries, we must intensify research on the causes of both ageing and age-related diseases. This distinction between processes of ageing and diseases of ageing has to be made since diseases of old age are not inevitable. Diseases are diseases and must be considered as such and a priori not as a normal process and this holds also true for the diseases of old age. Intensive attempts must be made to find out the causes of these diseases in order to come up with preventive strategies in the near future. Molecular biology and genetic approaches will provide important clues to these causes, to the treatment and diagnosis of age-related conditions.

Intellectual abilities do not change significantly with ageing provided that there is an absence of ill health and of conditions such as untreated high blood pressure. Working capacity and learning power remain but old people may take longer than the young to achieve the same task. But again, as will be pointed out below, this only applies to old people in the absence of age-related diseases such as Alzheimer's disease.

Today, the most common age-dependent disease is arteriosclerosis, resulting in heart diseases and stroke, which are the major causes of death followed in the given order by the other age-related conditions like cancer, diabetes and Alzheimer's disease. Eighty years ago, at the turn of the century, the chief causes were pneumonia, tuberculosis and infectious diarrhoea. If the presently

observed steady increase in deaths from Alzheimer's disease continues, the 21st century will have a new leading cause of death that strikes now already 1% of the population of Europe and 100% of the individuals with "aged" Down's syndrome.

Other age-related problems of the future are the increased susceptibility of the elderly to infectious diseases such as pneumonia and influenza and to bone fractures. The incidence and prevalence of hip fractures are related to osteoporosis, the loss of bone with ageing. Interestingly, this condition may be delayed by therapy with vitamin D, calcium and oestrogen.

What are we going to do about the diseases that will be the leading causes of death in the 21st century? We need to know more about the way we become more susceptible to specific diseases as we grow older. We need to understand the causes. We have to identify the molecules and genes involved in the disease processes in order to develop rational strategies for treatment. Equally important are new strategies for diagnosis and prevention of age-related conditions, since one of the problems with the prospective "top killer" Alzheimer's disease is the difficulty of making the diagnosis during life as early as possible. At present, it is diagnosed by exclusion. It is expected that techniques of molecular biology and genetics will provide exciting clues into the nature of age-dependent diseases and this will have a significant impact on health and welfare of older Europeans.

Recent developments in thinking about Alzheimer's disease and Down's syndrome are the results of molecular genetics. Major contributions came from the laboratories of EURAGE members (30-38). This work has substantiated that Down's syndrome represents the most common model of Alzheimer's disease and of accelerated ageing. Furthermore, the chromosomal abnormality associated with the familial form of Alzheimer's disease, when finally localized, will serve as a handle for identifying gene products or basic principles that may be involved in the molecular pathogenesis of Alzheimer's disease (36-38).

Research on Alzheimer's disease has long been characterized by an imbalance of theories and experimental data. This was partly due to the lack of appropriate animal models and to limitations set by the quality of post mortem brain tissue. The situation is now changing, and one reassuring sign has been the isolation and sequence analysis of amyloid A4 (also referred to as β-protein) that is formed in the neurofibrillary tangles, senile plaques and cerebrovascular amyloid deposits of patients with Alzheimer's disease and Down's syndrome (31-34,39,40). Subsequently, "reversed-genetics" based on the sequence of amyloid A4 has indicated that the amyloid is encoded as part of a larger protein by a gene on chromosome 21. The amyloid was shown to be derived from the part of the amyloid A4 precursor that represents the transmembrane domain and the junction piece connecting membrane domain and the extracellular domains of the precursors (34). Since the amyloid A4 precursor is an integral glycosylated membrane protein which spans the bilayer once (41), membrane injury by oxydative damage is suggested to be a primary event that preceeds the release of the small aggregating amyloid A4 subunit. Amyloid formation is therefore a secondary event that may represent selective neuronal dysfunction in Alzheimer's disease. Oxydative damage is also an event that is suggested to contribute to the acceleration of ageing in Down's syndrome (30).

The improved prognosis for life in Down's syndrome, that has resulted from control of infections and cardiac surgery, has uncovered a clinical and pathological pattern that resembles Alzheimer's disease. As such, Down's syndrome and the inherited autosomal dominant form of Alzheimer's disease represent the most useful models to study the molecular biology and genetics of Alzheimer's disease.

Research on the molecular biology and genetics of Down's syndrome and Alzheimer's disease - which we consider not only as single entities but also as models for age-related diseases in general - needs to be intensified in European countries to develop successful interventions. The emphasis is not the cure or prevention of age-related diseases. The aim is delaying its onset beyond life expec-

tancy and thus to eliminate those conditions that will otherwise become major financial, social and health-care problems in Europe in the 21st century.

Collaboration between the European groups active in research on genetics and molecular biology of age-related diseases and appropriate models such as Alzheimer's disease, Parkinson's disease and Down's syndrome needs to be intensified to face this challenge.

V MOLECULAR BIOLOGY OF CELLULAR DEFENCES

The deterioration of defence systems against adverse effects of the environment can represent a major factor in the ageing process. Much emphasis has been placed on the immune system on the organismic level in this regard. However, defence systems on the cellular level could be of equal importance. This has been recognized for DNA repair and a considerable effort has been devoted to the question whether cellular ageing is accompanied or caused by repair deficiencies. So far, no final answer has been given but most of the evidence argues against a primary defect in DNA repair in cellular ageing. Nevertheless, the deterioration of the structural and functional integrity of DNA and other macromolecules remains a plausible mechanism for ageing on the molecular level but it may result from overexposure to damaging agents which overwhelm the cellular defences rather than deficient repair. Therefore, the identification of adverse agents, whether of endogenous or xenobiotic origin, and the elucidation of the corresponding cellular defence and detoxification systems represent important topics of research in the molecular biology of ageing. Such studies include functional, biochemical and genetic aspects and major topics for future research are listed below.

Detoxification of xenobiotics

Studies on the occurrence of age-related changes as observed in oxidative drug-metabolism by the cytochrome P-450 systems, glucuronidation etc., should be initiated While many pharmacological studies have been carried out in the past, genetic investigations are now accessible with the cloning of P-450's and aryl hydrocarbon hydroxylases (42).

Antioxidant defence

Detoxification of active oxygen (superoxide, hydrogen-peroxide) and organic hydroperoxides is accomplished by CuZn- and Mn-

superoxide dismutase, catalase, glutathione-peroxidase and enzymes related to the cellular redox state (e.g. glutathione-reductase) as well as low-molecular weight radical-scavengers (e.g. cystein and glutathione). Several studies on changes in the activities of the major antioxidant enzymes as a function of ageing have been carried out in animals and in cell systems. The results, in general, have not been convincing because of experimental difficulties. The fact that the genes for these enzymes have recently been cloned and that antibodies have been produced now allows molecular analysis and genetic manipulation. The molecular approach will supply clear answers to an attractive theory of ageing which postulates that insufficient antioxidant defence results in the accumulation of macromolecular damage, in particular, damage of DNA and cell membranes (43).

Cellular genetic defence systems: cellular interfering factors and suppressor genes

Inactivation of master genes which normally serve to suppress or regulate the expression of families of "undesirable" genes may lead to cytopathology and ageing-related cellular degeneration (44). Examples for such genes derive from work with papilloma viruses where cellular factors have to be inactivated for the expression of integrated viral genetic functions, and from research of hereditary forms of cancer (e.g. retinoblastoma and Wilm's tumor) where defects in suppressor genes have been identified. The study of these and other cellular genetic mechanisms may yield important insights into the molecular biology of ageing.

Cellular anti-stress mechanisms

Cells may be exposed to many other potentially damaging conditions during ageing, besides toxic oxygen radicals. Cellular anti-stress mechanisms, such as for example the production of "heat-shock" proteins (45-50), appear to be induced by a variety of physical and chemical stressors, which may be interconnected with other

classical defence mechanisms such as DNA repair (51). The fact that the genes controlling these mechanisms appear to be highly conserved during evolution and that many of them have been cloned allows studies at the molecular level on their possible involvement in the ageing process. An analysis of such anti-stress mechanisms during ageing is also recommended by the fact that they appear to be involved in suicide mechanisms at the cellular level, which in turn may be responsible for the age-associated cell death and cell loss, in various organs and tissues.

Ageing and molecular effects of nutrition

Nutrition has profound effects on many age-related pathological processes (52-55) and an extension of the maximum lifespan in short and long lived strains can be obtained in caloric-restricted rodents, in comparison with ad libitum fed animals (56-62). Recent evidence indicates that membrane lipoperoxidation (63) and DNA repair capability (64) are positively influenced by caloric restriction, suggesting that the ageing process itself may be modulated by nutritional interventions. An alternative point of view would consider high caloric intake as the most striking factor able to decrease longevity. According to this point of view, ad libitum fed animals taken as controls in many studies should in fact be considered as animals that received high caloric intake and, paradoxically, caloric-restricted animals as animals closer to physiological nutritional conditions.

As nutritional aspects are involved in many of the above mentioned topics (e.g. detoxification of xenobiotics, antioxidant defence), and considering that nutrition affects the composition of cellular structures (membranes) and of intracellular organelles (mitochondria among others), it will be of great interest to assess the effects of nutritional factors and of different nutritional regimens at the molecular level (e.g. gene mutations, DNA repair responses, DNA rearrangements). These could be suitably studied in aging (transgenic) rodents kept under various conditions.

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VII FUTURE ACTIVITIES

On the basis of the scientific programme outlined above the institutes participating in the EURAGE molecular biology group will systematically study the molecular basis of ageing, age-related defects in physiological functions and age-related diseases. Research efforts will be collaborative. That is, information will be exchanged, tasks will be divided and biological materials such as cell lines, DNA probes, antisera and specimens from families with rare hereditable diseases will be shared. This approach will lower the costs and dramatically improve European competitiveness, for example as compared to the United States. It is anticipated that a great deal of the research will be performed in association with other EURAGE TORGs. For example, for molecular biological studies on Alzheimer's disease the complementary expertises of the molecular biology group and the group "Aging of the Brain" are required.

Further streamlining and coordination of ageing research in Europe will also open up new research areas that could thusfar only be explored in larger nations like the United States. This is especially true for studies that require materials from certain categories of individuals, such as families with specific inherited disorders or other characteristics that make them important study objects in ageing research. The necessary contacts for such collaborative studies will be guaranteed by organizing meetings that will be held frequently. To meet the high standards aimed at by the programme, international experts will be invited when necessary.

A second major task for the new EURAGE group is to provide information, reagents and certain technical facilities to EURAGE investigators. The general philosophy behind this is to encourage application of modern molecular biological techniques in the different research laboratories and clinics of EURAGE members. For example, the use of specialized equipment and facilities (e.g. chromosome flow sorting, oligonucleotide synthesis, production of transgenic animals) can be offered by certain EURAGE members. In addition, certain rea-

gents like DNA probes, monoclonal antibodies, cell lines, can be made available to those who need them.

An important aspect of the collaborative programme is the organization of courses, varying from basic level for transferring molecular biological knowledge to MD's, to advanced courses for learning special techniques. In general, such activities should remove hindrances to the application of molecular biological techniques at all levels.

Finally, the rapid improvements in molecular diagnosis of age-related disorders and the possibilities emerging from molecular biological approaches to intervene in patterns of ageing on a rational basis pose ethical problems. These will not be neglected. Indeed, the molecular biology group is determined to take its responsibility by initiating proper discussions of this topic, both during the regularly held meetings and in an open forum when that is more appropriate.

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