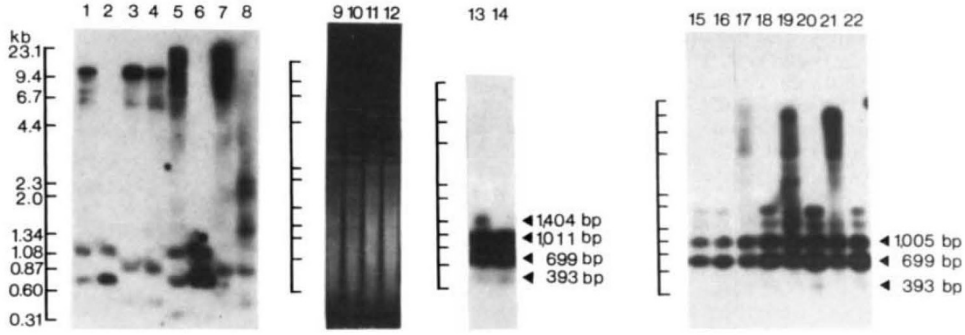

Fig. 4 Amplified transforming DNA is heavily methylated and 5-azacytidine prevents methylation. DNA samples were from the following strains (non-standard growth conditions are indicated if relevant; lane numbers in parentheses): wild-type STA4 (13, 14; the hybridizing bands of wild-type DNA were ~15-fold less intense than those of the transformant DNAs, but the autoradiographs were exposed for longer to give a similar appearance); transformants BC3 (1–4); E222 (5–12); E221 (15, 16; DNA prepared from mycelium grown for 16 h in medium containing 300 μM 5-azacytidine); E222 mycelium from 4 h liquid culture (17, 18); E222 mycelium from 12 h liquid culture (19, 20); E222 mycelium from 24 h liquid culture (21, 22). Restriction enzymes used were (lane numbers in parentheses): *Sau*3A (1, 5, 9, 13, 15, 17, 19, 21); *Mbo*I (2, 6, 10, 14, 16, 18, 20, 22); *Msp*I (3, 7, 11); *Hpa*II (4, 8, 12). Lanes 9–12 show the ethidium bromide-stained gel from which the blot of lanes 5–8 was made. For all Southern blots the probe was the 2.03-kb *Bam*HI–*Eco*RI fragment of *N. crassa* DNA encompassing the *am* gene (Fig. 1). Size markers (vertical bars) were a mixture of phage λ *Hin*dIII fragments and bacteriophage ΦX174 *Hae*III fragments. Arrows indicate the sizes of hybridizing fragments expected from the sequenced region probed (see the map of GATC sites indicated in Fig. 1) and the expected 1,005-bp boundary fragment that includes bacterial sequences. The 1,404-bp fragment marked (lane 13) results from the failure of *Sau*3A to cleave the GATC site between the 393- and 1,011-bp fragments. Other clearly defined fragments of ~1,300 and 1,600 bp (from E221 and E222 DNA, lanes 15–22) are probably new boundary fragments generated by deletion or rearrangement of DNA during amplification.

Methods: Standard medium for liquid cultures was Vogel's minimal medium (Fig. 2 legend) containing 20 mM glycine and 0.1% w/v *myo*-inositol. 300 μM 5-azacytidine was added as indicated; preliminary experiments showed that 3 μM 5-azacytidine, as commonly used to inhibit DNA methylation in animal cells[1–4], had no effect in *N. crassa*. Methods of DNA preparation and Southern blot analysis, and criteria for completeness of restriction digestion were as described in Fig. 2 legend. DNA methylation was tested by means of the isoschizomer pairs *Sau*3A (which does not cleave GATmC) and *Mbo*I (which cleaves GATmC)[40] and *Hpa*II (which does not cleave CmCGG or mCmCGG but cleaves mCCGG) and *Msp*I (which cleaves CmCGG, except in GGCmCGG sequences[41], but does not cleave mCCGG or mCmCGG, ref. 40) where mC denotes 5-methylcytosine. Completeness of *Mbo*I digests shows that N^6-methyladenine is not present at GATC sites.

with repair has also been suggested for UV-irradiated bacteria[28] and some recombinogenic lesions of 'hyper-*rec*' strains of *E. coli*[29]. In animal systems, DNA amplification, a frequent mechanism by which cells meet demands for increased amounts of gene products[10–16], does not generally[10] involve abnormally heavy methylation. However, *de novo* methylation of transfected foreign DNA sequences[30] has been found, and heavy hypermethylation of amplified rRNA genes occurs in rat hepatoma and some human cell lines[31] which, together with our results, suggests that methylation *de novo* of some types of amplified or rearranged DNA might be a widely distributed phenomenon.

We thank the SERC (research grant GR.B.34423 to J.C.W.) for financial support.

Received 5 April; accepted 21 June 1984.

1. Razin, A. & Riggs, A. D. *Science* **210**, 604–610 (1980).
2. Ehrlich, M. & Wang, R. Y.-H. *Science* **212**, 1350–1357 (1981).
3. Adams, R. L. P. & Burdon, R. H. *CRC Crit. Rev. Biochem.* **13**, 349–384 (1982).
4. Doerfler, W. *A. Rev. Biochem.* **52**, 93–124 (1983).
5. Hattman, S., Kenny, C., Berger, L. & Pratt, K. *J. Bact.* **135**, 1156–1157 (1978).
6. Feher, Z., Kiss, A. & Venetianer, P. *Nature* **302**, 266–268 (1983).
7. Achwal, C. W., Ganguly, P. & Chandra, H. S. *EMBO J.* **3**, 263–266 (1984).
8. Urieli-Shoval, S., Gruenbaum, Y., Sedat, J. & Razin, A. *FEBS Lett.* **146**, 148–152 (1982).
9. Bird, A. P. *Nature* **307**, 503–504 (1984).
10. Schimke, R. T. *Gene Amplification* (Cold Spring Harbor Laboratory, New York, 1982).
11. Beach, L. R. & Palmiter, R. D. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2110–2114 (1981).
12. Spradling, A. C. *Cell* **27**, 193–201 (1981).
13. Dalla-Favera, R., Wong-Staal, F. & Gallo, R. C. *Nature* **299**, 61–63 (1982).
14. Schwab, M., Alitalo, K., Varmus, H. E. & Bishop, J. M. *Nature* **303**, 497–501 (1983).
15. Roberts, J. M. & Axel, R. *Cell* **29**, 109–119 (1982).
16. Roberts, J. M., Buck, L. B. & Axel, R. *Cell* **33**, 53–63 (1983).
17. Kinnaird, J. H. & Fincham, J. R. S. *Gene* **26**, 253–260 (1983).
18. Kinnaird, J. H., Keighren, M. A., Kinsey, J. A., Eaton, M. & Fincham, J. R. S. *Gene* **20**, 387–396 (1982).
19. Kinsey, J. A. & Rambosek, J. A. *Molec. cell. Biol.* **4**, 117–122 (1984).
20. Case, M. E., Schweizer, E. M., Kushner, S. R. & Giles, N. H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5259–5263 (1979).
21. Case, M. E. in *Genetic Engineering of Microorganisms for Chemicals* (ed. Hollaender, A.) 87–100 (Plenum, New York, 1981).
22. Buxton, F. P. & Radford, A. *Molec. gen. Genet.* **190**, 403–405 (1983).
23. Perkins, D. D. & Barry, E. C. *A. Rev. Genet.* **11**, 133–225 (1977).
24. Bird, A. P. & Taggart, M. H. *Nucleic Acids Res.* **8**, 1485–1497 (1980).
25. Cooper, D. N., Taggart, M. H. & Bird, A. P. *Nucleic Acids Res.* **11**, 647–658 (1983).
26. Drahovsky, D., Lacko, I. & Wacker, A. *Biochim. biophys. Acta* **447**, 139–143 (1976).
27. Hilliard, J. K. & Sneider, T. W. *Nucleic Acids Res.* **2**, 809–819 (1975).
28. Ryan, A. M. & Borek, E. *Biochim. biophys. Acta* **240**, 203–214 (1971).
29. Korba, B. E. & Hays, J. B. *J. molec. Biol.* **157**, 213–235 (1982).
30. Pollack, Y., Stein, R., Razin, A. & Cedar, H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6463–6467 (1980).
31. Tantravahi, U., Guntaka, R. V., Erlanger, B. F. & Miller, O. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 489–493 (1981).
32. Jimenez, A. & Davies, J. *Nature* **287**, 869–871 (1980).
33. Wootton, J. C., Fraser, M. J. & Baron, A. J. *Neurospora Newslett.* **27**, 33 (1980).
34. Schweizer, M., Case, M. E., Dykstra, C. C., Giles, N. H. & Kushner, S. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5086–5090 (1981).
35. Vogel, H. J. *Am. Nat.* **98**, 435–440 (1964).
36. Metzenberg, R. L. & Baish, T. J. *Neurospora Newslett.* **28**, 20–21 (1981).
37. Stevens, J. N. & Metzenberg, R. L. *Neurospora Newslett.* **29**, 27–28 (1982).
38. Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
39. Hohn, B. *Meth. Enzym.* **68**, 299–309 (1979).
40. McClelland, M. *Nucleic Acids Res.* **11**, r169–r173 (1983).
41. Busslinger, M., de Boer, E., Wright, S., Grosveld, F. G. & Flavell, R. A. *Nucleic Acids Res.* **11**, 3569–3571 (1983).

Erratum

Functions of the canal system in the rotaliid foraminifer, *Heterostegina depressa*

R. Röttger, M. Spindler, R. Schmaljohann, M. Richwien & M. Fladung

Nature **309**, 789–791 (1984)

ON page 789, the last sentence of the second paragraph should read: We have examined here the symbiont-bearing Recent nummulitid, *H. depressa*, in which the canal system located within the test walls replaces the primary aperture seen in other foraminifera.