that only about one sequence in 200 of length 22 would show at least the ($5_s5_p1_h$) symmetry observed in the λ *cos* site. It would seem likely that this symmetry reflects some underlying mechanism. The significances of the symmetries at sites in the repressor-binding region of λ DNA vary considerably. When considered alone, these sites all appear to be quite rare, but this is misleading. If λ cohesive ends are generated by a symmetrical recognition-catalysis complex, the symmetry axis should, as it does, lie equidistant from the two sites at which nicks are to be introduced (Fig. 1*a*). There is no such *a priori* basis for site selection, however, in the case of the λ repressor-binding region: symmetries were considered wherever found. If the symmetry axis lies either on a base pair (as in Fig. 1*b*) or between two base pairs (as in Fig. 1*c*), then the number of sites comprised of two sequences of length N separated by zero or one base pair which can be tested for symmetry in a total sequence of length L is given by $2L-4N+1$. When each per-site expectation of symmetry is multiplied by the number of available sites, the results (Table 1, column 6) reveal that sites having at least ($8_s3_p1_h$) symmetry, or ($7_s6_p2_h$) symmetry, would be expected to occur in regions of 35 base pairs with a frequency of 0.7%, or 2%, respectively, but that sites having at least ($7_s3_p3_h$) symmetry would be expected to occur with an 18% frequency. Hence the symmetries shown in Fig. 1*b* and *d* would seem likely to be functionally significant, but the symmetry of Fig. 1*c* could well be due to chance.

It can be shown that in a random DNA sequence the occurrence of symmetry about one rotation axis does not affect the probability of symmetry about a second, distinct axis (my unpublished work). Two sites symmetrical about different axes can therefore be considered independently, even if the sites overlap. This independence does not generally hold for more than two overlapping sites.

The lack of statistical significance in the observation of a symmetry does not necessarily imply that the symmetry is not functionally significant. Trial calculations reveal that it may be impossible to distinguish statistically certain functional symmetries from spurious symmetries unless the region under consideration can be restricted independently to considerably fewer than 100 base pairs.

This analysis determines the position of an observed symmetrical site in the symmetry-ordered distribution of only those sites which are of the same length. Although this comparison is limited, it provides an easily-computed estimate of the rarity of the symmetry. A similar approach could be used in the statistical evaluation of other types of sequence anomalies such as purine-pyrimidine orientation bias.

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Single-stranded regions in DNA of old mice

INVESTIGATIONS have shown that senescence is accompanied by molecular changes in the structure of the eukaryotic genome. Some of these changes have been reported to result in the loss of some genetic material in the brain cells of ageing beagles[1]. Furthermore, the molecular weight of rat liver DNA decreases as a function of senescence[2]. If such changes occurred on a large scale, the resulting genetic damage could affect severely some of the crucial gene functions of an organism. We are studying the physical state of the DNA of senescing mice, and report here the accumulation of an increasing amount of single-stranded regions in the DNA with age.

DNA was isolated from the livers of 1, 6, 15, 20, 25, and 30-month-old CBF_1 mice (Charles River Breeding Laboratories). Single-strand-specific nuclease S_1 was used to investigate the presence of single-stranded regions in the native DNA of mice of different ages. Figure 1 shows the absence of nuclease S_1 sensitivity in the DNA of mice aged 1–15 months. That of mice 20 months of age or older was increasingly sensitive to the enzyme, which digested 14–25% of it. We cannot rule out the possibility that this sensitivity began before the mice were 20 months old, since we did not test the DNA of 15–20-month-old mice. These results are based on three experiments in each of which the DNA from different animals of the same age group were used.

There are already two sets of indirect evidence for single-stranded regions in the DNA of ageing animals. Samis *et al.*[5] reported increased incorporation of ^{3}H-thymidine into the nuclear DNA of senescent rats in the absence of a corresponding increase in mitotic index. This implies that the thymidine, after conversion to dTTP, was used in the repair of nuclear DNA. Calf thymus DNA polymerase has been shown to catalyse a greater incorporation of DNA precursors into the nuclei of old mouse neurones, astrocytes, Kupffer cells and heart muscle fibres[6]. This reaction is known to require single-stranded regions of primer DNA along which the complementary strand can be synthesised[7]. Presumably these single-stranded regions of the DNA from senescent mice were hydrolysed by nuclease S_1 in our experiments.

As we do not know the ultimate fate of the nuclease S_1-sensitive regions in the DNA of old mouse liver, we suggest that

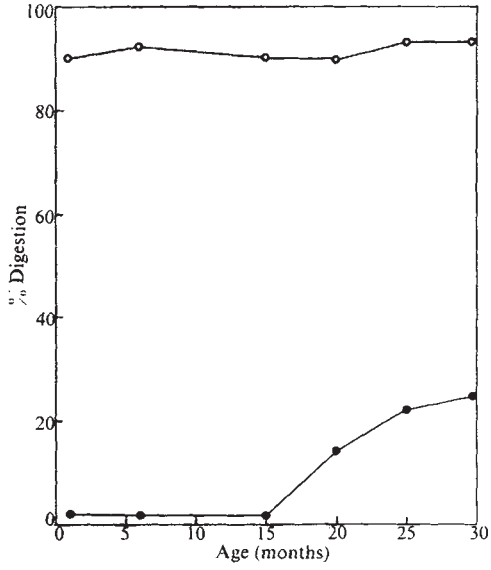

Fig. 1 Nuclease S_1 digestion of 1–30-month-old mouse liver DNA. DNA was prepared essentially by the method of Marmur[3] except that a phenol-chloroform (1:1) mixture was used for extracting the proteins. Nuclease S_1 was prepared from *Aspergillus orzae* crude α-amylase (Sigma) by the method of Sutton[4]. The reaction mixture consisted of 30 μg of native or heat-denatured DNA, and 10 μg nuclease S_1 all in 1.5 ml of KZS (0.1 M KCl, 0.1 mM $ZnSO_4$, 0.025 M sodium acetate *p*H 4.5). Incubation was at 37° C for 30 min. After chilling, 25 μg of carrier calf thymus DNA was added followed by 0.5 ml of 2 N perchloric acid. The control system (1.5 ml) consisted of 30 μg of liver DNA, 10 μg enzyme in KZS to which 25 μg carrier DNA and 0.5 ml of 2N chilled perchloric acid were added without incubating. The acid-insoluble material was collected by centrifuging at 17,000 r.p.m. for 20 min. The A_{260} of the supernate was measured. The amount of DNA rendered acid-soluble by nuclease S_1 was obtained by subtracting the A_{260} of the control supernate from that of the corresponding experimental sample. This value represented the amount of DNA digested by nuclease S_1 and was used to calculate the percentage of hydrolysed DNA. ●, Native DNA; ○, denatured DNA.

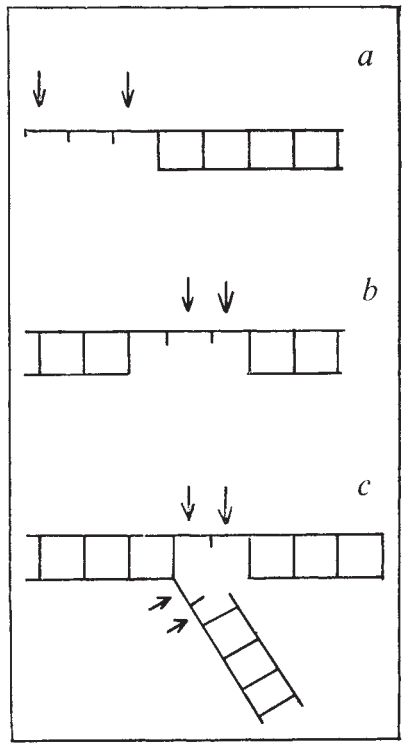

Fig. 2 Postulated model of the regions of nuclease S_1 attack on the DNA of senescent mice. DNA with single-stranded region (*a*) terminally located, and (*b*) internally located; (*c*) cross-linked DNA with single-stranded regions in the junction region. Arrows indicate points of possible nuclease attack.

the formation of regional single-stranded stretches in the DNA of senescent animals represents a transitional stage in the process leading to the elimination of nucleotide sequences that are to be discarded[1]. Figure 2 shows models of the possible structures of the regions in DNA attacked by nuclease S_1. There may be many areas of nuclease S_1 attack on crosslinked DNA molecules of which we present a simpler model in Fig. 2*c*. The metabolic significance of the existence of single-stranded regions in the DNA of older mice remains unknown. These regions may be the result of increased nuclease activity coinciding in time with a senescence-associated decline in repair enzyme activity in the cells. Loss in enzyme activity during senescence has been observed in other systems[8]. Thermodynamically, this has the advantage of making the energy previously reserved for repair functions available for hydrolytic processes. If the defective regions of the DNA occur in the genes for constitutive functions and are not repaired at the appropriate time, the biochemical lesions in the metabolic pathways of the particular cells are likely to result in the death of the organism; the harm can be particularly severe in the cells of non-replenishing tissues such as those of the muscular and nervous systems.

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Is mRNA transcribed from the strand complementary to it in a DNA duplex?

We have tested the assumption that the messenger RNA sequence is uniquely determined by the DNA sequence complementary to it. Because RNA is usually transcribed from a DNA duplex, there is the possibility that the DNA strand that is not complementary to the mRNA influences the RNA base selection. This possibility exists in models that postulate like-with-like base pairs[1-3], triple helix models of DNA-DNA-RNA[4], triple base interactions such as those described in tRNA[5], and models in which RNA polymerase may confer a special type of specificity (non-Watson–Crick) to RNA base–DNA duplex interactions, as well as in experiments in which separated are compared with non-separated strands[6-7].

The logic of our experiment is as follows. If one makes DNA heteroduplexes in which one strand has the coding for the premature termination of a given protein, while the other has the coding for the complete protein, and then uses these heteroduplexes as templates for transcription-translation in a coupled[8] cell-free protein synthesising system, then one can ask, by fractionating the radioactive protein products on Ornstein-Davis[9-10] acrylamide gels containing SDS, which product is made—the complete protein, a fragment or neither? If coding for RNA synthesis were solely a function of the nucleotide sequence of only one strand of a DNA duplex, then no matter

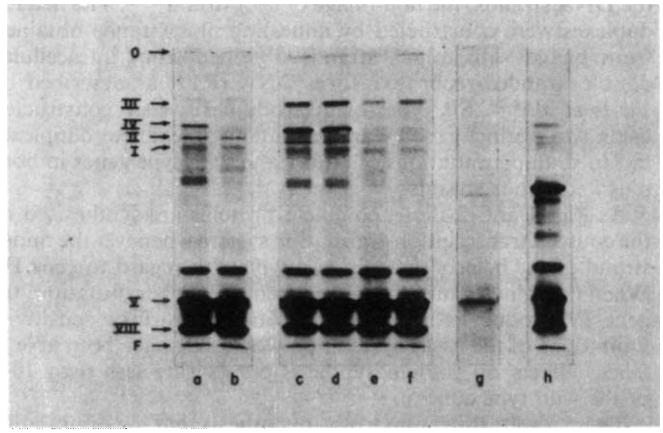

Fig. 1 Autoradiograph of urea SDS-Tris-glycine acrylamide gels showing the *in vitro* protein products stimulated by various homo-and hetero-duplex DNAs. Gene products were identified as described before[18] and are marked with Roman numerals. DNAs used as template for incorporations shown in (*a*), (*b*) and (*h*) were homoduplex (had not been subjected to cleavage, denaturation and renaturation). DNAs used were: (*a*) WT homoduplex; (*b*) amber IV_{12} and VII homoduplex; (*c*) amber IV_{12} and VIII +, WT −; (*d*) amber IV_{17} +, WT −; (*e*) WT +, amber IV_{12} and VII −; (*f*) WT +, amber IV_{17} −; (*g*) background (no added DNA: the bands reflect the synthesis of *E. coli* proteins from endogenous message); (*h*) amber IV_{17} homoduplex, showing prominent fragment produced when this DNA is used as template. DNA was isolated as described before[18]. Heteroduplex molecules were constructed by cleaving RFI with endonuclease R·*Hin*d, isolating the cleavage products on sucrose gradients, denaturing the product in alkali, annealing to a 20-fold molar excess of the appropriate phage (+) strand DNA, and re-annealing at 65° C for 1.5 h, all as described by Vovis *et al.*[13-14]. The circular reannealed molecules were purified by neutral sucrose gradient centrifugation and used as templates in the coupled transcription-translation system. All subsequent steps were as described previously[18].