Table 1 Transforming activity of chemically induced skin papillomas and carcinomas

Source of DNA	Mouse strain	Samples tested	Positive samples‡
Normal epidermis	NIH/Swiss	2	0
	Sencar	2	0
TPA-treated epidermis	NIH/Swiss	1	0
	Sencar	1	0
Papilloma	Sencar	5*	4*
Carcinoma	Sencar	3	2
Papilloma	NMRI	2†	2†
Liver	NMRI	1	0
Brain	NMRI	1	0

Frozen tissues were ground in liquid nitrogen and DNA samples were prepared by resuspension in guanidinium thiocyanate and spinning through CsCl as previously described[7]. Transfections were carried out by adding 20 μg high molecular weight DNA to 25 cm^2 flasks seeded 24 h earlier with 3×10^5 cells per flask. Cells were maintained and foci scored as described earlier[7]. Epidermal cells were prepared from groups of 10–20 mice by scraping with a rounded scalpel after heating the skins in water at 55 °C for 30 s. Epidermal fractions were immediately frozen in liquid nitrogen.

* One DNA sample was prepared from three pooled papillomas.

† Both DNA samples were prepared from 8–10 pooled papillomas.

‡ Samples positive in transfection assay.

with the exception of the RNA from the papilloma in lane *e* and from the TPA-treated epidermis, approximately equivalent amounts of undegraded RNA were present in each lane (data not shown). Similar relative transcript levels have been found using RNA dot hybridization with probes specific for ras^H, 7S RNA and ribosomal RNA sequences (data not shown). We conclude that ras^H transcripts are elevated to a varying degree in different papillomas. The elevation is not due simply to an increase in the proportion of proliferating cells within the tumours because the hyperplastic epidermis induced by TPA does not contain substantially higher ras^H transcript levels.

An increase in the concentration of c-ras^H trancripts with respect to NIH/3T3 controls was also observed in transformed foci induced by transfection of DNA from papillomas or carcinomas (Fig. 2*B*). No reproducible correlation was found between steady-state concentrations of ras^H-related transcripts and the source of DNA used to induce the transformants.

The elevation of c-ras^H transcripts which is observed in the chemically induced primary tumours contrasts with the observations reported on the transcription of the equivalent human gene in the EJ/T24 bladder carcinoma cell line. Tabin *et al.*[16] compared c-ras^H transcript levels in EJ cells and in a normal human bladder cell line, and found no detectable differences. A possible explanation of this apparent contradiction is that the establishment of normal cells in culture leads to elevated ras^H gene expression, thus disguising any true increase during tumour development *in vivo*. In this context, it is interesting that elevated ras^H transcription has recently been found in several primary human tumours of the colorectum[17]. Alternatively, prolonged culture of tumour cells may result in lower levels of ras^H transcripts. It is also possible, if somewhat less likely, that differential extraction of RNA from basal or supra-basal cells could have occurred in our experiments, leading to artificially low ras^H transcript levels in control epidermis.

The number of independent genetic events involved in the transformation of a normal cell into a malignant tumour cell is unknown, but estimates range from two[18,19] to about seven[20]. It seems likely that the acquisition of dominant transforming properties by the DNA of malignant cells constitutes an important step in tumour progression. It has previously been speculated that this event occurs relatively late in tumour development[21]. This was based on the assumption that NIH/3T3 cells may already exist in an 'initiated' state, and require only one additional (late) event to acquire malignant properties. The data presented here, however, demonstrate that during chemical carcinogenesis *in vivo*, activation of the ras^H gene is a relatively early event. The proportion of papilloma DNAs which exhibited transforming activity (>85%) is much higher than the percentage of benign tumours which progress to carcinomas in this system (5–7%)[5]. This indicates that the mutational event which induces the transforming capacity of the DNA does not take place at the transition to malignancy, but already exists in most or all of the pre-malignant lesions.

Our results demonstrate that no significant differences are found between papillomas and carcinomas with respect to transforming activity of high molecular weight DNA and the degree of transcription of the activated c-ras^H gene. The progression of some, but not all, papillomas to malignant carcinomas may therefore be determined by another genetic event which does not involve the c-ras^H gene.

We thank Kl. Goerttler, H. Loehrke and T. Slaga for supplying tumour material, E. Hecker and G. Furstenberger for TPA, Gail Cole for technical assistance, and I. Kerr, G. D. Birnie and J. Paul for discussions and support. The Beatson Institute is supported by grants from the Cancer Research Campaign.

Received 28 July; accepted 15 December 1983.

1. Pierce, G. B. & Fennell, R. H. in *Cancer Medicine* (eds Holland, J. F. & Frei, E.) 149–167 (Lea & Febiger, Philadelphia, 1982).
2. Knudson, A. G. *Cancer Invest.* **1,** 187–193 (1983).
3. Hecker, E., Fusenig, N. E., Kunz, W., Marks, F. & Thielmann, H. W. (eds) *Carcinogenesis* Vol. 7 (Raven, New York, 1982).
4. Boutwell, R. K. *Crit. Rev. Tox.* **2,** 419–443 (1974).
5. Burns, F. J., Vanderlaan, M., Snyder, E. & Albert, R. E. In *Carcinogenesis* Vol. 2 (eds Slaga, T. J., Sivak, A. & Boutwell, R. K.) 91–96 (Raven, New York, 1978).
6. Cooper, G. M. Science **217,** 801–806 (1982).
7. Balmain, A. & Pragnell, I. B. *Nature* **303,** 72–74 (1983).
8. Digiovanni, J., Slaga, T. J. & Boutwell, R. K. *Carcinogenesis* **1,** 381–389 (1980).
9. Hennings, H. *et al. Cancer Res.* **41,** 773–779 (1981).
10. Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S. & Barbacid, M. *Nature* **298,** 343–347 (1982).
11. Der, C. J., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79,** 3637–3640 (1982).
12. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* **297,** 474–478 (1982).
13. Shimizu, K., Goldfarb, M., Perucho, M. & Wigler, M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 383–387 (1983).
14. Pulciani, S. *et al. Nature* **300,** 539–542 (1982).
15. Balmain, A., Krumlauf, R., Vass, J. K. & Birnie, G. D. *Nucleic Acids Res.* **10,** 4259–4277 (1982).
16. Tabin, C. J. *et al. Nature* **300,** 143–149 (1982).
17. Spandidos, D. & Kerr, I. B. *Br. J. Cancer* (submitted).
18. Armitage, P. & Doll, R. *Br. J. Cancer* **11,** 161–169 (1957).
19. Moolgavkar, S. H. & Knudson, A. G. *J. natn. Cancer Inst.* **66,** 1037–1051 (1981).
20. Cook, P. J., Doll, R. & Fellingham, S. A. *Int. J. Cancer* **4,** 93–112 (1969).
21. Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. *Nature* **300,** 149–152 (1982).
22. Ellis, R. W. *et al. J. Virol.* **36,** 408–420 (1980).

Errata

In the letter 'A resetting of Phanerozoic community evolution' by L. M. Van Valen, *Nature* **307,** 50–52 (1984), the words 'It is therefore not feasible' in paragraph 5 should be replaced by 'This sort of analysis cannot be used'; and the reference to the 'Red Queen hypothesis' on page 51 should read 'Red Queen's hypothesis'. In addition, Fig. 1 was poorly reproduced: a correct version is shown below.