ules for antineoplastic drugs and polyamine biosynthesis inhibitors should be determined in the laboratory before the compounds are used in the clinical setting.

PHILIP J. TOFILON
STINA M. OREDSSON
Brain Tumor Research Center of the Department of Neurological Surgery, School of Medicine, University of California, San Francisco 94143
DENNIS F. DEEN
Brain Tumor Research Center of the Department of Neurological Surgery and *Department of Radiation Oncology, School of Medicine, University of California, San Francisco*
LAURENCE J. MARTON
Brain Tumor Research Center of the Department of Neurological Surgery, and *Department of Laboratory Medicine, School of Medicine, University of California, San Francisco*

References and Notes

1. H. G. Williams-Ashman and Z. N. Canellakis, *Perspect. Biol. Med.* **22**, 421 (1979).
2. D. R. Morris and L. J. Marton, Eds., *Polyamines in Biology and Medicine* (Dekker, New York, 1981).
3. V. A. Bloomfield and R. W. Wilson, in (*2*), pp. 183–206.
4. U. Bachrach and G. Eilon, *Biochim. Biophys. Acta* **179**, 494 (1969).
5. P. E. Brown, *Radiat. Res.* **34**, 24 (1968).
6. M. Tsuboi, *Bull. Chem. Soc. Jpn.* **37**, 1514 (1964).
7. A. M. Liquori, L. Costantino, V. Crescenzi, V. Elia, E. Giglio, R. Puliti, M. DeSantis Savino, V. Vitagliano. *J. Mol. Biol.* **24**, 113 (1967).
8. D. T. Hung, L. J. Marton, R. H. Shafer, in preparation.
9. A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. van der Marel, A. Rich. *Nature (London)* **282**, 680 (1979).
10. A. Rich, personal communication.
11. P. S. Mamont, M. Duchesne, A. Joder-Ohlenbusch, J. Grove, in *Enzyme-Activated Irreversible Inhibitors*, N. Seiler, M. J. Jung, J. Koch-Weser, Eds. (Elsevier/North-Holland, Amsterdam, 1978), pp. 43–53.
12. J. Seidenfeld, J. W. Gray, L. J. Marton. *Exp. Cell Res.* **131**, 209 (1981).
13. D. T. Hung, D. F. Deen, J. Seidenfeld, L. J. Marton, *Cancer Res.* **41**, 2783 (1981).
14. R. A. G. Ewig and K. W. Kohn, *ibid.* **37**, 2114 (1977).
15. S. M. Oredsson, A. E. Pegg, D. F. Deen, L. J. Marton, in preparation.
16. S. M. Oredsson, D. F. Deen, L. J. Marton, *Cancer Res.* **42**, 1296 (1982).
17. H. C. Harder and B. Rosenberg, *Int. J. Cancer* **6**, 207 (1970).
18. G. Laurent, L. C. Erickson, N. A. Sharkey, K. W. Kohn, *Cancer Res.* **41**, 3347 (1981).
19. G. H. W. Milburn and M. R. Truter, *J. Chem. Soc. Abstr.* **1966** (No. 11), 1609 (1966).
20. R. Langridge, L. D. Wilson, C. W. Hooper, M. H. F. Wilkins, L. D. Hamilton, *J. Mol. Biol.* **2**, 19 (1960).
21. D. M. L. Goodgame, I. Jeeves, F. L. Phillips, A. C. Skapski, *Biochim. Biophys. Acta* **378**, 153 (1975); S. Mansy, G. Rosenberg, A. J. Thomson, *J. Am. Chem. Soc.* **95**, 1633 (1973); I. A. G. Roos, A. J. Thomson, S. Mansy, *ibid.* **96**, 6484 (1974); P. J. Stone, A. D. Kelman, F. M. Sinex, *J. Mol. Biol.* **104**, 793 (1976).
22. E. Solomon and M. Bobrow, *Mut. Res.* **30**, 273 (1975).
23. S. Wolff, *Annu. Rev. Genet.* **11**, 183 (1977).
24. P. Perry and S. Wolff. *Nature (London)* **251**, 156 (1974).
25. Supported by NIH grants CA 13525 and CA 31867, ACS grant RD-137 and travel grants to S.M.O. from the Swedish National Science Research Council (R-RA 4685-100) and from the Swedish Medical Research Council (B81-04R-6065-504106065). P.J.T. is supported by an Aaron Silvera Fellowship, and NIH training grant CA 09215.

20 May 1982

Human β-Globin Gene Sequences Injected into Mouse Eggs, Retained in Adults, and Transmitted to Progeny

Abstract. *Foreign gene sequences were retained in two adult mice (out of 62 analyzed) from fertilized eggs injected with a recombinant plasmid containing the human β-globin genomic region and the herpes simplex viral thymidine kinase gene. The intact human and viral genes were found in DNA of one of the animals and, in the other, at least part of the human globin gene was present. The latter individual transmitted these sequences to its progeny in a Mendelian ratio. Thus, human DNA may be incorporated into the germ line of mice for in vivo studies of regulation of gene expression in development, genetic diseases, and malignancy.*

In a recent study, we demonstrated that human and viral genes introduced into fertilized mouse eggs by microinjection of recombinant DNA into a pronucleus could later be found intact in DNA prepared from fetuses near the end of their prenatal development (*1*). In the present report, eggs injected with the same genes were allowed to develop to adulthood. Persistent foreign genetic material was detected in some adults, and biological evidence for integration in host DNA was obtained through Mendelian transmission of human β-globin sequences to progeny.

Approximately 3000 copies of the bacterial plasmid PtkHβ1 were injected into the male pronucleus of (C3H × DBA/2)F_1 fertilized mouse eggs, as previously described (*1*). The plasmid consists of a pBR322 vector with a 7.6-kb Hind III insert carrying the human β-globin (HβG) genomic region and a 3.6-kb Bam HI fragment containing the thymidine kinase (tk) gene of herpes simplex virus (HSV) (*2*, *3*). Of 394 eggs injected, 304 survived the injection and were surgically transferred to the oviducts of pseudopregnant Icr random-bred females. At 8 to 10 weeks of age, partial splenectomy was performed on 62 survivors, and the DNA was extracted (*4*, *5*).

Some of the DNA was digested with the restriction endonuclease Pst I and tested by the Southern procedure (*6–8*) for hybridization with the ^{32}P-labeled 7.6-kb globin gene insert. The DNA's of two of the spleens hybridized with the HβG probe and therefore contained some gene sequences of human origin. One of the positive experimental animals (designated Exp. 1), a female, had the diagnostic 4.4-kb Pst I fragment that spans the entire coding region and also the neighboring 3.9-kb fragment (*9*), thereby indicating retention of the entire intact human globin insert, as seen by comparison with a control digest of the injected plasmid (Fig. 1A). When the animal was ultimately autopsied, after breeding, the DNA digests from its five other tested organs (liver, kidney, heart, lung, brain) showed the same result as the spleen, with approximately two copies of the HβG per cell in all tissues. The second experimental animal with HβG sequences (Exp. 2), a male, had a higher molecular weight band of 13 kb, which hybridized with the 7.6-kb human gene insert. This band was present in all seven organs eventually tested, at an estimated copy number of one per cell (Fig. 1C). In subsequent experiments aimed at identifying these sequences, Bam HI and Eco RI digests were examined for hybridization with the same HβG probe (Fig. 2A, lanes F_1 a′ and F_1 a″); they did not display the bands expected if the coding region were present. In addition, no hybridization with an HβG complementary DNA (cDNA) probe was observed (data not shown). The 13-kb band was, however, seen when the probe was a 3.6-kb Eco RI fragment including most of the 3′ end of the genomic region. Therefore, the HβG sequences in this second animal included at least some of the 3′ sequences flanking the coding region of the human β-globin gene.

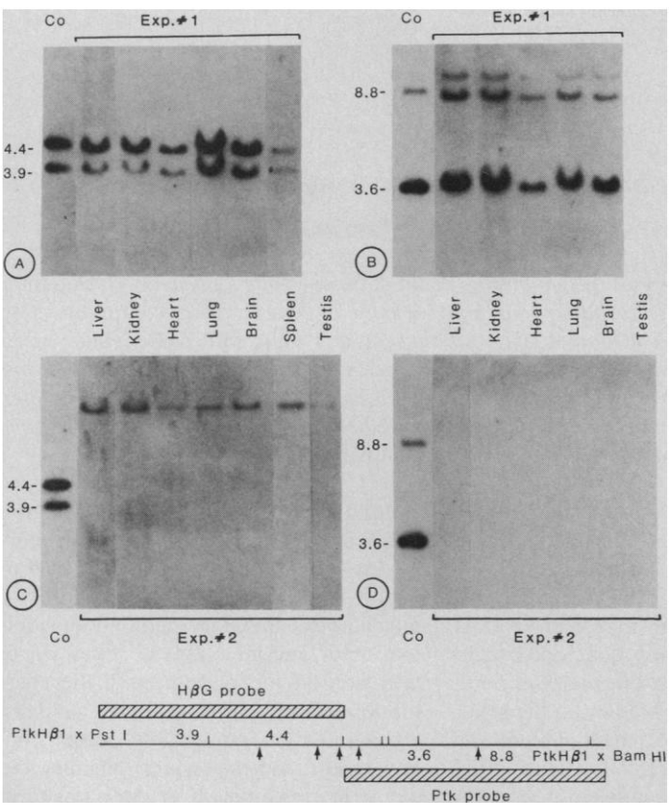
DNA's from the same spleen samples were also analyzed for presence of HSV tk gene and pBR322 sequences by digestion with Bam HI and hybridization with ^{32}P-labeled Ptk. This probe represents the vector after insertion of the HSV tk gene into the Bam HI site of pBR322 (*3*) and before introduction of the HβG fragment. The animal (Exp. 1) that was positive for the intact HβG gene also exhibited Ptk-hybridizing sequences, at approximately two copies per cell, in all five organs eventually analyzed (Fig. 1B). The other HβG-positive individual, Exp.

0036-8075/82/0910-1046$01.00/0

2, was negative for Ptk (Fig. 1D). The DNA of mouse Exp. 1 showed not only the 3.6-kb Bam HI band diagnostic for the intact HSV tk gene but also two additional bands: One, of 8.8-kb size, comigrated with a band in the control digest; the other, of approximately 12 kb, had no counterpart in the control lane (Fig. 1B). The presence of the 12-kb fragment is consistent with integration of some foreign DNA into the host genome, that is, loss of a Bam HI site in plasmid DNA and cleavage at a similar site in mouse flanking DNA. This conclusion was supported by occurrence of one extra high molecular weight band in a Pst I digest of DNA after hybridizing with Ptk, and of two extra bands in an Eco RI digest probed with Ptk (data not shown).

To learn whether foreign gene sequences in the two positive experimental animals could be passed on through the germ line, the mice were mated to DBA/2 strain controls, and liver samples were obtained from the progeny by partial hepatectomy at 4 to 6 weeks. Analyses of the DNA digests revealed that the male (mouse Exp. 2) had in fact transmitted to eight offspring, out of 14 examined, an HβG-hybridizing band with the same molecular weight, after Pst I digestion, as in the parent (Fig. 2A). Similarity to the parent's HβG sequences was further indicated by comigration after Eco RI digestion (data not shown). Offspring, like parent, lacked detectable HSV tk and pBR322 sequences (Fig. 2B). This transmission of HβG sequences to approximately half of the progeny follows a Mendelian pattern of inheritance for a single gene at a heterozygous locus and is strong evidence for the chromosomal integration of the injected sequences.

In contrast, the female (mouse Exp. 1) did not transmit any plasmid sequences (HβG, tk, or pBR) to any of the 15 offspring whose spleen DNA was tested. Therefore, this animal was a mosaic with donor sequences present in at least some cells of the various organs analyzed but either lacking in the germ cells or present in only a very small percentage. The disparity between somatic and germinal results could be due to delayed integration of the injected genes until after the developmental point at which the germinal lineage, originating in very few precursor cells (*10*), diverged from the soma. A comparable explanation was offered for some differences found in fetal as compared to placental DNA's (*1*). An alternative possibility is that introduced sequences may sometimes be lost, as noted in experiments with *Xenopus* (*11*). Losses might also account for the lower frequency of adult mice (3 percent) than of fetuses (15 percent)



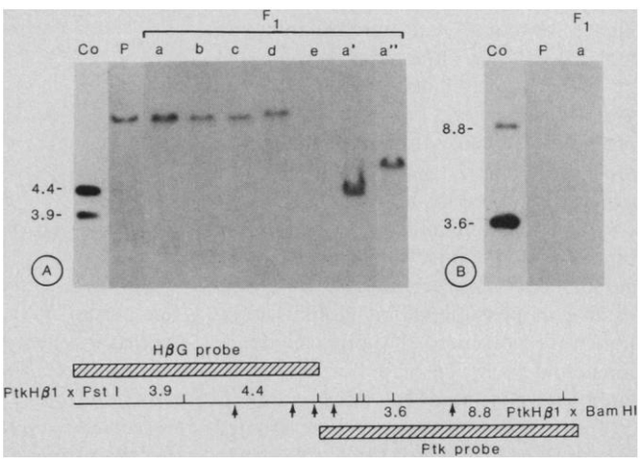


Fig. 1 (left). DNA hybridization evidence (shown in composites of results) for human β-globin (HβG) and herpes simplex viral (HSV) thymidine kinase (tk) gene sequences in adult mice derived from fertilized eggs injected with DNA of the recombinant plasmid PtkHβ1 containing both genes. In panels A and C, high molecular weight DNA (approximately 15 μg) extracted from the specified organs was probed, after Pst I digestion, for hybridization with the ^{32}P-labeled HβG insert; in panels B and D, the DNA was tested, after Bam HI digestion, for hybridization with the labeled Ptk probe representing the tk and pBR322 components of the plasmid. In each panel, the DNA in the control (*Co*) lane is the plasmid PtkHβ1 (100 pg) digested with the same restriction enzyme and hybridized with the same probe as used in the accompanying experimental lanes. The results in (A) show that the first animal, a female designated Exp. 1, has retained intact both diagnostic restriction fragments (4.4 kb and 3.9 kb) spanning the entire coding region of the HβG gene, in all organs analyzed. The same animal (in B) has also retained the diagnostic fragments (8.8 kb and 3.6 kb) indicating the presence of the intact HSV tk gene; the additional higher molecular weight (12 kb) band seen in the experimental samples (but not in the control) is consistent with integration of some sequences in endogenous mouse DNA. The results in (C) show that the second animal, a male designated Exp. 2, has, in all organs analyzed, a single band of approximately 13 kb hybridizing to the HβG probe; further tests (see text) revealed this to be at least in part from the 3′ (noncoding) end of the genomic region. This animal has no sequences that hybridized detectably with the Ptk probe (D). Fig. 2 (above). Transmission of HβG gene sequences through the germ line of an experimental male (Exp. 2) to its progeny. In (A), 20 μg of high molecular weight DNA from the livers of this parent (*P*) and of five of his F_1 progeny from the first litter (F_1, a to e), were digested with Pst I and hybridized to the HβG insert; four of these five offspring had the same positive 13-kb band (see also Fig. 1C) as their father. The DNA from the first of the positive F_1 progeny was also digested with Bam HI (F_1 a′) and Eco RI (F_1 a″) and hybridized to the HβG probe. In (B), liver DNA's (20 μg) from the same experimental parent (*P*) and from the first of the positive progeny (F_1 a) were digested with Bam HI and hybridized to the Ptk plasmid; the offspring, like the parent (see also Fig. 1D), displayed no bands hybridizing to the Ptk probe. The DNA in the control (*Co*) lanes is from the plasmid PtkHβ1 (100 pg) digested with Pst I (A) or Bam HI (B) and hybridized to the same probe as used in the respective experimental lanes.

found to have donor DNA from the same plasmid source, as described here and in our previous study (*1*).

Some in vivo instability of DNA of exogenous origin may be indicated by the fact that, whereas all positive fetuses in our earlier series had numerous intact copies of both marker genes, we now observe in one of the positive adults only partial sequences of a single-copy HβG gene. Other examples have been recently reported of retention of intact gene sequences in mice after injection into eggs of recombinant DNA including the rabbit β-globin (*12*) or HSV tk (*13*) genes. Retention of only rearranged or partially deleted sequences of other genes has also been shown (*14, 15*). In some instances, germ line transmission of the foreign DNA was observed (*12, 15*).

Of importance for further work based on gene injections into eggs is whether any of these newly introduced genes can function. We have demonstrated that it is possible to obtain mouse fetuses that synthesize a functional HSV thymidine kinase protein after pronuclear injection with PtkHβ1 (*1*). Others have shown that by using a fusion construct containing the structural gene for HSV tk and the promoter/regulatory region of the mouse metallothionein-I gene, viral enzyme expression could be detected in the eggs (*16*) and in adult mice (*13*). In another report involving injection of the rabbit β-globin gene (*17*), preliminary data suggestive of possible rabbit globin formation were presented, although evidence for actual presence of donor DNA was cited for only one of the mice.

With respect to the animal designated mouse Exp. 1, in which the HSV tk gene was present (Fig. 1B), we tested for viral thymidine kinase activity in liver homogenates with the ^{125}I-labeled deoxycytidine assay in the presence of tetrahydrouridine (*18*); 0.1 percent of the enzyme as viral-type would be detectable. No viral-specific enzyme activity was found (data not shown). Evidence for HβG-specific transcripts was sought in both experimental animals with HβG sequences by analyzing total liver messenger RNA's (mRNA's) with the S1-nuclease mapping technique (*19–21*) capable of detecting one copy of mRNA per cell. The diagnostic fragment of human β-globin–specific RNA (211 bases in size) was not found (data not shown) when a 3′-end-labeled Eco RI restriction fragment was used for hybridization; therefore, the occurrence of correct 3′ human β-globin-specific transcripts is unlikely. S1 assays on RNA's from hematopoietic organs, for example, spleen, were not performed in this series; therefore, the possibility of tissue-specific mRNA expression was not ruled out.

Our combined observations on mouse fetuses (*1*) and adults, following introduction of PtkHβ1 DNA into eggs, have thus disclosed instances in which foreign gene sequences are present (usually intact), fully functional (in one case), and transmissible (in another case) to progeny. These results support the expectation that introduction of foreign DNA early in mammalian development may prove useful for in vivo analyses of gene regulation in differentiation, genetic diseases, and malignancy.

TIMOTHY A. STEWART
ERWIN F. WAGNER
BEATRICE MINTZ

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

References and Notes

1. E. F. Wagner, T. A. Stewart, B. Mintz, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5016 (1981).
2. E. F. Fritsch, R. M. Lawn, T. Maniatis, *Cell* **19**, 959 (1980).
3. F. Colbere-Garapin, S. Chousterman, F. Horodniceanu, P. Kourilsky, A.-C. Garapin, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3755 (1979).
4. N. Blin and D. W. Stafford, *Nucleic Acids Res.* **3**, 2303 (1976).
5. F. Polsky, M. H. Edgell, J. G. Seidman, P. Leder, *Anal. Biochem.* **87**, 397 (1978).
6. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
7. G. M. Wahl, M. Stern, G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3683 (1979).
8. P. S. Thomas, *ibid.* **77**, 5201 (1980).
9. In this plasmid, the HβG insert was oriented in the reverse direction, that is, 3′ to 5′, relative to the tk gene insert.
10. B. Mintz, *Soc. Exp. Biol.* **25**, 345 (1971).
11. S. Rusconi and W. Schaffner, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5051 (1981).
12. F. Costantini and E. Lacy, *Nature (London)* **294**, 92 (1981).
13. R. L. Brinster, H. Y. Chen, M. Trumbauer, A. W. Senear, R. Warren, R. D. Palmiter, *Cell* **27**, 223 (1981).
14. J. W. Gordon, G. A. Scangos, D. J. Plotkin, J. A. Barbosa, F. H. Ruddle, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7380 (1980).
15. J. W. Gordon and F. H. Ruddle, *Science* **214**, 1244 (1981).
16. R. L. Brinster, H. Y. Chen, R. Warren, A. Sarthy, R. D. Palmiter, *Nature (London)* **296**, 39 (1982).
17. T. E. Wagner, P. C. Hoppe, J. D. Jollick, D. R. Scholl, R. L. Hodinka, J. B. Gault, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6376 (1981).
18. W. C. Summers and W. P. Summers, *J. Virol.* **24**, 314 (1977).
19. A. J. Berk and P. A. Sharp, *Cell* **12**, 721 (1977).
20. R. F. Weaver and C. Weissman, *Nucleic Acids Res.* **6**, 1175 (1979).
21. P. Mellon, V. Parker, Y. Gluzman, T. Maniatis, *Cell* **27**, 279 (1981).
22. Supported by U.S. Public Health Service grants HD-01646, CA-06927, and RR-05539, and by an appropriation from the Commonwealth of Pennsylvania. Address reprint requests to B.M., Institute for Cancer Research, 7701 Burholme Avenue, Fox Chase, Philadelphia, Pa. 19111.

26 April 1982; revised 28 July 1982

Plasmodium falciparum Gametocytes from Culture in vitro Develop to Sporozoites That Are Infectious to Primates

Abstract. *Gametocytes of two strains of the human malaria parasite* Plasmodium falciparum *have been produced in high density by means of a continuous-flow cultivation system. The gametocytes of these two strains infected a mean of 36 percent and 71 percent, respectively, of* Anopheles freeborni *mosquitoes that fed on a suspension of red blood cells containing the cultured gametocytes. Sporozoites harvested from the infected mosquito salivary glands were infective to the chimpanzee* (Pan troglodytes) *and the owl monkey* (Aotus trivirgatus).

The gametocyte stage of malaria represents the evolutionary link by which the microorganism developing in the vertebrate host undergoes sexual differentiation and infects the invertebrate host (mosquitoes). Recently developed techniques for cultivating *Plasmodium falciparum* in vitro allow studies on the sexual stage of this most malignant of the human malaria parasites. Our initial demonstration of the culture of gametocytes in vitro that could infect mosquitoes (*1*) established that biologically mature gametocytes would develop outside the primate. Subsequently, modification of the prime culture conditions (*2, 3*) and supplementation of the culture medium with adenosine 3′,5′-monophosphate (cyclic AMP) (*4*) or hypoxanthine (*5*) have been reported to enhance gametocyte production or maturation.

Although these studies have defined conditions conducive to the production of infective gametocytes, not all the morphologically mature gametocytes so produced have proved to be infective to mosquitoes. Similarly, not all mosquitoes with gut infections (oocyst stage) have consistently supported further maturation of the parasite to the salivary gland infection (sporozoite stage). We now report studies in which the complete extrinsic development of the parasite in anopheline mosquitoes has been repeatedly accomplished, producing sporozoites fully infective to two species of nonhuman primate hosts for falciparum malaria.

The Indochina III/CDC strain of *P. falciparum* (*6*) was cultured from a Lao refugee in August 1980. Gametocyte stimulation studies with this strain were performed during the initial 12 months of cultivation in vitro. The Tanzania I/CDC

0036-8075/82/0910-1048$01.00/0