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Phylogeny of age-related fitness decline in the wild and of related phenomena

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ABSTRACT

Apoptosis, the telomere-telomerase system, cell senescence and replicative senescence, a characteristic of cell senescence, are ubiquitous in eukaryotic species. Moreover, in some eubacterial species, "proapoptosis", a type of cell suicide, is determined by molecules homologous to apoptotic proteins, suggesting a common phylogenetic origin.
The sophisticated mechanisms and regulators underlying these phenomena are genetically determined.
A common feature is that they are always harmful for the individual cell or for the multicellular organism or for the single cell in a multicellular organism in which they act. However, they are probably advantageous for kin cells or individuals.
In particular, in some eukaryotic species, a significant effect is that they may cause, in natural conditions, an age-related fitness decline, which is also referred to as "aging", an imprecise term.
Here I suggest that their evolutionary meanings lie in kin selection, and the analogies between their action in monocellular and multicellular eukaryotes are underlined.
A phylogenetic reconstruction based on their adaptive meanings is proposed.

Keywords: IMICAW, IMICAC, aging, telomere, telomerase, apoptosis, proapoptosis

Preliminary remarks

Some preliminary consideration are indispensable to avoid misunderstandings.
The phenomenon of an "increasing mortality with increasing chronological age in populations in the wild" ("IMICAW" [Libertini, 1988]), alias "actuarial senescence in the wild" [Holmes and Austad, 1995], alias "age-related fitness decline in the wild", is a real and well documented phenomenon [Deevey, 1947; Laws and Parker, 1968; Spinage, 1970, 1972; Finch, 1990; Holmes and Austad, 1995; Ricklefs, 1998].
By definition, according to its presence in wild conditions, IMICAW phenomenon is subject to natural selection and should not be mixed up with the "increasing mortality with increasing chronological age in captivity" ("IMICAC" [Libertini 1988]), which is found in laboratory conditions at ages not existing in the wild for species that in natural conditions do not show IMICAW phenomenon. By definition, according to its absence in wild conditions, IMICAC is not subject to natural selection. In particular, the "fitness" in "age-related fitness decline in the wild" definition is unsuitable to the artificial conditions defined in IMICAC concept.
This paper regards only IMICAW, alias aged-related fitness decline, and related phenomena and not IMICAC phenomenon. This remark is important as in current scientific literature and in the prevailing ideas about age-related fitness decline both phenomena are confused in a single imprecise term, namely "aging" (or "senescence").
The concepts and the results referred to "aging" in its imprecise meaning but, in fact, to IMICAC phenomenon (e.g., the numberless papers regarding the survival in laboratory conditions and at ages not existing in the wild of *C. elegans* and *D. melanogaster*) will

not be considered in this paper, not for inaccuracy or for the sake of brevity but as not regarding the topic.
Moreover, in this paper, the term "aging" will be used only making reference to current ideas where a precise meaning is not defined.

INTRODUCTION

If species separated by different evolutionary histories of hundreds of millions of years show equal or similar features that are clearly of common phylogenetic origin, it is necessary to inquire about an equality or analogy of functions explaining their evolutionary persistence and similarity.
Phenomena such as apoptosis, the telomere-telomerase system, cell senescence and replicative senescence (that is, in relation to the number of cell replications, in a single cell: abrupt decline of cell functions and block of duplication capacities; in a cell culture: overall progressive decline of cell functions and of duplication capacities), which will be discussed in the next section, exist in yeast, a monocellular eukaryote, and in multicellular eukaryotic species with a divergent evolutionary history at least from the beginning of Cambrian period, about 600 millions of years ago [Minkoff, 1983].
Moreover, "proapoptosis" [Hochman, 1997], a form of eubacterial cell suicide with mechanisms clearly related to eukaryotic apoptosis [Koonin and Aravind, 2002], indicates a much older evolutionary persistence and similarity.
The present paper expounds the main common features of these phenomena, trying to explain their general evolutionary meanings and phylogenetic relations.

EMPIRICAL EVIDENCE

A) LIMITS IN DUPLICATION CAPACITIES

A-1) In multicellular eukaryotes

Normal eukaryotic non-germ cells of multicellular organisms with limited lifespan can, in general, duplicate themselves only a limited number of times both *in vitro* [Hayflick, 1965; Hayflick and Moorhead, 1961] and *in vivo* [Schneider and Mitsui, 1976]. This phenomenon (Hayflick limit), well documented for many types of cells [Rheinwald and Green, 1975; Bierman, 1978; Tassin et al., 1979], shows an inverse relation with the ages of donors of origin [Martin et al., 1970] and, with exceptions that will be discussed later, a rough direct correlation with the life span of the species from which cells are derived [Röhme, 1981].
The main cause of the phenomenon, for many years known to be caused by something acting in the nucleus [Wright and Hayflick, 1975], was suggested to be a result of the incomplete action of DNA polymerase, which at each duplication leaves out a part of the terminal portion of DNA, the telomere [Watson, 1972]. This incomplete replication leads to a progressive shortening of the DNA molecule with a related increase in duplication impairment [Olovnikov, 1973].
Telomeres are highly conserved repetitive sequences of DNA (e.g., TTGGGG in a protozoan [Blackburn and Gall, 1978], TTAGGG in mammals [Moyzis et al., 1988] and many other species [Blackburn, 1991]). Telomeres shorten with every duplication event [Harley et al., 1990], but an enzyme, telomerase, can elongate telomeres at each replication, thereby compensating for the incomplete action of DNA polymerase. The action of telomerase explains why some cells, such as those of germ line, have unlimited duplication capacities [Greider and Blackburn, 1985]. With telomerase deactivation, telomeres shorten at each duplication and, in a cell culture or in a tissue, overall duplication capacity is reduced [Yu et al., 1990]. On the other hand, telomerase

activation elongates telomeres and cells become capable of numberless duplications [Bodnar et al., 1998; Counter et al., 1998; de Lange and Jacks, 1999; Vaziri, 1998; Vaziri and Benchimol, 1998]. Moreover, active telomerase was demonstrated in immortal human cell lines [Morin, 1989], while in other cells it was proven to be repressed by regulatory proteins [van Steensel and de Lange, 1997].
In a cell culture, the final incapability of a cell to duplicate (replicative senescence) was shown not to be an abrupt event for all the cells at the same time, but a progressive reduction of cell culture growth potential that depended on the reduction of telomere length [Jones et al., 1985; Pontèn et al., 1983].
According to Blackburn's model [Blackburn, 2000], particular protective nucleoproteins cap telomeres, which oscillate between capped and uncapped conditions: the duration of the first state directly correlates with telomere length while the other state is vulnerable to the passage to "noncycling state" or final stage of replicative senescence (fig. 1).

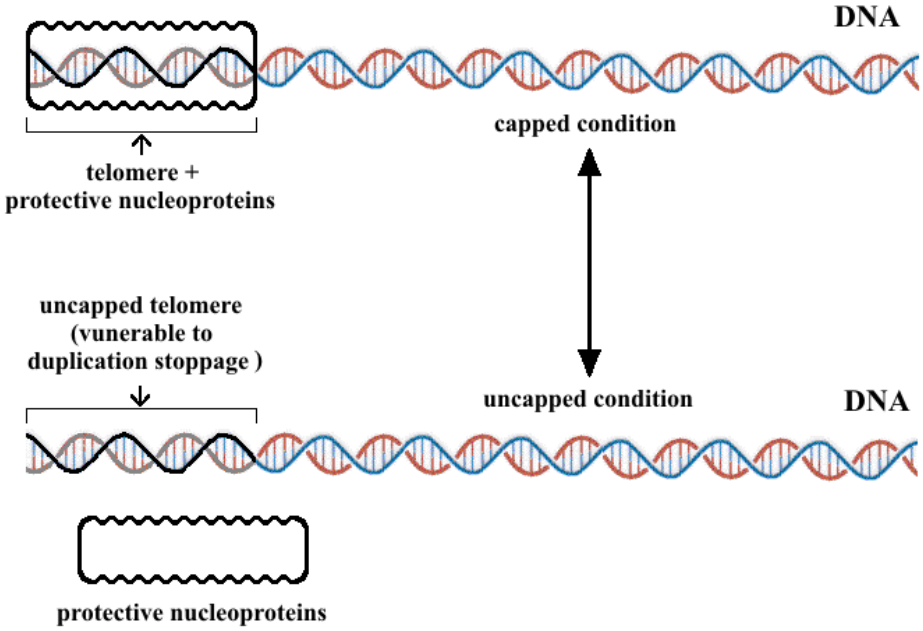

Figure 1 – Telomere oscillates between capped and uncapped conditions. The probability of uncapped condition increases at each duplication in relation to telomere shortening. Uncapped telomere acts as a broken end that can cause an end-to-end joining and a block of cell duplications.

A population of cells with telomeres at their maximum length, but inactivated telomerase, shows a progressive decline in replication capacities. Even cells with telomerase activated and so telomeres constantly at maximum length, should show a small percentage of cells passing to noncycling state at each division. Moreover, it has been proposed that stem cells, unlike germ cells, have levels of telomerase activity that are only partially able to stabilise telomere length [Holt et al., 1996] and for this reason they cannot indefinitely replace the apoptotic elements for cell populations in renewal [Fossel, 2004].
The absolute length of telomeres does not enable one to predict a species life span. Species, such as the mouse and the hamster have long telomeres [Slijepcevic and Hande, 1999], yet they age more precociously than species such as man, which have shorter telomeres. Moreover, in rodents, telomerase activity is not related to maximum lifespan [Gorbunova et al., 2008]. However, Blackburn's hypothesis does not postulate for different species a fixed ratio between telomere length or telomerase activity and the stability of telomere-capping nucleoproteins complex: it is easy to suppose that the

stability of the complex and, in general, the modulation of telomere-telomerase system is different from species to species. What is likely important is the species-specific critical telomere length and the relative rather than absolute telomere shortening [Fossel, 2004].
In relation to the mean number of duplications in cell culture or in a tissue, there is an increasing probability of cell senescence, a "fundamental cellular program" [Ben-Porath and Weinberg, 2005], which is characterized by an altered expression of many genes usually active in the cell, compromising cell overall functionality, and by replicative senescence. A senescent cell has deleterious consequences on the extracellular matrix as well as other cells that are physically near or physiologically interdependent. Cell senescence, and replicative senescence that is one of its characteristic, certainly derive somehow from the relative shortening of telomere (Fossel's "cell senescence limited model") [Fossel, 2004].
About the mechanism underlying cell senescence:
"One model of telomere-gene expression linkage is an altered chromosomal structure (Ferguson et al., 1991), such as a heterochromatin 'hood' that covers the telomere and a variable length of the subtelomeric chromosome (Fossel, 1996; Villeponteau, 1997; Wright et al., 1999). As the telomere shortens, the hood slides further down the chromosome (the heterochromatin hood remains invariant in size and simply moves with the shortening terminus) or the hood shortens (as the telomere is less capable of retaining heterochromatin). In either case, the result is an alteration of transcription from portions of the chromosome immediately adjacent to the telomeric complex, usually causing transcriptional silencing, although the control is doubtless more complex than merely telomere effect through propinquity (Aparicio and Gottschling, 1994; Singer et al., 1998; Stevenson and Gottschling, 1999). These silenced genes may in turn modulate other, more distant genes (or set of genes). There is some direct evidence for such modulation in the subtelomere ..." [Fossel, 2004].
These statements are largely based on experiments in yeast, but possible deductions for monocellular eukaryotes must consider the invariability of telomere length with duplications in these organisms (see next paragraph).
On the other hand, the likelihood that a mechanism of this type is true for multicellular eukaryotes is widely discussed by Fossel (see pages 45-56 in Fossel, 2004; a plausible scheme is illustrated in fig. 2).

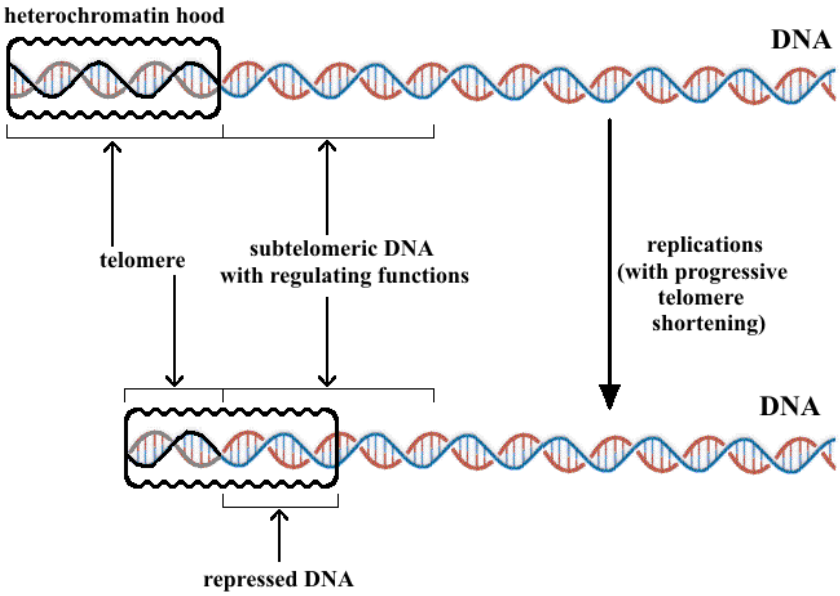

Figure 2 – The expression of many genes is impaired in relation with telomere progressive shortening. As likely hypothesis, a subtelomeric DNA tract regulates overall cell functionality and its action is impaired by the progressive sliding of the heterochromatin 'hood' caused by telomere shortening [Fossel, 2004].

Heterochromatin 'hood' [Fossel, 2004] and capping nucleoproteins [Blackburn, 2000] are most likely the same thing because: 1) they are supposed in the same part of the chromosome; 2) telomerase activation and the consequent telomere lengthening cause the reversal both of manifestations of cell senescence and of replicative senescence [Bodnar et al., 1998; Counter et al., 1998; de Lange and Jacks, 1999].
For germ line cells and for donor somatic cells that originate a cloned animal, the resetting of telomere clock is indispensable [Fossel, 2004]. The starting length of telomere must be established as with each subsequent shortening of the telomere, the probability of cell senescence and replicative senescence will increase. The absolute value of "telomere length is irrelevant" [Fossel, 2004]: two *Mus* strains with different telomere length (10 and 20 kb, respectively) show the same life span and an equal timing of cell senescence; analogously the same is true for donor animals and for cloned animals derived from cells with shortened telomeres [Fossel, 2004]. An appropriate shaping of the heterochromatin hood depending on telomere length could explain the equal timing of cell senescence and replicative senescence in spite of different telomere lengths (fig. 3).

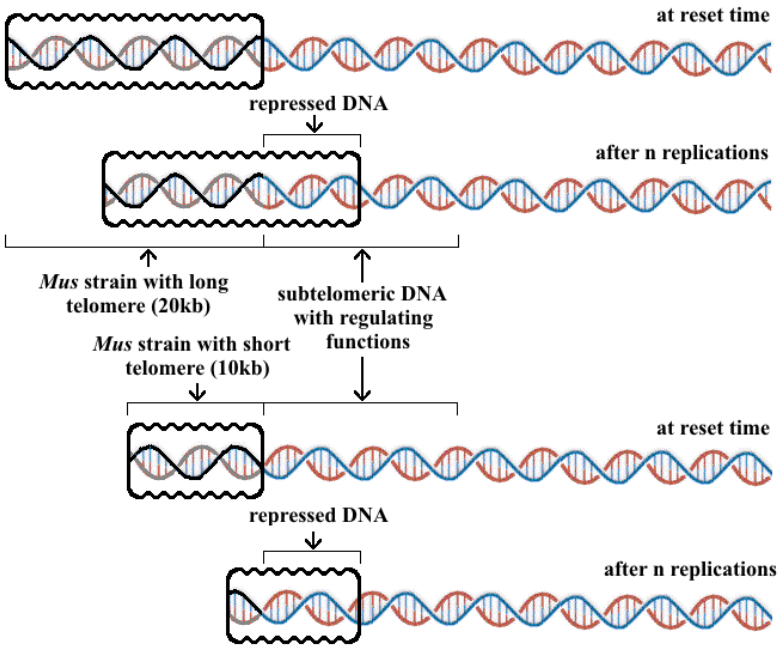

Figure 3 – In the reset of telomere clock, the heterochromatin hood is shaped proportionally to telomere length and does not vary for all the cell life. Telomere shortening in relation to the number of duplications causes the sliding of heterochromatin hood over subtelomeric DNA that regulates both overall cell functionality and telomere capped / uncapped condition equilibrium. The progressive repression of subtelomeric DNA increases the degree of cell senescence and the probability of replicative senescence. This hypothetical model could explain the large irrelevance of initial telomere length for the consequences of its subsequent shortening [Fossel, 2004].

Mice and other animals have a shorter life span, despite a baseline telomerase activity in most somatic cells [Prowse and Greider, 1995] and much longer telomeres than our species [Slijepcevic and Hande, 1999]. (But, in mice microglia cells, telomeres shorten with age and "the low levels of telomerase activity present may be preferentially

recruited to maintain the shortest telomeres while allowing the longer ones to shorten more rapidly" [Flanary, 2003].) Moreover, in knockout (mTR-/-) mice, which have telomerase genetically inactivated, only after four [Herrera et al., 1999] to six [Blasco et al., 1997] generations, with very shortened telomeres, fertility and viability are jeopardized, although organs with high cell turnover show dysfunctions in early generations [Herrera et al., 1999; Lee et al., 1998]. The model of fig. 3, as expounded in fig. 4, could explain this apparently paradoxical phenomenon.

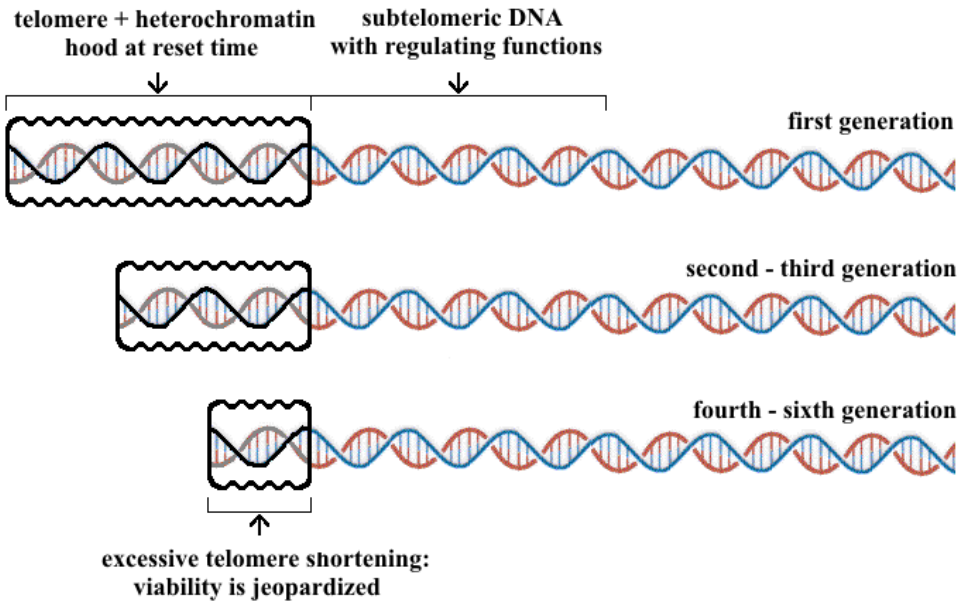

Figure 4 – For the model of fig. 3, the length of heterochromatin hood in knockout mice, defined in the reset phase, is proportional to telomere length. Subsequent sliding of the heterochromatin hood over subtelomeric DNA and the consequent genetic repression is independent from the length of the hood. With an excessive telomere shortening, the mechanism is compromised and cell viability is lost. The short life span of mice and other species with long telomeres is explained by a species-specific low degree of telomere + heterochromatin hood complex stability.

Subtelomeric DNA appears to have both a pivotal importance for overall cell functionality and a position vulnerable to inactivation by telomere shortening itself. Excluding the possibility of an absurd evolutionary illogicality, this coincidence can be explained only as something favoured by natural selection to determine cell senescence and replicative senescence. A possible scenario for the evolution of the telomere-cell senescence system is proposed below.

A-2) In a monocellular eukaryote

Yeast (*Saccharomyces cerevisiae*), a well studied eukaryotic monocellular species, reproduces by asymmetric division between mother and daughter cells. The mother lineage can reproduce a limited number of times only, specifically between 25 and 35 generations in about 3 days [Jazwinski, 1993].
Both in mother and daughter yeast cells, telomere length does not decrease with duplications [D'Mello and Jazwinski, 1991; Smeal et al., 1996]. "Budding [= daughter] yeast cells express telomerase and divide indefinitely." [Maringele and Lydall, 2004]

In mother cells of wild-type yeast, extrachromosomal ribosomal DNA circles (ERCs) accumulate in proportion to the number of duplications [Sinclair and Guarente, 1997] and "several lines of evidence suggest that accumulation of ERCs is one determinant of life span" [Lesur and Campbell, 2004].
ERCs, or some other unknown factor, interfere with gene expression and mutants such as *dna2-1*, which show abnormalities in the replication of DNA and therefore increased rates of ERCs accumulation, suffer by precocious alterations of gene expression. Specifically, transcriptome of older (18-generation-old) individuals of wild-type yeast are similar to those of young (8-generation-old) individuals of *dna2-1* mutants [Lesur and Campbell, 2004].
Telomerase-deficient mutants (tlc1Δ mutants) show, both in mother and daughter cells, telomere shortening. Additionally, older individuals of daughter cell lineages, which have no ERCs accumulation, show an overall expression of genes (transcriptome) similar to that of older individuals of wild-type yeast, and of young individuals of *dna2-1* mutants [Lesur and Campbell, 2004]. It is possible that in telomerase-deficient yeast mutants, as in cells of multicellular eukaryotes, telomere shortening causes the sliding of a telomere heterochromatin hood that interferes with a critical part of subtelomeric DNA, while in wild-type yeast subtelomeric DNA is somehow repressed by ERCs, or by some other unknown factor (fig. 5).
In old yeast cells, besides the replicative senescence, there are increasing metabolic alterations [Lesur and Campbell, 2004], which can be defined as cell senescence.

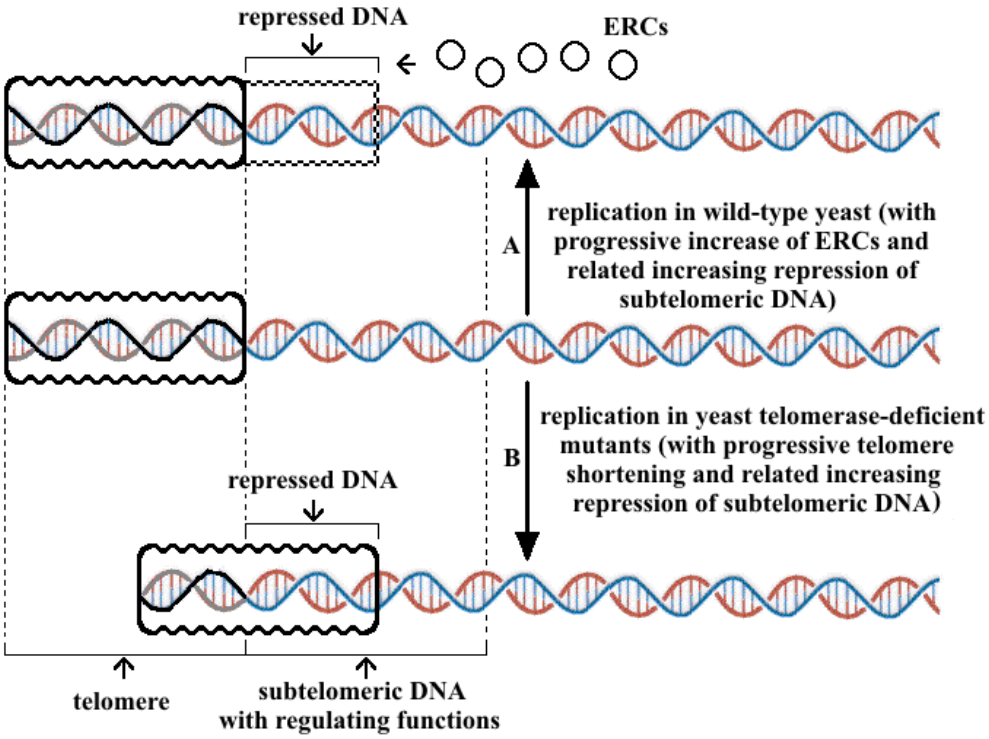

Figure 5 – A) In wild-type yeast, ERCs, or some other unknown factor increasing at each duplication, interfere with subtelomeric DNA, e.g. adding something to the telomere heterochromatin hood. This process is accelerated in *dna2-1* mutants; B) In telomerase-deficient mutants, in daughter cell line without ERCs accumulation, telomere shortening cause a sliding of telomere heterochromatin hood with subtelomeric DNA repression similar to that of case A.

B) APOPTOSIS

In contrast with necrosis, which is the cell death caused by acute cellular injury, apoptosis is an ordered form of cell self-destruction, which is ubiquitous in eukaryotic species [Longo et al., 2005].

Apoptosis was characterised and clearly differentiated from necrosis for the first time during observations of normal liver hepatocytes [Kerr et al., 1972]. It is described as a definite series of biochemical events leading to specific morphological changes (blebbing, loss of membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, etc.).

B-1) In multicellular eukaryotes

Programmed cell death by apoptosis, selectively triggered for some cells in specific times, is essential for morphogenetic mechanisms (e.g., embryo neural development [Nijhawan et al., 2000], wound healing [Greenhalgh, 1998]), lymphocyte selection [Cohen, 1993; Opferman, 2008], cell turnover in healthy adult organs [Israels and Israels, 1999; Lynch et al., 1986; Medh and Thompson, 2000; Wyllie et al., 1980] (as documented for many tissues and organs [Libertini, 2006]), removal of damaged or infected cells [Tesfaigzi, 2006; White, 2006], etc.

Apoptotic cellular debris does not damage other cells because phagocytes remove such cell fragments in an orderly manner without eliciting an inflammatory response [Erwig and Henson, 2008].

Inactivated telomerase and short telomeres increase the probability of apoptosis [Fossel, 2004; Ozen et al., 1998; Holt et al. 1999; Seimiya et al., 1999; Ren et al., 2001].

B-2) In a monocellular eukaryote

In yeast, a phenomenon closely resembling apoptosis of multicellular eukaryotes was described quite recently [Madeo et al., 1997]. It was soon evident that the overexpression of human Bcl-2, an apoptosis inhibiting factor, in yeast delays processes leading to the phenomenon [Longo et al., 1997], while the overexpression of an apoptosis inducing factor in mammalians (BAX) could elicit it [Ligr et al., 1998].

A growing body of evidence has documented similarities between this phenomenon in yeast and apoptosis in multicellular eukaryotes, to the extent that both deserve the same name. This data thus suggest that the two phenomena share a common phylogenetic origin [Kaeberlein et al., 2007; Longo et al., 2005; Madeo et al., 1999]: "... since the first description of apoptosis in a yeast (*Saccharomyces cerevisiae*) strain carrying a CDC48 mutation (Madeo et al., 1997), several yeast orthologues of crucial mammalian apoptotic proteins have been discovered (Madeo et al., 2002; Fahrenkrog et al., 2004; Wissing et al., 2004; Qiu et al., 2005; Li et al., 2006; Walter et al., 2006), and conserved proteasomal, mitochondrial, and histone-regulated apoptotic pathways have been delineated (Fig. 1; Manon et al., 1997; Ligr et al., 2001; Ludovico et al., 2002; Fannjiang et al., 2004; Ahn et al., 2005a; Gourlay and Ayscough, 2005; Pozniakovsky et al., 2005)." [Büttner et al., 2006]

In yeast, there is an increasing vulnerability to apoptosis and replicative senescence, when the number of duplications increases, together with the metabolic alterations of cell senescence [Büttner et al., 2006; Fabrizio and Longo, 2008; Herker et al., 2004; Laun et al., 2001]. The age-related death rate increments in yeast follow exponential dynamics [Laun et al., 2007], as they also do for multicellular organism [Ricklefs, 1998].

Apoptosis is also triggered or favoured by: a) unsuccessful mating [Büttner et al., 2006]; b) dwindling nutrients [Granot et al., 2003]; c) chemical alterations [Madeo et al., 1999]; and d) killer toxins secreted by competing yeast tribes [Büttner et al., 2006].

When a yeast individual dies by apoptosis, cellular fragments do not damage other cells and are usefully phagocytised by other cells, which, consequently, "are able to survive longer with substances released by dying cells" [Herker et al., 2004].
A schematic comparison between apoptosis in yeast and multicellular eukaryotes is illustrated in fig. 6.

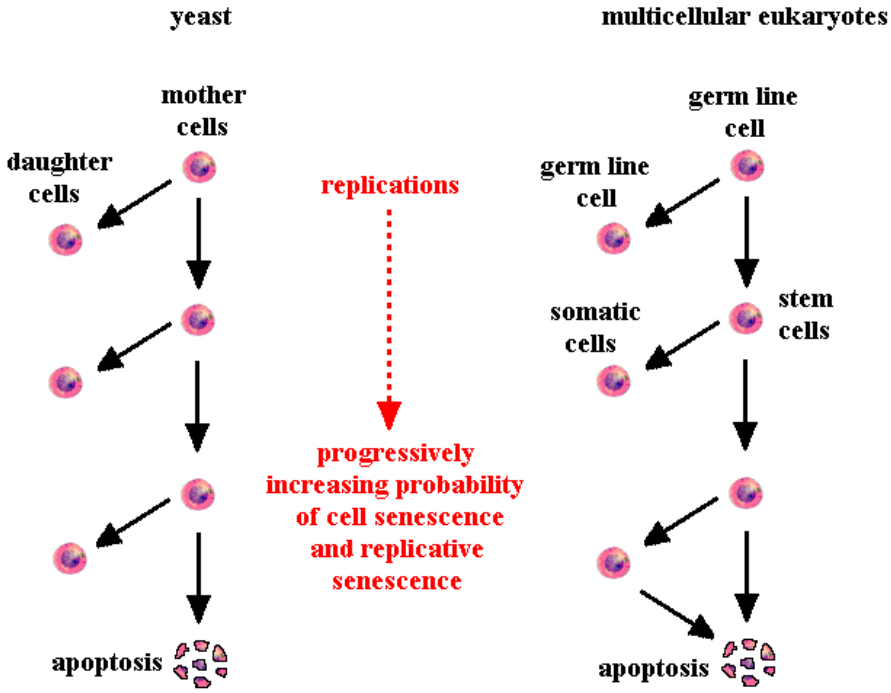

Figure 6 - A schematic comparison for apoptosis between yeast and multicellular eukaryotes.

EVOLUTIONARY INTERPRETATIONS

C) IN MONOCELLULAR EUKARYOTES

C-1) Apoptosis

Apoptotic patterns in yeast have been interpreted as adaptive because they are useful to the survival of the clone, which is likely made up of kin individuals [Fabrizio et al., 2004; Herker et al., 2004; Longo et al., 2005; Mitteldorf, 2006; Skulachev, 1999, 2002, 2003; Skulachev and Longo, 2005]. An exception is apoptosis triggered by toxin secreted by competing yeast tribes, where apoptotic mechanisms are exploited by competitors for increasing their fitness [Büttner et al., 2006].
The adaptive hypothesis appears plausible when a species is divided in many small demes, each of which is made up of one or a few clones, previously derived from as many individuals, and in conditions of K-selection, that is with population size "at or near [or over] carrying capacity of the environment" [Pianka, 1970]. In fact, in such conditions, the sacrifice of part of the population increases the survival probabilities of the remaining individuals, which are kin individuals (coefficient of relationship, *r*, equal to 1 in the case of a deme made up of a single clone, and greater than zero in the case of a deme made up of few clones). In terms of inclusive fitness [Hamilton, 1964, 1970; Trivers, 1971; Trivers and Hare, 1976], suicide individuals - by action of a hypothetical gene C - reduce their individual fitness but increases it for surviving kin individuals, in which there is a probability *r* of the existence of a copy of C. The inclusive fitness of C (F_C) is given by the sum of individual fitness reduction of suicide individuals plus the

sum of individual fitness increase of survivors each multiplied by the probability that C is present in them:

$$FC = \sum_{x=1}^{n_1} (r_x S_x) + \sum_{x=1}^{n_2} (-S'_x) \quad (1)$$

where n_1 = number of surviving individuals; S_x = advantage for a surviving individual; r_x = coefficient of relationship between a surviving individual and suicide individuals; n_2 = number of suicide individuals; $-S'_x$ = disadvantage for each suicide individual.
If F_C is positive, C is favoured by selection.
The possibility that the suicide of an individual, called "phenoptosis" by analogy to the term apoptosis [Skulachev, 1999], is also favoured by natural selection in prokaryote organisms, is necessary to explain the existence of "programmed death in bacteria" [Lewis, 2000; Skulachev, 2003]: e.g., bacterial phytoplankton mass suicide as defence against viruses [Lane, 2008], bacterial suicide triggered by phage infection "thereby curtailing viral multiplication and protecting nearby E. coli from infection" [Raff, 1998] and the "built-in suicide module" activated by antibiotics in E. coli [Engelberg-Kulka et al., 2004]. Interestingly, these mechanisms have been defined as "proapoptosis" and hypothesised as phylogenetic precursors of eukaryotic apoptosis [Hochman, 1997], as they share with it various features: "Several key enzymes of the apoptotic machinery, including the paracaspase and metacaspase families of the caspase-like protease superfamily, apoptotic ATPases and NACHT family NTPases, and mitochondrial HtrA-like proteases, have diverse homologs in bacteria, but not in archaea. Phylogenetic analysis strongly suggests a mitochondrial origin for metacaspases and the HtrA-like proteases, whereas acquisition from Actinomycetes appears to be the most likely scenario for AP-ATPases. The homologs of apoptotic proteins are particularly abundant and diverse in bacteria that undergo complex development, such as Actinomycetes, Cyanobacteria and alpha-proteobacteria, the latter being progenitors of the mitochondria." [Koonin and Aravind, 2002].

C-2) Cell senescence and replicative senescence

In yeast, increasing vulnerability to apoptosis in relation to the number of duplications, a feature of cell senescence [Fabrizio and Longo, 2008; Herker et al., 2004], determines, or contributes to determining, which cells will die in conditions in which the sacrifice of part of the population may allow the survival of the others.
Cell senescence and replicative senescence may be explained by a mechanism similar to that justifying apoptosis but with a different evolutionary advantage. In fact, Büttner et al. suggested that "apoptosis coupled to chronological and replicative aging limits longevity that would maintain ancient genetic variants within the population and, therefore, favor genetic conservatism." [Büttner et al., 2006]
This is not a new argument. Yeast ecological life conditions, if they are of the K-selection type, allow one to hypothesise that cell senescence and replicative senescence are adaptive and explainable with the same evolutionary mechanism proposed for age-related fitness decline in multicellular species subject to K-selection [Libertini, 1988, 2006]. This is the same above-mentioned suggestion of Büttner et al., but formulated in terms of individual selection.
In short, the diffusion of a gene G is dependent both on its advantage S over a neutral allele and on the inverse of the mean duration of life (*ML*), or generation time (fig. 7).

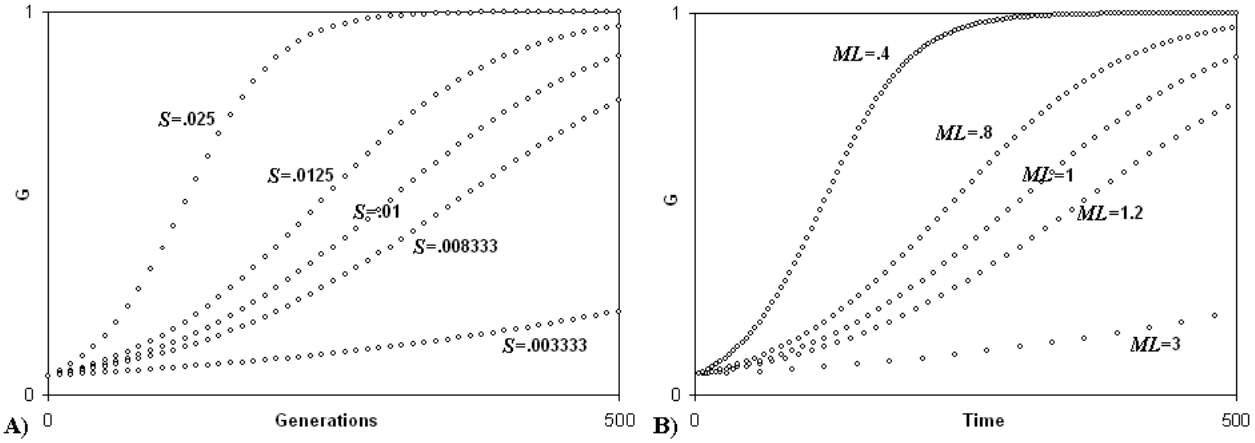

Figure 7 - A) Spreading of a gene (G) according to the variation of *S*; B) Spreading of a gene according to the variation of *ML*. An increase / decrease of *S* or of 1/*ML* have the same effects on the spreading of a gene G within a species [Libertini, 2006].

A gene C that causes the premature death of an individual I, where C is present, and so reduces its *ML* and causes a disadvantage *S'*, accelerates the spreading of any favourable gene in the individual (I') that takes the place of I. If I' is kin to I, the inclusive fitness (F_C) will be positive and C will be favoured by selection, if:

$$F_C = r \cdot \sum_{x=1}^{n} (S_x) \cdot (1/ML_C - 1/ML_{C'}) - S' > 0 \qquad (2)$$

where: ML_C and $ML_{C'}$ are the *ML* of individuals with the gene C and the neutral allele C', respectively; $\Sigma(S_x)$ is the summation notation of the advantages of the *n* favourable genes spreading within the species; *-S'* is the disadvantage of a smaller *ML*; *r* is the mean coefficient of relationship between I and I'. (The use of kin selection to explain the age-related fitness decline should not be confused with the use of the same type of selection to explain the survival in the post-reproductive period, as suggested in other papers [Lee, 2008].)

This hypothesis was formulated for multicellular organisms, but there is no theoretical argument against its application to monocellular eukaryotes. Büttner et al. do not express alternative evolutionary explanations for cell senescence and replicative senescence besides the above-mentioned suggestion [Büttner et al., 2006], which is a short reformulation of the theory described.

In contrast with this hypothesis, Lewis argues against the "suggestion that yeast cells provide a precedent for programmed death" [Lewis, 2000], proposed by others AA. [Sinclair et al., 1998], with the following observation: if a yeast cell of the mother lineage dies after *n* duplication (*n* = 25-35 in laboratory conditions [Jazwinski, 1993]), the death of a single individual among 2^n descendants (= 10^7-10^{10} individuals) appears insignificant for any theory of programmed death that is somehow favoured by natural selection. In fact, in natural conditions the probability that an individual of the mother lineage dies by apoptosis after *n* duplications is practically zero and the phenomenon, being observable in laboratory conditions only, cannot have selective value. However, this argument misses a pivotal point: it is important not the death after *n* duplications of a single individual among innumerable descendants, but the exponentially progressive - in relation to the number of duplications - increasing probability of apoptosis, coupled with a difference in mortality rates and capability of having offspring between "younger" and "older" individuals ("in a population of [yeast] cells the lifespan distribution follows the Gompertz law" [Laun et al., 2007], that is an age-related

exponential increase of mortality; "The probability that an individual yeast cell will produce daughters declines exponentially as a function of its age in cell divisions or generations (Jazwinski et al., 1998)." [Lesur and Campbell, 2004]) and, therefore, a faster generation turnover caused by the preferential death of "older" individuals. If cell senescence and replicative senescence manifest themselves in natural conditions and reduce significantly wild yeast *ML*, Lewis' objection does not invalidate the hypothesis that yeast fitness decline related to duplication number may have a selective value and may be favoured by natural selection. However, Lewis' objection is very interesting because it echoes a similar argument against programmed aging theories for multicellular organisms that will be discussed in the next section.

D) IN MULTICELLULAR EUKARYOTES

D-1) Apoptosis

In multicellular organisms, apoptosis is essential for many physiological functions as outlined above. The evolutionary justification for these phenomena is evident and will not be discussed.

D-2) Cell senescence and replicative senescence

As underlined in the preliminary remark, an age-related increasing mortality, or fitness decline, is documented for many species in wild conditions (fig. 8).

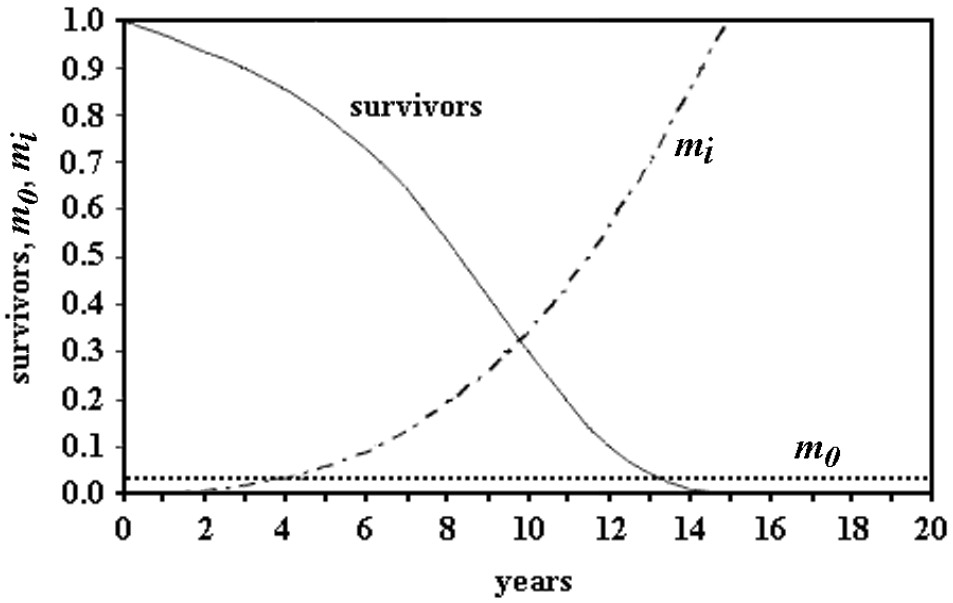

Figure 8 - An example of species with age-related fitness decline in the wild. Life table of *Panthera leo* after the early stages of life: survivors, basal mortality (m_0) and age-related increasing mortality (m_i). Weibull's equation ($m_t = m_0 + \alpha \cdot t^{\beta}$) and data ($m_0 = 0.032$, $\alpha = 2.52E\text{-}4$, $\beta = 3$), utilised to define the curves, are from Ricklefs [Ricklefs, 1998].

A plausible mechanism for this fitness decline is the progressive slowdown of cell turnover, that is a progressive prevalence of programmed cell death (PCD), by apoptosis or other forms of PCD, on cell substitution by duplication of stem cells (Fossel's "cell senescence general model of aging" [Fossel, 2004; Libertini, 2006]). A hypothesis of this type was suggested for the first time by Weissmann [Kirkwood and Cremer, 1982] while the concept of senescence as a result of decrease in cellularity of organs was discussed by Szilard [Szilard, 1959], although in the context of a theory that attributed

the cell loss to the accumulation of somatic mutations. In support of this thesis, for some species, as Rockfish and lobsters, both telomere length and mortality rate are unvaried with the age [Klapper, Heidorn et al., 1998; Klapper, Kühne et al., 1998].
There is empirical evidence for an adaptive meaning of the age-related fitness decline phenomenon [Libertini, 2008], which in its more advanced expression, common in protected conditions, is usually called 'aging', an imprecise term [Libertini, 2006]. A theory, the same as the above-mentioned to elucidate cell senescence and replicative senescence in yeast, explains this fitness decline as evolutionarily advantageous by a mechanism of kin selection that, in consequence of a quicker generation turnover, allows a faster spreading of any advantageous mutations. According to this theory, the advantage exists in conditions of K-selection (species divided in demes, populated by kin individuals, and with saturated habitats in which only the death of an individual gives space to a new individual) [Libertini, 1988, 2006].
The main objection against this theory, analogous to Lewis' argument above-mentioned, is that "As a rule, wild animals simply do not live long enough to grow old. Therefore, natural selection has limited opportunity to exert a direct influence over the process of senescence." [Kirkwood and Austad, 2000]. This objection, analogous to Lewis' argument, misses a pivotal point: the existence or absence in the wild of "old" individuals (e.g., individuals of *P. leo* older than 15 years) is not important. Individuals of *P. leo* younger than 15 years are "not old" individuals, yet they show an increasing fitness reduction at ages present in the wild: this significantly reduces *ML* with a consequent faster generation turnover and a possible selective advantage.
"Senescence reduces average life span ... by almost 80% when $m_0 = 0.01\ yr^{-1}$" [Ricklefs, 1998]. For the fraction of a population that survived the high mortality risk of the early stages of life, the ratio between the residual *ML*s without and with age-related increasing mortality has been estimated to be in the range 2.5-5 for eight mammal species in wild conditions. Without the subtraction of the early stages of life, the ratio has been estimated in the range 1.55-3.21 [Libertini, 1988].
In short, in wild conditions, *ML* reduction caused by age-related increasing mortality is not irrelevant, although the equivalents of septuagenarian or older men for animal species are likely inexistent in the wild.

PHYLOGENETIC CORRELATIONS

The empirical evidence and the above-mentioned arguments suggest a phylogenetic correlation between phenomena observed in colonies of kin yeast cells and analogous phenomena in multicellular organisms. These phenomena require the formulation of a general phylogenetic hypothesis of apoptosis, telomere-telomerase system, cell senescence, replicative senescence and the age-related fitness decline, which is commonly but imprecisely called "aging". Correlated phenomena in bacteria must be also considered in the phylogenetic model.
In particular (see Table 1 and fig. 9):
a) Phenomena described in eubacteria as "proapoptosis", activated in particular conditions and probably favoured by the mechanism of kin selection (e.g., for bacterial phytoplankton: "As most plankton in a bloom are near identical genetically, from the perspective of their genes, a die-off that creates enough scorched earth to stop the viral advance can make sense" [Lane, 2008]), have been interpreted as plausible phylogenetic precursors of eukaryotic apoptosis [Hochman, 1997]. Proapoptosis, a form of "suicide useful in critical conditions", is necessarily derived from a previous condition in which this pattern was inexistent.
b) Eubacteria evolved in monocellular eukaryotes. Apoptosis of monocellular eukaryotes, likely derived from a form of eubacterial proapoptosis, is in yeast triggered

by starvation, damaged cell conditions, unsuccessful mating, etc. In these cases, it is favoured by kin selection because cell suicide increases survival probability of kin cells [Herker et al., 2004] ("suicide useful in critical conditions").
c) Both for proapoptosis and for apoptosis, a mechanism that triggers the suicide pattern, but kills only a part of the population - proportional to the severity of stress condition - is indispensable. Yeast evolved an efficient mechanism based on the number of previous duplications and a telomere-telomerase-ERCs clock [Büttner et al., 2006; Fabrizio and Longo, 2008; Herker et al., 2004; Laun et al., 2007].
d) Apoptosis of multicellular eukaryotic species has a clear phylogenetic relationship with monocellular eukaryotic apoptosis [Longo and Finch, 2003]. In most species, the evolved clock does not use ERCs [Fossel, 2004]. Considering each multicellular individual as a clone having all cells with the same genes (coefficient of relationship, r, equal to 1) but with differentiated functions, apoptosis of less fit cells may be considered as favoured by analogous mechanisms of kin selection.
e) In multicellular organisms, apoptosis as part of morphogenetic mechanisms (e.g., embryogenesis, tissue development or reshaping, tissue turnover) and of lymphocyte selection is clearly a derived function, being impossible in monocellular organisms;
f) In yeast, apoptosis, cell senescence and replicative senescence, genetically determined by mechanisms based on telomere-telomerase system, appear to contrast "genetic conservatism" [Büttner et al., 2006]. Furthermore, these phenomena might be explained as favoured by kin selection, as for multicellular organisms [Libertini, 1988, 2006]. Suicide-predisposition passes from a pattern useful only in emergency conditions to a pattern useful in non-stress conditions too ("suicide useful in non-critical conditions").
g) In multicellular organisms, apoptosis, cell senescence and replicative senescence, cause age-related limits in cell turnover with consequent age-related fitness decline [Fossel, 2004; Libertini, 2006] ("senile state" in its more advanced expressions [Libertini, 2006]), and this has been explained by kin selection in conditions of K-selection [Libertini, 1988, 2006].

In short, "aging" mechanisms in yeast, a monocellular eukaryote, and in multicellular eukaryotic species, separated by about 600 millions of distinct evolution, are incredibly similar in their basic physiological components and selective explanations. Moreover, apoptosis, the core of these mechanisms, has its phylogenetic roots in eubacterial proapoptotic phenomena.

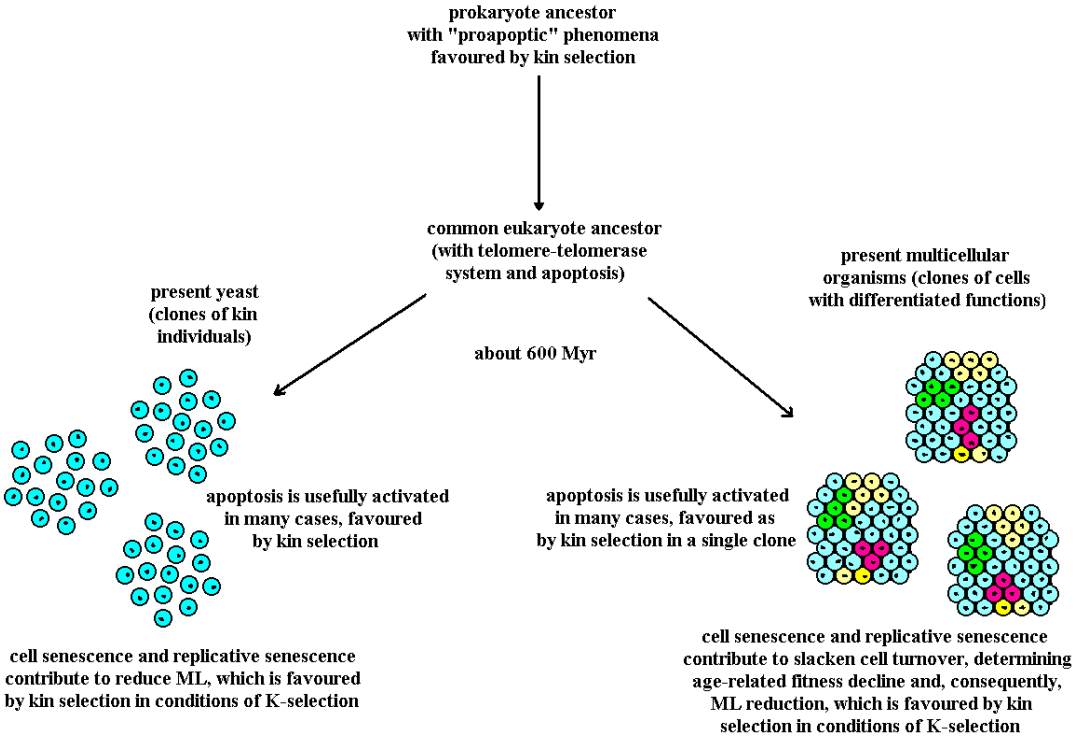

Figure 9 – Proapoptosis, apoptosis, cell senescence and replicative senescence in a phylogenetic scheme.

Table 1 - Comparison of three functions in bacteria, yeast (monocellular eukaryote) and multicellular eukaryotes

Phenomenon	Description	Function in bacteria	Function in yeast (and other monocellular eukaryotes)	Function in multicellular eukaryotes
Proapoptosis	**Various type of bacterial self-destruction mechanisms**	**Activated by various conditions (Note 1)**	**-**	**–**
Apoptosis	**Ordinate process of self-destruction with modalities allowing the use of cell components by other cells**	**–**	**Activated when nutrients are scarce, mating is not successful and in old individuals (Note 1)**	**Eliminates damaged cells (Note 2) Essential for morphogenesis and similar phenomena (Note 2) Essential to determine cell turnover whose progressive impairment contributes to age-related fitness decline (Note 1)**
Cell senescence and replicative senescence	**In relation to the number of replications, in a cell culture, progressive impairment of cell functions, increasing probability of apoptosis and of losing duplication capacity, determined by the repression of subtelomeric DNA**	**–**	**Reduce *ML*, causing a faster generation turnover (Note 1)**	**Contribute to progressively slacken cell turnover, determining age-related fitness decline (defined "senile state" in its more advanced expressions) and, therefore, *ML* reduction and faster generation turnover (Note 1)**
Note 1 = altruistic behaviour(s) favoured by kin selection in conditions of K-selection Note 2 = altruistic behaviour considering the multicellular individual as a clone				

CONCLUSION

Aging in yeast is considered adaptive while, for multicellular eukaryotes, this idea is excluded by the current gerontological paradigm [Kirkwood and Austad, 2000], which is contrasted both by theoretical arguments and empirical evidence [Goldsmith, 2003; Libertini, 1988, 2006, 2008; Longo et al., 2005; Mitteldorf, 2006; Skulachev, 1997]. Figure 10 shows that even authoritative Authors, not restrained by current paradigm, do not state openly that apoptosis is part of aging mechanisms in our species, while for other species this is maintained [Longo and Finch, 2003; Longo et al., 2005].

Apoptosis and the telomere-telomerase system are sophisticated mechanisms, necessarily determined and highly regulated by genes forged by natural selection. They

are ubiquitous in the eukaryotic world and the many variations among the different phyla do not obscure their single origin [Longo et al., 2005].
This strongly suggests that they have significant evolutionary meanings that are related to cell senescence (Fossel's "cell senescence limited model" [Fossel, 2004]) and, likely, to the age-related fitness decline of the whole organism (Fossel's "cell senescence general model of aging" [Fossel, 2004; Libertini, 2006]). In contrast with this evidence, current gerontological theories state that age-related fitness decline, a phenomenon certainly observable at ages existent in the wild [Libertini, 2008], is determined by random factors (harmful mutations, unpredictable effects of pleiotropic genes or of conflicting evolutionary exigencies [Edney and Gill, 1968; Hamilton, 1966; Kirkwood, 1977; Kirkwood and Holliday, 1979; Medawar, 1952; Mueller, 1987; Partridge and Barton, 1993; Rose, 1991; Williams, 1957]). This excludes the aforementioned mechanisms, which are sophisticated and highly regulated, as causes of the phenomenon.

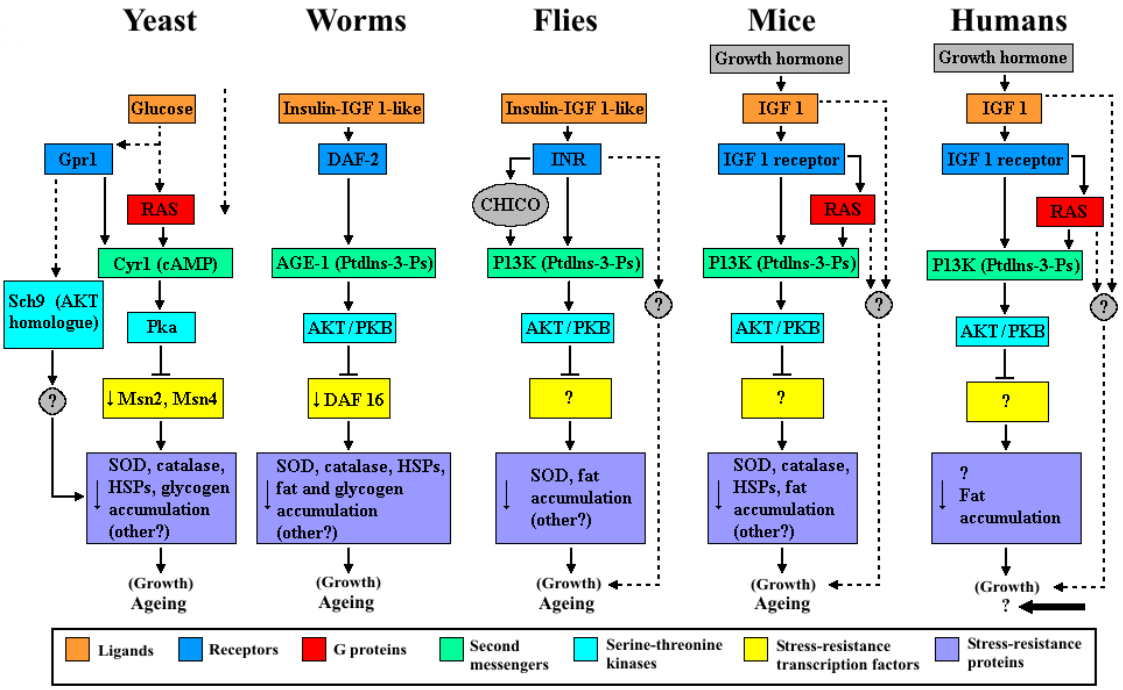

Figure 10 – Scheme of trigger mechanisms for apoptosis in various eukaryotic phyla. Part of a figure (redrawn) from Longo et al. [Longo et al., 2005], obtained with modifications from Longo and Finch [Longo and Finch, 2003]. Apoptosis is considered part of the aging mechanism, but only for our species this is considered doubtful (see the horizontal arrow, added to the original scheme) without a rational explanation.

It is important to underline that the life-limiting effects of telomere-telomerase system are currently explained as a general defence against cancer [Campisi, 1997, 2003; Troen, 2003; Wright and Shay, 2005] but there are strong arguments and evidence against this hypothesis [Fossel, 2004; Libertini, 2008; Milewski, 2010] (e.g., senescent cells secrete substances that increase mutation rates and the risk of oncogenesis [Parrinello et al., 2005; Coppé et al., 2008]). The steady affection to defence-against-cancer hypothesis by the supporters of non-adaptive aging theories may be explained by the fact that there is no other proposed explanation compatible with non-adaptive hypotheses and using philosophical and historical knowledge [Milewski 2010].
Current gerontological theories contrast strongly with the functions of apoptosis, the telomere-telomerase system, cell senescence and replicative senescence, in their phylogenetic schematisation outlined in this paper, which is based on the concept that

all these phenomena are certainly adaptive. This contrast should be solved by current gerontological theories or, on the other hand, these theories should be dropped and substituted by the alternative paradigm that the age-related fitness decline is a function with an evolutionary advantage and its physiological mechanisms.
Moreover, the thesis maintained in this paper, namely that genetically regulated active mechanisms, based on telomere-telomerase system and determining the death of an organism, have a very ancient phylogenetic history should not be considered a surprise if we consider the numberless well-known cases of phenoptosis through rapid senescence and sudden death widely described elsewhere [Finch, 1990].

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