synchronized with respiration. These spectral methods have been useful primarily for determining the effects of pharmacologic doses of potent cardiotropic drugs. Synchronization of breathing with pulse results in immensely less nonpredictable variability and a far more precise evaluation of the sinus arrhythmia.

Our curve-fitting method of assessment of the resting RSA, during voluntary cardiorespiratory synchronization, had reasonably small test-to-test variability within the same individual who had been tested at roughly the same times of day before and after venipuncture. The resting RSA amplitude is an easily measured index that correlates well with age. The timing of the peak of the function best described by the individual data also changes predictably with advancing age. These predictable relationships allow the construction of nomograms that correlate chronologic age, the RSA amplitude or timing of peak value, and "biologic cardiac age." This technology provides an objective measure of physiologic cardiovascular aging.

Data obtained on a patient after cardiac transplantation and a patient with severe neuropathy lead us to agree with the hypothesis put forward by Bainbridge (*17*) in 1920, stating that a negative pressure-dependent increase of the diastolic filling of the heart increases cardiac rate primarily through intracardiac, rather than extracardiac, sympathetic and parasympathetic reflex arcs. This is not to say that these heart rate changes cannot be overridden by sympathetic or parasympathetic discharge, by circulating chronotropes like epinephrine or norepinephrine, or by other cardioactive drugs.

Our results in patients with absolutely and relatively denervated hearts lead us to believe that the resting RSA is primarily a measure of cardiac suppleness, elasticity, or tissue compliance. For this to be proved, concurrent, more direct measurements of cardiac compliance need to be correlated with this index. Chest wall compliance also needs to be considered by measuring and correcting for respiratory volume and for the rate of and amount of negative pressure induced during inspiration.

If some basic property of the heart tissue, such as myocardial compliance, is the primary variable being measured, longitudinal assessment of cardiac status would seem to be pertinent for individuals suffering from many cardiovascular diseases and possibly in routine health maintenance. This technology will allow quantitative objective assessment of aging, as well as any drug effect, disease, or behavior that affects the RSA. The instrument may also be useful for screening drugs for cardiotoxicity or general cardioactivity.

W. J. M. HRUSHESKY
D. FADER
Departments of Medicine and Laboratory Medicine and Pathology, University of Minnesota Medical School, Masonic Cancer Center, Minneapolis 55455
O. SCHMITT
Department of Biophysics, University of Minnesota Medical School
V. GILBERTSEN
Department of Surgery, University of Minnesota Medical School

References and Notes

1. S. Hales, *Haemastaticks* (Innings and Manby, London, 1733).
2. C. Ludwig, *Archiv fur Anatomie and Physiologie und Wissenschofliche Medicin* (1847), p. 242.
3. E. Hering, *Sitzungsber. Akad. Wiss. Wien. Math. Naturwiss. Kl. Abt.* **64**, 333 (1871).
4. J. J. Almasi and O. H. Schmitt, *Ann. N.Y. Acad. Sci.* **170**, 509 (1970).
5. J. Almasi, thesis, University of Minnesota (1972).
6. O. Schmitt and J. Almasi, *IEEE Trans. Biomed. Eng.* **21**, 264 (1974).
7. S. Akselrod, D. Gordon, F. A. Ubel, D. C. Shannon, A. C. Barger, R. J. Cohen, *Science* **213**, 220 (1981).
8. B. Womack, *IEEE Trans. Biomed. Eng.* **18**, 339 (1971).
9. F. Halberg, E. A. Johnson, W. Nelson, W. Runge, R. Sothern, *Physiol. Teach.* **1**, 1 (1972).
10. S. W. Porges *et al.*, *Psychol. Bull.* **88**, 580 (1980).
11. S. W. Porges, R. E. Bohrer, G. Keren, M. N. Cheung, G. J. Franks, F. Drasgow, *Psychophysiology* **18**, 42 (1981).
12. C. Bingham, G. Arbogast, G. Cornelissen, J. K. Lee, F. Halberg. *Chronobiologia* **9**, 397 (1982).
13. G. Chess, R. Tam, F. Calaresu, *Am. J. Physiol.* **228**, 775 (1975).
14. E. Fischmann, J. Cosma, H. V. Pipberger, *Am. Heart J.* **75**, 465 (1968).
15. A. Melcher, *Acta Physiol. Scand.* **435**, 6 (1976).
16. I. Starr and C. Friedland, *J. Clin. Invest.* **25**, 53 (1946).
17. F. A. Bainbridge, *J Physiol. (London)* **54**, 192 (1920).
18. No federal, state, or private foundation funding has been used in the development or testing of this invention to date. A U.S. patent is pending. Patent applications have also been filed in the United Kingdom, Canada, Europe, and Japan. Inquiries about this instrument should be addressed to: Sine-o-graph Corp., 3538 Fremont Avenue South, Minneapolis, Minn. 55408.

21 November 1983; accepted 23 March 1984

Transient Expression of Homologous Genes in *Drosophila* Cells

Abstract. *A cloned* Drosophila *heat shock protein 22 gene was transfected into two independently established* Drosophila *cell lines. Each line carried a different heat shock protein 22 allele, distinguishable by electrophoresis of the protein. The transfected gene was not expressed at 25°C but could be induced at 36°C. In one line, two heat shock protein 22 electromorphs were synthesized.*

Cloned genes have been introduced into cultured somatic cells, unfertilized eggs, or zygotes under conditions that lead to their expression and regulation (*1*). Such transformation and transient expression systems provide a functional assay for defining DNA sequences flanking structural genes that are necessary for control of their expression (*2*). One limitation to these systems is that the introduced gene and recipient cell are seldom homologous—that is, they are usually derived from different species—because in most cases one could not distinguish qualitatively the expression of the transfected gene from the expression of the equivalent endogenous gene. As a consequence, several strategies have been developed to study the regulated expression of transfected genes. One is to use a completely heterologous system consisting of a cloned gene and a recipient cell from different species (*3*). The foreign gene, when expressed, produces an RNA and protein molecule whose presence can then be assayed directly and with no interfering signal from endogenous gene expression. A second strategy is to link physically the putative regulatory DNA sequence of a homologous gene to a heterologous structural gene (*4*). In this chimera the homologous regulatory region then drives the transcription and subsequent translation of a foreign gene whose expression can then be assayed directly. This approach introduces some homology. A third approach is to use a recipient cell that contains a mutationally defective or functionally inactive gene so that the expression and regulation of a gene introduced by transfection can be measured unambiguously (*5*). Such systems are limited by the availability of the necessary mutant line.

We now describe our study of homologous gene expression in a transient expression system. Although our experiments were performed with cultured *Drosophila* cells as recipients for transfection, the same principles would work for gene transfer into eggs (transformation) and would apply for any species. Our approach exploits the presence of ubiquitous genetic polymorphism, which appears in the form of electrophoretic mobility variants (*6*). We first identified two independently isolated cell lines that expressed different electrophoretic variants at some protein coding locus. The

two cell lines were then transfected with cloned DNA containing the gene in question. If the transfected gene was expressed, one of the cell lines would express a new electrophoretic variant of the protein. If the allele in the cloned gene was different from that in either cell line, both of the cell lines would acquire a new variant.

For example, the heat shock protein (hsp) 22 gene of *Drosophila melanogaster* belongs to a small multigene family located on chromosome 3 in the polytene band 67B1 (*7*). The gene has been isolated and cloned (*8*), and the entire nucleotide sequence of the coding and flanking regions has been determined (*9*). The hsp 22 gene is not normally expressed in *Drosophila* adults or in cultured cell lines. In cell lines, hsp 22 expression can be induced by either heat shock (*10*) or exposure to physiological doses of the steroid molting hormone ecdysterone (*11*). In whole animals hsp 22 expression can be induced by heat shock (*10*), and in pupae the hsp 22 gene is developmentally regulated presumably in response to an elevation of endogenous ecdysterone titers (*12*).

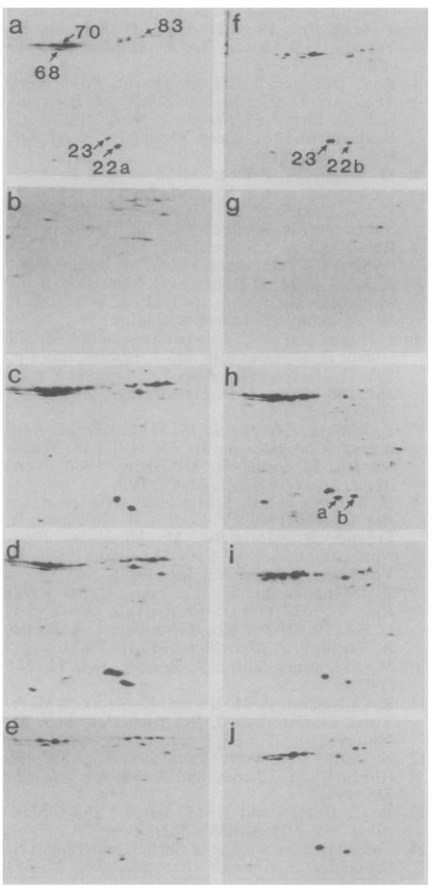
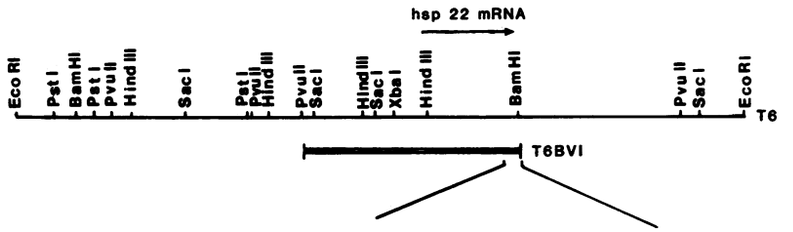


Fig. 1. Two-dimensional gel electrophoresis (*11, 13*) and autoradiography of [^{35}S]methionine-labeled polypeptides synthesized in line S3 cells (a to e) and line Kc cells (f to j) during a 90-minute period. (a and f) Controls heat shocked at 36°C; (b and g) samples labeled at 25°C 2 hours after transfection with T6BV1; (c and h) samples labeled at 36°C 2 hours after transfection with T6BV1; (d and i) samples labeled at 36°C 2 hours after transfection with pBR322; (e and j) samples labeled at 36°C 22 hours after transfection with T6BV1. The hsp's 83, 70, 68, and 23 and the two electrophoretic variants hsp 22a and 22b are indicated.



TAG AAATAAGTTG AGATTATACT AAAACCGATA AAATGCTAGT GAACTCCTAT GTTTAGATAT TCCAAAACCT ATCAAATTTA AGTTCTTGTT
AAATTAACAA GTTAATTTTA AAACAATTGT GATTCGGTAG CCCGCAGCCC AATAATTTTA TTTAGAAGAA AATAAATATT CGAAAAGACT
ATGATCAAAA TATTTACTTT CATTGGTTGG GTTGGGAACA CATTTGATAT GGATAGTATT ATAGATTATT ATATATATCT GTCAAGTCAT
GAAAATGGAT CC

Fig. 2. Partial restriction digest map of the recombinant plasmid T6 and the subclone T6BV1 used for transfection (*8*). The DNA sequence of the 3′ region from the translation stop signal (the underlined TAG) to the terminal Bam HI site of T6BV1 is indicated (*9*). The consensus poly-A addition sequence AATAAA is underlined, and the terminal nucleotide transcribed 14 bases toward 3′ is indicated (T, thymine; A, adenine; G, guanine; C, cytosine).

Earlier we showed that hsp 22 is polymorphic in two independently established cell lines (*13*) designated S3 (*14*) and Kc (*15*). When cells of the S3 line and the Kc line were heat shocked for an hour at 36°C in the presence of [^{35}S]methionine, the autoradiograph of extracted proteins analyzed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) revealed the presence of hsp 22 variants that differed in net charge but not molecular weight (Fig. 1). One-dimensional peptide maps of the two hsp 22 variants, constructed by the Cleveland procedure (*16*), produced indistinguishable patterns (data not shown) indicating that they are allelic variants. In S3-Kc hybrid cell lines, both hsp 22 variants were induced simultaneously by heat shock or ecdysterone treatment (*13*).

The recombinant plasmid used for transfection, designated T6BV1, contains a 2.15–kilobase pair (kbP) segment of genomic DNA inserted into the Bam HI–Pvu II sites of pBR322 (Fig. 2). The downstream Bam HI site is 282 ± 1 base pairs (bp) beyond the translation-terminating TAG stop codon and 116 bp beyond the consensus AATAAA poly-A addition signal (T, thymine; A, adenine; G, guanine). The upstream Pvu II site is about 1.0 kbP from the transcriptional initiation site (Fig. 2).

The T6BV1 plasmid DNA was transfected into both S3 and Kc cells by a modification of the polyornithine method (*17*) for *Drosophila* cells. Under these conditions we detected significant amounts of intact plasmid DNA within the cells 2 hours after transfection and a reduced but detectable amount by 20 hours (Fig. 3). Cells transfected with DNA in the absence of polyornithine or with DNA complexed with DEAE-dextran contained little or no plasmid (Fig. 3). Higher levels of transfection with DNA-$CaCl_2$ precipitates have been found (*18*). Control transfection with vector DNA, pBR322, alone was also performed.

We examined the constitutive and regulated expression of the transfected genes in both cell lines by 2D-PAGE analysis after 90 minutes of [^{35}S]methio-

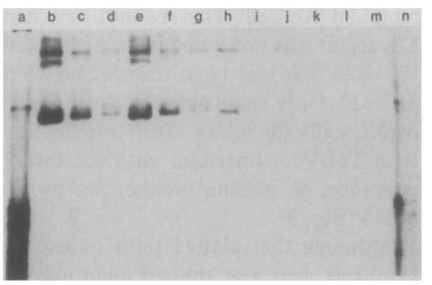


Fig. 3. Southern blot analysis (*21*) showing the presence of intact recombinant plasmid DNA in transfected *Drosophila* S3 cells. Cells were transfected for 2 hours with recombinant plasmid DNA complexed with polyornithine (50 μg/ml final concentration) (b, c, and d); with polyornithine (30 μg/ml final concentration) (e, f, and g); with DEAE-dextran (500 μg/ml final concentration) (h, i, and j); or with no carrier (k, l, and m). All transfections were done with recombinant plasmid DNA at a final concentration of 0.625 μg/ml. Cells were harvested immediately after transfection (b, e, h, and k); 4 hours after transfection (c, f, i, and l); and 20 hours after transfection (d, g, j, and m). Cells were treated with proteinase K (100 μg/ml, final concentration) in 0.01*M* tris (*p*H 7.8), 0.5*M* EDTA, and 0.5 percent sarcosyl. The DNA's were extracted with phenol and a mixture of chloroform and isoamyl alcohol (24:1) and precipitated with ethanol. DNA's were subjected to electrophoresis on a 1.0 percent agarose gel in tris-acetate buffer, blotted onto nitrocellulose, and hybridized to pBR322 in 50 percent formamide and 10 percent dextran sulfate. The blot was washed under conditions of moderate stringency. (a and n) DNA size-markers.

nine incorporation. In no case where labeling was carried out in cells maintained at 25°C was there any evidence of hsp synthesis (Fig. 1). This indicates that the transfection procedure and subsequent manipulation did not induce a stress response and that the transfected genes were not expressed constitutively at 25°C. Cells labeled during heat shock at 36°C displayed the characteristic stress response (Fig. 1), which included the synthesis of the large hsp's 68, 70, and 83, the small hsp's 23 and 22, as well as hsp 26 and 27, which were not resolved in these gels (*13*). In line S3 cells, whether untransfected or transfected with pBR322 or T6BV1, there was only a single hsp 22 variant expressed (Fig. 1). In line Kc cells labeled at 36°C 2 hours after polyornithine transfection with T6BV1, two hsp 22 variants were synthesized (Fig. 1). One was characteristic of the endogenous hsp 22 induced in control or sham transfected cells, and the other new hsp 22 variant comigrated with the hsp 22 found in S3 cells. This is evidence that the T6BV1 clone contains a functional hsp 22 gene that encodes a protein whose electrophoretic mobility is indistinguishable from the hsp 22 found in S3 cells. This also implies that the transfected gene has acquired a pattern of hsp 22 regulation characteristic of the recipient Kc cell. The second, new hsp 22 variant was not found in heat-shocked Kc cells that had been transfected with pBR322. Only small amounts were found in Kc cells 20 hours after transfection with T6BV1, consistent with the disappearance of plasmid sequences by 20 hours (Fig. 3).

Although the relative rates of hsp 22 synthesis were not studied quantitatively, qualitative inspection of hsp 22 labeling in Fig. 1 indicated that the expression of the two hsp 22 variants in T6BV1 transfected Kc cells at 2 hours occurred at similar levels. Since there appears to be no method of determining how many transcriptionally active genes have become transfected, or what the level of their expression is when active, it is not possible to comment on the significance of the observed pattern. Further, the relative intensity of hsp 22 labeling in S3 cells induced at 36°C 2 hours after transfection with T6BV1 did not differ noticeably from that induced in untransfected control cells. This implies the absence of a gene dosage effect on hsp 22 synthesis in this system. The absence of a gene dosage effect was also noted in experiments with Northern blot analysis (*19*) to determine the relative abundances of hsp 22 and 70 messenger RNA (mRNA) in control and transfected Kc cells subjected to heat shock (data not shown). Although several different hypotheses can explain this pattern (a rate-limiting amount of hsp 22 inducer or limiting RNA polymerase activity), there are indications that hsp 22 transcription and translation are autoregulated by the accumulated level of hsp 22 protein. Autoregulation of hsp 22 was indicated by the results of another experiment. When *Drosophila* S3 cells were heat shocked for an hour and returned to 25°C to prevent cell death, the level of hsp 22 mRNA reached a plateau and then declined (Fig. 4). When the same experiment was performed in the presence of cycloheximide at a concentration that inhibited protein synthesis by 95 percent, the level of hsp 22 mRNA continued to increase for several hours (Fig. 4). This suggests that the repression of hsp 22 transcription, which was initially triggered by heat shock, may be autoregulated. In *Drosophila* cells, hsp 70 transcription and translation is also thought to be autoregulated (*20*).

Genetic polymorphisms can be used by molecular biologists interested in defining DNA sequences that are important in homologous gene expression and regulation. In principle, allelic variation can be used in any system where the RNA or protein products can be distinguished.

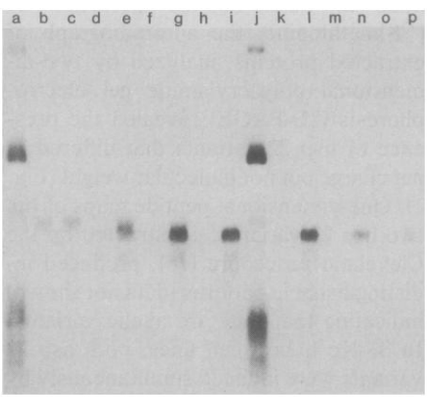


Fig. 4. Northern blot analysis (*19*) of hsp 22 RNA accumulation during recovery from heat shock. *Drosophila* S3 cells were incubated for 30 minutes at 25°C in the presence of cycloheximide at 1 μg/ml (c, e, g, i, l, n, and p) or in the absence of cycloheximide (b, d, f, h, k, m, and o); subjected to a 1 hour heat shock at 36°C; and allowed to recover at 25°C for 0 hours (b and c), 1 hour (d and e), 2 hours (f and g), 3 hours (h and i), 4 hours (k and l), 5 hours (m and n), or 6 hours (o and p). Cells were extracted in 7.5*M* guanidine hydrochloride, 10 m*M* dithiothreitol, and 20 m*M* sodium acetate. RNA's were isolated by centrifuging through a 5.7*M* CsCl cushion, extracted with phenol and a mixture of chloroform and isoamyl alcohol (24:1), and precipitated with ethanol. Total RNA's were glyoxalated and subjected to electrophoresis on a 1.6 percent agarose gel (40 μg of RNA per lane) in 10 m*M* sodium phosphate buffer, *p*H 6.8. The blot was hybridized to T6BV1 plasmid DNA in 50 percent formamide and 10 percent dextran sulfate and was washed under conditions of moderate stringency. (a and j) Glyoxalated size-markers.

CHRISTINE M. MORGANELLI
EDWARD M. BERGER
Biology Department,
Dartmouth College,
Hanover, New Hampshire 03755

References and Notes

1. R. C. Mulligan and P. Berg, *Science* **209**, 1422 (1980); *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2072 (1981); T. E. Wagner, P. C. Hoppe, J. D. Jollick, D. R. Scholl, R. L. Hodinka, J. B. Gault, *ibid.*, p. 6376; F. Constantini and E. Lacy, *Nature (London)* **294**, 92 (1981); R. D. Palmiter, H. Y. Chen, R. L. Brinster, *Cell* **39**, 701 (1982); R. D. Palmiter *et al.*, *Nature (London)* **300**, 611 (1982); M. V. Chao, P. Mellon, P. Charnay, T. Maniatis, R. Axel, *Cell* **32**, 483 (1983); J. H. Sinclair, J. H. Sang, J. F. Burke, D. Ish-Horowicz, *Nature (London)* **306**, 198 (1983).
2. H. R. B. Pelham, *Cell* **30**, 517 (1982); ——— and M. Bienz, *EMBO J.* **1**, 1473 (1982); M. Bienz and H. R. B. Pelham, *ibid.*, p. 1583.
3. V. Corces, A. Pellicer, R. Axel, M. Meselson, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7038 (1981); R. Voellmy and D. Rungger, *ibid.* **79**, 1776 (1982); J. F. Burke and D. Ish-Horowicz, *Nucleic Acids Res.* **10**, 3821 (1982); H. Kondoh, K. Yasuda, T. S. Okada, *Nature (London)* **301**, 440 (1983).
4. P. P. DiNocera and I. B. Dawid, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7095 (1983); M. Bourouis and B. Jarry, *EMBO J.* **2**, 1099 (1983); C. Benjayati and J. F. Dray, *Proc. Natl. Acad. Sci. U.S.A.* (in press).
5. M. Wigler, A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, L. Chasin, *ibid.* **76**, 1371 (1979); I. Lowy, A. Pellicer, J. F. Jacobson, G. K. Sim, S. Silverstein, R. Axel, *Cell* **22**, 817 (1980); G. M. Rubin and A. C. Spradling, *Science* **218**, 348 (1982); A. C. Spradling and G. M. Rubin, *ibid.*, p. 341; *Cell* **34**, 47 (1983); S. B. Scholnick, B. A. Morgan, J. Hirsh, *ibid.*, p. 37; P. A. Goldberg, J. W. Posakony, T. Maniatis, *ibid.*, p. 59.
6. J. Hubby and R. C. Lewontin, *Genetics* **54**, 577 (1966); R. C. Lewontin and J. Hubby, *ibid.*, p. 595; H. Harris, *Proc. Roy. Soc. London Ser. B.* **164**, 298 (1966); J. R. Powell, *Evol. Biol.* **8**, 79 (1975).
7. F. Ritossa, *Experientia* **18**, 571 (1962); M. Ashburner, *Chromosoma* **31**, 356 (1970); S. Wadsworth, E. Craig, B. McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2134 (1980).
8. E. A. Craig and B. J. McCarthy, *Nucleic Acids Res.* **8**, 4441 (1980); V. Corces, R. Holmgren, R. Freund, R. Morimoto, M. Meselson, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5390 (1980); R. Voellmy *et al.*, *Cell* **23**, 261 (1981).
9. T. D. Ingolia and E. A. Craig, *Nucleic Acids Res.* **9**, 1627 (1981); *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2360 (1982); R. Southgate, A. Ayme, R. Voellmy, *J. Mol. Biol.* **165**, 37 (1983).
10. M. Ashburner and J. J. Bonner, *Cell* **17**, 241 (1979).
11. R. C. Ireland, E. M. Berger, K. Sirotkin, M. A. Yund, D. Osterbur, J. Fristrom, *Dev. Biol.* **93**, 498 (1982).
12. K. Sirotkin and N. Davidson, *ibid.* **89**, 196 (1982); C. M. Cheney and A. Shearn, *ibid.* **95**, 325 (1983).
13. R. C. Ireland and E. M. Berger, *Proc. Natl. Acad. Sci. U.S.A.* **855** (1982).
14. The S3 line is a subline of the Schneider line 3 [I. Schneider, *J. Embryol. Exp. Morphol.* **27**, 353 (1972)].
15. G. Echalier and A. Ohanessian, *C. R. Acad. Sci. Paris* **268**, 1771 (1969).
16. D. Cleveland, S. G. Fischer, M. W. Kirschner, U. K. Laemmli, *J. Biol. Chem.* **252**, 1102 (1977).
17. C. Wyss, *Experientia* **37**, 665 (1981).
18. C. M. Morganelli and E. M. Berger, unpublished data.
19. P. S. Thomas, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201 (1980).
20. B. J. DiDomenico, G. E. Bugalsky, S. Lundquist, *Cell* **31**, 593 (1982).
21. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).

22. We thank E. Craig for the T6BV1 clone and the complete restriction map of the T6 insert, and T. Ingolia for the data on the 3′ hsp 22 gene sequence in Fig. 2. Supported in part by American Cancer Society grant 1N157.

31 January 1984; accepted 23 February 1984