become more tenuous as we understand more about how they function. Note that the first orientation-independent promoter element was found when a restriction fragment upstream of the sea-urchin H2A histone gene was inverted[26].

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Note added in proof: Recent experiments carried out in collaboration with Angelo Tognoni and Walter Schaffner have shown that in CV1 (Monkey) cells the *Xenopus* U2 distal sequence element can functionally replace the SV40 enhancer to allow replication and T-antigen expression in SV40 mutants lacking the SV40 enhancer.

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1. Banerji, J., Rusconi, S. & Schaffner, W. *Cell* **27**, 299-308 (1981).
2. Moreau, P., Hen, R., Wasylyk, B., Everett, R., Gaub, M. P. & Chambon, P. *Nucleic Acids Res.* **9**, 6047-6068 (1981).
3. Cereghini, S. *et al. Cold Spring Harb. Symp. quant. Biol.* **47**, 935-944 (1982).
4. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729-740 (1983).
5. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 717-728 (1983)
6. Borrelli, E., Hen, R. & Chambon, P. *Nature* **312**, 608-612 (1984).
7. Wildemann, A. G., Sassone-Corsi, P., Grundström, T., Zenke, M. & Chambon, P. *EMBO J.* **3**, 3129-3133 (1984).
8. Ephrussi, A., Church, G. M., Tonegawa, S. & Gilbert, W. *Science* **227**, 134-140 (1985).
9. Ares, M. Jr, Mangin, M. & Weiner, A. M. *Molec. cell. Biol.* (in the press).
10. Mattaj, I. W. & Zeller, R. *EMBO J.* **2**, 1883-1891 (1983).
11. Roop, D. R., Kristo, P., Stumph, W. E., Tsai, M. J. & O'Malley, B. W. *Cell* **23**, 671-680 (1981).
12. Murphy, J. T., Burgess, R. R., Dahlberg, J. E. & Lund, E. *Cell* **29**, 265-274 (1982).
13. Zeller, R., Carri, M.-T., Mattaj, I. W. & De Robertis, E. M. *EMBO J.* **3**, 1075-1081 (1984).
14. Mattaj, I. W. *Trends biochem. Sci.* **9**, 435-437 (1984).
15. Skuzeski, J. *et al. J. biol. Chem.* **259**, 8345-8353 (1984).
16. Westin, G., Lund, E., Murphy, J. T., Pettersson, U. & Dahlberg, J. E. *EMBO J.* **3**, 3295-3301 (1984).
17. Bogenhagen, D. F., Wormington, W. M. & Brown, D. D. *Cell* **28**, 413-421 (1982).
18. Brown, D. D. *Cell* **37**, 359-365 (1984).
19. Reeder, R. H., Roan, J. G. & Dunaway, M. *Cell* **35**, 449-456.
20. Parslow, T. G., Blair, D. L., Murphy, W. J. & Granner, D. K. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2650-2654 (1984).
21. Falkner, F. G. & Zachau, H. G. *Nature* **310**, 71-74 (1984).
22. Gruss, P., Dhar, R. and Khoury, G. *Proc. natn. Acad. Sci. U.S.A.* **78**, 943-947 (1981).
23. Krol, A., Lund, E. & Dahlberg, J. *EMBO J.* (in the press).
24. Ciliberto, G., Buckland, R., Cortese, R. & Philipson, L. *EMBO J.* (in the press).
25. Labhart, P. & Reeder, R. H. *Cell* **37**, 285-289 (1984).
26. Grosschedl, R. & Birnstiel, M. L. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7102-7106 (1980).
27. Tani, T., Watanabe-Nagasu, N., Okada, N. & Oshima, Y. *J. molec. Biol.* **168**, 579-584 (1983).
28. Messing, J. & Vieira, J. *Gene* **19**, 269-276 (1982).
29. Mattaj, I. W. & De Robertis, E. M. *Cell* **40**, 111-118 (1985).
30. Mattaj, I. W. *et al.* in *The Oxford Surveys on Eukaryotic Genes* (Oxford University Press, in the press).

Amplification of *inter-Alu* extrachromosomal DNA during cellular ageing: retraction and explanation

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We reported previously[1,2] the age-dependent appearance of extrachromosomal circular DNA bands hybridizing to a human DNA fragment ('*inter-Alu*'), isolated from a genomic cluster of *Alu*I repeats[3]. Such bands appeared or increased at late passage in four out of six human fibroblast strains (six out of nine cell expansions); moreover, all DNAs (9/9) obtained from peripheral lymphocytes of aged donors, but none (0/8) from young donors, revealed a non-genomic *inter-Alu* band at ≃4.8 kilobases (kb)[1,2]. Subsequent data extended these numbers to 16/24 aged donors compared with 0/18 young donors[4]. These results were interpreted as evidence of age-dependent DNA rearrangement in normal human cells[1,2]. We now report that the 'extra' bands were of microbial origin, although clearly occurring in an age-dependent manner.

The hybridizations reported[1,2,4] primarily used the entire *inter-Alu*-containing plasmid, pλH15A, as ^{32}P-DNA probe. Gel-purified insert DNA also hybridized to the extrachromosomal species, which was then believed to be an adequate control for plasmid contamination. Such hybridization, however, could still be due to residual traces of vector DNA, "invariably present in [insert] probes . . . even after two cycles of electrophoretic purification" (ref. 5). Control hybridizations against many of the same DNA preparations, with a pCR1 fragment or pBR322 plasmid containing other inserts, revealed no extra bands. However, when we obtained the parental vector for *inter-Alu*, pACYC184, we observed hybridization of this probe to the age-dependent bands in both fibroblast and lymphocyte DNA samples, indicating that such bands must be due to low levels of microbial plasmid DNA ($\simeq 10^{-6}$) in our human DNA samples. We discussed the dangers of microbial contamination[1], but were misled by unwarranted confidence in the controls, the pervasive extent and polymorphic nature of the contamination, and its remarkable age dependence.

In attempting to account for our data, we have considered the possibility of deliberate contamination and regard it as extremely unlikely. Inadvertent operator contamination, however, is difficult to reconcile with the marked age bias observed, in particular for the multiple fibroblast DNA bands seen in many independent samples, prepared by six persons over a 2-yr period. Biological contamination is more plausible in the light of recent reports of microbial plasmid DNA in biopsy and necropsy tissue samples and in body fluids[5-7]. Our preliminary evidence indicates that plasmid-homologous DNA can be detected in filter-sterilized serum and trypsin, two common reagents for cell culture. Thus, the correlation between culture age and plasmid bands may reflect cumulative exposure of fibroblasts to plasmid DNA.

We apologize for any inconvenience to other investigators resulting from this error. Our experience, along with other reports[5,6,8], underscores the danger of systematic plasmid contamination of DNA samples, and the necessity for control hybridizations using the appropriate vector alone as probe.

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1. Shmookler Reis, R. J., Lumpkin, C. K. Jr, McGill, J. R., Riabowol, K. T. & Goldstein, S. *Nature* **301**, 394-398 (1983).
2. Shmookler Reis, R. J., Lumpkin, C. K. Jr, McGill, J. R., Riabowol, K. T. & Goldstein, S. *Cold Spring Harb. Symp. quant. Biol.* **47**, 1135-1139 (1983).
3. Calabretta, B., Robberson, D. L., Barrera-Saldana, H. A., Lambrou, T. P. & Saunders, G. D. *Nature* **296**, 219-225 (1982).
4. Goldstein, S., Lumpkin, C. K. Jr, McGill, J. R., Riabowol, K. T. & Shmookler Reis, R. J. *Clin. Res.* **31**, 478A, abstr. (1983).
5. Barbacid, M. *Science* **225**, 670 (1984).
6. Hino, O., Kitagawa, T. & Sugano, H. *Science* **225**, 670-671 (1984).
7. Holmberg, S. D., Osterholm, M. T., Senger, K. A. & Cohen, M. L. *New Engl. J. Med.* **311**, 617-622 (1984).
8. Muschel, R. J., Khoury, G., Lebowitz, P., Koler, R. & Dhar, R. *Science* **219**, 853 (1983).