The Herpes Simplex Virus Thymidine Kinase Gene Is Not Transcribed in Saccharomyces cerevisiae

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The herpes simplex virus thymidine kinase gene has been cloned into a chimeric yeast plasmid cloning vehicle and transformed into appropriate yeast strains. Plasmids carrying the herpes simplex virus thymidine kinase gene can be propagated as autonomously replicating plasmids, but no RNA specific to the thymidine kinase coding sequence was detected.

Since the establishment of yeast transformation (15), a number of genes of procaryotic origin have been introduced into yeasts, and their expression has been demonstrated (6, 7, 16, 24, 27). Genes of eucaryotic origin have also been studied in yeasts transformed with chimeric plasmids. Beggs et al. (5) have demonstrated that genomic rabbit β-globin is transcribed but not correctly processed in yeasts. Mercereau-Puijalon et al. (23) have demonstrated the synthesis of a chicken ovalbumin-like protein in S. cerevisiae transformed with a plasmid containing cDNA complementary to chick ovalbumin mRNA. In the latter case, the lac promoter was probably utilized for transcription. Henikoff et al. (14) have reported the expression of a gene from Drosophila melanogaster in yeasts. We have been interested in using S. cerevisiae as a host to study the expression of nonyeast genes. We have chosen initially to study the herpes simplex virus (HSV) thymidine kinase (tk) gene. This gene is readily obtainable on a BamHI fragment (11), and it does not contain an intron (21, 22), which should reduce the complexity of studying its expression in a heterologous host. Also, since S. cerevisiae do not contain a thymidine kinase (13), the expression of a heterologous tk gene in S. cerevisiae would be of great practical importance in allowing for specific labeling of DNA in S. cerevisiae with thymidine. This report shows that the HSV tk gene carried on a BamHI fragment (11) is not transcribed in S. cerevisiae, nor is any antigenically cross-reacting protein or functional tk protein produced. A preliminary report of some of these data has been presented (17).

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MATERIALS AND METHODS

Plasmid and strains. The HSV tk gene carried on a BamHI fragment and cloned in pBR322 was supplied by L. Enquist. The chimeric yeast plasmid cloning vehicle pYF91 was as described by Storms et al. (28). Yeast strain 308/6C (ρ+ a tmp1-6 tup his [1, 7] ilv1-92 lys1 trp5) (19) was from J. G. Little, and strain LL20 (ρ+ α leu2-3 leu2-112 his3-11 his3-15) was from G. Fink.

DNA and RNA isolation and characterization. Plasmids propagated in Escherichia coli were amplified with 250 μg of chloramphenicol or 300 μg of spectinomycin per ml. Plasmid DNA was isolated and purified by CsCl-ethidium bromide density gradient centrifugation (8). Plasmid DNA was isolated from S. cerevisiae as described by Cryer et al. (10). This DNA was used to transform E. coli selecting for appropriate genetic markers. For rapid isolation and characterization of plasmids from E. coli, the method described by An and Friesen (2) was used. Restriction endonucleases were purchased from Boehringer Mannheim Canada Ltd., Bethesda Research Laboratories, or New England Biolabs. Digestions were carried out according to the suppliers' specifications, and digestion products were analyzed by electrophoresis through agarose gels and visualized by staining with ethidium bromide.

Poly(A)⁻, poly(A)⁺, and total RNA were isolated from yeasts by the method of Warner and Gorenstein (29). The isolated RNA was electrophoresed through an agarose gel under denaturing conditions, as described by Bailey and Davidson (4), and then blotted to diazobenzyloxymethyl paper. RNA was visualized by hybridization with DNA probes made radioactive with ³²P by nick translation (25). Hybridization was at 42°C in 50% formamide for 72 h. Hybridization and wash conditions were as described by Alwine et al. (1). Autoradiography was for 5 days at −70°C with an intensifying screen.

Transformation. Calcium-treated E. coli cells were transformed essentially as described by Mandel and Higa (20). S. cerevisiae was transformed as described by Hinnen et al. (15), with modifications according to Storms et al. (28). Mouse L-cells were transformed by using a modification of the calcium phosphate precipi-

tation method of Bacchetti and Graham (3). Each 100-mm tissue culture dish was seeded with 5×10^5 LMTK$^-$ cells in 10 ml of α-minimal essential medium supplemented with 10% fetal calf serum and incubated at 34°C for 24 h. A total of 20 μg of DNA (salmon sperm or plasmid, 2 μg) plus salmon sperm (18 μg) in 1 ml of HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid) saline phosphate buffer (140 mM NaCl, 20 mM HEPES [pH 7.2], 0.75 mM Na$_2$HPO$_4$) was precipitated by the addition of 0.05 ml of 2.5 M CaCl$_2$. The precipitate was mixed and allowed to stand at room temperature (25°C) for 30 min. The entire contents of the tube were then added to the LMTK$^-$ cells. The DNA-cell mixture was incubated at 34°C for 24 h. The medium was removed by aspiration and was replaced with 12 ml of α-minimal essential medium supplemented with 10% fetal calf serum plus 6.0×10^{-7} M methotrexate, 1.6×10^{-5} M thymidine, 5.0×10^{-5} M adenosine, and 5.0×10^{-5} M guanosine (MTAG medium; selective for TK$^+$ cells). The petri plates were incubated at 34°C. The medium was replaced with fresh MTAG medium every 7 days. The colonies were fixed and stained on day 17.

Enzyme assays. For assay of tk activity, yeast cultures were grown to approximately 2×10^7 cells per ml in defined medium plus growth requirements. Cells were pelleted by centrifugation at $7,000 \times g$ for 2 min, washed, and suspended in 1.2 ml of 10 mM Tris-hydrochloride (pH 8.0)–50 mM thymidine–1.4 mM β-mercaptoethanol–0.2 mM ATP. A 0.5-ml portion of glass beads (diameter, 0.45 to 0.5 mm) was made up to 1.5-ml final volume by the addition of the appropriate volume of cell suspension. The cells were lysed in an Eppendorf tube in a Braun homogenizer for 6 min with CO$_2$ cooling. The extract was then centrifuged at $2,000 \times g$ for 7 min, and the supernatant was centrifuged at $17,000 \times g$ for 30 min. The clarified supernatant (50 μl) was used for enzyme assays in a total volume of 200 to 250 μl, as described by Klemperer et al. (18), Wigler et al. (30), or Cornish and Pearlman (manuscript in preparation). Uridine kinase activity was determined as described by Cornish (M.Sc. thesis, York University, Downsview, Ont., Canada, 1980). A total of 56.2 Ci of 5-[*methyl*-^{3}H]thymidine and 35.2 Ci of [6-^{3}H]uridine) per mmol was used. The reaction was stopped by boiling for 2 min. The sample was then centrifuged for 2 min in a bench-top centrifuge to remove precipitated protein. A 50-μl portion of the supernatant was spotted on a 2-cm-diameter disk of DEAE-cellulose ion-exchange paper and was dried for 5 min. The disks were washed three times in 4 mM ammonium formate (10 ml per disk) and once in 95% ethanol (10 ml per disk). They were then dried, and the radioactivity on each disk was determined.

RESULTS

The HSV *tk* gene, carried on a *Bam*HI fragment that had been cloned in pBR322, was transferred in both orientations to the chimeric yeast plasmid cloning vehicle, pYF91 (28), by in vitro *Bam*HI digestion and ligation. The plasmids thus constructed (pGY14, pGY15) contain a 3.6-kilobase pair (kbp) *Eco*RI fragment of the yeast 2-μm circle, a 6.0-kbp *Bam*HI-*Hind*III fragment of the *Saccharomayces cerevisiae*

LEU2 gene, almost the entire *E. coli* plasmid pBR322, and the 3.4-kbp *Bam*HI fragment carrying the HSV *tk* gene. The structure of pGY14 is shown in Fig. 1. The structure of pGY15 is the same as pGY14 except the 3.4-kbp *Bam*HI fragment is in the opposite orientation. The presence of the HSV *tk* gene on pGY14 was verified by transformation of a TK$^-$ strain of mouse L-cells to TK$^+$. From the results of the transformation experiments (Table 1), we conclude that plasmid pGY14 carries a functional *tk* gene which can be expressed in L-cells under appropriate conditions.

Plasmids pGY14 and pGY15 were used to transform *S. cerevisiae* to obtain evidence for expression of the HSV *tk* gene in *S. cerevisiae*. *S. cerevisiae* LL20 was transformed by pGY14 to Leu$^+$ at a frequency of 6.1×10^2 transformants per g of plasmid DNA and by pYF91 at a frequency of 10×10^2 Leu$^+$ transformants per μg of plasmid DNA. Transformed colonies were purified by restreaking. The presence of pGY14 or pYF91 in the transformed yeast strains (designated GY700 and GY703, respectively) was verified by isolating DNA from the transformed cultures and using this to transform *E. coli*, selecting for ampicillin resistance. Crude DNA preparations from several of these transformed
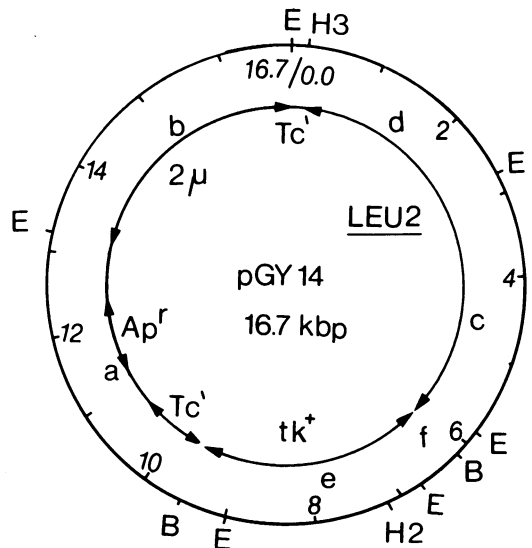

FIG. 1. Structure of plasmid pGY14. Symbols: tk$^+$, *Bam*HI fragment of HSV DNA carrying the *tk* gene; *LEU2*, a *Bam*HI-*Hind*III fragment of *S. cerevisiae* DNA carrying the *LEU2* gene (28); 2μ, 3.6-kbp *Eco*RI fragment of the yeast plasmid 2-μm circle; Ap, ampicillin resistance gene of pBR322; Tc, tetracycline resistance gene of pBR322, E, *Eco*RI; B, *Bam*HI; H2, *Hinc*II; H3, *Hind*III. Lowercase letters (a, b, c, d, e, f) refer to the *Eco*RI fragments of pGY14.

TABLE 1. Transformation of L-cells with tk^+ DNA[a]

Transforming DNA	No. of TK$^+$ transformants/μg of DNA per 5 × 10^5 TK$^-$ cells
pGY14	20.8
pBR322 carrying tk^+	37.5
pYF91	0[b]
Salmon sperm	0[c]

[a] Transformation of mouse L-cells was as described in the text.

[b] No TK$^+$ transformant was observed when a total of 4 μg of DNA and 10^6 cells was used.

[c] No TK$^+$ transformant was observed when a total of 60 μg of DNA and 1.5 × 10^6 cells was used.

E. coli cultures were analyzed by digestion with *Eco*RI followed by electrophoresis through an agarose gel. An example of these data is shown in Fig. 2. The data indicate that all plasmids recovered from the transformed *E. coli*, and therefore present in the originally transformed *S. cerevisiae* strain GY700 or GY703, had the same structure as the initial plasmid, pGY14 or pYF91.

Since *S. cerevisiae* LL20 will take up exogenous thymidine from the medium (J. B. McNeil, personal communication), we attempted to force strain GY700 to become dependent upon exogenous thymidine by blocking endogenous production of dTMP with sulfanilamide plus aminopterin. We reasoned that if the HSV *tk* were expressed in strain GY700, then exogenous thymidine might restore growth to the drug-treated cells. This proved not to be the case, either in complete or in defined growth medium. We next constructed a yeast strain, GY712 (ρ$^+$ a *tmp1-6 tup leu2-3 leu2-112*), to avoid the use of sulfanilamide and aminopterin in the expression studies. The construction was carried out by crossing strains 308/6C and LL20. After mating, spore formation, and plating, a colony (GY705) having the Leu$^-$ Ilv$^-$ Trp$^-$ Tmp$^-$ Tup$^-$ phenotype was selected, and Ilv$^+$ and Trp$^+$ spontaneous revertants were consecutively isolated. The resulting strain GY712 was Leu$^-$ Tmp$^-$ Tup$^-$ and could grow on complete medium supplemented with 100 μg of dTMP, 100 μg of aminopterin, and 5 mg of sulfanilamide per ml or on minimal medium (yeast nitrogen base) with dTMP and leucine. A thymidylate synthetase (*tmp1*) mutant of *S. cerevisiae* can grow in the presence of exogenous dTMP only if a mutation for thymidylate uptake (*tup*) is carried by the strain.

Plasmids pGY14 and pGY15 were used to transform *S. cerevisiae* GY712 selecting for complementation of *leu2*. Leu$^+$ transformants appeared on selective plates (yeast nitrogen base

supplemented with 100 μg of dTMP per ml) at a frequency of 1 to 10 colonies per μg of plasmid DNA. Transformed colonies were purified by restreaking, and two colonies, GY780 and GY782, carrying pGY14 and pGY15, respectively, were studied further.

The Leu$^+$ phenotype segregated very rapidly to Leu$^-$ when GY780 and GY782 were grown for 10 generations under nonselective conditions. This is consistent with these strains carry-
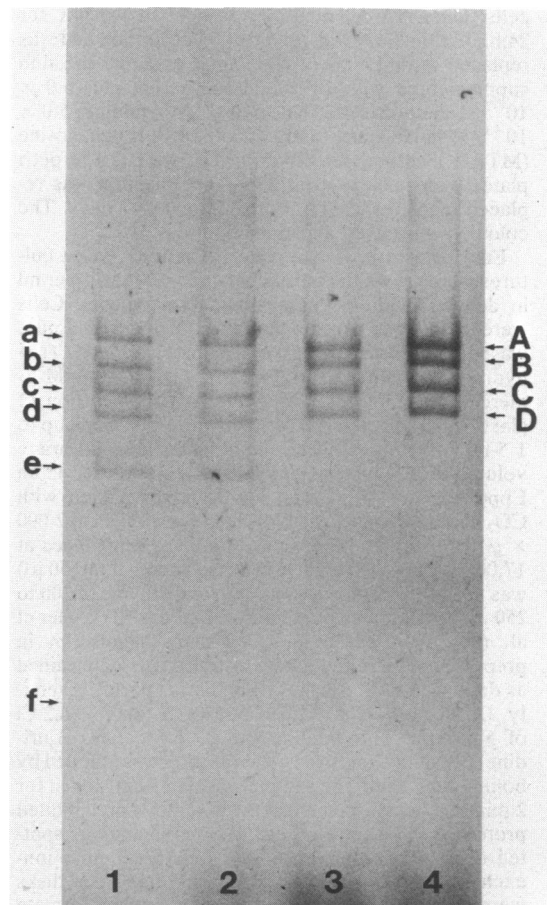

FIG. 2. *Eco*RI restriction fragments of pGY14 and pYF91 plasmid DNA. Lane 1, *Eco*RI-digested pGY14; lane 4, *Eco*RI-digested pYF91; lane 2, *Eco*RI-digested DNA from ampicillin-resistant *E. coli* that had been transformed with DNA isolated from *S. cerevisiae* GY700; lane 3, *Eco*RI-digested DNA from ampicillin-resistant *E. coli* that had been transformed with DNA isolated from *S. cerevisiae* GY703. Uppercase letters (A, B, C, D) indicate *Eco*RI fragments of pYF91. Lowercase letters (a, b, c, d, e, f) indicate *Eco*RI fragments of pGY14. The *Bam*HI fragment carrying the HSV *tk* gene was inserted into the largest (A) fragment of pYF91 in the construction of pGY14 (see Fig. 1 [28]). Electrophoresis was carried out on a 1.0% agarose gel.

ing an autonomously replicating plasmid. The presence of pGY14 and pGY15 in the transformed yeast strains was verified by isolating DNA from GY780 and GY782 cultures grown under selective conditions. These DNA preparations were used to transform *E. coli*, selecting for ampicillin resistance and Leu$^+$. Crude DNA preparations from several of these transformed *E. coli* cultures were analyzed by digestion with *Eco*RI followed by electrophoresis through an agarose gel. The digestion pattern of plasmids recovered from the transformed *E. coli*, and therefore present in the original transformed *S. cerevisiae* strains GY780 and GY782, were the same as the initial plasmids pGY14 and pGY15 (data not shown). We attempted to force GY780 and GY782 to become dependent upon exogenous thymidine, omitting dTMP from the minimal medium. As described previously, if HSV *tk* were actively expressed in strain GY780 or GY782, then exogenous thymidine might restore dTMP production by the *tk* activity. This proved not to be the case. To test for second site mutations allowing expression of *tk*, we tried to select, by spontaneous mutation, yeast cells having *tk* activity by plating 5×10^9 to 8×10^9 cells from both GY780 and GY782 on minimal plates plus thymidine. After 2 weeks no colony appeared on any plate.

Total, poly(A)$^-$, and poly(A)$^+$ RNA from strains GY780 and GY782 were isolated as described in Materials and Methods and analyzed by hybridization with DNA probes from various regions of the plasmids that had been used to transform the strains. Data from these experiments are presented in Fig. 3. With nick-translated pYF91 as a probe (Fig. 3A), a number of poly(A)$^-$ RNA bands were observed. At least six specific poly(A)$^+$ RNA species were also seen. With the *Bam*HI HSV *tk*-containing fragment as a probe (Fig. 3B), a smear of poly(A)$^-$ RNA, but no poly(A)$^+$ RNA, was visualized. Using an 840-bp *Pst*I fragment from within the *Bam*HI HSV *tk*-containing fragment (Fig. 3C), no RNA, either poly(A)$^-$ or poly(A)$^+$, complementary to this probe was visualized. This *Pst*I fragment begins 20 bp downstream from the presumptive 5' mRNA initiation site and 90 bp upstream from the initiation codon for HSV *tk* and includes 67% of the *tk* coding sequence (21). Similar results were obtained when RNA from strain GY700 was analyzed in the same way (data not shown). Thus, although a smear of poly(A)$^-$ RNA homologous to the HSV tk-containing *Bam*HI fragment is made in transformed *S. cerevisiae*, no RNA is made that is homologous to the 5' two-thirds of the *tk*-coding sequence.

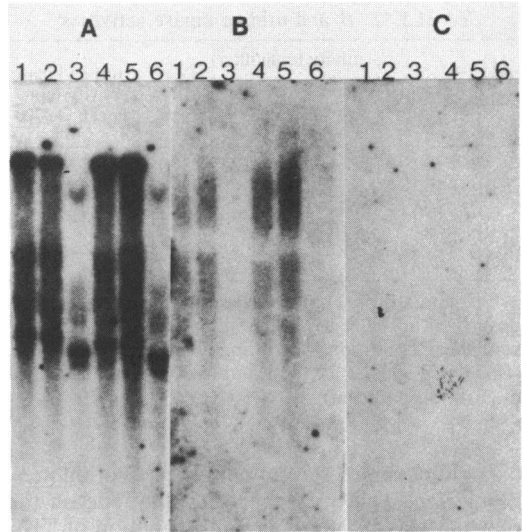To obtain further evidence regarding the expression, or lack of expression, in *S. cerevi-*

FIG. 3. Autoradiogram of RNA extracted from strains GY780 and GY782 and hybridized to nick-translated DNA. Total, poly(A)$^-$, and poly(A)$^+$ RNA was isolated from strains GY780 and GY782, electrophoresed under denaturing conditions, and transferred to diazobenzyloxymethyl paper as described in the text. The specific activity of probes was 5×10^7 to 8×10^7 cpm/μg, and hybridization was in sealed plastic bags at 42°C in 50% formamide for 72 h as described by Alwine et al. (1), using about 3×10^4 cpm/cm^2 and about 100 μl of hybridization mix per cm^2. After washing (1), autoradiography was for 5 days with an intensifying screen. (Lane 1) 12.4 μg of total RNA from GY780; (lane 2) 12.6 μg of poly(A)$^-$ RNA from GY780; (lane 3) 2.6 μg of poly(A)$^+$ RNA from GY780; (lane 4) 11.4 μg of total RNA from GY782; (lane 5) 13.2 μg of poly(A)$^-$ RNA from GY782; (lane 6) 3.0 μg of poly(A)$^+$ RNA from GY782. (A) Nick-translated pYF91 as probe; (B) nick-translated 3.4-kbp *Bam*HI fragment containing HSV *tk* gene as probe; (C) nick-translated 840-bp *Pst*I fragment from the coding region of HSV *tk* (21) as probe.

siae of the HSV *tk*, we assayed for *tk* activity in crude cell extracts of transformed yeast strains. Under three different assay conditions, no *tk* enzymatic activity above background was detected with either strain GY700 or GY703. On the other hand, uridine kinase activity was detected in both strains (Table 2). In agar diffusion studies, antibody prepared against HSV *tk* (kindly provided by Sheldon Gervitz, McMaster University, Hamilton, Ontario) and crude cell lysates of strains LL20, GY700, and GY703 were analyzed by the method of Scheidegger (26). For all three strains, a weak band of precipitation, but no band(s) unique to strain GY700, was observed (data not shown).

DISCUSSION

We have cloned a 3.4-kbp *Bam*HI fragment containing the HSV *tk* gene onto a chimeric

TABLE 2. *tk* and uridine kinase activities[a]

Time (min)	*tk* activity (cpm/filter)					Uridine kinase activity (cpm/filter), GY700
	GY700			GY703		
	a	b	c	a	b	
0	260	310	2,140	220	300	970
30	180		2,020	180		25,560
60	190	330	2,060	200	350	29,230
120		360			370	
150	220					

[a] *tk* and uridine kinase assays were performed as described in the text. The column designations represent assay conditions according to (a) Klemperer et al. (18), (b) Wigler et al. (30), and (c) Cornish and Pearlman (in preparation).

plasmid capable of autonomous replication in *S. cerevisiae*. The presence of the *tk* gene on the plasmid was verified by transformation of TK⁻ mouse L-cells to TK⁺. Although the plasmids we constructed contained the *tk* gene (22) and replicated autonomously in *S. cerevisiae*, yeast cells transformed with these plasmids contained neither *tk* enzymatic activity nor protein cross-reacting with antibody to HSV *tk*. These yeast cells also could not grow in the presence of thymidine when the synthesis of dTMP was blocked with drugs or by mutation. To ascertain whether the absence of *tk* function in transformed *S. cerevisiae* was at the level of transcription or translation, we analyzed RNA made in the transformed yeast strains. Both poly(A)⁻ and poly(A)⁺ RNA complementary to the cloning vehicle pYF91 were made. These represented pBR322, 2-μm circle, and *LEU2*-specific RNAs. At least some of the poly(A)⁺ RNA represented polyadenylation of pBR322 mRNA in yeast, in agreement with the results of Chevallier et al. (7). No poly(A)⁺ RNA complementary to the *Bam*HI HSV *tk* fragment was made, but a heterogeneous smear of poly(A)⁻ RNA was seen. This broad band of hybridization might result from the random initiation, termination, or stability of the RNA molecules. No RNA complementary to the HSV *tk* coding sequence was made in the transformed yeast strains. The coding sequence probe incudes the 5' two-thirds of the *tk* protein as well as 90 bp of *tk* mRNA 5' to the initiation codon (21). Thus, yeast transcription terminators must occur outside the *tk* coding sequence, and no promoters recognized by yeast RNA polymerases are present within the HSV *tk*-coding region. Consistent with this is the observation (J. B. McNeil and J. D. Friesen, Mol. Gen. Genet., in press) that HSV *tk* is expressed in *S. cerevisiae* when the region 5' to the *tk* initiation codon is removed and the *tk*-coding sequence is fused to a yeast promoter. Our results differ, however, from those of Beggs

et al. (5), who demonstrated that genomic rabbit β-globin sequences cloned into a chimeric plasmid capable of autonomous replication in *S. cerevisiae* are transcribed but are not processed correctly.

LITERATURE CITED

1. **Alwine, J. C., D. J. Kemp, B. A. Parker, J. Reiser, J. Renart, G. R. Stark, and G. M. Wahl.** 1980. Detection of specific RNAs or specific fragments of DNA by fractionation in gels and transfer to diazobenzyloxymethyl paper. Methods Enzymol. **68:**220–242.
2. **An, G., and J. D. Friesen.** 1979. Plasmid vehicles for direct cloning of *Escherichia coli* promoters. J. Bacteriol. **140:**400–407.
3. **Bacchetti, S., and F. L. Graham.** 1977. Transfer of the gene for thymidine kinase to thymidine kindase-deficient human cells by purified Herpes Simplex viral DNA. Proc. Natl. Acad. Sci. U.S.A. **74:**1590–1594.
4. **Bailey, J. M., and N. Davidson.** 1979. Methylmercury as a reversible denaturing agent for agarose gel electrophoresis. Anal. Biochem. **70:**75–85.
5. **Beggs, J. D., J. van den Berg, A. van Ooyen, and C. Weissmann.** 1980. Abnormal expression of chromosomal rabbit β-globin gene in *Saccharomyces cerevisiae*. Nature (London) **283:**835–840.
6. **Chevallier, M. R., and M. Aigle.** 1979. Qualitative detection of penicillinase produced by yeast strains carrying chimeric yeast *E. coli* plasmids. FEBS Lett. **108:**179–180.
7. **Chevallier, M. R., J. C. Bloch, and F. Lacroute.** 1980. Transcriptional and translational expression of a chimeric bacterial-yeast plasmid in yeasts. Gene **11:**11–19.
8. **Clewell, D. B., and D. R. Helinski.** 1970. Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry **9:**4428–4440.
9. **Cohen, J. D., T. R. Eccleshall, R. B. Needleman, H. Federoff, B. A. Buchferer, and J. Marmur.** 1980. Functional expression in yeast of the *Escherichia coli* plasmid gene coding for chloramphenicol acetyltransferase. Proc. Natl. Acad. Sci. U.S.A. **77:**1078–1082.
10. **Cryer, D. R., R. Eccleshall, and J. Marmur.** 1975. Isolation of yeast DNA. Methods Cell Biol. **12:**39–44.
11. **Enquist, L. W., M. J. Madden, P. Schlop-Stansly, and G. F. Vande Woude.** 1979. Cloning of Herpes Simplex Type 1 DNA fragments in a bacteriophage lambda vector. Science **203:**541–544.
12. **Enquist, L. W., G. F. Vande Woude, M. Wagner, J. R. Smiley, and W. C. Summers.** 1979. Construction and characterization of a recombinant plasmid encoding the gene for the thymidine kinase of *Herpes Simplex* type 1 virus. Gene **7:**335–342.
13. **Grivell, A. R., and J. F. Jackson.** 1968. Thymidine kinase: evidence for its absence from *Neurospora crassa* and some other micro-organisms and the relevance of this to the specific labelling of DNA. J. Gen. Microbiol. **54:**307–317.
14. **Henikoff, S., K. Tatchell, B. H. Hall, and K. A. Nasmyth.** 1981. Isolation of a gene from *Drosophila* by complementation in yeast. Nature (London) **289:**33–37.
15. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. U.S.A. **75:**1929–1933.
16. **Hollenberg, C. P.** 1979. The expression of bacterial antibiotic resistance genes in the yeast *Saccharomyces cerevi-*

siae, p. 481–492. *In* K. N. Timmis (ed.), Plasmids of medical, environmental and commercial importance. Elsevier/North-Holland Biomedical Press, Amsterdam.

17. **Kiss, G. B., K. V. Cornish, R. E. Pearlman, and J. D. Friesen.** 1980. The Herpes Simplex Virus thymidine kinase gene is not expressed in *Saccharomyces cerevisiae*. Recomb. DNA Tech. Bull. **3:**21–24.

18. **Klemperer, H. G., G. R. Haynes, W. I. H. Shedden, and D. H. Watson.** 1967. A virus-specific thymidine kinase in BHK21 cells infected with Herpes Simplex Virus. Virology **31:**120–128.

19. **Little, J. G., and R. H. Haynes.** 1979. Isolation and characterization of yeast mutants auxotrophic for 2'-deoxythymidine 5'-monophosphate. Mol. Gen. Genet. **168:**141–151.

20. **Mandel, M., and Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. **73:**453–471.

21. **McKnight, S. L.** 1980. The nucleotide sequence and transcript map of the herpes simplex virus thymidine kinase gene. Nucleic Acids Res. **8:**5949–5964.

22. **McKnight, S. L., and E. R. Gavis.** 1980. Expression of the herpes thymidine kinase gene in Xenopus laevis oocytes: an assay for the study of deletion mutants constructed *in vitro*. Nucleic Acids Res. **8:**5931–5948.

23. **Mercereau-Puijalon, O., F. Lacroute, and P. Kourilsky.**

24. **Panthier, J. J., P. Fournier, H. Heslot, and A. Rambach.** 1979. Expression of a bacterial gene cloned in the yeast *Saccharomyces cerevisiae*. C.R. Acad. Sci. Paris Ser. D **289:**773–775.

25. **Rigby, P. W. J., M. Dieckman, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick-translation with DNA polymerase I. J. Mol. Biol. **113:**237–251.

26. **Scheidegger, J. J.** 1955. Micromethode d'immunoelectrophorese. Int. Arch. Allergy Appl. Immunol. **7:**103–110.

27. **Storms, R. K., E. W. Holowachuck, and J. D. Friesen.** 1981. Genetic complementation of the *Saccharomyces cerevisiae leu2* gene by the *Escherichia coli leuB* gene. Mol. Cell. Biol. **1:**836–842.

28. **Storms, R. K., J. B. McNeil, P. S. Khandekar, G. An, J. Parker, and J. D. Friesen.** 1979. Chemeric plasmids for the cloning of deoxyribonucleic acid sequences in *Saccharomyces cerevisiae*. J. Bacterial. **140:**73–82.

29. **Warner, J. R., and C. Gorenstein.** 1977. The synthesis of eurkaryotic ribosomal proteins *in vitro*. Cell **11:**201–212.

30. **Wigler, M., A. Pellicer, S. Silverstein, and R. Axel.** 1978. Biochemical transfer of single-copy eurkaryotic genes using total celluar DNA as a donor. Cell **14:**725–731.