tageous introduction of such DNA). The benefit, at each generation, was increased efficiency, but the price was the loss of potential for further evolution, at a level above that of point mutational change.

The first to pay this price were the ancestors of modern bacteria; their streamlined genomes are organised and expressed with an efficiency surpassed only by that of the genomes of the viruses which infect them, but their morphological and functional complexity is limited. Lower protists, which show among themselves a variety of different approaches to genomic streamlining[23], and which may retain traces of a genes-in-pieces organisation[24], were perhaps next. There will of course have been at each stage cells which did not succumb to the selective advantage of genomic simplification, although they resisted such change accidentally, not purposively. The 'higher' forms are not more complex because they have recently increased genetic plasticity. They are, rather, 'higher' and more complex because their ancestors were, at each generation, those very organisms which accidentally (and at some selective disadvantage to their immediate descendants) retained the genetic plasticity inherent in the genomes of the primitive ancestors of all cells. □

1. Gilbert, W. *News and Views* **271**, 501 (1978).
2. Williamson, B. *News and Views* **270**, 295 (1977).
3. Marx, J. L. *Science* **199**, 517–518 (1978).
4. Jeffrey, A. J. & Flavell, R. A. *Cell* **12**, 1097 (1977).
5. Breathnach, R. *et al. Nature* **270**, 314–319 (1977).
6. Glover, D. M. & Hogness, D. S. *Cell* **10**, 167–176 (1977).
7. Tonegawa, S. *et al. Proc. natn. Acad. Sci. U.S.A.*, in press (1978).
8. Tilghman *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 725 (1978).
9. Britten, R. J. & Davidson, E. H. *Q. Rev. Biol.* **46**, 111 (1971).
10. Lewin, B., *Cell* **4**, 77 (1975).
11. Ohno, S. *Evolution by Gene Duplication* (Springer-Verlag, New York, 1970).
12. Wilson, A. C. *et al. A. Rev. Biochem.* **46**, 573 (1971).
13. Margulis, L. *Origin of Eukaryotic Cells* (Yale University Press, New Haven, 1970).
14. Schopf, J. W. & Oehler, D. Z. *Science* **193**, 47 (1976).
15. Knoll, A. H. & Barghoorn, E. S. *Science* **190**, 52 (1975).
16. Kleckner, N. *Cell* **11**, 11 (1977).
17. Woese, C. R. & Fox, G. E. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5088 (1977).
18. Woese, C. R. & Fox, G. E. *J. molec. Evol.* **10**, 1 (1977).
19. Bonen, L. & Doolittle, W. F. *J. molec. Evol.*, in press (1978).
20. Schopf, J. W. *Origins of Life* **5**, 119 (1974).
21. Knoll, A. H. & Barghoorn, E. S. *Science* **198**, 396 (1977).
22. Reanney, D. C. *J. theor. Biol.* **48**, 243 (1974).
23. Roberts, T. M. *et al. CRC Crit. Rev. Biochem.* **3**, 349 (1971).
24. Valenzuela, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 190 (1978).

Incorporation of uracil into DNA

from Gerard O'Donovan

WHY does DNA contain thymine and not uracil? Over the past few years mechanisms preventing the incorporation of uracil into DNA have been uncovered in bacteria but more recently, several groups have shown that if these constraints are circumvented an apparently functional DNA containing uracil can be produced.

The incorporation of uracil into DNA is prevented by at least two mechanisms. The first is the presence of dUTPase activity which hydrolyses dUTP to dUMP thereby keeping the endogenous dUTP pool low while providing the dUMP substrate for thymidylate synthetase (see figure). The second is the presence of uracil-DNA glycosidase activity which removes uracil from single and double-stranded DNA (Lindahl *Proc. natn. Acad. Sci. U.S.A.* **71**, 3649; 1974).

Viable dUTPase mutants (*dut* mutants) were isolated by Hochauser and Weiss (*Fed. Proc.* **35**, 1492; 1976) These mutants are identical with previously described *dnaS* and *sof* mutants. As expected these mutants incorporate uracil into their DNA (Tye *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 154; 1977). They also accumulate labelled transient 4-5S DNA fragments after 5-10 s pulses of ^{3}H-thymidine (Tye *et al. op. cit.*). These fragments result from the increased incorporation of uracil into DNA as a consequence of the dUTPase defect, followed by excision repair of the incorporated uracil, probably by uracil-DNA glycosidase.

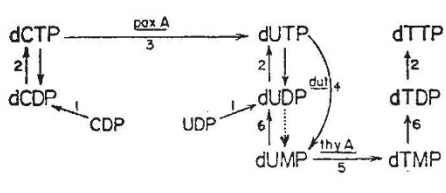
Viable mutants deficient in uracil-DNA glycosidase (*ung* mutants) have been isolated by Duncan *et al.* (*Fed. Proc.* **35**, 1493; 1976). The next step was obviously to examine *dut ung* double mutants for uracil incorporation into DNA. Warner and Duncan have recently reported (*Nature* **272**, 32; 1978) the presence of ^{3}H-uridine in the DNA of such mutants of *E. coli* and in the DNA of T4 phage propagated in them. Up to 30% of the thymine in T4 DNA seems to have been replaced by uracil and this DNA is functional as judged by the following findings.

When *ung*$^-$ cells of *E. coli* are infected they are killed equally well by the uracil-containing T4 phage DNA (T4·U) and the uracil-free phage (T4·T). The base composition of the total DNA of the infected cell and of the DNA packaged into the phage are the same. When the T4·U DNA is injected into an *ung*$^-$ cell (unable to initiate uracil-specific degradation) the T4·U DNA is normally functional as evidenced by its ability to be replicated and to direct phage synthesis.

When T4·U phage DNA is injected into wild-type *E. coli* (*ung*$^+$) the T4·U phage DNA is degraded so rapidly that it is not functional.

So why then does DNA contain thymine? Since the frequent random substitution of uracil for thymine during T4 DNA synthesis seems to have no serious consequences for the subsequent phage replication, the occasional misincorporation of uracil may have only minor effects on replication and transcription. Although it is believed to be attacking the A-U base pairs in the altered DNA the most important function of the uracil-DNA glycosidase normally *in vivo* therefore may not necessarily be to remove misincorporated uracil but to excise uracil produced by deamination of cytosine *in situ* by cytosine deaminase, thus preventing transition mutations. Warner and Duncan explain the importance of the presence of thymine rather than uracil in DNA by proposing that the absence of A-U base pairs allows specific recognition and excision of the potentially mutagenic mismatches produced by cytosine deaminase. Indeed Duncan has recently been able to show that *ung* mutations are mutagenic particularly when C→U transitions were specifically studied.

One final point seems pertinent. In a recent collaborative study Tye *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 233; 1978) have shown that uracil persists in the DNA of the *dut*$^-$ *ung*$^-$ double mutant through several generations. The *dut*$^-$ *ung*$^-$ mutant seems unaffected by levels of uracil in its DNA up to 1 per 100 nucleotides. This finding, taken together with the 30% substitution in phage T4 DNA reported here, strongly suggests that uracil incorporation into DNA is a common phenomenon. Experiments are in progress to determine how much uracil can be substituted for thymine in phage DNA as well as in *E. coli*. As pointed out by Warner, T4 DNA has to interact with far fewer proteins than does the DNA of bacterial and eukaryotic cells. Thus it seems likely that cells will be rather less tolerant of thymine replacement by uracil than are viruses. □



Gerard O'Donovan is Professor of Biochemistry and Genetics at Texas A&M University, College Station.