

Instability and decay of the primary structure of DNA

Tomas Lindahl

Although DNA is the carrier of genetic information, it has limited chemical stability. Hydrolysis, oxidation and nonenzymatic methylation of DNA occur at significant rates *in vivo*, and are counteracted by specific DNA repair processes. The spontaneous decay of DNA is likely to be a major factor in mutagenesis, carcinogenesis and ageing, and also sets limits for the recovery of DNA fragments from fossils.

ALL biological macromolecules spontaneously decompose. Proteins that renature completely after heat-induced unfolding in neutral solution are susceptible to slow and irreversible inactivation at elevated temperatures which is independent of concentration. The intact covalent structure of the unfolded form of a typical enzyme such as egg-white lysozyme has a half-life of only a few minutes at pH 7.4 and 100 °C, largely due to deamidation of asparagine residues¹. Such simple chemical processes occur also at lower temperatures, with a typical roughly threefold decrease in reaction rate for each 10 °C interval, and deamidated asparagine residues not protected by tertiary structure have been observed in trypsin crystals after prolonged growth periods². Nucleic acids also undergo spontaneous decomposition in solution, RNA being particularly vulnerable. Because of the presence of the 2'-hydroxyl group of ribose, the phosphodiester bonds of RNA molecules are very susceptible to hydrolysis, particularly in the presence of divalent cations such as Mg²⁺ and Ca²⁺ (ref. 3). Transition metals, for example Zn²⁺, have an even greater deleterious effect. This property poses a major problem for predictions of the early existence of an aqueous 'RNA world' in connection with the origin of life⁴. Although RNA can serve as an adequate but relatively short-lived and mutation-prone carrier for a limited amount of genetic information, as seen in RNA viruses, the development of autonomously replicating cells probably depended on reduction of the ribose moiety in nucleotides to the unusual sugar, deoxyribose. This event provided genomes of greatly improved chemical stability.

DNA hydrolysis

The chemical price paid for the greatly increased resistance of the nucleic acid phosphodiester bond (gained by removal of the sugar 2'-OH group) is a labile *N*-glycosyl bond. Base-sugar bonds of ribonucleosides are much less susceptible to hydrolysis than those of deoxyribonucleosides. The instability of DNA glycosyl bonds under physiological solvent conditions was investigated 20 years ago; it was measured by using DNA with ¹⁴C-labelled purine or pyrimidine residues and determining the rates of release of free bases as a function of temperature, pH, ionic strength and nucleic acid secondary structure⁵⁻⁷. A more sensitive method involves registering the introduction of apurinic sites in covalently closed circular DNA molecules as they become sensitive to cleavage by alkali or specific DNA repair enzymes, AP endonucleases⁸. Guanine and adenine are liberated from DNA at similar rates, with guanine being released slightly more rapidly, whereas cytosine and thymine are lost at 5% of the rate of the purines. The difference in depurination velocity between single-stranded and double-stranded DNA is only four-fold, so the double helical structure does not provide much protection. As is the case for deoxyribonucleosides⁹, the reaction is mainly acid-catalysed under physiological conditions⁵. Effective charge-shielding of DNA with Mg²⁺ does not measurably influence the rates of hydrolytic release of free bases. At a site of base loss, the DNA chain is weakened and undergoes

cleavage by a β -elimination process within a few days⁸⁻¹⁰. This reflects the fact that the base-free sugar residue exists in an equilibrium between the major cyclic form and about 1% of the acyclic reactive aldehyde form.

In metabolically active cells, DNA occurs in the fully hydrated B form and would be expected to undergo depurination (with accompanying loss of genetic information) at a rate similar to that of DNA in solution. Fortunately, ubiquitous AP endonucleases rapidly initiate a DNA repair process (Fig. 1) by introducing DNA strand breaks on the 5' side of base-free sites. The sugar-phosphate residue is subsequently removed by a separate phosphodiesterase, followed by filling-in of the one-nucleotide gap by DNA polymerase and DNA ligase^{10,11}. This excision-repair pathway can handle much larger loads of DNA depurination than that produced by spontaneous hydrolysis. Thus, an *Escherichia coli* cell growing at 37 °C in the presence of a high, but nonlethal, concentration of the alkylating agent methyl methanesulphonate would suffer (and successfully repair) several thousand apurinic sites per generation, caused by release of the alkylation products 3-methyladenine and 7-methylguanine. In contrast, only a single apurinic site would emerge every second generation by spontaneous depurination of the *E. coli* chromosome. This reaction must, nevertheless, be of significance for mammalian cells in view of their very large genome size and slow replication, and it can be estimated that in each human cell 2,000-10,000 DNA purine bases turn over every day owing to hydrolytic depurination and subsequent repair⁵.

In addition to the problem of the intrinsic lability of glycosyl bonds, DNA base residues are susceptible to hydrolytic deamination. As expected from data on nucleosides⁹, cytosine and its homologue 5-methylcytosine are the main targets for this reaction. The deamination rates of these bases were defined experimentally by following their conversion to uracil or thymine residues in suitably radioactively labelled DNA¹²⁻¹⁴ as a function of temperature, pH and DNA secondary structure. Because the reaction is slow at 37 °C, the rate constants at various higher temperatures have been measured. This procedure, which yielded the activation energy of the reaction, allowed the cytosine deamination rate in DNA at 37 °C to be deduced. Recently, a very sensitive genetic reversion assay measuring the rate of deamination at a single DNA cytosine residue in the *E. coli lacZ* gene was used to reinvestigate this problem¹⁵. The cytosine deamination rate was determined directly for both single-stranded and double-stranded DNA at 37 °C and 30 °C. This genetical approach validated the extrapolation from elevated temperatures in the biochemical experiments in that the estimated cytosine deamination rates at 37 °C were the same within experimental error. For single-stranded DNA in solution, the half-life of an individual cytosine residue is about 200 years at 37 °C and pH 7.4. In such DNA, loss of purine residue occurs at a similar or slightly slower rate. In contrast to depurination, however, the double helix structure affords very good protection

against hydrolytic cytosine deamination, and this reaction occurs at only 0.5–0.7% of the rate of single-stranded DNA, that is, with a half-life of about 30,000 years for each cytosine residue. Heat-induced deamination of the 5-hydroxymethylcytosine residues found in bacteriophage T4 DNA to 5-hydroxythymine takes place at a somewhat higher rate¹⁶.

The mechanism of hydrolytic conversion of cytosine to uracil in DNA at neutral pH involves direct deamination by alkali-catalysed hydrolysis and also attack by water on the protonated base in a general acid-catalysed reaction⁹. Consequently, there is no strong pH dependence in the vicinity of pH 7.4 (ref. 12). An alternative mechanism involving an addition–elimination reaction⁹, analogous to the deamination of cytosine residues by bisulphite treatment, seems less likely because it would yield relatively long-lived pyrimidine hydrates as reaction intermediates; these are recognized by a different DNA repair enzyme than that acting at uracil residues. Furthermore, if the addition–elimination reaction occurred, for both steric and electrostatic reasons, 5-methylcytosine should be more refractory to deamination than cytosine residues. Instead, 5-methylcytosine moieties are deaminated three to four times more rapidly than cytosines^{12,14}. In a mammalian cell with 3% of its DNA cytosine residues in methylated form, about 10% of hydrolytic deamination would occur at 5-methylcytosine residues and 90% at cytosine residues. This relatively small difference in deamination rates is by itself unremarkable, and would hardly make 5-methylcytosine residues greatly preferred targets for spontaneous mutagenesis. But the effect is amplified by different rates of DNA repair. The deaminated form of cytosine is very rapidly excised by the abundant and ubiquitous uracil-DNA glycosylase to generate a base-free site, which is efficiently corrected (Fig. 1). This DNA repair enzyme cannot act on deaminated 5-methylcytosine, that is, thymine. The G·T base-pair in DNA is, instead, a substrate for mismatch correction systems, but these processes are slower than repair of G·U base pairs. A correspondingly greater risk for mutation fixation, therefore, will occur. In wild-type *E. coli*, 5-methylcytosine residues are mutational hot spots, whereas all cytosines are hot spots in a mutator *ung*⁻ strain deficient in uracil-DNA glycosylase¹⁷. In agreement with these data, *Streptococcus pneumoniae* and *Saccharomyces cerevisiae* strains deficient in uracil-DNA glycosylase also have a 10–20-fold increased spontaneous mutation rate caused by deamination of DNA cytosine residues^{18,19}. The hydrolytic reaction occurs without any apparent sequence specificity. Interestingly, the rate of spontaneous deamination of DNA cytosine residues is estimated to be 40-fold higher in *S. cerevisiae* than in *E. coli*, possibly because the slower rate of eukaryotic transcription might keep DNA locally in a single-stranded form for a more extended period¹⁹. The higher rate of deamination of 5-methylcytosine, combined with its relatively slow repair in mammalian cells, contribute to making methylated CpG sequences preferential (about 40-fold) targets for spontaneous point mutations; such G·C→A·T transitions account for one-third of single-site mutations observed in inherited human disease²⁰. Direct genomic sequencing of the p53 tumour suppressor gene showed that cytosine residues known to have undergone mutation were methylated in normal and tumour tissues, indicating that “5-methylcytosine functions as an endogenous mutagen and carcinogen in humans”²¹. This pattern of reduced correction of lesions arising from deamination of 5-methylcytosine may also account for the underrepresentation of CpG dinucleotides in mammalian genomes.

A separate factor contributing to the deamination of CpG sequences in mammalian cells might be ascribed to the catalytic mechanism in which cytosine is converted to 5-methylcytosine by the DNA methyltransferase²². The enzyme forms a covalent bond with the C6 position of the cytosine in CpG, thereby generating a transient dihydropyrimidine reaction intermediate that is highly susceptible to spontaneous deamination. So far, an enzyme-induced deamination reaction of this type has only

been demonstrated in cells with an *ung*⁻ genotype at levels of the S-adenosylmethionine cofactor far below the physiological concentration (~40 μM), but such active deamination might also occur occasionally in normal cells²³. This could explain the finding that apparently unmethylated CpG doublets in α-globin gene sequences exhibit an increased mutation frequency²⁴. Furthermore, enzyme-promoted deamination provides an attractive explanation for the very rapid alteration of extensively methylated, recently duplicated DNA sequences in *Neurospora crassa*, designated repeat induced point mutations (RIP, ref. 22).

By comparison with the hydrolytic conversion of cytosine to uracil, the deamination of DNA purines is a minor reaction. Adenine is converted to hypoxanthine (the base residue in deoxyinosine) in DNA at 2–3% of the rate of cytosine deamination²⁵. Because this moiety forms a more stable base pair with C than with T, the product of hydrolytic deamination of adenine is a mutagenic lesion. It appears to be repaired in an analogous fashion to uracil in DNA but by a separate DNA glycosylase²⁶. The repair reaction is, however, less efficient because of the low cellular level of hypoxanthine-DNA glycosylase²⁷. The rate of deamination of guanine to xanthine in DNA has not been precisely determined but is similar to, or slower than, adenine deamination. No specific repair enzyme for xanthine residues in DNA has been identified. Both hypoxanthine and xanthine base-pair preferentially with cytosine, albeit with reduced coding specificity, so rare deamination of adenine residues may be much more deleterious than that of guanine from the point of view of generating premutagenic lesions. In addition, the xanthine-deoxyribose bond is particularly susceptible to spontaneous hydrolysis, indicating that a dXMP residue generated by a rare guanine deamination event in a mammalian cell would be converted to an apurinic site within days or weeks at 37 °C in a nonenzymatic reaction. In view of the relatively poor cellular response mounted to deaminated purines compared to uracil residues, nitrosative deamination of DNA is unlikely to have been of general importance during evolution, because this process acts similarly on guanine, adenine and cytosine. A recent proposal suggesting that ‘nitrosative deamination of DNA by nitric oxide may represent an important mechanism of genomic alteration’²⁸ therefore seems unlikely. Possibly, endogenously produced nitric oxide could be of occasional mutagenic relevance in organs exposed to large amounts of this short-lived signal compound.

Other types of hydrolytic damage may conceivably result in lesions in DNA, but there is no biochemical evidence for such reactions. A mutagenic guanine derivative resulting in G→T transversions was observed in phage T4 DNA after prolonged incubation, and it was speculated that this might have resulted from heat-induced glycosyl bond migration¹⁶. Alternatively, this event might reflect oxidative formation of 8-hydroxyguanine residues.

DNA oxidation

Aerobically growing cells are exposed to active oxygen during normal metabolism. This represents an important source of endogenous damage to the genome. Bacteria possess elaborate inducible responses to oxidative stress, including the overproduction of DNA repair enzymes that remove blocked 3' termini at strand breaks²⁹. The major mutagenic base lesion generated by hydroxyl radicals is 8-hydroxyguanine, which base-pairs preferentially with adenine rather than cytosine and thus generates transversion mutations after replication^{30,31}. This lesion is excised by a specific DNA glycosylase, present both in *E. coli* and in mammalian cells, which also removes other altered purine residues with ring-opened rather than oxidized imidazole moieties, that is formamidopyrimidines³². The 8-hydroxyguanine and formamidopyrimidine base derivatives are generated in similar amounts by hydroxyl and superoxide radicals³³, but 8-hydroxyguanine is of greater interest because it has a directly mutagenic effect. Inactivation of the *E. coli fpg* (*mutM*)

gene encoding the relevant DNA glycosylase leads to a 10-fold increase in spontaneous mutation frequency³⁴. The same relative increase is seen in *E. coli ung* mutants that are unable to excise uracil from DNA. It seems likely that hydrolytic deamination of cytosine to uracil in DNA, and oxidation of guanine residues to 8-hydroxyguanine, are the two major types of spontaneous, directly premutagenic events in living cells. Furthermore, they occur at similar frequencies. (By comparison, apurinic sites are mainly cytotoxic lesions that block DNA polymerases.) The data on the rate of cytosine deamination in double-stranded DNA suggest that each type of event takes place 100–500 times per day in a human cell. Oxidation of the guanine moiety of dGTP poses another serious mutagenic threat to cells, because

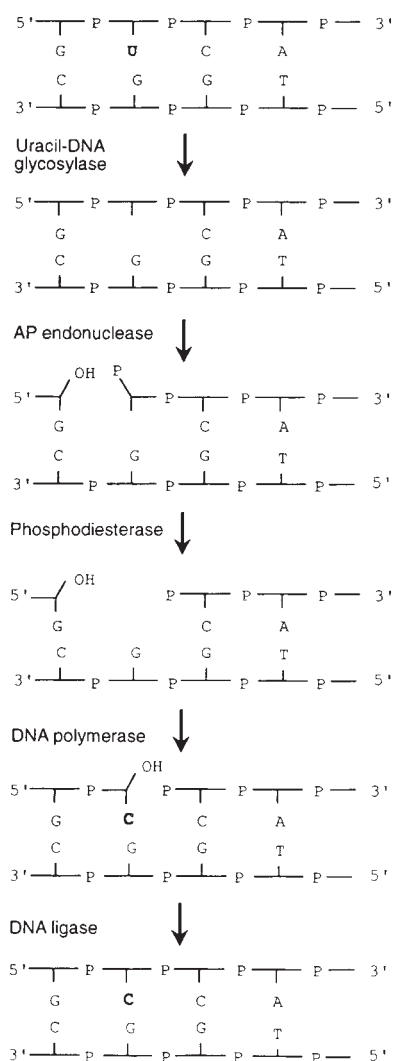


FIG. 1 The base excision-repair pathway for removal of endogenous damage from cellular DNA. The scheme is a minor modification of a previous model⁸². Apurinic/aprimidinic (AP) sites in DNA can be introduced by nonenzymatic hydrolysis of base-sugar bonds or by the action of several DNA glycosylases, specific for various types of DNA damage. Shown here is the excision of a deaminated cytosine residue (uracil) and the base-free deoxyribose-phosphate moiety, followed by replacement and correction of the missing nucleotide residue by DNA repair synthesis. Other forms of base damage generated by hydrolysis or by nonenzymatic methylation are recognized by separate DNA glycosylases, but the subsequent steps are identical. Two DNA glycosylases that counteract oxidative damage have an associated AP lyase chain-breaking activity and thus may be able to sidestep the requirement for an AP endonuclease. Attempts to construct an *E. coli* strain unable to incise DNA at AP sites have been unsuccessful, strongly indicating that such a cell would not be viable⁸³ and illustrating the importance of this DNA repair pathway.

8-hydroxy-dGMP residues can be misincorporated opposite adenine residues during DNA replication. This replication problem is dealt with by rapid degradation of 8-hydroxy-dGTP to a monophosphate by the small MutT protein, thus preventing the use of the triphosphate as a precursor for DNA synthesis³⁵. Moreover, any adenine residue that is misincorporated opposite an 8-hydroxyguanine in template DNA is excised by a specific DNA glycosylase, the product of the *E. coli mutY* gene³⁴. The cellular dUTP pool is also kept very small by a specific dUTPase. But the occasional use of dUTP instead of TTP as a precursor for DNA synthesis is harmless, because the resulting A-U base pair is equivalent to an A-T base pair. The uracil residue would in any event be rapidly removed by uracil-DNA glycosylase.

Published measurements of rates for generation of oxidative DNA damage are indirect and imprecise. Thus the estimate for 8-hydroxyguanine formation given above is 1–3 orders of magnitude lower than that in a recent proposal³⁶. The data in the latter study were of two kinds, neither of which was wholly compelling. Thus, it was reported that 8-hydroxyguanine could be detected in DNA from several rat organs. Because the lesion, being so mutagenic, is effectively repaired, it is unlikely that it would accumulate in nuclear DNA to a measurable degree, so this result may be attributable to minor oxidation of the DNA during isolation with solvents such as phenol and chloroform, and air-saturated buffers. Secondly, 8-hydroxydeoxyguanosine and smaller amounts of 8-hydroxyguanine were detected and measured in rat urine. It is a common misconception that such occurrence of the altered nucleoside reflects DNA repair. In fact, the nucleoside is not a reaction product in the base excision-repair pathway initiated by DNA glycosylase. Instead, it is presumably derived by degradation of DNA from dead cells by unspecific nucleases and phosphatases, and oxidation could have occurred during passage through the kidney.

Another type of extensively studied DNA base lesion generated by oxidation damage is a ring-saturated derivative of a pyrimidine. This lesion occurs in several forms, in particular as thymine and cytosine glycols and pyrimidine hydrates. These derivatives have lost the 5, 6 double bond and, in consequence, their planar ring structures; they are noncoding bases^{37,38}. The lesions may decompose further into fragments of bases. *In vitro*, ring-saturated pyrimidines are formed in DNA at rates marginally higher than that of 8-hydroxyguanine. They are excised by a specific DNA glycosylase with an associated AP lyase activity that promotes cleavage of the sugar-phosphate chain by β -elimination. This enzyme, which in *E. coli* has received the erroneous name 'endonuclease III', is an iron-sulphur protein; it is the first of the ubiquitous DNA repair enzymes for which the three-dimensional structure has been determined³⁹. Attempts to estimate the rates of conversion of DNA pyrimidines to ring-saturated forms *in vivo*⁴⁰ meet with the same difficulties as encountered with regard to 8-hydroxyguanine.

A particularly interesting form of oxygen radical-induced DNA damage which has received relatively little attention is that causing major helical distortion. Such lesions include 8, 5' cyclopurine deoxyribonucleosides generated by an additional covalent bond, formed between the base and the sugar-phosphate backbone⁴¹, and covalent intrastrand purine dimers⁴². These are unlikely to be substrates for DNA glycosylases and, therefore, would not be expected to be removed by the base excision-repair pathway. Recently, a major oxygen radical-induced DNA lesion of this type was found to remain unrepaired in biochemical experiments using cell extracts of xeroderma pigmentosum origin, whereas control extracts were repair-competent⁸⁴. Xeroderma pigmentosum cells are specifically defective in nucleotide excision-repair and normal with regard to base excision-repair.

Stimulated polymorphonuclear leukocytes and monocytes, and human tumour cell lines, produce substantial amounts of H₂O₂ (ref. 43). This might result in oxidative DNA damage and resulting death in adjacent cells, and chromosome instability in

tumour cells, in spite of cellular catalase activity. Some of the peroxide-generated DNA damage may proceed through a non-radical pathway to generate distinct DNA lesions, for example adenine-N1-oxide⁴⁴. The relative significance of such events compared to more abundant hydroxyl radical-induced DNA damage remains to be investigated.

The cell nucleus is a very poorly oxygenated intracellular compartment which lacks detectable O₂ metabolism⁴⁵. The delegation of the metabolism of reactive oxygen to mitochondria must protect nuclear DNA against oxidative damage; this may be a major reason for the evolution of distinct nuclei in eukaryotic cells. A corollary to this is that mitochondrial DNA should be uniquely sensitive to such damage. The situation is further aggravated by the fact that mitochondria do not contain histones which quench the generation of oxygen radical-inflicted DNA lesions⁴⁶. Thus, DNA from mitochondria contains higher levels of 8-hydroxyguanine than DNA isolated from cell nuclei. Interestingly, the amounts of this lesion are even higher in DNA from isolated mitochondria exposed to oxidative stress⁴⁷. Oxygen radical-induced DNA damage in mitochondria could be largely responsible for the high mutation rate, rapid evolution and decline with age of this organelle, as well as contributing to several maternally inherited human degenerative diseases, such as Leber's hereditary optic neuropathy^{48,49}.

Nonenzymatic DNA methylation

In addition to oxygen, living cells contain several other small reactive molecules that might cause DNA damage and act as endogenous genotoxic agents⁵⁰. The best characterized and probably most important of these is *S*-adenosylmethionine (SAM). This efficient methyl group donor is used as cofactor in most cellular transmethylation reactions. As for other simple methylsulphonium compounds, nonenzymatic transmethylation from SAM to various nucleophiles occurs at a slow rate. Thus, SAM is a weak alkylating agent and its nonenzymatic methylation of proteins⁵¹ and DNA^{52,53} can readily be demonstrated by using ³H-methyl-labelled SAM. The reaction is analogous to that of simple alkylating agents (such as methyl methanesulphonate or dimethyl sulphate) with DNA, the main targets being ring nitrogens of purine residues. 7-Methylguanine and 3-methyladenine are the major DNA lesions (Fig. 2). The former compound seems relatively harmless, because this guanine modification does not alter the coding specificity of the base. By contrast, 3-methyladenine is a cytotoxic DNA lesion that blocks replication, so it poses a major threat to the cell. It may be estimated that about 600 3-methyladenine residues per day are generated in DNA of a human cell in this reaction⁵², an alkylation level that would be achieved by continuous cellular exposure to 20 nM methyl methanesulphonate. There is no obvious protection against this DNA modification by cellular compartmentalization, because SAM is present in the cell nucleus and serves as cofactor for enzymatic DNA methylation. But both eukaryotic cells and bacteria contain a highly efficient 3-methyladenine-DNA glycosylase that rapidly excises the altered base, generating an apurinic site. 7-Methylguanine, by contrast, is poorly repaired and might be expected to accumulate to a measurable degree in the DNA of mammalian cells, although the chemical lability of the 7-methylguanine-deoxyribose bond ensures that a steady state of base modification and loss would be achieved within a few days. In agreement with this notion, direct measurements of 7-methylguanine adducts in rat liver DNA by a sensitive electrochemical detection procedure demonstrated the presence of small but significant amounts of the alkylation product⁵⁴.

Enzymatic methylation of DNA is widespread and is an important mechanism for modulating gene expression in mammalian cells. Such methylation occurs at the C5 position of cytosine, or in bacteria at the amino group of adenine. These are extremely poor acceptor sites for alkylating agents, so there is virtually no overlap between targets of enzymatic versus

nonenzymatic DNA methylation. This arrangement ensures that an enzymatically modified base residue serving as a biological signal is not accidentally removed by a specific DNA repair enzyme that counteracts alkylation damage. Similarly, the selection of thymine rather than uracil as a DNA base aids the repair of deaminated cytosine residues.

The enzyme cofactor for TMP synthesis, *N*⁵-methyltetrahydrofolic acid, has a poor transfer potential and is present at low concentrations in cells, so it is presumably much less important than SAM as a potential DNA methylating agent. Neither is likely to generate the highly mutagenic DNA lesion *O*⁶-methylguanine except in trace amounts. The production of this miscoding base accounts for the very strong mutagenic effect of alkylating agents such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and methylnitrosourea. Interestingly, *E. coli* cells specifically defective in repair of *O*⁶-methylguanine exhibit a threefold increased frequency of spontaneous mutation in stationary phase but not in logarithmic growth phase, compared to wild-type cells⁵⁵. These data imply that nongrowing *E. coli* cells may produce small amounts of an endogenous highly mutagenic alkylating compound. The identity of this agent remains unknown, but a survey of possible endogenous or environmental sources has revealed tentative candidates⁵⁶. For example, the simple metabolites carbamyl phosphate and methylamine may condense to form methylurea, which can be nitrosated to generate methylnitrosourea⁵⁷.

Many other cellular compounds, including metabolites of common amino acids, may form covalent adducts with DNA to a limited extent. This topic requires further investigation. The nonenzymatic reaction of reducing sugar compounds such as glucose-6-phosphate with amino groups of DNA bases is of particular interest⁵⁸. Under *in vivo* conditions, such adducts would only be generated at 0.1–1% of the rate of the other spontaneous DNA alterations discussed above, and the lesions would probably be repaired by the nucleotide excision–repair pathway. Nevertheless, a detectable mutagenic effect on plasmid DNA was demonstrated in *E. coli* cells with elevated glucose-6-phosphate levels⁵⁹.

Spores and thermophiles

There are biological strategies that overcome the intrinsic instability of DNA. Many microorganisms respond to hostile environmental conditions by sporulation, and DNA in some bacterial spores exhibits impressively increased resistance to heat- and radiation-induced damage. A recent review⁶⁰ summarizes the strategies used to achieve this goal. Prolonged heating of suspensions of *Bacillus subtilis* spores induces a very high mutation frequency among survivors, indicating that the introduction of lethal mutations is a major cause of loss of viability. In order to reduce hydrolytic depurination, the double-stranded DNA in spores exists in a partly dehydrated state and exhibits an A-like rather than B conformation. Moreover, a special group of small acid-soluble DNA-binding proteins is synthesized on sporulation; these proteins bind tightly and specifically to the A-form of DNA and reduce by at least 20-fold its rate of depurination. As an additional precaution, extensive DNA repair occurs during the first few minutes of spore germination. Oxygen-induced DNA damage in spores may be minimized by the DNA-binding proteins as well as by the absence of active metabolism; the lack of nucleoside triphosphates (and the low water content) in spores prevents DNA repair before germination.

In contrast to the special mechanisms devised to protect DNA in bacterial spores, there is no obvious need for unusual or exaggerated DNA repair processes in thermophilic bacteria growing at high temperatures. In fact, the efficient DNA repair mechanisms present in *E. coli* for correcting depurination and cytosine deamination would be just sufficient to withstand the roughly 3,000-fold increase in DNA decay and allow growth at 100 °C instead of 37 °C if the bacterial proteins could tolerate

the elevated temperature. But above 100–110 °C, serious difficulties would arise, both because of the chemical lability of the DNA structure to hydrolysis and the problem of retaining appropriate hydrogen-bonding between the two DNA strands. In the latter regard, high pressure affords some protection, in that the melting temperature (T_m) of the double helix is about 10 °C higher at 5,000 atmospheres. A report⁶¹ that thermophilic methanogenic bacteria are proliferating in deep-sea sulphide chimneys at temperatures above 250 °C seems amazing and is very difficult to reconcile with the known chemistry of DNA: the molecule would decompose so rapidly that no known DNA repair systems could conceivably counteract all the damage, and the preservation of the double helix would require stabilizing factors with a capacity far beyond any known compounds. Subsequent studies on this phenomenon indicate that the apparent bacterial growth at 250 °C may have been due to a chemical artefact produced at high temperatures rather than to cellular proliferation⁶².

Ancient DNA

Recovery of DNA fragments from extinct animals or plants in museum collections or from archaeological excavations can permit direct comparison with related contemporary material by DNA sequencing. This approach offers a valuable complement to taxonomic studies. Short mitochondrial DNA sequences from the extinct quagga have been cloned and compared with homologous sequences⁶³ from zebra and other related animals. Similarly, 2,400-year-old DNA fragments from an Egyptian mummy have been cloned and characterized⁶⁴. The advent of the polymerase chain reaction (PCR) has greatly aided such investigations because minute amounts of short DNA fragments

can be selectively amplified⁶⁵. Thus, DNA samples from archaeological bones up to 5,000 years old have been recovered and analysed⁶⁶. A problem with the hypersensitive PCR technique, as encountered by most newcomers to the method, is that trace amounts of contaminating DNA accidentally derived from laboratory glassware, or even the experimenter, readily produce false-positive results. This is a particularly difficult problem in work with ancient DNA, which is often highly degraded or possibly non-existent in available specimens and the occasional dramatic 'success' in this area should be viewed with skepticism.

From the data on hydrolytic and oxidative decomposition reviewed above, it can be predicted that deprived of the repair mechanisms provided in living cells, fully hydrated DNA is spontaneously degraded to short fragments over a time period of several thousand years at moderate temperatures. The most important route of decay for hydrated DNA is depurination. A 5–10-fold reduction in the rate of this process can be achieved at very high ionic strength⁵. Furthermore, adsorption of DNA to hydroxyapatite results in a twofold decrease in the rate of depurination (P. Robins and T.L., unpublished data); this could slightly improve the chances of recovery of useful DNA from old bones. Thus, in connection with favourable preservation conditions, it seems feasible that useful DNA sequences tens of thousands of years old could be recovered, particularly if the fossil has been retained at low temperature. Preliminary findings have been made on the identification of short mitochondrial DNA sequences from 40,000-year-old mammoth tissue (R. Higuchi, unpublished data). The data on bacterial spores would suggest that further increased stabilization of the DNA in fossils would be achieved by partial dehydration, and by the exclusion of oxygen. Note in this regard that DNA in air-dried tissues

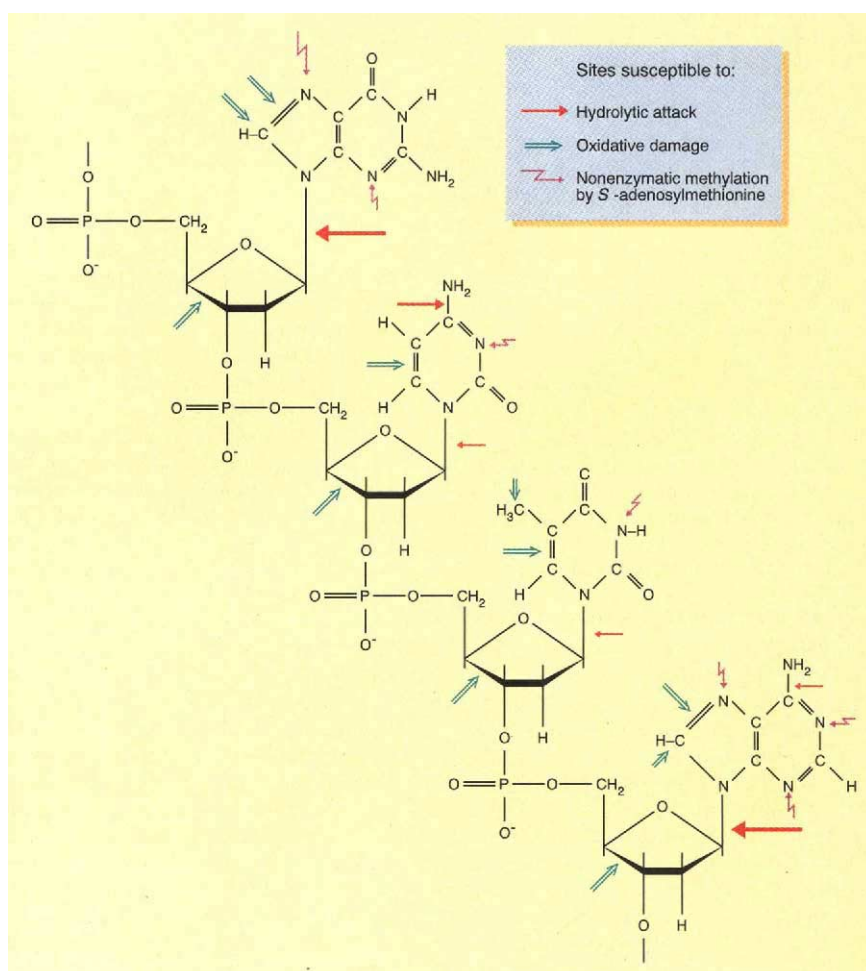


FIG. 2 Target sites for intracellular DNA decay. A short segment of one strand of the DNA double-helix is shown with the four common bases (from top: guanine, cytosine, thymine, adenine). Sites susceptible to hydrolytic attack are indicated by solid red arrows, oxidative damage by open green arrows, and nonenzymatic methylation by *S*-adenosylmethionine as zig-zagged purple arrows. Major sites of damage are indicated by the large arrows. Hydrolytic and oxidative damage, but not methylated residues would accumulate in fossil DNA.

remains partly hydrated and is still susceptible to decay. Because some of the water molecules in the grooves of the DNA double helix are structurally essential, 'dry DNA' obtained by storing DNA fibres over the efficient drying agent phosphorous pentoxide does not retain a double-helical conformation, making the bases more vulnerable to damage; such DNA is extremely hygroscopic and is rapidly rehydrated on exposure to air.

Recently, much excitement has been generated by claims of PCR amplification of DNA tens of millions of years old. The most intriguing of these reports concerns DNA from a 25–30-million-years-old termite preserved in amber⁶⁷. The authors tried with commendable care to avoid the contamination artefacts that have marred much of the work in this novel area of research. Minute amounts of highly degraded DNA were extracted from the preserved termite under sterile conditions in a laboratory where previous work on insect DNA had not been done. A number of control PCR amplifications were done to exclude any high-molecular mass DNA from the analysis, because such DNA would probably be of recent origin. In spite of these precautions, traces of several types of insect DNA sequences were recovered from the fossil material, which were 'dipteran in general and drosophilid in particular'. The most highly degraded DNA (<250 base pairs) contained some termite-like sequences as deduced from analysis of mitochondrial and nuclear ribosomal DNA; these were somewhat arbitrarily assigned as being representative of the fossil, whereas the other insect DNAs were deemed to be contaminants. It will now be important to assess the reproducibility of such findings. Amber preservation appears to provide a uniquely advantageous way of retaining ancient DNA sequences, because the DNA is largely dehydrated, partly protected from atmospheric oxygen, and not exposed to microbial contamination. It is disappointing in this context that no verified recoveries of DNA fragments have been made from less ancient (10^5 – 10^6 years old) but similarly entombed dehydrated fossils before attempts to isolate DNA 10^7 – 10^8 years old. In contrast to the careful study of the termite in amber, a widely publicized report on the recovery of 17–20-million-year-old DNA from an ancient magnolia leaf⁶⁸ exhibits serious deficiencies in that stringent PCR controls were not done. The leaf had been recovered from a wet deposit, and it was observed that high-molecular mass chloroplast DNA was recovered after a small number of PCR cycles. A reinvestigation of magnolia leaf material from the same *Clarkia* deposit, done elsewhere in a laboratory not working on chloroplast DNA, indicated that no plant DNA sequences could be recovered by PCR, although some (probably recent) high-molecular mass bacterial DNA was present⁶⁹. The apparent observation that fully hydrated plant DNA might be retained in high-molecular mass form for 20 million years is incompatible with the known properties of the chemical structure of DNA; it is, therefore, as incredible as the report on bacterial growth at 250 °C. Much better experimental documentation (preferably provided by meticulous nucleic acid biochemists) is required before such claims can be seriously considered.

Mutagenesis, carcinogenesis and ageing

Because DNA repair processes are not completely effective, the decay of the covalent structure of DNA may be expected to contribute in a major way to spontaneous mutagenesis⁷⁰ and by inference to carcinogenesis. Investigations of spontaneous mutation patterns in *E. coli*⁷¹ and mammalian cells indicate that both misreading of the DNA template during normal replication, and the occasional occurrence of damaged nucleotides in the template, are contributing factors. Several mechanisms for generation of spontaneous DNA lesions may be involved. Oxidative damage would appear to make only a minor contribution under most conditions: the experimentally determined oxygen- and hydrogen peroxide-derived mutational spectra in the human *hprt* gene are quite different from that of spontaneous mutagenesis⁷², and a DNA repair-deficient yeast strain exhibited

similarly elevated spontaneous mutation frequencies during aerobic and anaerobic growth⁷³. In addition to miscoding base derivatives, several types of primarily cytotoxic DNA lesions can generate occasional single-site mutations; these alterations include apurinic sites⁷, thymine glycol⁷⁴ and 3-methyladenine residues⁷⁵. The accumulated effect of replication and transcription of such damaged DNA templates has been proposed to be an important contributory factor in spontaneous carcinogenesis^{50,76}.

During evolution, cells acquired efficient DNA repair processes to permit very rapid correction of the predominant forms of spontaneous DNA damage. Water, oxygen and SAM resemble all other so-called 'group-specific agents' in that, in addition to the major ones, several distinct minor DNA lesions are generated. Some of these may only become relevant after slow accumulation, and for this reason may not be well repaired. One case in point may be deaminated 5-methylcytosine, which occurs less frequently but is more troublesome to cells than deaminated cytosine. It seems likely that certain very minor DNA lesions may have unexpectedly large biological effects due to their inefficient removal by DNA repair. It has often been speculated that cellular ageing may be a consequence of the gradual deterioration of the covalent structure of DNA. If this were correct, one might expect to find an accumulation of one or several unusual DNA base derivatives in very long-lived human cells such as neurons. Note that it is difficult to propose reasonable chemical models in this regard. Such a hypothetical DNA component must be chemically stable under physiological conditions, and this rules out many spontaneous lesions such as deoxyxanthosine or 7-methyldeoxyguanosine. Furthermore, the base derivative should be refractory to known versatile DNA repair processes. The complex nuclease that initiates nucleotide excision–repair of lesions causing major helix distortion also has some ability to act as a backup system in the repair of discrete lesions handled more effectively by other repair enzymes, for example apurinic/apyrimidinic sites, thymine glycol, and single-strand breaks with blocked 3'-termini⁷⁷. Few DNA lesions would escape survey by this activity. With regard to experimental studies, the ³²P postlabelling technique devised to detect damage in nonradioactive DNA⁷⁸ seems primarily suited to reveal bulky lesions which should be readily corrected by the nucleotide excision–repair pathway. Nevertheless, several compounds of unknown structures (designated 'I compounds') have been observed in nuclear DNA from aged tissue as well as in mitochondrial DNA by this method⁷⁸. Cells deficient in nucleotide excision–repair would be expected to be much more vulnerable to some forms of spontaneous DNA damage, because there is no known backup system to this excision–repair pathway. In this context, it is interesting that repair-defective cells from severe cases of xeroderma pigmentosum seem unable to remove a major oxygen radical-induced form of DNA damage, which might accumulate⁸⁴, because such patients suffer from a gradual but massive loss of neurons with accompanying mental deterioration over a period of decades and also exhibit a significantly increased frequency of internal cancers⁷⁹. Even in normal human cells, there would be little or no evolutionary reason to repair accumulating DNA lesions in stem cells or in neurons that might only become affected after 40–100 years. Chemically defined models for cellular ageing may be proposed along these lines, in addition to other hypotheses such as telomere shortening⁸⁰. For example, nonenzymatic DNA methylation by SAM generates the minor pyrimidine lesions 3-methylthymine and 3-methylcytosine. These are chemically stable, and active DNA repair of N3-methylated pyrimidines by DNA glycosylases has not been detected⁸¹. Unless they are removed by the nucleotide excision–repair pathway, such derivatives could accumulate in DNA *in vivo* over several decades and contribute to human ageing. □

T. Lindahl is in the Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Hertfordshire EN6 3LD, UK.

1. Ahern, T. J. & Klibanov, A. M. *Science* **228**, 1280–1284 (1985).
2. Kossiakoff, A. A. *Science* **240**, 191–194 (1988).
3. Lindahl, T. *J. Biol. Chem.* **242**, 1970–1973 (1967).
4. Pace, N. R. *Cell* **65**, 531–533 (1991).
5. Lindahl, T. & Nyberg, B. *Biochemistry* **11**, 3610–3618 (1972).
6. Lindahl, T. & Karlström, O. *Biochemistry* **12**, 5151–5154 (1973).
7. Loeb, L. A. & Preston, B. D. *A. Rev. Genet.* **20**, 201–230 (1986).
8. Lindahl, T. & Andersson, A. *Biochemistry* **11**, 3618–3623 (1972).
9. Shapiro, R. in *Chromosome Damage and Repair* (eds Seeberg, E. & Kleppe, K.) 3–18 (Plenum, New York, 1981).
10. Doetsch, P. W. & Cunningham, R. P. *Mutat. Res.* **236**, 173–201 (1990).
11. Dianov, G., Price, A. & Lindahl, T. *Molec. cell. Biol.* **12**, 1605–1612 (1992).
12. Lindahl, T. & Nyberg, B. *Biochemistry* **13**, 3405–3410 (1974).
13. Lindahl, T. *Prog. Nucleic Acid Res. molec. Biol.* **22**, 135–192 (1979).
14. Ehrlich, M., Zhang, X.-Y. & Inamdar, N. M. *Mutat. Res.* **238**, 277–286 (1990).
15. Frederico, L. A., Kunkel, T. A. & Shaw, B. R. *Biochemistry* **29**, 2532–2537 (1990).
16. Krickner, M. C. & Drake, J. W. *J. Bact.* **172**, 3037–3039 (1990).
17. Duncan, B. K. & Miller, J. H. *Nature* **287**, 560–561 (1980).
18. Chen, J.-D. & Lacks, S. A. *J. Bact.* **173**, 283–290 (1991).
19. Impellizzeri, K. J., Anderson, B. & Burgers, P. M. J. *J. Bact.* **173**, 6807–6810 (1991).
20. Cooper, D. N. & Youssoufian, H. *Hum. Genet.* **78**, 151–155 (1988).
21. Rideout, W. M. III, Coetzee, G. A., Olumi, A. F. & Jones, P. A. *Science* **249**, 1288–1290 (1990).
22. Selker, E. U. A. *Rev. Genet.* **24**, 579–613 (1990).
23. Shen, J.-C., Rideout, W. M. III, & Jones, P. A. *Cell* **71**, 1073–1080 (1992).
24. Perutz, M. F. *J. molec. Biol.* **213**, 203–206 (1990).
25. Karran, P. & Lindahl, T. *Biochemistry* **19**, 6005–6011 (1980).
26. Dianov, G. & Lindahl, T. *Nucleic Acids Res.* **19**, 3829–3833 (1991).
27. Hill-Perkins, M., Jones, M. D. & Karran, P. *Mutat. Res.* **162**, 153–163 (1986).
28. Wink, D. A. *et al. Science* **254**, 1001–1003 (1991).
29. Demple, B. A. *Rev. Genet.* **25**, 315–337 (1991).
30. Kasai, H. & Nishimura, S. *Nucleic Acids Res.* **12**, 2137–2145 (1984).
31. Shitubani, S., Takeshita, M. & Grollman, A. *Nature* **349**, 431–434 (1991).
32. Tchou, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **88**, 4690–4694 (1991).
33. Aruoma, O. I., Halliwell, B. & Dizdaroglu, M. *J. Biol. Chem.* **264**, 13024–13028 (1989).
34. Michaels, M. L., Cruz, C., Grollman, A. P. & Miller, J. H. *Proc. natn. Acad. Sci. U.S.A.* **89**, 7022–7025 (1992).
35. Maki, H. & Sekiguchi, M. *Nature* **355**, 273–275 (1992).
36. Fraga, C. G., Shigenaga, M. K., Park, J.-W., Degan, P. & Ames, B. N. *Proc. natn. Acad. Sci. U.S.A.* **87**, 4533–4537 (1990).
37. Wallace, S. S. *Envir. molec. Mutag.* **12**, 431–477 (1988).
38. Breimer, L. H. *Molec. Carcinog.* **3**, 188–197 (1990).
39. Kuo, C.-F. *et al. Science* **258**, 434–440 (1992).
40. Wagner, J. R., Hu, C.-C. & Ames, B. N. *Proc. natn. Acad. Sci. U.S.A.* **89**, 3380–3384 (1992).
41. Dirksen, M. L., Blakely, W. F., Holwitz, E. & Dizdaroglu, M. *Int. J. Radiat. Biol.* **54**, 195–204 (1988).
42. Carmichael, P. L., Shē, M. N. & Phillips, D. H. *Carcinogenesis* **13**, 1127–1135 (1992).
43. Szatrowski, T. P. & Nathan, C. F. *Cancer Res.* **51**, 794–798 (1991).
44. Mouret, J. F., Odin, F., Polyverelli, M. & Cadet, J. *Chem. Res. Toxicol.* **3**, 102–110 (1990).
45. Joenje, H. *Mutat. Res.* **219**, 193–208 (1989).
46. Ljungman, M. & Hanawalt, P. C. *Molec. Carcinog.* **5**, 264–269 (1992).
47. Richter, C., Park, J.-W. & Ames, B. N. *Proc. natn. Acad. Sci. U.S.A.* **85**, 6465–6467 (1988).
48. Wallace, D. C. *et al. Science* **242**, 1427–1430 (1988).
49. Linnane, A. W., Marzuki, S., Ozawa, T. & Tanaka, M. *Lancet* **i**, 642–645 (1989).
50. Lutz, W. K. *Mutat. Res.* **238**, 287–295 (1990).
51. Paik, W. K., Lee, H. W. & Kim, S. *FEBS Lett.* **58**, 39–42 (1975).
52. Rydberg, B. & Lindahl, T. *EMBO J.* **1**, 211–216 (1982).
53. Barrows, L. R. & Magee, P. N. *Carcinogenesis* **3**, 349–351 (1982).
54. Park, J.-W. & Ames, B. N. *Proc. natn. Acad. Sci. U.S.A.* **85**, 7467–7470 (1988).
55. Rebeck, G. W. & Samson, L. J. *Bact.* **173**, 2068–2076 (1991).
56. Vaughan, P., Sedgwick, B., Hall, J., Gannon, J. & Lindahl, T. *Carcinogenesis* **12**, 263–268 (1991).
57. Kodama, M. & Saito, H. *Cancer Lett.* **10**, 319–324 (1980).
58. Bucala, R. P., Model, P. & Cerami, A. *Proc. natn. Acad. Sci. U.S.A.* **81**, 105–109 (1984).
59. Lee, A. T. & Cerami, A. *Proc. natn. Acad. Sci. U.S.A.* **84**, 8311–8314 (1987).
60. Setlow, P. *J. Bact.* **174**, 2737–2741 (1992).
61. Baross, J. A. & Deming, J. W. *Nature* **303**, 423–426 (1983).
62. Trent, J. D., Chastain, R. A. & Yayanos, A. A. *Nature* **307**, 737–740 (1984).
63. Higuchi, R., Bowman, R., Friedberger, M., Ryder, O. A. & Wilson, A. C. *Nature* **312**, 282–284 (1984).
64. Pääbo, S. *Nature* **314**, 644–645 (1985).
65. Pääbo, S., Higuchi, R. G. & Wilson, A. C. *J. Biol. Chem.* **264**, 9709–9712 (1989).
66. Hageberg, E. & Clegg, J. B. *Proc. R. Soc. B244*, 45–50 (1991).
67. De Sille, R., Gatesy, J., Wheeler, W. & Grimaldi, D. *Science* **257**, 1933–1936 (1992).
68. Golenberg, E. M. *et al. Nature* **344**, 656–658 (1990).
69. Sidow, A., Wilson, A. C. & Pääbo, S. *Phil. Trans. R. Soc. B333*, 429–433 (1991).
70. Smith, K. C. *Mutat. Res.* **277**, 139–162 (1992).
71. Schaaper, R. M. & Dunn, R. L. *Genetics* **129**, 317–326 (1991).
72. Oller, A. R. & Thilly, W. G. *J. molec. Biol.* **228**, 813–826 (1992).
73. Ramotar, D., Popoff, S. C., Gralla, E. B. & Demple, B. *Molec. cell. Biol.* **11**, 4537–4544 (1991).
74. Basu, A. K., Loehler, E. L., Leadon, S. A. & Essigmann, J. M. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7677–7681 (1989).
75. Klungland, A., Fairbairn, L., Watson, A. J., Margison, G. P. & Seeberg, E. *EMBO J.* **11**, 4439–4444 (1992).
76. Loeb, L. A. *Cancer Res.* **49**, 5489–5496 (1989).
77. Snowden, A., Kow, Y. W. & van Houten, B. *Biochemistry* **29**, 7251–7259 (1990).
78. Randerath, K., Leibr, J. G., Gladek, A. & Randerath, E. *Mutat. Res.* **219**, 121–133 (1989).
79. Cleaver, J. E. & Kraemer, K. H. in *The Metabolic Basis of Inherited Disease* 6th edn. (eds Scriver, C. R. *et al.*) 2949–2971 (McGraw-Hill, New York, 1989).
80. Harley, C. B., Futcher, A. B. & Greider, C. W. *Nature* **345**, 458–460 (1990).
81. McCarthy, T. V., Karran, P. & Lindahl, T. *EMBO J.* **3**, 545–550 (1984).
82. Lindahl, T. *Nature* **259**, 64–66 (1976).
83. Saporito, S. M., Geden, M. & Cunningham, R. P. *J. Bact.* **171**, 2542–2546 (1989).
84. Satoh, M. S., Jones, C. J., Wood, R. D. & Lindahl, T. *Proc. natn. Acad. Sci. U.S.A.* (in the press).

Young formation age of a mantle plume source

Cornelia Class, Steven L. Goldstein, Stephen J. G. Galer & Dominique Weis*

Max-Planck-Institut für Chemie, Postfach 3060, W-6500 Mainz, Germany

* Université Libre de Bruxelles, 50 Avenue F. D. Roosevelt, B-1050 Brussels, Belgium

The Ninetyeast Ridge hotspot track displays strontium, neodymium and lead isotope variations over time that reflect simple radioactive decay in the plume source rather than a change in the mantle components present. The lead isotope variations indicate that the time spent by the plume source in a non-convecting mantle boundary layer was only a few hundreds of millions of years, contrary to the conventional view of individual plume sources as old (1–3 Gyr) or persistent features.

MANTLE plumes are the main mode by which material upwells from the deep mantle^{1–3}. When the lithosphere moves over a plume, a hotspot track is formed, which is manifested as linear chains of ocean-island volcanoes, seamounts or aseismic ridges, and continental flood basalts^{1,4,5}. Such tracks provide the only means available of examining the compositional evolution of a plume through time, stemming from a common source region deep in the mantle. This record extends back to ~200 Myr in some cases⁶. Distances along each track usually correspond to past loci and ages of the plume. Within individual tracks, changes in initial (age-corrected) isotope ratios over time have been interpreted, conventionally, as reflecting changes in the ‘mantle components’^{7,8} present in the source (compare with refs

9–11). Here we examine the Ninetyeast Ridge plume track in some detail. Initial Nd and Pb isotope ratios of the plume source show large increases with decreasing age. Strontium isotope ratios increase slightly by an amount which is insignificant compared with the range of Sr isotope ratios in ocean basalts. These isotope characteristics would be unlikely to arise if a change in the mixture of ‘mantle components’ had taken place in the plume source with time. Rather, they seem primarily to reflect radioactive decay with time in the source, which we term an ‘evolving plume source’. The changes in isotope ratios limit the timing of chemical fractionations involved; they indicate that the Ninetyeast Ridge plume source is a young, ephemeral feature persisting for <1 Gyr. If these observations are generally