alien genes into cultivated varieties which can be effective against more than one pathogen. Normally, genes transferred at the intervarietal level offer resistance only against one pathogen.

The salient features of the most promising wheat–rye recombinants developed in the present programme, as compared to Sonalika and Chinese Spring, are given in Table 1.

The efficacy of chromosome 5B manipulation in transferring desirable traits from rye into bread wheat has been demonstrated[6]. Earlier, a wheat-rye genetic stock (selection 111) was bred[11] which consistently gave 1000 grain weight of 64–68 g (the spike of this selection has about 50 seeds per spike). Such a high test weight is not available in the existing bread wheat germplasm.

The present programme laid emphasis on developing rust-resistant genetic stocks in wheat having rye genes. Since these genetic stocks cross easily with wheat varieties and the F_1s thus produced form 21 bivalents at metaphase I of meiosis, it is envisaged that these stocks could be useful in wheat bree ing programme.

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5-Methylcytosine residues in DNA decrease during ageing

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The degree of methylation of DNA is thought to be linked to many processes involving DNA, including transcriptional activity. Changes in DNA methylation are also believed to be a component of ageing of cells in culture and of organisms. We have estimated 5-methylcytosine in liver DNA of mice of ages between 11 days and 36 months using anti-5mC antibody and biotin–avidin peroxidase. We show that 5mC levels first decrease by 30% between 1 and 6 months of age, remain unchanged thereafter till 26 months, and then decrease again by 17% between 26 and 36 months. In differentiated liver, known to have decreased transcriptional activity, we find hypomethylation of DNA. On the basis of these and earlier data, we suggest that DNA in old liver is less accessible to methylases.

THE fidelity and efficiency of retrieval of genetic information depends on genome integrity, which may be causally related to the nature of lesion and the DNA repair function[1–4]. Furthermore, normal metabolic processes like methylation of cytosine may modify DNA structure[5] and alter DNA:protein interactions[6] In eukaryotes, chromatin proteins seem to control the accessibility of DNA to repair enzymes, so that linker DNA is preferentially repaired[7,8]. The presence of 5-methylcytosine (5mC) increases recombination frequency[9], while 6-methyladenine increases mismatch repair[10]. Razin and Riggs[11] have correlated the extent of methylation to the functional state and integrity of DNA. Transcriptionally active regions of chromatin seem to be undermethylated while inactive chromatin appears to be hypermethylated[12,13]. Wilson and Jones[14] have reported that, unlike in immortal cell lines, 5mC in diploid fibroblast DNA decreases with number of passages of the cultures, and suggested that survival and lifetime of diploid fibroblasts may depend on their ability to maintain a constant level of 5mC. Using an immunochemical assay[15,16], we have estimated 5mC in liver nuclear DNA of mice at several ages covering the entire life-span. We find that 5mC concentration decreases in two steps, first between 1 and 6 months, and later during the last quarter of life (26 to 36 months). This supports our preliminary observation[4].

Liver nuclei were isolated[17] from C57Bl × A/J F_1 hybrid mice at ages 11 days, and 1, 6, 7.5, 18, 26 and 36 months. DNA was purified[18], reextracted after treatment with ribonuclease A, and dissolved in TE (10 mM Tris–HCl, pH 7.8; 1 mM EDTA). The 260/280 absorption ratio was 1.82–1.87. DNA concentration was then adjusted to 100 $\mu g\,ml^{-1}$. It is well established that contaminating RNA raises the 260/280 ratio beyond 2.0, which was not the case in the preparation. Further, no RNA could be detected in these DNA preparations upon electrophoresis[19]. Nonmethylated DNA from bacteriophage lambda (grown in *dcm*⁻ *dam*⁻ *E. coli*, purchased from Sigma) was used as negative control. For each age, pooled liver DNA from six mice (three males and three females) was used.

DNA (100 ng in 1 μl) was spotted on nitrocellulose BA85 paper and baked for 4 hours at 70°C. The paper was incubated with 180 μg of rabbit

antibody[15] to 5mC dissolved in 10 ml of TBS (10 mM Tris–HCl, pH 7.5; 0.14 M NaCl) for 6 h at 25°C and then washed in TBS for 1 hour. It was then treated with biotinylated goat anti-rabbit IgG for 4 h and washed for 1 h. 5mC-antibody–antiantibody complex was detected by incubating with avidin and biotinylated peroxidase (Vector Laboratories, Burlingame, UK) for 1 h, washing again with TBS, and then staining for peroxidase using 0.1% diaminobenzidine tetrahydrochloride (DABT) as substrate and 0.02% H_2O_2. The stained paper was photographed and the negatives were scanned on a Kontron UVIKON 810 spectrophotometer with gel scanner. The experiment was repeated thrice and the integrated densitometric data used to compute 5mC content. Background was determined from equivalent areas adjacent to the DNA spots. The area under each densitometric peak was estimated by planimetry. Stained spots were also scanned in a single-beam photoacoustic spectrometer[16], with unmethylated lambda DNA as negative control. In a photoacoustic spectrometer, the sample is illuminated with chopped (23 Hz) monochromatic light (540 nm), and pressure oscillations in the gas phase in the sample chamber appear as acoustic signals. In this way, amounts of solid chromophores can be estimated with high accuracy.

The immunochemical reaction produces a dark spot on nitrocellulose[15]. We immobilised 100 ng of nuclear DNA from mice of different ages on BA85 paper and the 5mC-specific colour reaction (Figure 1) demonstrates that the highest spot intensity is for ages 11 days and 1 month while the lowest is for 36 months. The colour intensity was quantified by densitometry (inset in Figure 2) and the amount of 5mC relative to the amount of DNA per haploid genome (the 1C value)[20]

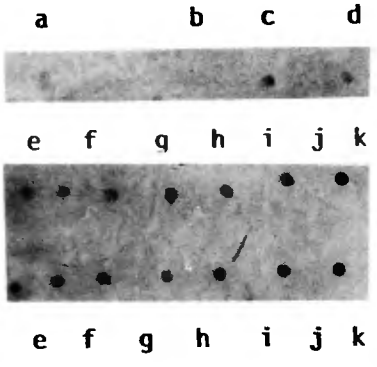

Figure 1. Immunochemical assay of 5-methylcytosine in ageing mouse liver nuclear DNA. One microlitre DNA (100 ng) from mice of ages (e) 11 days, and (f) 1, (g) 6, (h) 7.5, (i) 18, (j) 26 and (k) 36 months were spotted on nitrocellulose BA85 paper, reacted with rabbit antibody to 5mC, amplified with biotinylated goat anti-rabbit IgG, and visualized by the avidin–biotin-peroxidase assay. Positive controls are (a) 50 ng ϕX174 DNA and (c) 100 ng and (d) 50 ng methylated DNA from lambda bacteriophage grown in wild type *E. coli*. The reaction with 100 ng unmethylated lambda DNA is also shown (b).

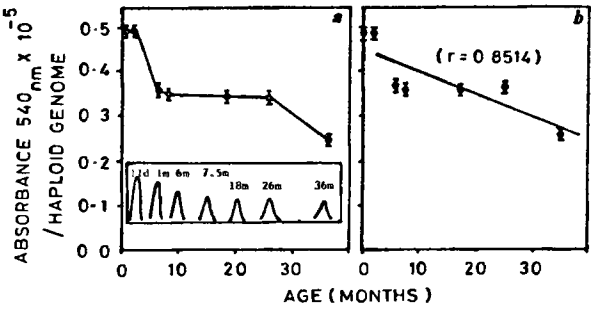

Figure 2. The relationship between 5mC content per haploid genome and age. From densitometric tracings of the colour reaction in DNA spots (inset) and the known average amount of DNA per nucleus[4,20], the amount of 5mC per haploid genome was estimated and plotted against age (in months). The standard deviation bars are based on analysis of at least three separate experiments. *a*, The relative amount of 5mC in liver DNA decreases in two steps during the lifetime of the mouse. The densitometric tracings of Polaroid negatives of stained DNA spots are shown in the inset. *b*, The data fitted to a regression line ($Y = mX + C$), which gives the coefficient of correlation $r = 0.8514$.

estimated (Figure 2, *a*). Unmethylated DNA gave no colour reaction (Figure 1). The relative amount of 5mC was highest at 11 days and 1 month, decreased by 31% by 6 months, then remained unchanged till 26 months, and decreased by another 17% between 26 and 36 months. It has been shown previously using photoacoustic spectroscopy that the amount of colour in such an immunochemical reaction is proportional to 5mC residues[16]. Therefore we also measured the photoacoustic signal intensity of the spots for ages 11 days, and 18 and 36 months and found them to be $1.045 + 0.02$, $0.692 + 0.02$ and $0.556 + 0.02$ respectively. Again we find a difference of 34% between ages 11 days and 18 months and a difference of 20% between 18 and 36 months. In the case of positive-control DNA the signal intensity increases with increasing concentration, and is comparable to values reported earlier[16].

We have thus shown that 5mC concentration in mouse liver DNA undergoes a two-step decrease during the lifetime of the mouse. According to Wilson *et al.*[21] the amount of 5mC in liver DNA decreases progressively by about 12%. A careful examination of the data published by Wilson *et al.*[21] is also suggestive of a two-step decrease in 5mC in the liver. On the other hand, fitting our data to a unique regression gives a progressive decrease of 35% in 5mC residues in liver DNA (Figure 2, *b*). In comparison, Singhal *et al.*[22] reported that the amount of 5mC first decreases progressively up to 26 months and increases thereafter. In conclusion, while there is a general agreement that 5mC residues decrease during the lifetime of the mouse, our data, as well as reexamination of the data of Wilson *et al.*[21], suggest that 5mC levels decrease in a step-wise manner.

It has been suggested[11-13,23] that transcriptionally active sequences are undermethylated. While we have no data to test this suggestion, we have shown that the concentration of 5mC is highest in actively proliferating newborn tissue, and that the step-wise decrease in 5mC is correlated with the decreased proliferative activity by 6 months of age. Furthermore, it is noteworthy that 5mC levels remain at a constant level during most of active adult life (6 to 26 months). In newborn mice, liver cells proliferate actively, with an average amount of DNA per nucleus of about 8 pg, or greater than the 2*C* value[20], which then decreases to about 5 pg by 3.5 months. Between 3.5 and 16 months, DNA per nucleus again increases to 11.7 pg owing to polyploidization[4,20]. The present data show that the first decrease in 5mC is temporally related to the decreased proliferative activity and that the polyploidization does not involve any change in the average level of methylated cytosine in DNA. During the lifetime of the mouse, the resistance of liver chromatin DNA to micrococcal nuclease increases in two steps: first from 35% at 10–11 days to 50% by 1.5 months, and then during the last quarter of life, between 28 and 35 months, from 50% to 63%[4,20]. Furthermore, resistance to micrococcal nuclease remained constant at 50% between 1.5 and 28 months of age. In another experiment[4], the relative number of free 3′-OH ends in DNA, which indicate single-strand breaks, were estimated for neurons in the visual cortex, and found to increase between 22 and 28 months of age. On the basis of the present data and previous work on DNA strand breaks[24-27], we suggest that the lower amount of 5mC in very old liver is due to decreased accessibility of chromatin DNA to methylases, with a consequent loss of steady state in the methylation process late in life.

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