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Introduction: aging research comes of age

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Understanding the molecular mechanism of aging is one of the most important issues in biology. In contrast to ontogenetic process as a series of programmed phenomena, aging is an essentially stochastic phenomenon because nobody can tell when and how one will start aging. This is probably one of the difficulties peculiar to understanding aging at the genetic and molecular levels. Nevertheless, we have witnessed marked progress in aging research in the past 5 years. The aim of this multi-author review is to describe some of these epoch-making accomplishments and discuss future research directions.

Since Hayflick and Moorhead [1] reported that normal cultured human fibroblasts are mortal and are only capable of a finite number of cell doublings, mortal cells were suggested to possess a 'counting machinery' that somehow documents how many times they divided. Telomeres are thought to be involved in the counting machinery because the length of telomeres shortens progressively each time the cell duplicates [2]. Since normal somatic cells lack telomerase activity, they progressively lose telomeric repeats in each cell cycle due to 'the end replication problem' that originates from the inability of DNA polymerase to replicate the very end of the template DNA [3]. After dividing for a limited number of times (the Hayflick limit), normal cells with short telomeres stop dividing and become senescent. Interestingly, the maximum population doubling of cultured human fibroblasts decreases in proportion to donor age [4]. In addition, the maximum longevity of various species is proportional to the maximum number of population doublings that their cultured cells can attain [5]. Based on these observations, cell senescence has been suggested to be directly related to senescence of the living organism, including humans.

Research on the molecular mechanism of the 'counting machinery' and of senescence in mammals has progressed enormously in recent years. The gene for the catalytic subunit of mammalian telomerase has been cloned [6]. In addition, reconstitution of telomerase activity by expression of this gene in normal human fibroblasts extended their replicative life-span beyond the Hayflick limit [7]. These findings clearly demonstrate that telomerase activity primarily regulates replicative senescence of normal cells, at least in vitro. Research is now focusing on the molecular mechanisms for 'watching' telomere length that eventually regulate cell cycle progression, and which are also closely related to cell immortalization. F. Ishikawa discusses recent progress in telomere biology in this multi-author review.

Mice deficient in telomerase activity were recently generated by targeted disruption of the gene for the telomerase RNA component [8]. These animals ($mTR^{-/-}$) exhibit abnormalities in organs containing highly proliferative cells, such as testis and bone marrow. Spermatogenesis is defective due to both increased apoptosis and decreased proliferation of spermatocytes. The proliferative capacity of hematopoietic cells is also impaired. These results indicate that telomerase plays an essential role in replicative senescence of highly proliferative cells in vivo as well as in vitro. In addition, $mTR^{-/-}$ mice showed a reduced capacity to respond to stresses such as wound healing and hematopoietic ablation and an increased incidence of spontaneous malignancies [9]. Although $mTR^{-/-}$ mice exhibit the phenotypes that clearly reflect important aspects of aging, another important result is that they do not exhibit any of the disease states commonly observed in human aging, such as arteriosclerosis, osteoporosis, and brain atrophy. These knockout mice suggest that cell senescence induced by telomere shortening in vivo does not directly lead to the pathological changes characteristic of human aging.

From a phenotypic point of view, aging has been defined as the age-related deterioration of physiological functions necessary for the survival and fertility of an organism [10]. In humans, common age-related diseases such as arteriosclerosis, osteoporosis, dementia, and

malignant neoplasia can be regarded as extremes of age-related physiological deterioration. Therefore, central to understanding human aging is the imperative to determine how these common age-related diseases develop and to search for evidence of common or distinct molecular mechanisms. In this review, Drouet and colleagues focus on Alzheimer's disease and discuss recent progress in understanding the molecular basis of this disease.

Another approach to understanding the molecular basis of human aging has been to search for genes that determine hereditary premature-aging syndromes. Recent investigations have revealed that several members of the helicase gene family, which participate in DNA replication, transcription, and/or repair, are involved in some premature-aging syndromes including Werner syndrome [11], Cockayne syndrome [12, 13], and Bloom syndrome [14]. A defect in helicase activity in these patients results in an accumulation of somatic gene mutations and significant chromosomal instability [15]. Another premature-aging syndrome, ataxia telangiectasia, is regulated by a gene involved in cell cycle regulation at the G1 → S transition [16]. These findings have led to the suggestion that mutations associated with premature-aging syndromes may have a mutator phenotype generating further genomic instability/mutations [17]. Nakura and colleagues summarize the relationships between helicases and premature-aging syndromes.

The generation of mouse models for premature-aging syndromes using target gene replacement (gene knockout) technologies is expected to be most useful for experimental analysis of the relationship between somatic mutations and age-related diseases, with special reference to carcinogenesis. Mice carrying a mutation in the ataxia telangiectasia gene recapitulate human phenotypes, including neurologic dysfunction, infertility, malignant thymic lymphoma, and sensitivity to X-ray irradiation [18]. Mice carrying a loss-of-function mutation in the Werner syndrome gene were generated and their fibroblasts showed premature loss of proliferative capacity; however, they appear almost normal during their first year of life and longer periods of observation are required [19]. Mice with null mutations in the Cockayne syndrome genes *CSA* and *CSB* are already available [20]. If these mutant mice also display phenotypes similar to those observed in human patients, they will provide important experimental models for investigating the molecular pathophysiology of age-related disease, including carcinogenesis.

New animal models of human aging and characterization of the genetic and biochemical defects in such animals are of fundamental importance. Senescence-accelerated mice (SAM) and their substrains were, until quite recently, the only mammalian models available for human aging. These strains exhibit several aging phenotypes such as amyloidosis, osteoporosis and cataracts, among others [21]. However, these aging phenotypes are distributed among various substrains and no single SAM mouse exhibits all of them. Furthermore, their genetics is very complex and has not yet been clarified. Multiple genes are implicated in the phenotypes observed in SAM.

Numerous animal models for individual human age-related diseases have also been generated. These include mice deficient in the LDL-receptor [22] or apolipoprotein E [23, 24] for atherosclerosis, mice defective in various DNA repair genes including xeroderma pigmentosum genes [20] and in tumor suppresser genes such as *p53* [25]. Transgenic mice that overexpress mutated amyloid precursor protein for Alzheimer disease have been constructed [26] and natural mutants with spontaneous hypertension (spontaneous hypertensive rat, SHR) [27] or salt-sensitive hypertension (Dahl rat) have been identified [28]. However, until recently, no animal model that exhibits an extensive subset of these different diseases in a single individual has been available. The phenotype of multiple age-related diseases in a single individual is indispensable to animal models for human aging because this is a common and essential feature of aging in humans.

In this respect, the *klotho* mutant mouse ($KL^{-/-}$ mouse) is the first laboratory animal model for human aging in general. $KL^{-/-}$ animals manifest multiple simultaneous aging phenotypes, including short lifespan, infertility, reduced activity, arteriosclerosis, osteoporosis, atrophy of the skin, ectopic calcification and pulmonary emphysema [29]. All these phenotypes are caused by defective expression of a single gene (*klotho*). The gene encodes a novel protein of 1014 amino acids containing a signal sequence in its N terminus and a single transmembrane domain near its C terminus, indicative of a single-pass membrane protein. The extracellular domain is composed of two internal repeats that exhibit weak homology to each other. Each repeat has homology to β-glucosidase enzymes of both bacteria and plants. This is a very different class of protein from those involved in previously described premature-aging syndromes and cell senescence, which function essentially in the nucleus. Studies on the *klotho* mouse and the *klotho* gene are thus expected to provide new insights into human aging. Two articles describe *klotho*. Kawaguchi and colleagues discuss a cellular mechanism of osteopenia observed in the *klotho* mice, which shares similar pathophysiology to that of senile osteoporosis in the aged. Nagai and colleagues describe their recent data on vascular dysfunction in the *klotho* mouse and provide direct evidence that the Klotho protein is involved in a humoral signaling pathway. Understanding Klotho protein function may validate the novel concept that a humoral factor(s) can regulate aging [30].

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