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Role of transposable elements in age-related genomic instability

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Summary

Genetic instability is associated with aging in many species. One of the initiating factors for genetic instability is the movement of transposable elements (TEs), which occur in all prokaryotic and eukaryotic organisms. The hypothesis that TEs could be involved in the aging process is discussed and the correlation between aging and activity of TEs is analysed in a variety of biological systems.

1. Introduction

It has been widely stated that biological aging is associated with progressive deterioration of genetic information (Medvedev, 1972; Finch, 1990), and genomic instability has been reported to increase with age for a variety of organisms (reviewed in Shmookler Reis, 1989, and in Slagboom & Vijg, 1989). Processes that may contribute to progressive loss of genomic stability include age-related changes in gene expression, decreases in the efficiency of DNA repair, increases in the frequency of gene mutations, gene inactivation by insertion of mobile DNA elements, reduction of repetitive sequences, chromosomal rearrangements and chromosomal breakage, and excision and amplification of mitochondrial DNA (mtDNA) segments (Gensler & Bernstein, 1981; Kirkwood, 1988; Slagboom & Vijg, 1989; Cummings *et al.*, 1990; Finch, 1990; Gaubatz, 1990; Osiewacz, 1990, 1995; Shmookler Reis, 1990; Rice, 1993; Vijg & Gossen, 1993).

Among the principal factors that cause genetic instability in living organisms are mobile genetic elements, or transposable elements (TEs). TEs are widely distributed in essentially all taxa of bacteria, plants and animals. They play a major role in spontaneous genetic damage, including various gene mutations, modifications in gene expression, alterations of gene sequence, site-specific recombination, deletion and duplication of DNA sequences, chromosomal aberrations and rearrangements, and chromosomal breakage (Spradling & Rubin, 1981; Vijg *et al.*, 1985; Finnegan & Fawcett, 1986; Green, 1988; Berg & Howe, 1989; Finnegan, 1989). Where there is active TE transposition, such as in *Caenorhabditis elegans* strains with active mutator loci, TEs can account for the great majority of spontaneous mutations (Eide & Anderson, 1985). It has also been shown that TEs are directly involved in expression and determination of quantitative genetic characters in the genus *Drosophila*, including fitness and its components (Mackay, 1985, 1986, 1988, 1989; Pasyukova *et al.*, 1986; Vasilyeva *et al.*, 1988; Mackay *et al.*, 1992; Ratner & Vasilyeva, 1992*a*). On the basis of their effect on quantitative traits, it has been suggested that TEs may represent components of an autonomous genetic regulation system that modifies the function, expression and evolution of the genome (Ratner & Vasilyeva, 1992*a*, *b*).

Somatic transposition of mobile elements may also contribute to the aging process. In the past decade there have been reports on correlations between TE activity and aging in various living systems. In fungi, one finds the most conclusive information on the age-associated activity of mobile DNA sequences (reviewed in Dujon & Belcour, 1989; Osiewacz, 1990, 1995). Furthermore, a significant amount of work on the subject of TEs and aging has been done in *Drosophila* species. Among the TEs studied with respect to aging are the P elements (Driver & McKechnie, 1992; Woodruff, 1992; Woodruff & Nikitin, 1995), *412* and *copia* (Driver & McKechnie, 1992) in *Drosophila melanogaster*, *mariner* in *D.*

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simulans and *D. melanogaster* (Nikitin & Woodruff, 1995), and *hobo* in *D. melanogaster* (A. G. Nikitin & R. C. Woodruff, unpublished data). Age-specific excision of the Tc1 TE has also been studied in the nematode *C. elegans* (Egilmez & Shmookler Reis, 1994). In addition, there have been reports in mammalian systems that activation of TEs may play a role in a type of programmed cell death (Servomaa & Rytömaa, 1988, 1990). Possible involvement of mobile DNA sequences in the aging process has been discussed before (Shmookler Reis *et al.*,' 1983; Cummings, 1984; Esser, 1985; Kirkwood, 1988; Murray, 1990; Shay & Werbin, 1992; Woodruff, 1992; Egilmez & Shmookler Reis, 1994; Osiewacz, 1995), although we are not aware of any comprehensive reviews of this area. Therefore, the purpose of this paper is to review critically the available information about the age-associated activity of TEs in different organisms and to examine the hypothesis that the activity of TEs is one of the causes of aging (Shmookler Reis *et al.*, 1983; Murray, 1990; Woodruff, 1992; Egilmez & Shmookler Reis, 1994).

2. TE-associated aging in fungi

Numerous reports indicate that fungal senescence is associated with age-dependent activity of plasmids of mitochondrial origin (see Esser *et al.*, 1986; Nevers *et al.*, 1986; Gaubatz, 1990; Osiewacz, 1990, 1995; Griffiths, 1992 for extensive reviews on this topic). Most of these senescence-associated plasmids possess characteristics of TEs. Studies on fungal senescence provide convincing evidence that TEs are directly involved in the onset of this 'aging' process and in some cases are the primary factor that restricts mitotic lifespan in these organisms.

The difference in aging between fungi and higher organisms, such as plants and animals, should be noted. As emphasized by Griffiths (1992), senescence in fungi is not a necessary or a useful life history trait, as fungi have a potentially unlimited capacity for vegetative proliferation. However, there are certain senescent patterns described in fungi, also termed 'clonal attenuation' or 'vegetative death', for which the involvement of mobile genetic sequences has been convincingly implicated.

In baker's yeast, *Saccharomyces cerevisiae*, there exists a group of petite mutants that are unable to synthesize respiratory enzymes and consequently lack normal respiratory functions (Bernardi, 1979). Molecular analysis has shown that in these mutants a non-coding region of the mitochondrial genome excises from its original location and undergoes tandem amplification, forming a petite mitochondrial genome. These sequences are flanked by short direct nucleotide repeats and they excise from the host genome in a similar fashion to the excision of bacterial transposons (Bernardi, 1979). The overall structure of these mobile segments shows homology with the foldback TEs. In addition, their terminal repeats resemble the structure of the corresponding regions of the IS*1* and Tn*9* transposons of *Escherichia coli* (Bernardi, 1979; Gaillard *et al.*, 1980; Esser, 1985). Another interesting characteristic is that the region responsible for the petite mutation in *S. cerevisiae* displays homology with the senescence-associated mobile α-intron of *Podospora anserina* (Wright *et al.*, 1982; see below).

One of the organisms in which mobile DNA sequences play a major role in aging is the ascomycete fungus *Podospora anserina*. Early studies with *P. anserina* revealed an alteration of the mitochondrial genome during senescence (see Stahl *et al.*, 1978; Cummings *et al.*, 1979; Cummings, 1984; Esser, 1985; Osiewacz, 1990, and references therein). At the onset of senescence, the normal mitochondrial genome of *P. anserina* is replaced by plasmid-like circular DNA sequences of mitochondrial origin, so-called senDNAs (Wright *et al.*, 1982; Cummings, 1984; Turker *et al.*, 1987; Kück, 1989). The most abundant of all senDNAs is a 2·6 kb circular plasmid, so-called α-senDNA or plDNA. It constitutes the first intron of subunit I of a mitochondrial gene that codes for cytochrome *c* oxidase in juvenile mycelia (Osiewacz & Esser, 1984). In aging mycelia, this mobile intron (α-intron) excises and undergoes amplification (Osiewacz & Esser, 1984; Kück *et al.*, 1985; Kück, 1989). The amplification of α-senDNA, in turn, is associated with the distortion of normal metabolic activity and with the development of different morphological defects, which lead to irreversible arrest of vegetative growth (Esser, 1985; Dujon & Belcour, 1989). Molecular analysis of the plasmid revealed the presence of an open reading frame (ORF), a putative autonomously replicating sequence (ARS) within the ORF, and inverted terminal repeats (Osiewacz & Esser, 1983, 1984). It has also been shown that α-senDNA encodes the enzyme reverse transcriptase – a necessary component of the replication system of retroviruses and retrotransposons (Michel & Lang, 1985; Steinhilber & Cummings, 1986; Fassbender *et al.*, 1994) – which may, in turn, have evolved from the reverse transcriptase sequence possessed from ancient retrotransposons by mobile introns of early mitochondria (Xiong & Eickbush, 1990). Further experiments directly demonstrated the transposition ability of this intron sequence (Sellem *et al.*, 1993; Osiewacz, 1994). It should also be noted that in young mitochondria of a rapidly senescing race of *P. anserina* the α-senDNA plasmid exists in a high copy number, whereas in a slowly senescing race of the same organism no increase in copy number of this plasmid was observed (Wright & Cummings, 1983).

Mutants of *P. anserina* that either lack the α-intron, have novel DNA rearrangements in or near the α-intron region, or have a delayed amplification of that plasmid, display prolonged lifespans (Belcour & Vierny, 1986; Schulte *et al.*, 1988; Dujon & Belcour, 1989; Osiewacz *et al.*, 1989). Furthermore, in some of

these mutants an increase in longevity is also correlated with the presence of a linear plasmid, pAL2–1, either free or integrated into the mitochondrial genome (Osiewacz *et al*., 1989; Hermanns & Osiewacz, 1992; Hermanns *et al*., 1994). Structurally, pAL2-1 belongs to the invertrons, a class of linear mobile DNA molecules with identical inverted terminal repeats (Osiewacz *et al*., 1989; Sakaguchi, 1990; Hermanns & Osiewacz, 1992). This plasmid is able to integrate into mtDNA at a region that does not contain any essential genes and therefore does not disrupt vital functions of the organism (Osiewacz *et al*., 1989; Hermanns *et al*., 1994). Even when pAL2-1 integrates into an essential mtDNA region, such as an intron of the apocytochrome *b* gene, the resulting recombinant mtDNA molecules are not suppressive to the remaining wild-type mitochondrial genomes (Hermanns *et al*., 1994). In some mutant strains, this linear plasmid was found in the same genome with the α-senDNA circular plasmid which is responsible for the early onset of fungal vegetative aging. Hermanns & Osiewacz (1992, 1996) point out that coexistence of two antagonistic structures in the same genome may constitute a mechanism for extrachromosomal regulation of senescence in the genus *Podospora*. Although α-senDNA has been shown to influence the onset of aging in *Podospora*, the role of pAL2-1 as a positive controlling mechanism is ambiguous, considering the fact that this plasmid is not commonly found in wild-type strains of *P. anserina* (Hermanns & Osiewacz, 1992, 1996; Hermanns *et al*., 1994). Some of the wild-type strains with no mtDNA integration of pAL2-1, however, appear to be short lived (Hermanns *et al*., 1995*b*; Hermanns & Osiewacz, 1996). The mechanism of the effect of pAL2-1 integration on lifespan of *P. anserina* remains unclear (Hermanns & Osiewacz, 1996).

Another senescent plasmid commonly found in aging mycelia of *P. anserina* is β-senDNA (Wright *et al*., 1982). It represents a 9·8 kb plasmid that contains genetic information for subunit III of the cytochrome *c* oxidase (Wright *et al*., 1982; Wright & Cummings, 1983). It has been suggested that the β-senDNA plasmid, together with α-senDNA, can integrate into the nuclear genome during senescence in *P. anserina* (Wright & Cummings, 1983). Integration of the liberated plasmids into the nuclear genome, or homologous recombination between the plasmids and mtDNA, can lead to inactivation of essential nuclear and mitochondrial genes (e.g. the cytochrome *c* oxidase gene), causing disintegration of mtDNA and thus apparently contributing to the onset of senescence (Wright & Cummings, 1983; Esser, 1985; Osiewacz, 1990, 1992). On the other hand, overproduction of proteins coded by the amplified plasmids can contribute to the induction of mtDNA rearrangements (Belcour *et al*., 1981; Schulte *et al*., 1988). There is also the possibility that excision and amplification of senDNA is under the control of nuclear genes (Kück, 1989; Belcour *et al*., 1991; Sellem *et al*., 1993), which would be consistent with a chromosomally determined genetic program governing the onset of aging in *Podospora* species (Osiewacz & Nuber, 1997).

Plasmids similar to α-senDNA have been found in the genome of *P. curvicolla* (Böckelmann & Esser, 1986). They originate in senescent mycelia from a mtDNA gene for the large subunit of the mitochondrial rRNA. In a yeast transformation system, these plasmids show the capacity for autonomous replication, indicating the presence of an ARS region. In contrast to *P. anserina*, the consequences of the plasmids' amplification in *P. curvicolla* are reversible: a single hypha can resume vegetative growth after 'senescent' arrest, and subsequently will alternate between periods of growth and non-growth. These plasmids do not represent the entire intron and therefore cannot be defined as actual 'mobile introns'. A lack of homology between α-senDNA and the plasmids from *P. curvicolla*, and the fact that the latter are not complete mobile introns, could explain why the amplification of these plasmids does not ultimately result in senescence. This also poses a question of whether the TE-like mobile introns are more important in causing senescence than other fungal plasmids (Osiewacz, 1995).

Dependence of the onset of senescence on the presence of mitochondrial plasmids, comparable to that of *Podospora* species, has been observed in another fungal genus, *Neurospora*. In *Neurospora crassa* and *N. intermedia*, two circular transposon-like plasmids were found that exist as free molecules in wild-type non-senescing strains and become integrated into mtDNA in senescing mutants (Akins *et al*., 1986). This integration is thought to disrupt a mtDNA gene sequence and cause a failure of mitochondrial functions. On the other hand, the abundant presence of amplified plasmids can inhibit normal mitochondrial metabolism and protein synthesis, which may itself result in the appearance of a senescent phenotype (Akins *et al*., 1986; Osiewacz, 1990). These plasmids, like α-senDNA, code for reverse transcriptase and share other characteristics of retrotransposons (Akins *et al*., 1986; Kuiper & Lambowitz, 1988).

Another cytoplasmic senescence-inducing factor has been found in some Hawaiian wild-type strains of *N. intermedia* (Griffiths & Bertrand, 1984). This factor appears to be an extrachromosomal linear DNA plasmid that inserts into the mtDNA during senescence of the fungus (Bertrand *et al*., 1985, 1986). This plasmid, called *kalilo* or *kalDNA*, is about 9·0 kb long, has 1·364 bp inverted terminal repeats, and possesses other structural characteristics of invertrons (Bertrand *et al*., 1985; Chan *et al*., 1991). The insertion of *kalDNA* usually occurs within the mitochondrial gene for the large rRNA subunit (Bertrand *et al*., 1985, 1986). Unlike the pAL2-1 plasmid of *P. anserina*, integration of *kalDNA* into the mtDNA initiates

double-stranded breaks, generates long inverted repeats of mtDNA that flank the plasmid insertion site, and distorts normal ribosomal functions (Bertrand & Griffiths, 1989; Chan *et al*., 1991; Court & Bertrand, 1992). Since mtDNA molecules that contain the inserted plasmid accumulate in *N. intermedia* during the vegetative lifespan, the accrual of abnormal mitochondria and the depletion in number of functional mitochondrial molecules together lead to organismal malfunction and vegetative death (Bertrand *et al*., 1985).

A plasmid similar to the *kalDNA* has also been found to induce the senescence syndrome in the *maranhar* strains of *N. crassa* (Court *et al*., 1991; Court & Bertrand, 1992). The *maranhar* plasmid, or *marDNA*, is a 7 kb linear molecule that has 349 bp long inverted terminal repeats. The insertional mechanism and the consequences of *marDNA* insertion are identical to those of *kalDNA*. As in the case of *kalilo*, accumulation of defective mitochondria that have the *marDNA* inserts leads to premature senescence and loss of organismal functions. Despite having a similar structure and the same mechanism of insertion, *kalilo* and *maranhar* plasmids do not have extensive sequence homology and are not directly related to each other or to any other linear plasmids found in fungi (Bertrand & Griffiths, 1989; Court *et al*., 1991; Court & Bertrand, 1992).

Taken together, these lines of evidence strongly implicate the involvement of TEs in vegetative 'senescence' of fungi. Similar events, leading to substantial replacement of normal mtDNA with specific defective mitochondrial genomes, are not seen during cellular senescence of human fibroblasts. However, cells from older donors contain roughly twice as much mtDNA as those from young donors, suggesting compensation for a large fraction of variably defective molecules (Shmookler Reis & Goldstein, 1983). This topic will be discussed further in Section 8.

3. Tc1 TE and aging in nematodes

In the free-living nematode *Caenorhabditis elegans*, several related families of TEs have been recognized (Moerman & Waterston, 1989; Collins & Anderson, 1994, and references therein; Oosumi *et al*., 1995; Robertson, 1995). Among them, the Tc1 family is the most abundant in the *C. elegans* genome. It is a relatively small (1·6 kb) element with short inverted terminal repeats (Moerman & Waterston, 1989). Among the distinctive characteristics of Tc1 is that it is able to move somatically as well as in the germline. In fact, in wild-type strains of *C. elegans*, genetic instability of Tc1 is far greater in somatic cells than in the germline (Emmons & Yesner, 1984; Harris & Rose, 1986; Egilmez & Shmookler Reis, 1994). Tc1 elements are stable in the germline of wild-type strains that do not show a mutator genotype. Presence of a mutator genotype leads to increased germline transposition, although this genotype has much less effect on somatic transposition (Harris & Rose, 1986).

Egilmez & Shmookler Reis (1994) examined the excision rate of Tc1 in somatic cells of *C. elegans* at different stages of its lifespan. They monitored somatic excision of a Tc1 element from a locus to which it had been recently transposed with no detectable phenotypic effect. Excision increased with age from 1·2% at day 1 of the adult lifespan, to 17·4% at day 36, by which time 99% of the worms were dead. The maximum accumulation of excised Tc1 in older worms was demonstrated by two methods to reach approximately 17%, and this could not be accounted for by selection favouring those worms with germline excision of Tc1. Although excisions accumulated steadily throughout the lifespan, the rate of excision increased in the last third of life, suggesting an increase in transposon mobility with age of nematodes. These observations may imply the absence of effective mechanisms to suppress somatic transposition in this organism. While it appears likely that Tc1 transposition has a direct negative impact on the integrity of the *C. elegans* somatic genome with age, this remains to be demonstrated. Certainly, most or all mutator strains in *C. elegans* have somewhat reduced lifespans relative to non-mutators (Egilmez & Shmookler Reis, 1994), although Tc1 transposition cannot account for the longevity variation observed in *C. elegans* interstrain crosses (Ebert *et al*., 1996).

4. Age-dependent activity of TEs in *Drosophila*

The *Drosophila* genome harbours many different families of TEs. In fact, about 10% of the *Drosophila* genome is represented by TEs (Finnegan & Fawcett, 1986). Consequently, a great deal of attention has been devoted to the question of whether it suffers any consequences from the presence of this vast number of mobile elements (Lambert *et al*., 1988; Berg & Howe, 1989; Corces & Geyer, 1991), and whether their somatic transposition might play a role in *Drosophila* aging (Woodruff, 1992).

The *copia* retroposon family is one of the most abundant TE classes in *D. melanogaster* (Finnegan & Fawcett, 1986; Bingham & Zachar, 1989). *Copia*-like elements, which use reverse transcriptase for replication, are actively expressed in *Drosophila* tissue culture cells (Finnegan & Fawcett, 1986), and show increasing numbers of reverse transcription intermediates with *in vitro* age (Ilyin *et al*., 1984; Arkhipova *et al*., 1986). These and other observations suggest that retrotransposons may be active in somatic cells of *Drosophila* (Kim & Belyaeva, 1991; Jouan-Dufournel *et al*., 1996).

Somatic content of the *412* and *copia* transposable elements has been measured in *D. melanogaster* males

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(Driver & McKechnie, 1992). Using Southern blot analysis, the authors reported a quantitative increase with age in hybridizations to *copia* and *412* sequences in total DNA. Since only 3-day-old and 45-day-old males were compared, the possibility of differential replication of TEs in somatic tissues, relative to spermatogenesis, should be considered.

A recent study provided indirect evidence that retrotransposons may be active *in vivo* and that they may cause cellular genetic damage in *D. melanogaster* (Driver & Vogrig, 1994). *D. melanogaster* flies treated throughout life with various inhibitors of reverse transcriptase showed modest increases in lifespan under some conditions. Although inhibition of retrotransposition is only one of many possible effects of inhibitors of reverse transcriptase, these results suggest that lifespan may be in part affected by the activity of TEs. It is not clear, however, whether the inhibitors exerted their effect during aging or during development; certainly somatic transposition of TEs during embryonic, larval or imaginal development could produce random somatic genetic damage which could impair survival of the adults.

P DNA elements comprise another prominent class of *Drosophila* TEs. The intact P elements, although mobile in the germline, are stable in somatic cells; a molecular construct, P[ry^+ Δ2–3], has been engineered to mobilize P elements in the soma (Laski *et al*., 1986; Robertson *et al*., 1988).

The transpositional activity of P elements is temperature-dependent, appearing to increase at higher temperatures (Robertson *et al*., 1988; Engels, 1989). To investigate the temperature effect on lifespan of *Drosophila* strains containing 17 inactive P elements and a source of somatic transposase, P[ry^+ Δ2–3](99B), Driver & McKechnie (1992) compared the longevity of parental strains with their F_1 progeny, containing both somatic transposase and defective P elements, at different environmental temperatures. Lifespan of the F_1 progeny significantly decreased at 25 °C compared with the mean lifespan of parental strains. Although these results may be taken to suggest a correlation between somatic activity of TEs and the aging process in *Drosophila*, it is unclear why the lifespan of F_1 flies that had somatically active P elements was similar at 18 °C to the lifespans of the parental strains, since even the lowest developmental temperatures cannot prevent somatic induction of P elements in the presence of P[ry^+ Δ2–3](99B) (Engels *et al*., 1987; Engels, 1989).

Convincing evidence for a negative effect of TE movement on aging in *Drosophila* species comes from a lifespan comparison of *D. melanogaster* males with different numbers of somatically active P elements in the genome (Woodruff, 1992). A somatic source of P element transposase, P[ry^+ Δ2–3](99B), was used to induce transposition of a number of defective P elements present in the same genome. A significant log-linear decrease in lifespan was observed at 25 °C in flies that had 3–17 P elements per genome. The observed effects may be due to somatic chromosome breakage and recombination induced by transposase-mediated somatic movement of P elements (Gunn *et al*., 1989; Sved *et al*., 1991; Woodruff, 1992).

Even the presence of just one somatically active P element is sufficient to reduce lifespan of *D. melanogaster* males (Woodruff & Nikitin, 1995). Furthermore, it appears that somatic activity of P elements is the factor responsible for reduced lifespan, since the introduction of a somatic transposase suppressor P[ry^+ SalI](89D) (Robertson & Engels, 1989), eliminated the lifespan reduction in the corresponding group of flies (Woodruff & Nikitin, 1995).

The results from these experiments, relating lifespan to P element mobility, encouraged further studies with different TEs such as *mariner* and *hobo*. Whereas P elements had to be artificially modified to enable somatic transposition, the *mariner* and *hobo* TEs are naturally active in somatic cells, although the somatic transposition frequency of *hobo* is very low (Blackman & Gelbart, 1989; Hartl, 1989; Kim & Belyaeva, 1991; Calvi & Gelbart, 1994). Initial experiments indicated a significant reduction in lifespan of the males of a *D. simulans* line that had somatically active *mariners* (Nikitin & Woodruff, 1995). In these experiments, a single inactive *mariner* (w^{pch}) element was induced to somatic transposition by the presence in the same genome of a fully functional, transposase-producing *mariner* element (*Mos*). However, further experiments did not support a strong negative correlation between *mariner* somatic activity and lifespan in *D. simulans* males, nor was such a correlation found for the *hobo* TE in *D. melanogaster* males (A. G. Nikitin & R. C. Woodruff, unpublished data). On the other hand, parallel studies in *D. melanogaster* indicated a modest decrease in lifespan for the flies with somatically active *mariners* in the genome. It should be noted, however, that the interpretation of the *D. melanogaster* study was complicated by effects of inbreeding depression and hybrid vigour in the progeny of certain crosses used in the experiment; such effects arise inevitably when constructing experimental and control strains in a constant genetic background.

The overall conclusion from these experiments is that although active somatic transposition of a single P element can reduce lifespan in some backgrounds, there might not be enough genetic damage from the somatic activity of a single *mariner* or *hobo* TE to affect lifespan significantly in fruitflies. It should also be noted that no clear demonstration that TEs are normally active in adult (primarily postmitotic) tissues of *Drosophila* species has been presented as yet, and it remains possible to argue that the reduced survivals which have been observed are due to genomic damage from transpositions prior to emergence of the adult. On the other hand, Driver & McKechnie (1992) have shown that there may be an increase in copy number of TEs with adult age. Subsequent work by C. Driver

& D. Vogrig (personal communication) indicates that significant excision of TEs also occurs, which makes such measurements highly variable and difficult to interpret. Nevertheless, these data support the presence of transpositional activity in at least some tissues of adult flies.

Another attempt to correlate TEs and aging has been made in the study of *P–M* hybrid dysgenesis at different developmental temperatures (Konaç *et al.*, 1995). In one of the hybrid dysgenic crosses with strong P element activity, a significant reduction of lifespan was reported at 29 °C compared with a reciprocal cross where hybrid dysgenesis was repressed. However, when the same experiment was performed at 25 °C, dysgenic flies actually lived longer than their reciprocals, contrary to expectation. The authors did not address the observed discrepancy in their results or offer any hypothesis to explain the way a P-element-induced hybrid dysgenesis could shorten lifespan at 29 °C. A possible explanation would be that mildly dysgenic effects in flies developing at 25 °C reduce subsequent fecundity and thus indirectly extend lifespan, whereas more severe effects are produced at 29 °C resulting in adults of diminished fitness and longevity.

5. *LINE* and *SINE* sequences and their transposition

The largest group of human highly repetitive DNA sequences, the *Alu* family, comprises a highly diverged set of **s**hort **in**terspersed **e**lements (*SINE*s) with several features characteristic of retrotransposable elements. Primate *Alu* repeat elements, approximately 300 bp in length, are normally flanked by 6–20 bp target-site direct repeats; such repeats are characteristic of all classes of TEs. It is thought that these elements have most probably dispersed in the genome through retroposition, since they are highly transcribed and the consensus *Alu* sequence contains an internal RNA polymerase III promoter which could be transcribed and transposed with each RNA copy (Schmid & Jelinek, 1982). Due to approximately 20% divergence in the *Alu* family, repeat numbers were initially underestimated, but appear to exceed 10^6 per haploid human genome, or approximately 6% of total DNA. Other short interspersed repeat elements, many of them related to tRNA sequences, may together comprise a similar fraction of the genome in humans and other mammals.

Long **in**terspersed repeat **e**lements (*LINE*s) are found throughout the class Mammalia; they belong to the non-LTR (long terminal repeat) retrotransposons, the most ancient subset of the non-viral superfamily (Xiong & Eickbush, 1990; see also Weiner *et al.*, 1986). The largest mammalian *LINE* family, the *L1* or *KpnI* family, has a full length of 6·5 kb and contains two ORFs, one of which encodes a reverse transcriptase-like protein (Martin *et al.*, 1984; Fanning & Singer, 1987). Polyadenylated *L1* transcripts of 6·5 kb have been identified in nuclear, and occasionally in cytoplasmic, RNA (Weiner *et al.*, 1986). *LINE1* elements show evidence of transposition in the human genome (Singer *et al.*, 1993).

KpnI sequences appear to be enriched approximately threefold in polydisperse circular DNA of human cells (Riabowol *et al.*, 1985), which may represent intermediates in their retrotransposition. There is substantial evidence of age-dependent activation of endogenous retroviruses in rodents (Ono *et al.*, 1985; Gaubatz *et al.*, 1991); indeed, activation of murine leukaemia viruses (MuMLVs) can produce age-dependent coat greying in some mouse strains (see references in Finch, 1990). Such activation could mediate passive retroposition of other (non-viral) retroposons that serve only as target sequences for reverse transcriptases, or the latter elements might display age-dependent activation of transposition via their own reverse transcriptase genes, which are rarely but occasionally functional (Schukkink & Plasterk, 1990; Plasterk, 1993).

6. *LINE* activity and cell death of rat chloroleukaemia cells

Apoptosis (programmed cell death) is a component of a variety of normal physiological processes, such as embryogenesis, regulation of homeostasis and the immune response (Sen, 1992). Rat chloroleukaemia cells in culture undergo synchronous cell death resembling apoptosis, which appears to involve *LINE* activation (Servomaa & Rytömaa, 1988). When cell cultures reached about half the maximal population density, there was a sudden transcriptional activation of the *L1* retrotransposon family. A dramatic sevenfold increase in the copy number of *L1* elements was followed by random incorporation of *L1* copies into nuclear DNA, which induced lethal mutations and consequent cell death. The authors called this apoptosis merely to indicate the fact that the cells simultaneously commit a massive 'suicide', whereas the genetic program responsible for this event remains unclear. Although the initial observation was made *in vitro*, the authors also reported a set of events *in vivo* corresponding to the processes in cell culture. The authors further demonstrated that the application of ultraviolet light or ionizing radiation leads to premature and elevated induction of *L1* retrotransposition in the cell culture (Servomaa & Rytömaa, 1990). They concluded that stress conditions, such as an increase in the cell population density or elevated radiation levels, may trigger the transcriptional activation of retrotransposons, which might represent an ancient mechanism for apoptosis, i.e. an inducible genetic pathway leading to cell death (Servomaa & Rytömaa, 1988). (Stress-related expression of TEs and its role in aging will be discussed in Section 10.)

7. Transposition of interspersed repeat sequences in humans and its role in the aetiology of disease

Involvement of TE sequences has been implicated in the triggering of cancer and cancer-related diseases in humans and other mammals (see Morgan *et al*., 1996, and references therein). These sequences can become somatically activated, or they can act in the germline. Although in some respects TE insertion is equivalent to other types of mutagenesis, it has several distinctive features: (*a*) it generally cannot produce point or frameshift mutations except upon imprecise excision; (*b*) it is highly reversible, either at the DNA level (via excision) or at the RNA level (via splicing); and (*c*) TEs, at least those with LTRs, are capable of activating expression of adjacent or nearby genes.

An example of *de novo* insertional germline mutation is an insertion of the *Alu* sequence into an intron upstream from the gene associated with the neurofibromatosis type 1 disorder (NF1). This insertion results in frameshift and proliferation of the NF1 disorder, which is accompanied by a number of cancer-related diseases (Wallace *et al*., 1991). An X-chromosomal *de novo* insertion of an *L1* element has resulted in two related cases of muscular dystrophy (Narita *et al*., 1993) and in two unrelated cases of haemophilia A (Kazazian *et al*., 1988). Somatic insertion of the *L1* element can disrupt the DNA sequence of a colon cancer suppressor gene and thereby can promote the onset of colon cancer (Miki *et al*., 1992). A somatic *L1* insertion disrupting the *myc* locus has been reported in human breast carcinoma (Morse *et al*., 1988), and active *L1* transpositions have been detected in human testicular cancer (Bratthauer & Fanning, 1992). Bratthauer & Fanning (1992) even suggested that *L1* elements may transduce oncogenes, the products of which may participate in the induction or progression of certain cancers. While the analysis of oncogenesis is beyond the scope of this review (see Weinstein *et al*., 1988, for a discussion of the possible role of TEs in carcinogenesis), evidence indicates that *de novo* germline and somatic insertion of TEs occur in the human genome and can result in, or facilitate the occurrence of, certain cancers which are a common cause of mortality in aging humans and other vertebrates.

8. Age-dependent alterations of mammalian mtDNA: a possibility of TE involvement

As in the case of chromosomal DNA, mtDNA instability can be an important part of the age-related degenerative processes (Schon *et al*., 1989; Gaubatz, 1990; Wallace, 1992). A number of reports indicate the appearance and accumulation of mtDNA deletions in aged human and animal tissues (Pikò *et al*., 1988; Schon *et al*., 1989; Cortopassi & Arnheim, 1990; Mita *et al*., 1990; Yen *et al*., 1991; Baumer *et al*., 1994; Brossas *et al*., 1994, and references therein). Similar deletions in mtDNA occur in a variety of progressive diseases with maternal (mitochondrial) inheritance, including myopathies, neuropathies, diabetes and deafness (Wallace, 1992; Wallace *et al*., 1995). Many of these deletions are site-specific and they appear to be flanked by direct nucleotide repeats (see, for example, Tanhauser & Laipis, 1995, and references therein). In addition, a common mitochondrial deletion has been observed in many tissue samples from aged individuals (Schon *et al*., 1989; Baumer *et al*., 1994, and references therein). Insertions of mtDNA sequences into the nuclear genome, often associated with the deletions and amplifications of the mitochondrial genome in cytoplasm, have also been documented (Farrely & Buttow, 1983; Gellissen *et al*., 1983; Jacobs *et al*., 1983; Hadler *et al*., 1983; Corral *et al*., 1989; Kamimura *et al*., 1989; Shay & Werbin, 1990, 1992; Liang, 1996). These observations may indicate that certain mtDNA segments are potentially mobile, and can transpose from their mitochondrial locations into the nuclear genome by reverse transcription via RNA intermediates (Shay & Werbin, 1992). It was therefore suggested that these excised sequences might represent TEs that originated in the nucleus and later became incorporated into the mitochondrial genome through reverse transcription (Shay & Werbin, 1992). With age, such sequences could become activated (e.g. by reactive oxygen species), excise from their mitochondrial locations, and occasionally insert back into the nuclear genome. Consequences of such an event were described in the previous sections (see also Richter, 1988). At present, however, data are insufficient to confirm the existence of such TE sequences or their age-related excision from the mitochondrial genome. Even if the deleted sequences do not represent actual TEs but instead are the byproducts of other age-dependent processes, their excision from mtDNA and subsequent incorporation into the nuclear genome are unexpected and noteworthy 'transpositional' events. Although the frequencies of deletions increase during aging, they individually never comprise a large fraction of mtDNA; nevertheless, the total of all such events could be quite substantial (Wallace, 1992).

9. The relationship between TEs and aging: theoretical considerations

A theoretical rationale for transposon effects on aging has been proposed as a modification of the somatic mutation theory of aging (Murray, 1990). In this version, transposons are suggested to be the principal source of somatic mutations and it is argued that the rate of such mutations would increase exponentially with cell or animal age, in the manner of Orgel's 'error catastrophe' hypothesis (Orgel, 1963). The proposed mechanism of mutation is replicative transposition, so that transposon copy number is alleged to increase exponentially with time in somatic cells, leading to an

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increasing probability of transcription, and hence to a chance insertion in a structural or regulatory gene. This may lead to inactivation of essential genomic sequences and eventually cause senescence and death of the cell strain or organism.

Consequences of somatic transposition of TEs have been shown to be at least potentially negative for the lifespan of organisms with post-mitotic somatic cells, such as *Caenorhabditis elegans* and *Drosophila* species (see above). It is not clear, however, that the same process would reduce the lifespan in organisms with continuously dividing somatic cells. Although this theory is not without appeal, since it provides a plausible rationale for the time-course of cellular and organismal senescence, no evidence has been forthcoming of such an exponential increase with age in TE genomic abundance. Indeed, prior evidence indicates a pronounced loss of highly repetitive DNA sequences during cellular aging (Shmookler Reis & Goldstein, 1980). The appearance of extrachromosomal circular DNA (ecDNA) molecules in cultured cells and in animal tissues may include intermediates of DNA and RNA transposition, and the representation of repetitive sequences in ecDNA can change with age (Riabowol *et al*., 1985; Sunnerhagen *et al*., 1986; Gaubatz, 1990; Gaubatz & Flores, 1990). Indeed, *L1* sequences have been shown to be overrepresented in human fibroblast ecDNA (Riabowol *et al*., 1985). However, ecDNA accumulated only linearly with serial passage of cells (R. A. Jones, S. Goldstein & R. J. Shmookler Reis, unpublished data).

10. Conclusion

Aging is a complex trait that is influenced by diverse mechanisms, some of which are genetically determined while others may result from environmental processes such as induced somatic mutations and oxidative damage, and other sources of 'wear and tear'. If we ask only whether aging is associated with increased activity of TEs, the overall answer is affirmative. However, no conclusion can yet be drawn about the specific role TEs play in the aging process. It is clear that TEs would not be maintained in the genome if their transpositions were lethal to their hosts (Brookfield, 1995). In fact, the scarcity of mutator (actively transposing) strains among natural isolates of *C. elegans* implies that such activity is deleterious and probably appears only sporadically and transiently in evolution (Egilmez *et al*., 1995). It is nevertheless possible that somatic activity of TEs could be involved in the determination of certain traits, such as aging and age-dependent diseases. However, in most of the studied cases it remains unclear whether TEs are directly involved in the onset of aging, or whether genetic instability of TEs is just an age-associated process, where aging itself results from other factors. Even when it is shown that mobile genetic sequences do affect the aging process, the overall conclusion can sometimes be ambiguous. For example, in the discussion of the 'Mobile Intron Model', Esser (1985) hypothesizes that the liberation of the α-senDNA plasmid is an 'accident', where the plasmid slips away from the chromosomal genetic control. Whether this event is accidental or is, in fact, induced by nuclear genes, remains to be determined. In *Drosophila*, one can envisage more than one possible interpretation of the decrease in lifespan when somatically active TEs are present in the genome. For example, in the experiment with inhibitors of reverse transcriptase, the postponement of aging could result from inhibition of retroviral activity, rather than from inhibition of retrotransposon mobility (Driver & Vogrig, 1994). Furthermore, the influence of the somatic activity of a single P or *mariner* elements on lifespan also deserves a careful interpretation because of the differential effects in different genetic backgrounds (Woodruff, 1992; Nikitin & Woodruff, 1995; Woodruff & Nikitin, 1995), and the possibility of developmental transposition affecting the adult lifespan.

If the age-associated induction of TE activity were responsible for aging, what might be the mechanism of this induction? It has long been established that stress conditions, such as heat shock, chemical treatment, radiation or viral infection, can lead to increased transposition and excision of TEs in the genome (McClintock, 1978, 1984; Strand & McDonald, 1985; Kapitonov *et al*., 1987; McDonald *et al*., 1987; Green, 1988; Ratner & Vasilyeva, 1992*b*; Ratner *et al*., 1992*a*, *b*). This formed a basis for speculation that TEs might constitute a reactive cellular mechanism that provides a common response to many diverse stress factors (Kapitonov *et al*., 1987). Since TEs can become mobilized through stress, it is possible that aging itself (or an accompanying loss of homeostasis) can act as a stress factor that promotes mobilization of TEs. Once mobilized, TEs can in turn produce additional genetic damage, which may contribute to the 'age-stressed' condition. This idea, however, needs to be tested experimentally.

Overall, the material presented in this review demonstrates the existence of a negative correlation between the activity of mobile DNA sequences and aging in a variety of organisms. This correlation is strongest in fungi; among those higher eukaryotes which display an unambiguously limited and genetically determined lifespan, *C. elegans* has shown strong evidence of an age-dependent increase in somatic mobility of TEs, whereas *Drosophila* provided genetic evidence that elevated TE activity is associated with a decrease in lifespan. To determine whether TEs are the active inducers of aging or merely a side effect of the aging process in higher eukaryotes will require carefully designed genetic and reverse-genetic experiments to resolve cause from effect, and to distinguish between effects on development and mechanisms operating during senescence.

Future research in this direction could help to

define what causal role TEs actually play in aging. For example, it remains to be determined in *Drosophila* whether adult lifespan is affected by TE somatic mobility in larvae, in adults, or both. In *C. elegans*, although there is a clear progressive increase in transposon somatic mobility during aging of the adult, no studies have yet been conducted comparable to those in *Drosophila* to establish an effect of such TE movement on adult lifespan. Although the tools for such decisive studies, such as an inducible source of transposase, are not yet available, recent progress in this area has been encouraging (Van Luenen *et al*., 1993, 1994; Vos *et al*., 1996). Furthermore, a causal association has yet to be determined between TEs and deleted or amplified sequences of the mitochondrial genome, in the variety of organisms for which age-associated mitochondrial alterations have been observed. Without this we cannot assert that TEs play a role in mitochondrial changes with age, or that these changes contribute to the senescent phenotype. Experiments probing functional relationships are now possible through reverse-genetic approaches, and have the potential to advance our understanding of the genetic and epigenetic components which limit metazoan lifespan. It should also be added that, unlike X-rays or other mutagens used to study the aging process, TEs act directly on DNA, without generating any side-effects in other macromolecules (Woodruff, 1992). This confers a great advantage to using transposable DNA elements in the study of the genetics of aging.

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