MOLECULAR AND CELLULAR BIOLOGY, Aug. 1986, p. 2903–2909
0270-7306/86/082903-07$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 6, No. 8

Genetic Analysis of the Human Thymidine Kinase Gene Promoter

JORDAN A. KREIDBERG AND THOMAS J. KELLY*

Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Received 25 February 1986/Accepted 1 May 1986

The promoter of the human thymidine kinase gene was defined by DNA sequence and genetic analyses. Mutant plasmids with deletions extending into the promoter region from both the 5′ and 3′ directions were constructed. The mutants were tested in a gene transfer system for the ability to transform TK^- cells to the TK^+ phenotype. This analysis delimited the functional promoter to within an 83-base-pair region upstream of the mRNA cap site. This region contains sequences common to other eucaryotic promoters including G · C-rich hexanucleotides, a CAAT box, and an A · T-rich region. The CAAT box is in an inverted orientation and is part of a 9-base-pair sequence repeated twice in the promoter region. Comparison of the genomic sequence with the cDNA sequence defined the first exon of the thymidine kinase gene.

Thymidine kinase (TK) is a member of a group of enzymes whose expression is related to the growth state of cells (1). The activity of TK as well as that of other members of the group is highest in growing cells, because of elevated levels in cells that are in the S phase of the cell cycle (2, 21). Little is known about the mechanism by which the level of TK activity is regulated. The increase in activity during S phase has been shown to be sensitive to inhibitors of protein and RNA synthesis, suggesting that de novo synthesis of TK or a necessary cofactor is required for its regulation (17, 21). In several cell culture systems where quiescent cells can be stimulated to proliferate by the addition of serum, the level of TK mRNA has been shown to increase upon stimulation (33, 36). Schlosser et al. first showed that the growth-dependent regulation of TK was maintained when cellular DNA isolated from rat TK^+ cells was introduced into TK^- cells (32). This observation was subsequently confirmed after the isolation of the human TK gene by recombinant DNA techniques (4, 19, 20, 33), indicating that a genetic element(s) required for growth-dependent regulation is closely linked to the TK structural gene. It has also been observed that TK mRNA levels and activity decrease when TK^- myoblasts transformed with the chicken TK gene differentiate into myotubes and cease to divide (27). In the latter system, interchanging promoters between the herpes and chicken TK genes has led to the conclusion that the increase in activity is dependent on information contained in the mRNA-coding portion of the gene (27).

As an approach to understanding the growth-related regulation of TK, we undertook a genetic analysis of the human TK gene. Mutants with deletions in the 5′ region of the human TK gene were constructed and introduced into TK^- cells to define the elements involved in the expression of the gene. Our initial analysis defined a region upstream of the gene that is required for efficient expression. This region contains sequences found to be involved in the expression of several other genes, some of which are also expressed in a growth-dependent fashion.

MATERIALS AND METHODS

Plasmid and phage preparation. TK genomic clone λTK46 and cDNA clone pTK11 were the kind gift of H. Bradshaw. All plasmids were introduced into *Escherichia coli* DH1 by the $CaCl_2$ procedure (22). Plasmid DNAs were isolated by the alkaline lysis procedure (22) and purified on two sequential CsCl gradients in vertical rotors. Phage clones were prepared by published procedures (22).

Restriction digests. Restriction enzymes were purchased from Bethesda Research Laboratories, Inc., Gaithersburg, Md.; Boehringer Mannheim Biochemicals, Indianapolis, Ind.; and New England BioLabs, Inc., Beverly, Mass. Phage and plasmid DNAs were digested with restriction enzymes under conditions suggested by the supplier. Digests for mapping the λTK46 clone were subjected to electrophoresis on 0.4 and 1.0% agarose gels to obtain accurate sizes for large and small fragments.

DNA sequencing. The nucleotide sequence of the 5′ end of the TK gene was determined by the dideoxynucleotide chain termination method (31). A set of overlapping fragments was prepared for sequencing by subcloning the 1.5-kilobase (kb) *Sac*I fragment containing the 5′ end of the TK gene into the *Sac*I site of pUC13 (28), so that the *Eco*RI site of pUC13 was on the upstream side of the insert. The *Eco*RI fragment of this plasmid, containing sequences that hybridized to an oligonucleotide complementary to the 5′ end of the cDNA (5), was digested with BAL 31 (Bethesda Research Laboratories) for various amounts of time between 15 s and 12 min, under conditions suggested by the supplier. The exonuclease-treated DNA molecules were then repaired with Klenow fragment and digested with *Bam*HI, yielding fragments with a blunt-end deletion endpoint between the *Eco*RI site internal to the *Sac*I fragment and the *Bam*HI site in pUC13. The resulting fragments were then inserted between the *Bam*HI and *Sma*I sites of M13mp11, and clones with overlapping deletions were sequenced with a universal 17-base-pair (bp) primer (Collaborative Research, Inc., Waltham, Mass.). To determine the sequence of the noncoding strand, mutant plasmids were digested with *Sal*I, which cleaves the plasmids in the vector sequences 300 bp from the deletion endpoints in the 5′ direction. The linearized plasmids were digested with *Exo*III (New England BioLabs) for 20 min to generate single-stranded termini. The digested DNA was then annealed with a 16-base primer complementary to the noncoding strand at the 3′ end of the first exon (primer: 5′TGCCCCCGGGTCTTGC3′).

S1 nuclease mapping. An oligonucleotide complementary to the coding strand at the 5′ end of the cDNA was

* Corresponding author.

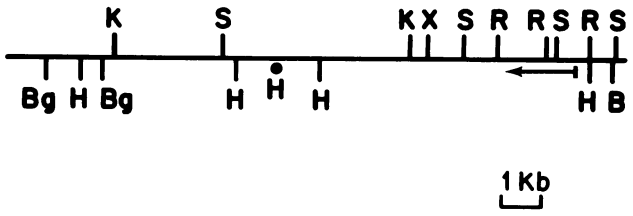

FIG. 1. Restriction map of the human TK gene. The map shows the location of restriction sites within the 16-kb insert of λTK46. Recognition sites of restriction endonucleases are abbreviated as follows: B, *Bam*HI; Bg, *Bgl*II; R, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; S, *Sac*I; X, *Xho*I. The precise location of the *Hin*dIII site noted by the dot is not known. There are two possibilities for the positioning of the 0.8- and 1.6-kb *Hin*dIII fragments. An arrow marks the start site and direction of transcription.

end-labeled with polynucleotide kinase, hybridized to the 1.5-kb TK *Sac*I fragment cloned in M13Mp11, and extended by Klenow fragment. The product of this primer extension reaction was cleaved with *Pst*I and denatured. Radioactive DNA containing primarily a 490-base single-stranded fragment was purified with a 6% acrylamide–8 M urea gel. This probe was hybridized to total HeLa cell cytoplasmic RNA in a buffer containing 80% formamide, 0.04 M PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], 0.4 M NaCl, and 0.001 M EDTA, in a volume of 40 μl. The hybridization was carried out at 47°C for 12 h after an initial incubation at 90°C for 5 min. The hybridization product was digested with S1 nuclease (P-L Biochemicals, Inc., Milwaukee, Wis.) for 1 h at 37°C in a mixture of 0.3 M NaCl, 0.06 M sodium acetate (pH 4.5), 3.75 mM $ZnSO_4$, and 50 μg of sheared denatured herring sperm DNA per ml, in a 300-μl reaction. The reaction mixture was then extracted with phenol and chloroform and precipitated with ethanol. The final product was subjected to electrophoresis on a 6% acrylamide–8 M urea gel.

Cell culture and gene transfer. Ltk$^-$ cells were obtained from G. Scangos and maintained on Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Hyclone Laboratories, Inc., Logan, Utah), penicillin (50 U/ml), streptomycin (50 μg/ml), and glutamine (2 mM). The gene transfer procedure was modified from several procedures (9, 13, 15). Cells (5×10^5) were plated in 6-cm dishes (Falcon 3002, Becton Dickinson and Co., Paramus, N.J.) 24 h before transfection. A 250 mM $CaCl_2$ solution containing 0.1 to 1 μg of plasmid DNA (linearized at the *Nru*I site in pBR322, 300 bp from the deletion endpoint) and 20 μg of sheared herring sperm DNA was added dropwise to a solution containing 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)–NaOH(pH 7.12), 250 mM NaCl, 1.5 mM Na_2HPO_4, and 11 mM dextrose (HEPES-buffered saline solution). After 10 min, 0.4 ml of this mixture was added to plates of cells containing 4 ml of medium. At 16 h after addition of precipitate, the dishes were washed twice with Dulbecco modified Eagle medium–10% fetal calf serum. A 10% glycerol–HEPES-buffered saline solution was then placed on the cells for 1 min, followed by a final wash with HEPES-buffered saline solution, and addition of fresh medium. After 4 to 6 h of incubation at 37°C, the cells were washed with phosphate-buffered saline, trypsinized, and transferred to 10-cm dishes. Hypoxanthine-aminopterin-thymidine medium (34) selection was started 48 h after transfection. Colonies appeared approximately 2 weeks later. The transfection frequency of wild-type plasmids was 10^3 colonies per μg.

Construction of plasmids. The TK gene was subcloned from the λTK46 clone by isolating the *Eco*RI-to-*Xho*I fragment and the *Xho*I-to-*Bam*HI fragment, containing the left and right halves of the phage genomic clone, respectively, and inserting them between the *Bam*HI and *Eco*RI sites of pBR322 in a three-fragment ligation reaction. This construct (pTK8) contains the entire λTK46 clone except for approximately 200 bp between the *Bam*HI site and the end of the genomic fragment inserted in λTK46.

Deletion mutants (5′) were constructed (see Fig. 5) by linearizing pTK8 at the unique *Bam*HI site and digesting with BAL 31 (Bethesda Research Laboratories) at a DNA concentration of 75 μg/ml and an enzyme concentration of 10 U/ml, for times from 5 to 15 min. The digested fragments were then repaired by Klenow fragment, ligated to phosphorylated *Bam*HI linkers (P-L Biochemicals), and digested with *Xho*I and *Bam*HI. Fragments containing different endpoints were gel purified and ligated to the *Xho*I-to-*Bam*HI fragment of pTK8 which contains the 3′ end of the TK gene and the vector. The deletions were mapped by digesting plasmids with *Sac*I and *Bam*HI and confirmed by sequencing.

Mutants with deletions extending into the promoter region from the 3′ side were constructed by using two 5′ deletion mutants (see Fig. 6). The −83 5′ deletion plasmid was linearized at the *Sma*I (see Fig. 2) site in the first exon and digested with BAL 31. The resulting ends were repaired with Klenow fragment and ligated to phosphorylated *Bgl*II linkers (New England BioLabs). The DNA was digested with *Bgl*II and with *Sal*I, which cuts at a unique site in the vector upstream of the promoter. The fragments extending from the *Sal*I site to the *Bgl*II linkers at the termini generated by BAL 31 were purified by gel electrophoresis. These fragments were ligated to the *Sal*I-to-*Bam*HI fragment of the +9 5′ deletion mutant (see Fig. 6) containing the TK structural gene and vector sequences. Mutants with deletions extending into the promoter region were selected for further study.

A plasmid containing the human TK cDNA under the control of the human TK promoter (pTK21; see Fig. 7) was constructed by making use of the unique *Xma*I site at the 3′ end of the first exon. The starting point for this construction was pTK11 (5), a full-length TK cDNA clone obtained by screening the Okayama-Berg expression library (29). It

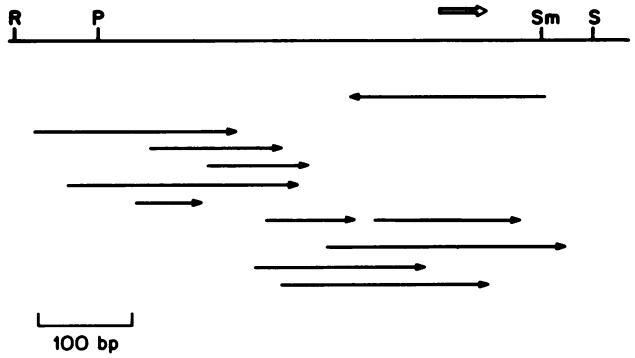

FIG. 2. Strategy for sequencing the 5′ region of the human TK gene. A restriction map of the *Eco*RI-to-*Sac*I restriction fragment is shown at the top of the figure. The start site of the mRNA is indicated by the open arrow. The restriction sites are abbreviated as follows: R, *Eco*RI; P, *Pst*I; Sm, *Sma*I; and S, *Sac*I. The sequences of the indicated regions were determined as described in the text.

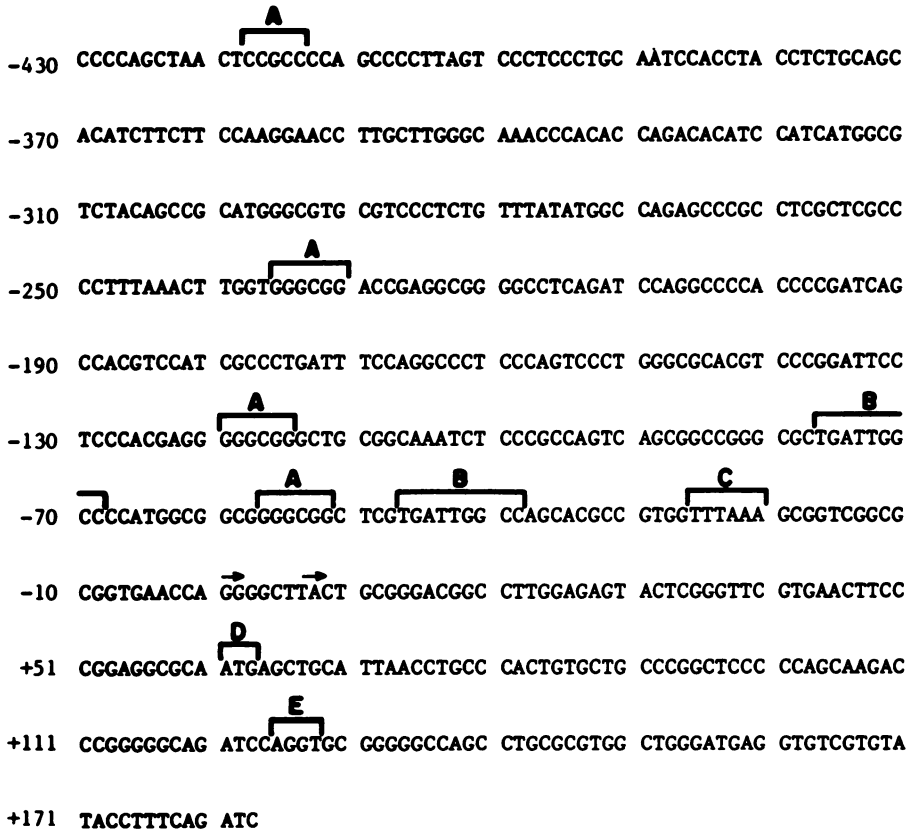

FIG. 3. Sequence of the 5′ region of the human TK gene. The nucleotides are numbered relative to the first mRNA start site. Sequences of interest are bracketed above and described in the text: (A) G · C hexanucleotides; (B) 9-bp repeats; (C) A · T-rich region; (D) translation start site; (E) splice site. Arrows mark the two mRNA cap sites.

contains the cDNA positioned between the simian virus 40 (SV40) early promoter and an SV40 poly(A) addition site. A sample of pTK11 DNA was linearized with *Sal*I, and the recessed 3′ ends were repaired with Klenow fragment. After the addition of *Sac*I phosphorylated linkers, the DNA was digested with *Sac*I and *Xma*I. The larger fragment that contains the TK cDNA sequences 3′ to the *Xma*I site, the SV40 poly(A) addition site, and pBR322 vector sequences was isolated by gel electrophoresis. The purified fragment was ligated to a *Sac*I-to-*Xma*I fragment derived from the genomic clone containing the TK genomic sequence upstream of the start site and mRNA coding sequences 5′ to the *Xma*I site.

RESULTS

Sequence organization. The human TK gene clone, λTK46, used in these studies was isolated and characterized by Bradshaw (4). As a first step in elucidating its structure, we developed a restriction map of this clone (Fig. 1). Several differences were found in comparison with the previously published maps of the TK gene (4, 19, 20, 33). In particular, the 1.5-kb *Sac*I fragment previously assigned to the left end of the clone is at the right end. Our map is largely in agreement with maps of clones obtained by Lin et al. and Stuart et al. (20, 33).

The only areas of divergence lie at the ends of the clones. Since in all cases the gene was cloned from a library derived from mouse Ltk$^-$ cells transformed with human DNA, the differences near the ends of the clones presumably correspond to different joints between human and mouse DNA. A comparison of our map with that of Stuart et al. (33) and the cDNA sequence of Bradshaw and Deininger (5) suggests that the λTK46 clone does not contain the extreme 3′ end of the gene, as it is missing the *Bam*HI site found both in the cDNA and in the left end of the map of Stuart et al. Our map and that of Stuart et al. also diverge at the right ends in a manner that suggests the human/mouse breakpoint may be near the right end of the 1.5-kb *Sac*I fragment.

5′ sequence. To study the human TK promoter, it was first necessary to localize the 5′ end of the gene. This was done by hybridizing an oligonucleotide containing 15 bp found near the 5′ end of the cDNA (5) to fragments produced by various restriction digests of the cloned gene. The 5′ end of the gene was localized to a *Sac*I-*Eco*RI fragment near the right end of the genomic insert (Fig. 1, data not shown). The nucleotide sequences of several overlapping subclones within this *Sac*I-*Eco*RI fragment were determined (Fig. 2). The sequence obtained is shown in Fig. 3. This sequence contains the entire first exon of the TK gene and the splice junction beween the first exon and first intron, as determined by a comparison with the published sequence of the putative full-length cDNA (5). It also contains 430 bp of sequence 5′ to the beginning of the cDNA and 54 bp of the first intron.

Several interesting features of the sequence 5′ to the start of the homology with the cDNA are apparent. There is a 6-bp A · T-rich sequence, TTTAAA, about 25 bp before the start of the cDNA homology. Further upstream, a 9-bp sequence, TGATTGGCC, is repeated twice. This sequence

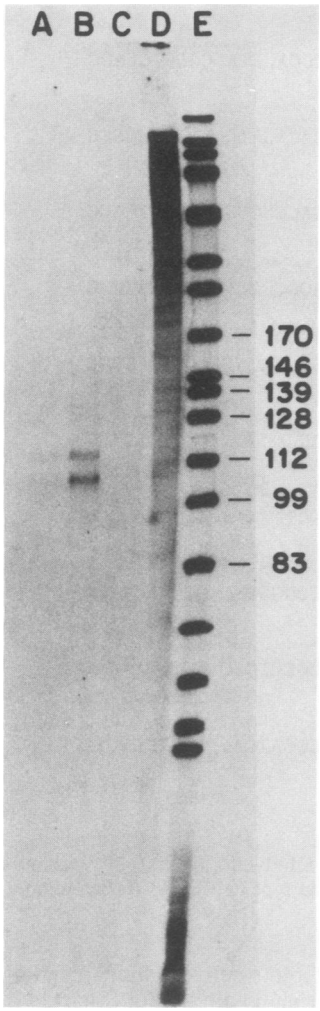

FIG. 4. S1 nuclease mapping of the 5′ end of the human TK transcript. The probe used was a single-stranded primer extension product extending from the first exon to the *Pst*I site, 377 bp upstream of the cDNA start site. Lanes show the products of S1 nuclease digestion of hybridization reactions with (lane A) 15 μg, (lane B) 45 μg, and (lane C) 0 μg of total HeLa cell cytoplasmic RNA. Lane D contains the probe, and lane E contains an end-labeled *Dde*I digest of simian virus 40 DNA that was denatured before loading.

contains the canonical CAAT box in inverted orientation. Between these two repeats and repeated three times further upstream, the hexanucleotide sequence GGGCGG is found; this sequence is found at sites that bind the transcription factor SP1 (14).

S1 analysis. To map the 5′ end of the TK mRNA coding region, human RNA was analyzed by using the S1 nuclease procedure (3) with a probe complementary to the coding strand from near the end of the first exon to the *Pst*I site 377 bp upstream of the start of homology with the cDNA. Cytoplasmic RNA from HeLa cells protected two fragments differing in length by 7 bp (Fig. 4). This indicates two start sites for the mRNA as shown in Fig. 3 and demonstrates that the cDNA clone is indeed full length, corresponding to the longer of the two possible mRNAs as defined by 5′ endpoints.

Genetic analysis. To define elements required for expression of the human TK gene, we undertook a genetic analysis with mutants that contained deletions in the 5′ region. These mutants were tested for their ability to confer the TK^+ phenotype when introduced into Ltk^- cells. TK^+ transformants were selected by growth in hypoxanthine-aminopterin-thymidine medium (34), which is normally lethal for Ltk^- cells. The wild-type human TK gene, defined in this study as the λTK46 clone or the plasmid subclone pTK8, transforms Ltk^- cells at a high frequency (10^3 colonies per μg). Mutations in the region upstream of the mRNA start site which impair the function of the promoter confer a decreased transformation frequency.

Successive deletion mutations extending from the *Bam*HI site in pTK8 towards the cap site were constructed (Fig. 5). The results of transfection experiments with these mutants are shown in Fig. 6. Mutants that retained at least 83 bp 5′ to the first cap site transformed cells with approximately the same frequency as the wild-type construct. The 83-bp region contains the two 9-bp repeats, the G · C hexanucleotide, and the A · T hexanucleotide. A mutant that retained 53 bp 5′ to the cap site appeared to transform at a frequency intermediate between those of the wild type and mutants retaining fewer than 53 bp. There was no difference observed among the mutants with deletions that extended further than −53, all of which transformed Ltk^- cells at a frequency 250-fold lower than the wild-type gene. Mutants with deletions extending into the translated portion of the first exon were unable to transform Ltk^- cells (data not shown).

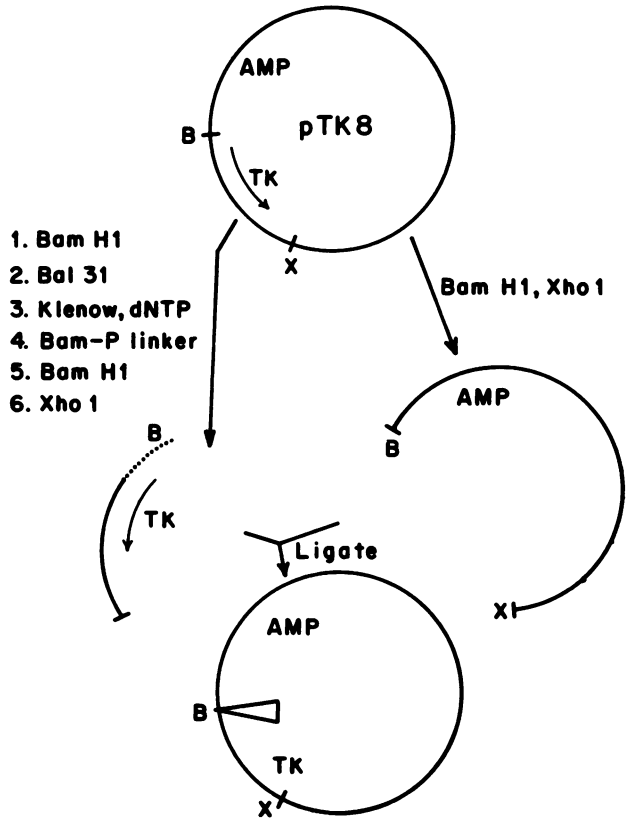

FIG. 5. Construction of 5′ deletion mutants. Deletion mutants were constructed as shown and are described in greater detail in the text. Restriction sites are abbreviated as in the legend to Fig. 1. The *Bam*HI site in pTK8 is the junction between vector and TK 5′ sequences. The 5′-to-3′ orientation of the TK gene is indicated by the arrow.

FIG. 6. Analysis of deletion mutants. The structure of the mutants studied and their transformation frequencies are shown. Symbols: ▬, TK sequence present in each mutant; ▩, locations of *Bam*HI (CGGATCCG) linker sequences; ▭, the location of *Bgl*II/*Bam*HI hybrid linkers (CAGATCCG). The extent of deletion is noted at the left of each mutant. The transformation frequency as a fraction of the wild-type level is shown at the right of each mutant (WT = 10^3 colonies per μg). The sequence of the 5′ region is shown at the top. Particular sequences of interest are underlined.

Since deletions extending beyond nucleotide −83 reduced transformation frequency to a barely detectable level, it is difficult to determine the significance of additional decreases in transformation frequency caused by further deletion. To establish whether any sequences closer to the mRNA cap site are required for expression, we also examined a series of deletions extending from the cap site towards the upstream limit of the promoter defined by the 5′ deletion analysis. These deletions extended from an 8-bp synthetic linker adjacent to nucleotide +9 toward the 5′ boundary defined by the −83 5′ deletion mutant, so that various amounts of DNA between this linker and the linker at −83 were deleted (see above for details). Deletion of nucleotides from −10 to +9 caused a slight decrease in transformation frequency, and a deletion extending to −39 which included the A · T hexanucleotide reduced transformation 14-fold. This suggests that the A · T hexanucleotide, or sequences near it, plays an important role in expression and that the 9-bp repeats and the G · C hexanucleotide alone are not sufficient for maximally efficient gene expression.

These deletion analyses define the limits of the human TK promoter required for efficient expression in the transformation assay. The 5′ limit is between −83 and −53, and the 3′ limit is between −10 and −39.

Analysis of pTK21. Since the minimum size of the TK gene is over 15 kb (33) and the TK mRNA is only 1.5 kb (4), there is the potential that a great deal of information exists in the introns that is related to the expression of the TK gene. One way of evaluating the importance of introns is to eliminate them and then determine whether the gene retains full function. We constructed a plasmid, pTK21, containing the human TK cDNA (5) under control of the human TK promoter (see above; Fig. 7). This construct also contained an SV40 poly(A) addition site downstream from the cDNA. This plasmid contains no introns and therefore allowed us to test whether sequences present in the introns of the TK gene were required for expression of TK. Both pTK8 and pTK21 transformed Ltk$^-$ cells with an efficiency of approximately 10^3 colonies per μg. Since there is no significant difference in transformation efficiency between these two plasmids, we conclude that sequences in the introns are not required for expression of TK.

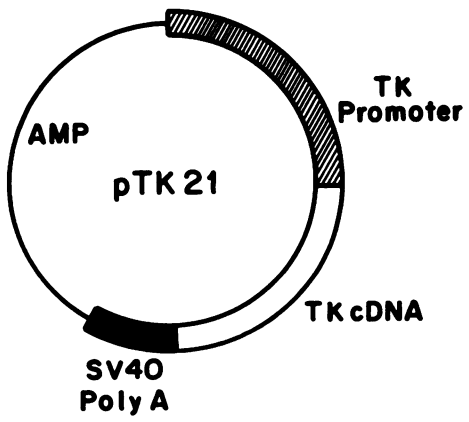

FIG. 7. Map of plasmid pTK21, containing the human TK cDNA and human TK upstream region. See the text for details of construction.

DISCUSSION

We have defined the promoter of the human TK gene through a genetic analysis which demonstrated a region upstream of the mRNA cap site that is required for efficient expression of the gene. The gene transfer system used in the present study tests the ability of a transfected TK gene to confer a TK$^+$ phenotype on Ltk$^-$ cells, as defined by their acquiring the ability to grow in hypoxanthine-aminopterin-thymidine medium (34). While the transfection frequency of a particular mutant may not be a direct linear measurement of the TK RNA level after transfection of that mutant, the gene transfer system provides a qualitative test as to whether a gene can be expressed efficiently. Our data demonstrate that only 83 bp 5′ to the cap site are required for the efficient expression of the gene.

The promoter region defined by the genetic analysis revealed several similarities with other eucaryotic genes transcribed by RNA polymerase II (6). There is an A · T-rich hexanucleotide sequence 25 bp upstream of the cap site. This region does not contain the canonical TATA box sequence (6); however, many variations of the canonical

sequence are found among the promoter regions sequenced thus far. The A · T-rich region is not absolutely required for expression, but deletions which remove the A · T box reduce promoter function to an intermediate level. In the absence of additional promoter sequences, the A · T-rich region is not capable of directing expression of the gene.

The 9-bp sequence TGATTGGCC is repeated twice, starting 76 and 46 nucleotides upstream of the cap site. Within this sequence is the canonical CAAT box in inverted orientation. In this orientation, the TK repeat shares an 8- or 9-bp homology with the globin CAAT sequence; detailed mutagenesis of globin gene promoters has revealed this sequence to be important in globin gene expression (7, 11). The herpes simplex virus TK also contains a CAAT box in inverted orientation (23). Linker-scanning mutants that lack this sequence exhibit decreased transcriptional efficiency from this promoter (24). A protein binding this sequence in the herpes simplex virus TK promoter has been partially purified (18). The specific function of this protein in transcription is unknown. The upstream regions of several other genes contain the CAAT homology in this inverted orientation. These include the human HSP70 gene (16), the hamster HMG-coenzyme A reductase (30), and the chicken TK gene (26). The chicken TK gene contains the GATTGGCC sequence repeated twice, 42 bp apart, although it is not known whether these are upstream of the cap site, as it has not yet been mapped (26). The repeats are separated by three and four full turns of the DNA double helix in the human and chicken TK genes, respectively. In the case of the simian virus 40 promoter, recent work has demonstrated that insertions resulting in nonintegral numbers of helical turns between certain promoter elements reduce promoter efficiency (35); thus, the specific spacing of the repeats in the human and chicken TK promoters may be important for efficient promoter function.

The sequence GGGCGG is found between the two 9-bp repeats in the human TK promoter. Studies on the simian virus 40 early promoter and the herpes simplex virus TK promoter have shown that this sequence is involved in the binding of the transcription factor SP1 (14). SP1 is required for in vitro transcription from the simian virus 40 early promoter (12). The GGGCGG sequence is also found upstream of several other genes, including the chicken TK gene (26), the mouse HGPRT gene (25), the mouse and human DHFR genes (8, 10), and the hamster HMG-coenzyme A reductase gene (30). In both the herpes simplex virus TK gene and the human TK gene, the GGGCGG sequence and the CAAT homology are close to each other, although the exact spacing is different. The two promoters also differ in that the herpes promoter has two GGGCGG sequences and one CAAT sequence, but the human promoter has two CAAT sequences and one GGGCGG sequence. Proteins binding these two sites may participate in a specific interaction required for the initiation of transcription.

Stuart et al. report that TK mRNA levels are elevated during conditions that stimulate growth (33). It is not known whether this regulation is at the level of mRNA synthesis and whether it is mediated through sequences upstream of the mRNA cap site. Since the upstream sequence of the human TK gene contains sequence motifs found upstream of many genes, not all of which are known to be regulated by growth conditions, it is likely that proteins binding these sequences are responsible for constitutive maintenance of transcription. There may, however, be unrecognized additional control elements in the human TK promoter conferring nonconstitutive regulatory patterns.

ACKNOWLEDGMENTS

We thank J. Li, D. Rawlins, R. Wides, M. Bolanowski, P. Rosenfeld, and E. O'Neill for their advice on this work. J. Sherley provided valuable advice and help throughout this project and reviewed the manuscript. We thank R. Reed for advice in sequencing the TK 5′ region and K. W. C. Peden for advice with transfection procedures. We thank G. Ketner and D. Weinberg for a critical reading of this paper. We are grateful to B. Athey for assistance with the preparation of the manuscript. D. Weinberg and C. Riley assisted in preparation of figures.

This work was supported by Pubic Health Service grant CA16519 from the National Institutes of Health. J.A.K. is a fellow of the Medical Scientist Training Program (GM07309).

LITERATURE CITED

1. **Baserga, R.** 1985. The biology of cell reproduction. Harvard University Press, Cambridge, Mass.
2. **Bello, L. J.** 1974. Regulation of thymidine kinase synthesis in human cells. Exp. Cell Res. **89**:263–274.
3. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease digested hybrids. Cell **12**:721–732.
4. **Bradshaw, H. D.** 1983. Molecular cloning and cell cycle-specific regulation of a functional human thymidine kinase gene. Proc. Natl. Acad. Sci. USA **80**:5588–5591.
5. **Bradshaw, H. D., Jr., and P. L. Deininger.** 1984. Human thymidine kinase gene: molecular cloning and nucleotide sequence of a cDNA expressible in mammalian cells. Mol. Cell. Biol. **4**:2316–2320.
6. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eukaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.
7. **Charnay, P., P. Mellon, and T. Maniatis.** 1985. Linker scanning mutagenesis of the 5′ flanking region of the mouse β-major-globin gene: sequence requirements for transcription in erythroid and nonerythroid cells. Mol. Cell. Biol. **5**:1498–1511.
8. **Chen, M.-J., T. Shimada, A. L. Moulton, A. Cline, R. K. Humphries, J. Maizel, and A. W. Nienhuis.** 1984. The functional human dihydrofolate reductase gene. J. Biol. Chem. **259**:3933–3943.
9. **Corsaro, C. M., and M. L. Pearson.** 1981. Enhancing the efficiency of DNA-mediated gene transfer in mammalian cells. Somatic Cell Genet. **7**:603–616.
10. **Crouse, G. F., C. C. Simonsen, R. N. McEwan, and R. T. Schimke.** 1982. Structure of amplified normal and variant dihydrofolate reductase genes in mouse sarcoma S180 cells. J. Biol. Chem. **257**:7887–7897.
11. **Dierks, P., A. van Ooyen, M. D. Cochran, C. Dobkin, J. Reiser, and C. Weissmann.** 1983. Three regions upstream from the cap site are required for efficient and accurate transcription of the rabbit β-globin gene in mouse 3T6 cells. Cell **32**:695–706.
12. **Dynan, W. S., and R. Tjian.** 1983. The promoter-specific transcription factor SP1 binds to upstream sequences in the SV40 early promoter. Cell **35**:79–87.
13. **Frost, E., and J. Williams.** 1978. Mapping temperature-sensitive and host-range mutations of adenovirus type 5 by marker rescue. Virology **91**:39–50.
14. **Gidoni, D., W. S. Dynan, and R. Tjian.** 1984. Multiple specific contacts between a mammalian transcription factor and its cognate promoters. Nature (London) **312**:409–413.
15. **Graham, F. L., and A. J. Van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
16. **Hunt, C., and R. J. Morimoto.** 1985. Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70. Proc. Natl. Acad. Sci. USA **82**:6455–6459.
17. **Johnson, L. F., L. G. Rao, and A. J. Muench.** 1982. Regulation of thymidine kinase enzyme level in serum-stimulated mouse 3T6 fibroblasts. Exp. Cell Res. **138**:79–85.
18. **Jones, K. A., K. R. Yamamoto, and R. Tjian.** 1985. Two distinct

transcription factors bind to the HSV thymidine kinase promoters *in vitro*. Cell **42**:559–572.
19. **Lau, Y.-F., and Y. W. Kan.** 1984. Direct isolation of the functional human thymidine kinase gene with a cosmid shuttle vector. Proc. Natl. Acad. Sci. USA **81**:414–418.
20. **Lin, P.-F., S.-Y. Zhao, and F. H. Ruddle.** 1983. Genomic cloning and preliminary characterization of the human thymidine kinase gene. Proc. Natl. Acad. Sci. USA **80**:6528–6532.
21. **Littlefield, J. W.** 1966. The periodic synthesis of thymidine kinase in mouse fibroblasts. Biochim. Biophys. Acta **114**: 398–403.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **McKnight, S. L., E. R. Gavis, R. Kingsbury, and R. Axel.** 1981. Analysis of transcriptional regulatory signals of the HSV thymidine kinase gene: identification of an upstream control region. Cell **25**:385–398.
24. **McKnight, S. L., and R. Kingsbury.** 1982. Transcriptional control signals of a eukaryotic protein-coding gene. Science **217**:316–324.
25. **Melton, D. W., D. S. Konecki, J. Brennand, and C. T. Caskey.** 1984. Structure, expression, and mutation of the hypoxanthine phosphoribosyl transferase gene. Proc. Natl. Acad. Sci. USA **81**:2147–2151.
26. **Merrill, G. F., R. M. Harland, M. Groudine, and S. L. McKnight.** 1984. Genetic and physical analysis of the chicken *tk* gene. Mol. Cell. Biol. **4**:1769–1776.
27. **Merrill, G. F., S. D. Hauschka, and S. L. McKnight.** 1984. tk Enzyme expression in differentiating muscle cells is regulated through an internal segment of the cellular *tk* gene. Mol. Cell. Biol. **4**:1777–1784.
28. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20.
29. **Okayama, H., and P. Berg.** 1983. A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. Mol. Cell. Biol. **3**:280–289.
30. **Reynolds, G. A., S. K. Basu, T. F. Osborne, D. J. Chin, G. Gil, M. S. Brown, J. L. Goldstein, and K. I. Luskey.** 1984. HMG Co A reductase: negatively regulated gene with unusual promoter and 5′ untranslated regions. Cell **38**:275–285.
31. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
32. **Schlosser, C. A., C. Steglich, J. R. DeWet, and I. E. Scheffler.** 1981. Cell cycle-dependent regulation of thymidine kinase activity introduced into mouse $LMTK^-$ cells by DNA and chromatin-mediated gene transfer. Proc. Natl. Acad. Sci. USA **78**:1119–1123.
33. **Stuart, P., M. Ito, C. Stewart, and S. E. Conrad.** 1985. Induction of cellular thymidine kinase occurs at the mRNA level. Mol. Cell. Biol. **5**:1490–1497.
34. **Szybalska, E. H., and W. Szybalski.** 1962. Genetics of human cell lines. IV. DNA-mediated heritable transformation of a biochemical trait. Proc. Natl. Acad. Sci. USA **48**:2026–2034.
35. **Takahashi, K., M. Vigneron, H. Matthes, A. Wildeman, M. Zenke, and P. Chambon.** 1986. Requirement of stereospecific alignments for initiation from the simian virus 40 early promoter. Nature (London) **319**:121–126.
36. **Thompson, C. B., P. B. Challoner, P. E. Neiman, and M. Groudine.** 1985. Levels of c-myc oncogene mRNA are invariant throughout the cell cycle. Nature (London) **314**:363–366.