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The genetic structure of mouse ornithine transcarbamylase

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ABSTRACT

The gene encoding the mouse urea cycle enzyme, ornithine transcarbamylase has been isolated on five partially overlapping bacteriophage lambda clones. We have characterized the gene and found that it is split between ten exons distributed over approximately 70 kb of the X chromosome. The introns range in size from 88 bases to the relatively unusual size of approximately 26 kilobases, while the splice donor/splice acceptor sequences conform to the consensus established for other eukaryotic genes.

INTRODUCTION

Ornithine transcarbamylase (OTC) (EC 2.1.3.3) catalyses the condensation of carbamyl phosphate and ornithine to form citrulline as the second step of the mammalian urea cycle (1,2). The 41 kD precursor is transported to the mitochondrial matrix where a 32 amino acid leader peptide is cleaved and trimers associate to form the active enzyme (3,4). The gene is located on the X chromosome (5) and is expressed primarily in liver and to a lesser extent in small bowel (6,7). The tissue specific regulatory sequences have been localized to a 750 bp 5' sequence (8). The OTC deficiency state is the most common urea cycle disorder in humans and is associated with a severe hyperammonemia evident within the first few days after birth (9). There are at least two OTC-deficient mouse mutants, *sparse fur* (*spf*) (10) and *sparse fur*/abnormal skin and hair (spf^{ash}) (11). We have recently identified the molecular defect responsible for the spf phenotype (12). The availability of a mouse mutant for a human hepatic deficiency provides a system to develop tissue-specific gene transfer therapy. We have undertaken the study of the mouse OTC gene as a step toward that objective. In this report, we describe the isolation, cloning and characterization of the mouse OTC gene.

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MATERIALS AND METHODS

Enzymes and Radioisotopes

Restriction endonucleases were obtained from New England Biolabs., Bethesda Research Laboratories, Boehringer Mannheim Biochemicals, or Pharmacia and were used as specified by the supplier. T4 polynucleotide kinase, *E. coli* ligase and DH-5α competent cells were obtained from Bethesda Research Laboratories; DNA polymerase I, holoenzyme and Klenow fragment, T4 ligase and Calf Intestinal Phosphatase were supplied by Boehringer Mannheim Biochemicals, and dideoxynucleotides, deoxynucleotides, ATP, oligolabelling kits, and sequencing primer were supplied by Pharmacia. Radioisotopes including α^{32}PdCTP, γ^{32}P ATP, α^{32}PdATP, and α^{35}SdATP were obtained from either Amersham Corp. or ICN Biomedicals, Inc.

Screening of the Genomic Library

The mouse genomic DNA library (kindly provided by J. Seidman, Harvard Medical School) was constructed as a partial Hae III digestion of C57BL/6J mouse DNA cloned into bacteriophage Charon 4A with Eco RI linkers and plated on Y1088 host. Plaque hybridization was performed as described by Benton and Davis (13) on nitrocellulose filters using various mouse OTC cDNA probes (12). Probes were prepared by separating the cDNA sequence of interest from vector sequences using appropriate restriction endonuclease digestion followed by agarose gel electrophoresis and electroelution (14). Probes were labelled using either nick translation (14) or random oligonucleotide labelling (15) with α^{32}PdCTP to a specific activity of 0.5 - 1.0 x 10^9 cpm/μg. Filters were prehybridized in 5X SSC (1X SSC is 0.15 M NaCl, 0.015M Na-Citrate, pH 7.0), 5X Denhardt's solution (1X Denhardt's is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 0.1% sodium dodecyl sulfate (SDS) and 100 μg/ml sheared herring sperm DNA for a minimum of 6 hours at 68° C. after prehybridization 5 x 10^5 cpm of probe was added per filter and allowed to hybridize overnight. The filters were washed in 2.0-0.1X SSC/.1% SDS at 50-60° C and exposed with a Dupont Cronex intensifying screen to Kodak XAR film for 12-36 hours at -70° C. Positives plaques were subjected to at least two more rounds of plaque purification before DNA was isolated by both plate lysis and liquid culture techniques (14).

Gel Transfer and Hybridization

DNA fragments in agarose gels were depurinated by acid or nicked with UV light, denatured in 0.5N NaOH/1.5M NaCl and neutralized in 1M Tris pH 7.5/1.5M NaCl before transferring to nitrocellulose, Zeta-bind, Zeta-probe, or Gene screen Plus charged nylon membranes by the method of Southern (16) or

bidirectionally by the method of Smith and Summers (17). Transfer buffers were ammonium acetate, 20X SSC, or .4N NaOH and the filters were baked *in vacuo* at 68 or 80° C for 2 hours. Filters were prehybridized in 45% Formamide, 5X SSC, 50 mM Tris pH 7.5, 0.1% sodium pyrophosphate, 10X Denhardt's, 1% SDS, and 100 μg/ml herring sperm DNA for at least 4 hours at 42° C. Hybridization buffers were identical except 1X Denhardt's was substituted and 10% Dextran Sulfate was added along with 2 x 10^6 cpm/ml of probe. After hybridization, the filters were washed in 2 - 0.1X SSC/.1% SDS at 25 - 65° C and exposed to X-ray film.

Oligonucleotide Hybridization

Approximately 150 ng of synthetic oligonucleotide (kindly provided by J. Habener, Massachusetts General Hospital) was end-labelled (14) with T4 polynucleotide kinase and γ^{32}P ATP to a specific activity of 5 x 10^8 cpm/μg. Formamide concentrations were lowered to 15-36% by volume and washing temperatures were reduced to 35-50° C depending on stringency requirements.

Phage Clone Mapping

Restriction mapping of the phage clones was carried out using the rapid cos-site oligo technique essentially as described by Rackwitz, *et al.* (18). Bacteriophage lambda digested with Hind III/Xho I and end-labelled with α^{32}P dATP and Klenow fragment served as DNA molecular weight standards. Discrepancies were resolved by double restriction endonuclease digestion. Clone overlap was confirmed by probing phage clone digestions with genomic probes by the modified technique of Southern (19).

Subcloning of Genomic Fragments and DNA Sequencing

Restriction fragments from phage clone inserts were electroeluted from agarose gels and ligated to dephosphorylated pUC8 or 9 and M13 single-stranded sequencing bacteriophage in 50mM Tris pH 7.6, 10mM $MgCl_2$, 1 mM ATP, 1 mM DTT, and 5% PEG. Ligations were used to transform competent TG-1 or DH-5 α bacterial hosts on ampicillin plates in the presence of X-Gal and IPTG. Transformants were confirmed by mini-prep methods and were followed by large scale preparations (14). M13 ligations were used to transform competent TG-1 hosts and positive plaques were grown up with single-stranded DNA isolated by the technique of Messing (20). DNA sequencing was performed by the dideoxynucleotide chain termination method as modified by Biggin, *et al.* (21) on 7M Urea, 5-20X TBE gradient acrylamide gels.

RESULTS

Isolation of OTC Recombinants from the Genomic Library

Approximately 10^6 phage were screened initially with the full length

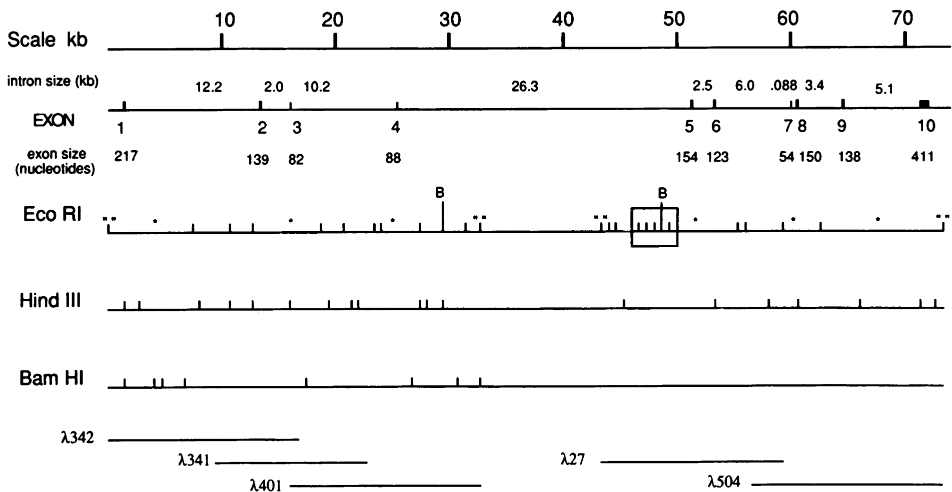

Fig. 1. Summary of the OTC gene organization. The overall structure of the gene is represented in the second line. Exons appear as vertical bars and their size is noted below their number. Intron sizes are denoted above the line. Composite Eco RI, Hind III and Bam HI restriction maps appear below the gene map and the lambda clones used to generate the maps appear at the bottom of the figure. The two sites labelled "B" in the Eco RI map represent Bgl I sites used to map the gap distance between clones λ401 and λ27. The asterisks denote the Eco RI fragments that were sub-cloned for localization of intron-exon boundaries. The boxed Eco RI sites are impossible to accurately order using the mapping techniques cited herein due to their number and size. The Eco RI sites in quotation marks are artificial sites created during construction of the lambda library.

mouse OTC cDNA and finally with a 850 bp 3′ cDNA fragment. Twelve positive clones were isolated, seven were unique and four were found to overlap without redundancy. The rapid restriction enzyme mapping technique of Rackwitz *et al.* (18) utilizing end-labelled oligonucleotides complementary to the 3′ vector cos-site was used to order the restriction fragments within the phage inserts. Accurate fragment sizes were determined by complete single restriction endonuclease digestion and ambiguities resolved by double digestion. Overlapping genomic sequences between clones were confirmed by hybridization with selected genomic fragment probes. A complete gene map is presented in Figure 1. The size of the gap between phage clones 401 and 27 was estimated by screening a series of mouse genomic DNA Southern blots with the 3′ end fragment from λ OTC 401 and the 5′ end fragment from λ OTC 27. A common Bgl I fragment of approximately 20 kb was found to hybridize with both probes and its ends were mapped within the clones yielding a gap distance of approximately 12 kb. Eco RI restriction fragments found to hybridize with

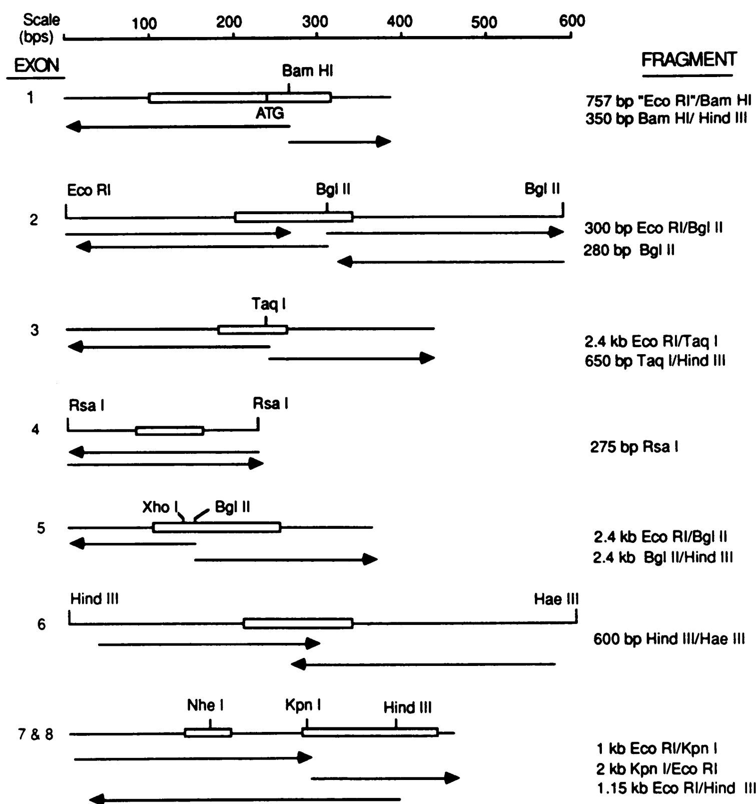

Fig. 2. Sequencing strategy used to delineate intron-exon boundaries. The exons are represented by bars. Arrows beneath each exon denote the direction and length of the sequence read. The genomic fragments inserted into M13 sequencing vectors are listed to the right. These fragments are derived from the following pUC based plasmid subclones: p342-7.3 contains exon 1, p342-4.6 contains exons 2 and 3, p401-3.8 contains exon 4, p27-6.0 contains exons 5 and 6, p504-3.0 contains exons 7 and 8, and p504-9.8 contains exons 9 and 10.

the OTC cDNA were subcloned into plasmid vectors for further characterization.

Intron-Exon Boundaries

Using convenient restriction endonuclease sites within both the cDNA and the genomic subclones (Figure 2), exon-containing fragments were further subcloned into single-stranded M13 sequencing vectors and sequenced by the dideoxynucleotide chain termination method to delineate the intron-exon boundaries as presented in Figure 3. The boundaries of the first exon were delineated during promoter analysis presented previously (8). One of the two

ATGCTGTCTAATTTGAGGATCCTGCTCAACAATGCAGCTCTTAGAAAGGGTCACACTTCTGTGGTTCGACATTTTTGgt
aagtgacagagactgggatctggtcaggagtaaaagttggaatcctgttctgcag . . . intron 1 12.2 kb . . . gaattcaaattaaaagtcattgcatatataa
aatggaaacaggactatactagttttattaatgctttgcaaagagacaatgctgcactatgtggtttgcttgtttgattccaagactaggccaatggactaccccacagca
ttgttcttgttttatacctactgtcctcttcttacttcccctttcttttgtagGTGTGGGAAGCCAGTCCAAAGTCAAGTACAGCTGAAAGGCCGTGACC
TCCTCACCTTGAAGAACTTCACAGGAGAGGAGATTCAGTACATGCTATGGCTCTCTGCAGATCTGAAATTCAGGATCAA
GCAGAAAGGAGAAgtatgcgatatttgccttttcttctcttccattgcaaaccaagggcccttttggaggcagctttccaaaagcagagggaaaagagagcaga
ttaaaattcccgaattgtagctaagtaatgaaacctcacaggcaaggtaattgcttagcactgagtgtccaggcatataaacaagcataaaaagcagttattcaatga
caagtccttgtaatttttctttcagcctaatgcttatatgcattagtaactagatct . . . intron 2 2.0 kb . . . attatcgttccggctcgtatgttgtgtggaa
ttgtgagcggataacaatttcacacaggaaacagctatgaccatgattagccaagcttggctgcaggtctatttttattagcattttgaaaaataatgcttgatgttgga
tagtgtaccttgcattttcctcctatttttgtctttggttttcagTATTTACCTTTATTGCAAGGGAAATCCTTAGGAATGATTTTTGAGAAAA
GAAGTACTCGAACAAGACTGTCCACAGAAACAGgtgagtctactgacaagctcgagcacttcgtgttgaagacgtggccaggaaggataggagac
cttggaaggcttaaccttgtggaggggattttgtgtgttcgtttcacgaaccacacgagagcaagcggtttcgtggaagccgcagtacagaggtgtctttcaattcatcg
ta . . . Intron 3 10.2 kb . . . attttggttaacattttagttctttcgttttcccctctcaatacattcactgtctttttcttttgttctagGCTTTGCTCTGCTGGG
AGGACACCCTTCCTTTCTTACCACACAAGACATTCACTTGGGTGTGAATGAAAGTCTCACAGACACCGCTCGgtttgta
aaacttttcttccttccaaagtttatttcaaactctgatgggttagtttaaaagagaagatgatgcttctccttagataatgcc . . . intron 4 approx. 26.3 kb . .
atatattaatgtagcttttcaaatctatcacattcctaggtatagccatacacacattttcatttattggcactcattcatgtctttaaggagctgtattaaagaagggt
tattgagaacaattgttaagaatgtctcttcttggtttgccatagTGTCTTATCTAGCATGACAGATGCAGTGTTAGCTCGAGTGTATA
AACAATCAGATCTGGACACCCTGGCTAAAGAAGCATCCATCCCAATTGTCAATGGACTGTCAGACTTGTATCATC
CTATCCAGATCCTGGCTGATTACCTTACACTCCAGgttggtgtatttctttgctttgcaaaacagaaagtttaccaaataattcctagctaccatca
agtggagccatttatttaaatgatgtttagtttttatgaataacttctatctttaaaagtatgtgtatgtgggaatactacaggtgtagaaacag . . . intron 5
2.5 kb . . . ttttgtgattgtatattcatgttaatgaatagaacttggcaaatgtagtctattccttccaataaggcatttatttggaggtaaatttttttcctttattttagctctagt
aactgatttactggcaagtgggttccctacccgtatctaactcccttttacctcttgtttgtagGAACACTATGGCTCTCTCAAAGGTCTTACCCTCA
GCTGGATAAGGGATGGGAACAATATCTTGCACTCTATCATGATGAGTGCTGCAAAATTCGGGATGCACCTTCAAG
CAGCTACTCCAAAGgtaggaacactgtcttgcctgaaaaaatatctctgtgatcagaggctattcccagtgtaaacaaagctcctgatgaaggagagcata
gctgaagccttgtaatttagccatattaccacttttctgttgaatctagtttcttaagagtgcaattttccatttttacagtggaaactagtcttgctgatgcctttattccatgagt
ctgtgatagcagcacatct . . . intron 6 6.0 kb . . . ggggatccgtctattcttaggcttttttttgttttaagatcatactagatagttgttaaatataagagctattaa
atactgtcatgtataattaaactaactaatcacctaaataagaagctaatgtttcctctttcagGGTTATGAGCCAGATCCTAATATAGTCAAGCTA
GCAGAGCAGTATGCCAAGGAGgtatgctttttttatttgtaatcttctaattgtctttctctgttccatcaagtttttgtgtttttaaccagcaaacttctctgtgctagA
ATGGTACCAAGTTGTCAATGACAAATGATCCACTGGAAGCAGCACGTGGAGGCAATGTATTAATTACAGATACTT
GGATAAGCATGGGACAAGAGGATGAGAAGAAAAAGCGTCTTCAAGCTTTCCAAGGTTACCAGGTTACGATGAAGgta
caaatggatgcttctctcga . . . intron 8 3.4 kb . . . tagtgatatatcatggccagtacaaagctgtgcttattacatgccaaagaagctgtatatgacactgcc
atgtgtagatagttcatactcaaaatcgtgatcttatctccacctctttagACTGCCAAAGTGGCTGCGTCTGACTGGACATTTTTACACTGT
TTGCCTAGAAAGCCAGAAGAAGTGGATGATGAAGTATTTTATTCTCCACGGTCATTAGTGTTCCCAGAGGCAGAGA
ATAGAAAGTGGACAATCATGgtaaggaagaagggggcaatggaagggtaagagactgtaagtgtgctttcaacttgacaattcatgcttttggggtaaa
gaataagtatccaaggttatccaattatatctgattgctcttctggcatctcttctacatttaaattgtatctattaattagttt . . . intron 9 5.1 kb . . . ccgtcaaaa
ttccaagcctgaaaaactgaaatctaattctcttggtttagttcccaaggcagactgttgctaatgtttatccatttgtttctctttcttttgtcatcccagGCTGTCATGG
TATCCCTGCTGACAGACTACTCACCTGTGCTCCAGAAGCCAAAGTTTTGATGCCTGTCAAAAGGAAAAAAACAGA
AAACAAAACAATAACAATAACAACAACAACAACAAAAACCCCTCTGTTCTTTAGCAATAGAATAAGTCAGTTTATG
TGGGAAAGAGAAGAATTTAAAATTGTAAACACATCCCTAGTGCATGGTATGATTATGTAATTGCTTTGCTATTATG
AGAATTTCTTAAAGCTTTTAGTTTAAGTGCCTGGCATTTTATTATCCTGCTTGACTTGGTTTAAACACTCTCTTCAA
TTTACAACCTCTGAATGACATTTGGGTATCATATTAATTATCATACACATTTCCTTCCACTAAACATTAAACACTT
TGCTTACAATGTCTAAGTCATtaaatgtaatctagagcttgggg

Fig. 3. Intron-exon organization of the mouse OTC gene. The sequences at the intron-exon junctions were determined using the sub-cloning and sequencing strategies depicted in Fig. 2. Coding sequences are uppercase and lowercase letters represent intron sequences. The translation initiation and termination codons are double underlined while the polyadenylation signal is boxed.

Bgl II sites in subclone p342-4.6 was found just 3' to the splice donor site of Intron 1 and to be correlated with the Bgl II site in the cDNA thus positioning Exon 2. p342-4.6 also contained a unique Taq I site which corresponded to a Taq I site in the cDNA identifying the position of Exon 3. Exon 4 was identified by digesting the p401-3.8 subclone with a series of 4 base recognition sequence restriction endonucleases and determining the sequence of a 370 bp Rsa I fragment which hybridized to the cDNA probe. Hind III digestion of the subclone p27-6.0 revealed two bands, which hybridized with the cDNA. The larger contains a Bgl II site which corresponded to the 3' Bgl II site in the cDNA thus localizing exon 5. Exon 6 was found by sequencing a 600 bp Hind III/Hae III fragment in both orientations. Exon 7 was localized by probing the 3' phage clones with a 50 nucleotide oligomer synthesized after delineating the 5' end of Exon 8. A Nhe I digest of the 1.0 kb Eco-RI/Kpn I fragment from p504-3.0 released a 90 bp fragment permitting Exon 7 to be sequenced from the Kpn I site. The Hind III site in p504-3.0 was found to correspond to the 5' Hind III site in the cDNA thus localizing Exon 8. A unique Hga I site within the 3.6 kb Eco RI-Hind III fragment from p504-9.8 was found to correlate with the remaining Hga I site in the cDNA thus localizing Exon 9. Sequencing of the hybridizing Aha III/Hind III fragments from p504-9.8 served to position and delineate the boundaries of Exon 10.

DISCUSSION

The gene encoding murine OTC is composed of ten exons distributed over approximately 70 kb of the mouse X chromosome. The sequences present at the splice donor/acceptor sites for these exons conforms closely to the consensus sequences compiled for other eukaryotic genes (22). Southern analysis has failed to detect the presence of any pseudogenes within the mouse genome. Surprisingly, the sequences coding for the thirty-two amino acid leader peptide, involved in mitochondrial vectoring and cleavage, extends into exon 2. However, the functionally relevant amino acid sequences thus far defined (23) are all encoded on exon 1. Of further note is the tryptophan residue at amino acid position 26 in place of the arginine residue present in both the rat (24) and human (25) leader peptides. This codon is interrupted by the splice donor/acceptor site between exons 1 and 2 and a C to T transition at the first base within this codon would explain the amino acid substitution present here.

Since OTC occurs as a mitochondrially localized trimer, secondary struc-

ture and hydrophobicity plots of the amino acid sequence have been examined. Three consecutive, highly hydrophilic alpha-helical domains are present in exons 8 and 9. The only cysteine present in the monomer lies between the first two of these domains.

A search for sequences homologous to OTC coding sequences did not reveal any homologies although a number of genes including insulin and Factor IX show homology to the 3′ untranslated region of the OTC transcript. The significance of this is unclear.

These untranslated sequences together with intronic sequences are currently being assessed for possible regulatory function. We have previously shown that 800 bp of the 5′ flanking region is sufficient to direct hepatic specific expression of this gene (8). While recent studies of α-fetoprotein by Tilgman, *et al.* (26) and OTC by Mullins, *et. al* (27) suggest that hepatic-specific regulatory regions may be located up to 12 kb upstream of the initiation codon, we have recently shown that the 800 bp sequence correctly directs expression of the CAT reporter gene in the liver and small intestine of transgenic mice (28).

The mouse OTC gene structure presented here is highly homologous to the rat OTC gene structure very recently delineated by Takiguchi, *et al.* (29). The exon break points were identical between the genes including the splice donor/acceptor site present in the leader peptide sequence. With the exception of introns 3 and 8, the intron size are very similar and the intronic sequences available are approximately 83% homologous. These data are highly suggestive that the structure of OTC in mammals, including man, is established. The generation of exon specific probes now enables us to elucidate the structure of the human gene. Human OTC genomic fragments may then be used to characterize known RFLP's deletions, and other mutations present at this locus. These studies together with further analysis of regulatory regions represent the first steps toward understanding the tissue-specific expression and the possibilities of gene replacement therapies involving this critical urea cycle enzyme.

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REFERENCES

1. Grisolia, S. and Cohen, P.P. (1952) J. Biol. Chem. **198**,561-571.
2. Jones, N.E., Anderson, A.D., Anderson, C. and Hodes, S. (1961) Arch. Biochem. Biophys. **95**,499-507.
3. Conboy, J.G., Kalousek, F. and Rosenberg, L.E. (1979) Proc. Natl. Acad. Sci. USA **76**,5724-5727.
4. Mori, M., Miura, S., Morita, T., Takiguchi, M. and Tatibana, M. (1982) Mol. Cell. Biochem. **49**,97-111.
5. Lindgren, V., Martinville, B., Horwich, A.L., Rosenberg, L.E. and Francke, U. (1984) Science **226**,698-700.
6. Raijman, L. (1974) Biochem. **138**,225-232.
7. Ryall, J., Nguyen, M., Bendayan, M. and Shore, G.C. (1985) Eur. J. Biochem. **152**,287-292.
8. Veres, G., Craigen, W.J. and Caskey, C.T. (1986) Jour. Biol. Chem. **261**,7588-7591.
9. Walser, M. (1983) In *The Metabolic Basis of Inherited Disease* (Stanbury, J.B., Wyngaarden, J.B., Friedrickson, D.S., Goldstein, J.L. and Brown, M.S., eds.) 5th Ed., pp. 402-419, McGraw-Hill, New York.
10. DeMars, R., LeVan, S.L., Trend, B.L. and Russel, L.B. (1976) **73**,1693-1697.
11. Doolittle, D.P., Hulbert, L.L. and Cordy, C. (1974) **65**,194.
12. Veres, G., Gibbs, R.A., Scherer, S.E. and Caskey, C.T. (1987) Science **237**,415-417.
13. Benton, W.D. and Davis, R. (1977) Science **196**,180-182.
14. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) In *Molecular Cloning*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
15. Feinberg, A. and Vogelstein, B. (1984) Anal. Biochem. **137**,266.
16. Southern, E.M. (1975) J. Mol. Biol. **98**,503-517.
17. Smith, G.E. and Summers, M.D. (1980) Anal. Biochem. **109**,123-129.
18. Rackwitz, H-R., Zehetner, G., Frischauf, A-M. and Lehrach, H. (1984) Gene **30**,195-200.
19. Sealy, P.G., Whittaker, P.A. and Southern, E.M. (1985) Nucl. Acids. Res. **13**,1905-1922.
20. Messing, J. (1983) In *Methods in Enzymology*, **Vol. 101**, pp.20-78. Academic Press, New York.
21. Biggin, M.D., Gibson, T.J. and Hong, C.F. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**,3963-3965.
22. Breathnach, R. and Chambon, P. (1981) Ann. Rev. Biochem. **50**,349-383.
23. Horwich, A.L., Kalousek, F., Fenton, W.A., Pollock, R.A. and Rosenberg, L.E. (1986) Cell **44**,451-459.
24. Takiguchi, M., Miura, S., Mori, M., Tatabana, M., Nagata, S. and Kaziro, Y. (1984) Proc. Natl. Acad. Sci. USA **81**,7412-7416.
25. Horwich, A.L., Fenton, W.A., Williams, K.R., Kalousek, F., Kraus, J.P., Doolitle, R.F., Konigsberg, W. and Rosenberg, L.E. (1984) Science **224**,1068-1074.
26. Hammer, R.E., Krumlauf, R., Camper, S.A., Brinster, R.L. and Tilghman, S.M. (1987) Science **235**,53-58.
27. Mullins, L.J., Veres, G., Caskey, C.T. and Chapman, V. (1987) Mol. Cell. Biol., in press.
28. Veres, G., Scherer, S.E. and Caskey, C.T. Manuscript in preparation.
29. Takiguchi, M., Murakami, T., Miura, S. and Mori, M. (1987) Proc. Natl. Acad. Sci. USA **84**,6136-6140.