as shear relaxation times this may suggest that a lower frequency mechanism, such as concentration fluctuations in an alloy, is responsible for the bulk viscosity.

If appreciable bulk attenuation occurs in the mantle and inner core, for example, by intergranular thermal currents, then the estimate of Q_K^{-1} in the outer core will be reduced and the estimate of the bulk viscosity will be an upper bound. The thermal relaxation time is not a strong function of temperature or pressure. It would, therefore, be a coincidence if it happened to be near the period of ${}_0S_0$. The viscous relaxation times, on the other hand, are strong functions of the external variables and it is not unreasonable that they sweep through the seismic band in the core.

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1. Anderson, Don L., Ben-Menahem, A. & Archambeau, C. B. *J. geophys. Res.* **70,** 505 (1965).
2. Anderson, Don L. & Hart, R. S. *Phys. Earth planet. Inter.* **16,** 289 (1978).
3. Anderson, Don L. & Hart, R. S. *J. geophys. Res.* **83,** 5869 (1979).
4. Sailor, R. V. & Dziewonski, A. M. *Geophys. J. R. astr. Soc.* **53,** (1978).
5. Knopoff, L., Zurn, W., Rydelek, P. & Yogi, T. *J. Geophys.* **46,** 89 (1978).
6. Cormier, V. & Richards, P. G. *J. geophys. Res.* **81,** 3066 (1976).
7. Suzuki, Y. & Sato, R. *J. Phys. Earth* **18,** 157 (1970).
8. Gutenberg, B. *Trans. Am. geophys. Un.* **38,** 750 (1957).
9. Flinn, J., Jarzynski, J. & Litovitz, T. *J. chem. Phys.* **54,** 4331 (1971).
10. Herzfeld, K. & Litovitz, T. *Absorption and Dispersion of Ultrasonic Waves* (Academic, New York, 1959).
11. Leppaluoto, D. A. in *High Pressure Physics and Planetary Interiors*, (ed. Runcorn, S. K.) (North-Holland, Amsterdam, 1972).
12. Evans, R. & Jain, A, in *High Pressure Physics and Planetary Interiors* (ed. Runcorn, S. K.) 141-145 (North-Holland, Amsterdam, 1972).
13. Furukawa, K., *Sci. Rep. Tohoku Univ., 1st Ser.* **12,** 368 (1960).
14. Gans, R. *J. geophys. Res.* **77,** 360 (1972).
15. Jordan, T.H. & Anderson, Don L. *Geophys. J.R. astr. Soc.* **36,** 411 (1974).
16. Bukowinski, M. & Knopoff, L. *The Physics and Chemistry of Minerals and Rocks* (ed. Strens, R. G. J.) (Wiley, London, 1976).
17. Toomre, A. *Geophys. J. R. astr. Soc.* **38,** 335 (1974).
18. Julian, B. R., Davies, D. & Sheppard, R. M. *Nature* **235,** 317 (1972).
19. Dziewonski, A. M. & Gilbert, F. *Nature* **234,** 465 (1971).
20. Zener, C. *Elasticity and Anelasticity of Metals* (University of Chicago Press, 1948).
21. Flinn, J., Gupta, P. & Litovitz, T. *J. chem. Phys.* **60,** 4390 (1974).
22. Buland, R., Berger, J. & Gilbert, F. *Nature* **277,** 358 (1979).

Isolation of the chicken thymidine kinase gene by plasmid rescue

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We have used the bacterial plasmid pBR322 as a vehicle to isolate genes coding for selectable markers from higher eukaryotes. In this way, we have obtained the chicken thymidine kinase (tk) gene as a 2.2-kilobase EcoRI/HindIII insert in pBR322. The cloned gene transforms tk⁻ animal cells with an efficiency equal to that of the cloned herpes simplex virus-1 tk gene.

SEVERAL genes of higher eukaryotes which encode major products of at least one tissue have been isolated, chiefly by using specific hybridization probes to screen genomic DNA fragments cloned into prokaryotic vectors[1-3]. Recently, an efficient method has been developed for the stable genetic transformation of cultured animal cells[4-7]. Genomic DNA from wild-type cells has been used as donor to transfer genes coding for selectable markers, such as thymidine kinase, adenine phosphoribosyl transferase and hypoxanthine phosphoribosyl transferase, to mutant recipient cells[8-11]. These developments make possible the isolation of genes coding for selectable markers. We describe here the isolation of a chicken thymidine kinase (tk) gene by means of 'plasmid rescue'. The chicken tk gene was chosen because an excellent recipient for DNA-mediated gene transfer was available in Ltk⁻, a tk-deficient mouse cell line, which has never been known to revert spontaneously to the tk⁺ phenotype[8-12]. In addition, this gene has the advantage that both the tk⁺ and the tk⁻ phenotypes can be selected[13].

Experimental design

In our scheme, chicken DNA is first digested with a restriction endonuclease which does not cleave tk and then ligated to similarly digested *Escherichia coli* plasmid pBR322 coding for ampicillin resistance (amp)[14]. This concatenated DNA is used to transform mouse Ltk⁻ cells to tk⁺. Some transformants can be expected to contain the chicken tk gene linked to pBR322 sequences. Next, the pBR322 sequences residing in the transformant are used to 'rescue' the flanking animal host sequences containing the tk gene. For this, DNA from the transformant is cleaved with a second restriction endonuclease, ligated in cyclization conditions and then used to transform *E. coli* to ampicillin resistance. Plasmid clones resulting from transformation of *E. coli* are then tested for the ability to transfer tk back into tk⁻ animal cells. This scheme is illustrated in Fig. 1, in which the restriction endonuclease *Hin*dIII is used for the first set of cleavage/ligations and *Eco*RI is used for plasmid rescue.

Transformation of Ltk⁻ cells

Restriction endonuclease-cleaved chicken DNA was tested for the ability to transfer tk to Ltk⁻ recipients to identify the enzymes that did not cut the chicken tk gene. Among these were *Hin*dIII, *Bam*HI and *Eco*RI. *Hin*dIII was chosen for initial constructions. *Hin*dIII-cleaved chicken DNA was ligated to *Hin*dIII-cleaved pBR322 at 1:1 mass ratios, and then used to transform Ltk⁻ cells by the calcium phosphate co-precipitation method[9,15]. We found that pBR322 sequences inhibited transformation in amounts exceeding 2 μg per 10^6 cells. Therefore, 1 μg of chicken DNA which was ligated to 1 μg of pBR322 was co-precipitated in calcium phosphate along with 18 μg of Ltk DNA. This mixture was added to each plate containing 10^6 cells. Cells were then selected in hypoxanthine/aminopterin/thymidine medium as reported before[7]. After 2 weeks, 12 tk⁺ colonies were cloned and cultured for further analysis.

All tk⁺ transformants were examined for the presence of pBR322 sequences by blot analysis[16,17] using ^{32}P-labelled nick-translated[18,19] plasmid as probe. Although 8 of 12 transformants contained 2–20 copies of pBR322, we could not assume that any of these was adjacent to the tk gene because far more pBR322 sequences than tk genes were present during transformation, and co-transformation with unlinked DNA fragments does occur[17].

To establish the relationship between pBR322 sequences and the chicken tk gene, a second round of transformation and

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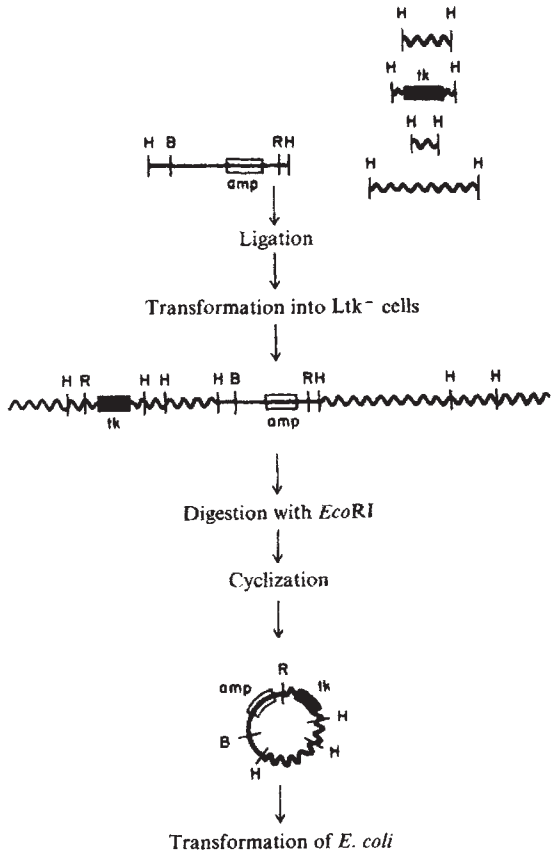

Fig. 1 A schematic illustration of the isolation of the chicken thymidine kinase gene (tk) by plasmid rescue. H, R and B refer to restriction endonuclease sites for *Hin*dIII, *Eco*RI and *Bam*HI, respectively (see text for details).

selection was necessary. Total DNA from each of four primary transformants containing pBR322 sequences was used to create eight secondary tk^+ transformants. Blot analysis showed that 4 of the resulting 32 transformants (derived from 3 primary transformants) contained a single pBR322 sequence. Such sequences were not present in vast excess in the genomic DNA of primary transformants and so we concluded that the pBR322 sequences present in the secondary transformants were adjacent to the tk gene.

Rescue of pBR322 sequences

To rescue pBR322 sequences from transformed animal cells, these sequences must contain plasmid replication functions and an antibiotic resistance gene. Rtll-1a, a secondary transformant derived from the primary transformant tll, contained a *Hin*dIII fragment which annealed with nick-translated pBR322 and co-migrated with *Hin*dIII-cleaved pBR322. We concluded that no loss of pBR322 sequences occurred during transformation of this cell line. *Eco*RI and *Bam*HI were each used to rescue pBR322 from this line because they do not cleave the chicken tk gene and each cleaves pBR322 only once, on either side of the single *Hin*dIII site of the circular pBR322 molecule[20].

DNA from Rtll-1a was digested, ligated in cyclization conditions, and used to transform *E. coli* χ1776 (ref. 21) by a high efficiency procedure (D.H., in preparation). The efficiency of transformation of χ1776 with this cellular DNA was about 100-fold lower than expected from reconstruction experiments. Two ampicillin-resistant colonies were obtained which contained pBR322 derivatives: a 9.2-kilobase plasmid, pR-1, derived from *Eco*RI cleavage, and an 8.5-kilobase plasmid, pB-1, derived from *Bam*HI cleavage. Restriction maps of these plasmids are presented in Fig. 2.

We next verified (by hybridization) that the rescued plasmids were derived from the transformed mouse cells. Rescued sequences were nick translated and used as probes in blot analysis of restriction endonuclease-cleaved Rtll-1a DNA. Figure 3*a* and *b* show that *Bam*HI-cleaved pB-1 co-migrated with an homologous *Bam*HI fragment of Rtll-1a. Furthermore, Fig. 3*c* and *d* show that *Hin*dIII/*Bam*HI-cleaved pB-1 co-migrated with homologous fragments in *Hin*dIII/*Bam*HI-cleaved Rtll-1a. Double digestion with *Hin*dIII and *Bam*HI yielded two fragments not resolved in these conditions. Similarly, Fig. 4*a–d* show that *Eco*RI-cleaved pR-1 co-migrated with an homologous *Eco*RI fragment of Rtll-1a and that *Eco*RI/*Hin*dIII cleavage products of pR-1 each co-migrated with homologous fragments present in *Eco*RI/*Hin*dIII-cleaved Rtll-1a. The 0.6-kilobase *Hin*dIII/*Hin*dIII fragment is not visible.

We conclude that both pR-1 and pB-1 were rescued from animal cells into *E. coli*. From their respective restriction maps, a restriction map was reconstructed of pBR322 and its flanking host sequences in Rtll-1a (Fig. 2). This map was verified independently by blot analysis after cleavage of Rtll-1a DNA with a variety of restriction endonucleases.

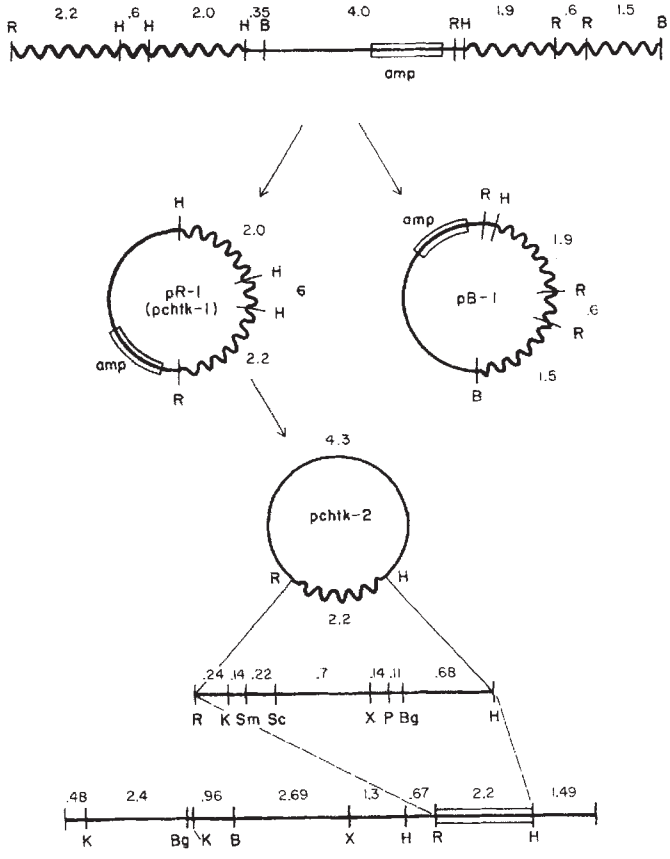

Fig. 2 Restriction maps and derivations of rescued plasmids. DNA from the secondary transformant Rtll-1a was prepared as previously described[17] and cleaved with either *Eco*RI or *Bam*HI. Digested DNA was then cyclized with T4 DNA ligase at a DNA concentration of 5 μg ml^{-1}. Cyclized DNA was used to transform *E. coli* χ1776 by a procedure to be described elsewhere (D.H., in preparation). Two plasmids, pR-1 and pB-1, were obtained from *Eco*RI-cleaved and *Bam*HI-cleaved, cyclized DNA, respectively. Restriction maps of these plasmids are shown, with pBR322 sequences denoted "⎯" and inserted sequences denoted "⏦". The numbers refer to kilobase pairs. From the maps of the two rescuants, a restriction map was reconstructed of the sequences flanking the pBR322 sequence resident in Rtll-1a (top line). pR-1 was found to encode tk and was renamed pchtk-1. pchtk-1 was cleaved with *Hin*dIII and cyclized to construct pchtk-2, which contains only the 2.2-kilobase *Eco*RI/*Hin*dIII insert encoding tk. Below is a restriction map (to scale) of the 2.2-kilobase insert. At the bottom is a restriction map (to scale) of the 12.5-kilobase insert of λchtk-1 isolated from a Charon 4A chicken library[22]. *Pvu*II, *Sma*I and *Sac*I sites are not mapped on the phage. Restriction enzymes are *Bam*HI (B), *Eco*RI (R), *Hin*dIII (H), *Kpn*I (K), *Sma*I (Sm), *Sac*I (Sc), *Xba*I (X), *Pvu*II (P) and *Bgl*II (Bg). Restriction enzyme digestions and ligations carried out in conditions described by the vendors (Bethesda Research Labs and New England Biolabs).

Table 1 Transformation data

Gene source	Restriction cleaved*	Total colonies†	Specific activity‡
pchtk-1	Uncut	1,628§	14.7
pchtk-2	Uncut	1,075§	7.6
pchtk-2	*Eco*RI	612§	4.3
pchtk-2	*Kpn*I	0§	0.00
pchtk-2	*Sma*I	3§	0.02
pchtk-2	*Sac*I	0§	0.00
pchtk-2	*Xba*I	0§	0.00
pchtk-2	*Pvu*II	227§	1.6
pchtk-2	*Bgl*II	618§	4.4
pchtk-2	*Hin*dIII	1,863§	13.0
λchtk-1	Uncut	255‖	55.0
pTK-2 #	*Sal*I	690‖	29.2
Chicken, genomic DNA	Uncut	51¶	63.7

* Three units of enzyme were used to digest 1 μg of DNA for 4 h at 37 °C in buffers recommended by the vendors.

† Transformations were carried out as previously described[9]. Ltk⁻ DNA was used as carrier for cloned genes at 20 μg per plate. For genomic chicken transformations, the DNA was its own carrier.

‡ Colonies per 10^8 molecules per 10^6 cells. The DNA content of chicken is taken as 2.5 pg per cell.

§ Total number of colonies in five plates, 20 ng gene per plate.

‖ Total number of colonies in five plates, 4 ng gene per plate.

¶ Total number of colonies in five plates, 20 μg per plate.

The 3.5-kilobase *Bam*HI fragment of HSV-1 strain F (ref. 7) containing the HSV-1 tk gene was cloned into the *Bam*HI site of pBR322. The orientation of the tk fragment is the same as the pFG5 clone of Colbere-Garapin *et al.*[25].

The sequences flanking pBR322 in the secondary transformant Rtll-1a could theoretically have arisen from chicken or mouse (or both), depending on events occurring during transformation. Blot analysis showed that the sequences contained in both pR- and pB-1 were derived entirely from chicken. The *Hin*dIII/*Bam*HI insert of pB-1 co-migrated with an homologous *Hin*dIII/*Bam*HI fragment of chicken DNA (Fig. 3*c, e*) whereas pB-1 showed no homology to mouse DNA in stringent hybridization conditions (Fig. 3*f–j*). Similarly, *Hin*dIII/*Eco*RI digestion of pR-1 generated one fragment of 4.3 kilobases containing only pBR322 sequences and three fragments of 2.2, 2.0 and 0.6 kilobases. These latter three fragments co-migrated with homologous fragments in chicken DNA cleaved with *Hin*dIII and *Eco*RI (Fig. 4*c, e*). Moreover, pR-1 showed no homology to mouse DNA (Fig. 4*j*).

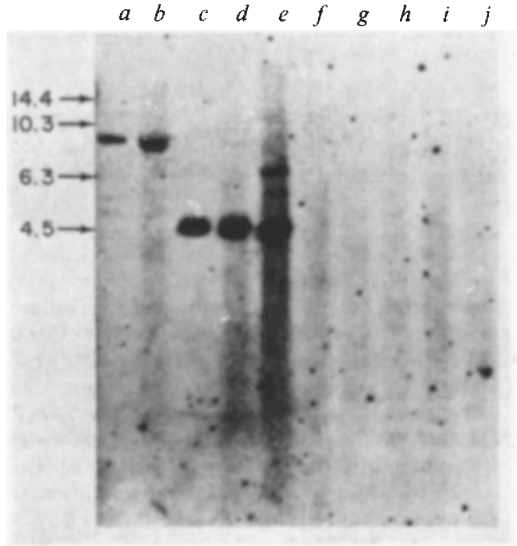

Fig. 3 Blot analysis of the pB-1 rescuant. pB-1 was ^{32}P labelled to high specific activity (2×10^8 c.p.m. per μg) by nick translation[16] and used as probe in Southern blots[17] to DNA electrophoresed in 1.0% agarose gels: *a*, *Bam*HI-cleaved pB-1 (40 pg with 2 μg salmon DNA carrier); *b*, *Bam*HI-cleaved Rtll-1a (10 μg); *Bam*HI- and *Hin*dIII-cleaved DNA from: *c*, pB-1 (40 pg, with carrier); *d*, Rtll-1a (10 μg); *e*, chicken (10 μg); *f*, *i*, various secondary, chicken tk⁻ mouse transformants (primary transformant tll used as donor) (10 μg each); *j*, Ltk⁻ (10 μg). Arrows on the left are sizes (in kilobases) of restriction fragments of adenovirus-2. Digestions were in 30 μl final volume, 2 units of enzyme per μg DNA for 3 h at 37 °C.

pR-1 contains the chicken tk gene

pR-1 and pB-1 were tested in transformation assays for ability to transfer tk activity to Ltk⁻ cells. Although pB-1 gave no tk⁺ colonies, treatment of mouse cells with pR-1 yielded approximately 10 tk⁺ colonies per ng, only slightly below the frequency observed with a pBR322 clone containing the 3.5-kilobase *Bam*HI fragment of HSV-1 which encodes the herpes simplex virus tk gene (pTK-2) (Table 1). These observations strongly suggested that pR-1 encoded the chicken tk gene, and this was confirmed by hybridization studies. As expected, pB-1 showed no homology to chicken tk⁺ mouse transformants (Fig. 3). On the other hand, pR-1 showed homology to chicken tk⁺ mouse lines but not to Ltk⁻ itself (Fig. 4*f–j*). Moreover, mouse cells transformed with pR-1 contained these chicken DNA sequences (Fig. 5*b, c, d*). No transformants contained HSV-1 tk sequences. Further confirmation was obtained by comparing the physical properties of the tk activity expressed in transformants with that of chicken, mouse and herpes tk. Isoelectric focusing of cytoplasmic extracts showed that transformants obtained using pR-1 as gene donor expressed a tk activity with the same isoelectric point (9.7) as that of 6-day-old chick embryo extracts and distinguishable from mouse (9.0) and herpes (6.1)[22]. We therefore conclude that pR-1 encoded the chicken tk gene. pR-1 was renamed pchtk-1.

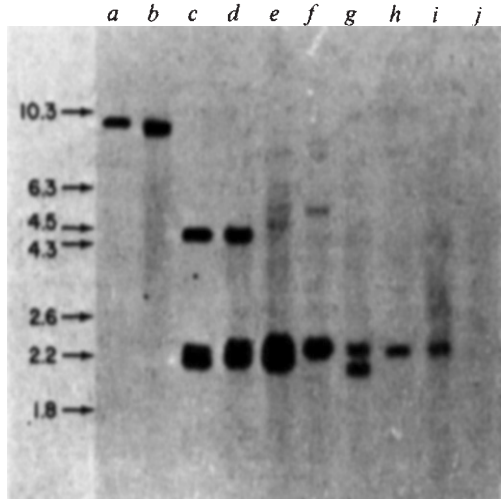

Fig. 4 Blot analysis of the pR-1 (pchtk-1) rescuant. pR-1 was used as probe in blots to DNA electrophoresed in 1.0% agarose gels: *a*, *Eco*RI-cleaved pR-1 (40 pg with 2 μg salmon carrier); *b*, *Eco*RI-cleaved Rtll-1a DNA (10 μg); *Eco*RI- and *Hin*dIII-cleaved DNA from: *c*, pR-1 (40 pg with carrier); *d*, Rtll-1a (10 μg); *e*, chicken (10 μg); *f*, *g*, *h*, various secondary chicken tk⁺ mouse transformants (primary transformant tll used as donor) (10 μg); *i*, a chicken tk⁺ primary transformant (10 μg); *j*, Ltk⁻ (10 μg). Arrows indicate sizes (in kilobases) of restriction fragments of adenovirus-2. Digestions were as in Fig. 3.

Localizing the chicken tk gene

pchtk-1 contains three chicken fragments bounded by *Eco*RI and/or *Hin*dIII sites: a 2.2-kilobase *Hin*dIII/*Eco*RI fragment, a 2.0-kilobase *Hin*dIII/*Hin*dIII fragment and a 0.6-kilobase *Hin*dIII/*Hin*dIII fragment. From its construction, we expected that the three chicken specific fragments of pchtk-1 were not contiguous in the chicken genome (Fig. 1). Thus, any of these fragments could have encoded the chicken tk gene. Blot analysis using nick-translated pchtk-1 as a probe showed that DNA from chicken and chicken tk⁺ mouse transformants contained in common only a 3.0-kilobase *Hin*dIII fragment. Thus, the 2.2-kilobase *Eco*RI/*Hin*dIII fragment contained the active gene. To test this rigorously, a second plasmid, pchtk-2, was derived from pchtk-1 by cleaving it with *Hin*dIII, cyclizing it and using it to transform *E. coli*. The resultant plasmid contained only the 2.2-kilobase *Hin*dIII/*Eco*RI fragment inserted into pBR322. This plasmid transformed Ltk⁻ to the tk⁺ phenotype with an

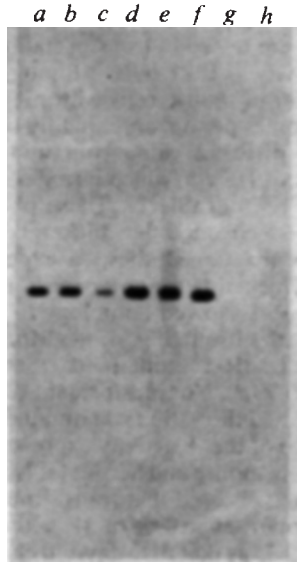

Fig. 5 Presence of the 1.4-kilobase *Eco*RI/*Pvu*II fragment in transformants, chicken, plasmid and phage DNA. DNAs from various sources were digested with *Eco*RI/*Pvu*II, electrophoresed in 1.5% agarose gels, transferred to nitrocellulose filters and hybridized *in situ* with ^{32}P-labelled, nick-translated, *Kpn*I/*Xba*I fragment of chicken tk (2×10^8 c.p.m. per μg). *a*, Charon 4A clone, λchtk-1 (200 pg); *b–d*, three different tk$^+$ mouse transformants obtained using pchtk-1 as gene donor (8 μg each); *e*, chicken (8 μg); *f*, pchtk-2 (50 pg with 2 μg salmon DNA carrier); *g*, HSV-1 tk$^+$ mouse transformant (8 μg); *h*, Ltk$^-$ (8 μg).

efficiency approximately equal to that of pchtk-1 (Table 1).

To localize the tk gene in more detail, we identified restriction sites on the 2.2-kilobase *Hin*dIII/*Eco*RI fragment using enzymes that cleave the fragment once (Fig. 2). pchtk-2 was cleaved to completion with each enzyme and the linearized plasmid was used in transformation assays (Table 1). By this procedure, we localized one end of the tk gene to the fragment bounded by the *Eco*RI and *Kpn*I sites, and the other end to the fragment bounded by the *Xba*I and *Pvu*II sites. Thus, a functional chicken tk gene is no larger than 1.4 kilobases and may be as small as 1.0 kilobase.

The plasmid carrying the biologically active chicken tk gene has had a complex history: it was passaged through mouse cells as well as through *E. coli*. To verify the structure of the tk gene independently, we have cloned it by an alternative and more familiar route. A library of chicken DNA[23,24] distributed into the bacteriophage λ vector, Charon 4A, was screened using nick-translated pchtk-2 as probe. DNA from one cloned phage (λchtk-1) contained a 12.5-kilobase chicken insert which transferred tk efficiently to Ltk$^-$ cells (Table 1). Restriction mapping and blot analysis (Figs 2, 5) confirmed the colinearity of the tk genes cloned in phage and by plasmid rescue.

The scheme we have demonstrated for the isolation of the chicken tk gene represents the first instance of the purification of a higher eukaryotic gene using essentially genetic techniques. In principle, this method is applicable to any higher eukaryotic gene coding for a selectable marker. We emphasize, however, that the isolation of tk has been greatly facilitated by its small size, a strong selection system and an excellent recipient cell used to detect its transfer.

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1. Tilghman, S. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **74,** 4406 (1977).
2. Tonegawa, S., Brack, C., Hozumi, N. & Scholler, R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 3518 (1977).
3. Maniatis, T. *et al. Cell* **15,** 687 (1978).
4. Graham, F. L. *et al. Cold Spring Harb. Symp. quant. Biol.* **39,** 637 (1974).
5. Maitland, N. J. & McDougall, J. K. *Cell* **11,** 233 (1977).
6. Bacchetti, S. & Graham, F. L. *Proc. natn. Acad. Sci. U.S.A.* **74,** 1590 (1977).
7. Wigler, M. *et al. Cell* **11,** 223 (1977).
8. Wigler, M., Pellicer, A., Silverstein, S. & Axel, R. *Cell* **14,** 725 (1978).
9. Wigler, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **76,** 1373 (1979).
10. Willecke, K., Klomfass, M., Mirau, R. & Dohmer, J. *Molec. gen. Genet.* **170,** 179 (1979).
11. Graf, L. H., Urlaub, G. & Chasin, L. *Somatic Cell Genet.* (in the press).
12. Kit, S., Dubbs, D., Piekarski, L. & Hsu, T. *Expl Cell Res.* **31,** 297 (1963).
13. Littlefield, J. *Science* **145,** 709 (1974).
14. Bolivar, F. *et al. Gene* **2,** 95 (1977).
15. Graham, F. L. & van der Eb, A. J. *Virology* **52,** 455 (1973).
16. Southern, E. M. *J. molec. Biol.* **98,** 503 (1975).
17. Wigler, M. *et al. Cell* **16,** 777 (1979).
18. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72,** 1184 (1975).
19. Weinstock, R., Sweet, R., Weiss, M., Cedar, H. & Axel, R. *Proc. natn. Acad. Sci. U.S.A.* **75,** 1299 (1978).
20. Sutcliffe, J. G. *Nucleic Acids Res.* **5,** 2721 (1978).
21. Curtis, R. III *et al.* in *Recombinant Molecules: Impact on Science and Society* (eds Beers, R. F. & Basset, E. G.) 45–56 (Raven, New York, 1977).
22. Kit, S., Leung, W.-C., Jorgensen, G. N., Trkula, D. & Dubbs, D. R. *Cold Spring Harb. Symp. quant. Biol.* **39,** 703 (1974).
23. Dodgson, J. B., Strommer, J. & Engel, J. D. *Cell* **17,** 879 (1979).
24. Benton, W. D. & Davis, R. W. *Science* **196,** 180 (1977).
25. Colbere-Garapin, F., Chousterman, S., Horodniceaunu, F., Kourilsky, P. & Garapin, A.-C. *Proc. natn. Acad. Sci. U.S.A.* **76,** 3755 (1979).

LETTERS

An upper limit on the EUV flux from HD192273

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Cash *et al.*[1] reported the possible detection of an extreme ultraviolet (EUV) source in the constellation Pavo. They suggested HD192273 as a possible candidate and cited a TD-1 satellite observation indicating that this star is also unexpectedly bright at 1,565 Å. Wegner[2] reported visual photometric and spectroscopic data for HD192273 and concluded that it is apparently a normal B-type star. Shipman and Wegner[3] have reviewed the available data on HD192273 and suggested that all of the observations could be explained if this object were an analogue of HZ22, an evolved, mass-exchange, binary. We report here a recent observation with the UV spectrometer (UVS) aboard the Voyager 1 spacecraft. An upper limit (2σ) of 8×10^{-3} photon cm^{-2} s^{-1} Å^{-1} is placed on the 534–776 Å flux from HD192273 at the time of observation. This is ~10% of the 500–780 Å flux reported from Pavo by Cash *et al.* The 1,565 Å flux observed by TD-1 is confirmed and is consistent with the stellar parameters determined by Wegner. Discounting the possibility that HD192273 is an EUV variable, it is probably not the potential source reported by Cash *et al.*

The Voyager 1 UVS is an objective grating instrument covering 534–1,701 Å with 126 contiguous photon counting pixels having maximum sensitivity shortward of 1,000 Å (refs 4, 5). Pre-flight laboratory calibrations were based on calibrated channeltron detectors, traceable to NBS standard photodiodes, and indicate a transfer error of ±15% from the photodiodes. The in-flight EUV sensitivity has been demonstrated in several

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