Proc. Natl. Acad. Sci. USA
Vol. 76, No. 10, pp. 4981–4985, October 1979
Biochemistry

Cloning and nucleotide sequence of DNA coding for bovine preproparathyroid hormone

(recombinant DNA/parathyroid hormone/mRNA/restriction map/codon usage)

HENRY M. KRONENBERG*†, BARBARA E. MCDEVITT*†, JOSEPH A. MAJZOUB*†, JEREMY NATHANS†, PHILLIP A. SHARP†‡, JOHN T. POTTS, JR.*, AND ALEXANDER RICH†

*Endocrine Unit, Massachusetts General Hospital, Boston, Massachusetts 02114; and †Department of Biology and ‡Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Contributed by Alexander Rich, July 10, 1979

ABSTRACT We have cloned in *Escherichia coli* a DNA copy of mRNA coding for bovine preproparathyroid hormone. Double-stranded DNA was inserted into the *Pst* I site in plasmid pBR322 by using the poly(dG)·poly(dC) homopolymer extension technique to join the DNA molecules. Recombinant plasmids coding for preproparathyroid hormone were identified by the plasmid's ability to arrest specifically the translation of preproparathyroid hormone mRNA. The nucleotide sequence of the largest recombinant was determined by using both chemical and enzymatic techniques. The parathyroid insert contains 470 nucleotides—102 nucleotides from the 5′ noncoding region of the mRNA, 345 nucleotides representing the entire coding region, and 23 nucleotides from the 3′ noncoding region. The coding sequence clarifies the hormone's amino acid sequence, which has been disputed. Codon usage is discussed.

Parathyroid hormone (PTH) regulates the blood level of calcium, and in turn, blood calcium regulates the secretion of PTH. Although modulators of PTH secretion have been extensively delineated (1), the regulation of synthesis of PTH is poorly understood. Pulse-chase studies of parathyroid gland slices (2) and translation of mRNA in cell-free systems (3) have shown that PTH (84 amino acids) is first synthesized as a 115-amino acid precursor, preproparathyroid hormone (PreproPTH), with a 31-amino acid NH_2-terminal extension. The first 25 amino acids are rapidly cleaved to yield the 90-amino acid proparathyroid hormone, which is subsequently converted to PTH. As a first step in studying further the regulation of PTH biosynthesis, we have cloned in *Escherichia coli* a DNA copy of PTH mRNA. Nucleotide sequence analysis of the cloned DNA demonstrates that one clone contains the entire coding region plus portions of both the 5′ and 3′ noncoding regions of the mRNA.

MATERIALS AND METHODS

Enzymes. Reverse transcriptase was provided by J. W. Beard (Life Sciences, St. Petersburg, FL). DNA polymerase I and polynucleotide kinase were purchased from Boehringer Mannheim, S1 nuclease and lysozyme were from Sigma, terminal transferase was from Bethesda Research (Cockeysville, MD), and restriction endonucleases and DNA polymerase I (large fragment) were from New England Biolabs.

Bacteria and Nucleic Acids. *E. coli* χ1776 (4), a certified EK2 host, was provided by R. Curtiss and *E. coli* carrying plasmid pBR322 (5) was provided by A. Bothwell. pBR322, a certified EK2 vector,§ after amplification with chloramphenicol was purified on two $CsCl_2$ gradients after use of the cleared lysate technique (6). PTH mRNA was partially purified as described (7). Vesicular stomatitis virus RNA was isolated as described (8).

Synthesis of Poly(dC)-Tailed Double-Stranded DNA. DNA complementary in sequence to PTH mRNA was synthesized by using minor modifications of the protocols of Friedman and Rosbash (9) and Efstratiadis *et al.* (10). mRNA (18 μg) was incubated in a 2.2-ml reaction mixture containing 169 units of reverse transcriptase (220 μl), 50 mM Tris·HCl (pH 8.3), oligo(dT) at 27 μg/ml, 200 μM [α-^{32}P]dATP and dTTP (both 68 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels), 900 μM dGTP and dCTP, 120 mM KCl, 10 mM dithiothreitol, and 10 mM $MgCl_2$. After 1 hr at 43°C the reaction mixture was extracted with an equal volume of phenol/chloroform/isoamylalcohol (25:24:1), 55 μg of *E. coli* tRNA was added, and the nucleic acids were precipitated with 2 vol of ethanol. The pellet was treated with NaOH, neutralized, and chromatographed over Sephadex G-100 as described (10).

The cDNA was made double-stranded with DNA polymerase I according to the method of Efstratiadis *et al.* (11), except that 1/15 vol of a HeLa cell S-100 fraction (12) provided by J. Rose was added to 1 vol of reaction mixture. The nucleic acid was then extracted with phenol/chloroform, precipitated with ethanol, passed over a column of Sephadex G-100, digested with S1 nuclease (11), extracted again with phenol/chloroform, and precipitated with ethanol. The DNA was then centrifuged through a gradient of 5–20% sucrose in 0.8 M NaCl as described (13). The largest molecules (20 ng) were dialyzed against 10 mM Tris·HCl, pH 7.6/0.1 mM Na_2 EDTA, 5 μg of tRNA was added, and the nucleic acids were ethanol-precipitated. Poly(dC) homopolymer extensions, approximately 20 nucleotides long, were added by using terminal transferase as described (14). Similarly, poly(dG) homopolymer extensions, 20 nucleotides long, were added to *Pst* I-cut pBR322 DNA after phenol extraction, dialysis, and ethanol precipitation of the DNA.

Transfection of *E. coli* χ1776. Poly(dG)-tailed pBR322 (400 ng) and 30 ng of poly(dC)-tailed parathyroid DNA were annealed in 0.1 M NaCl/10 mM Tris·HCl, pH 7.6/0.1 mM EDTA for 2 min at 65°C and then for 2 hr at 42°C. *E. coli* χ1776 was grown and treated with 70 mM $MgCl_2$/30 mM $CaCl_2$/40 mM Na acetate, pH 5.6, as described (15). Transformation was performed in a biological safety cabinet in a P3 physical containment facility according to the then current National Institutes of Health guidelines for recombinant DNA research.§

Hybrid-Arrested Translation. Individual clones were grown in 200 ml of FB medium (25 g of tryptone/7.5 g of yeast extract/1 g of glucose/6 g of NaCl/50 mM Tris·HCl, pH 7.6, taken to 1 liter) supplemented with thymidine 50 at μg/ml,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: PTH, parathyroid hormone; PreproPTH, preproparathyroid hormone

§ National Institutes of Health, *Federal Register*, July 7, 1976.

diaminopimelic acid at 100 μg/ml, and tetracycline at 20 μg/ml; then, they were amplified with chloramphenicol. The plasmid DNA was isolated by using the cleared lysate technique (6) followed by two centrifugations in CsCl/ethidium bromide equilibrium gradients. Hybrid-arrested translation was performed as described (16).

Restriction-Enzyme Analysis. Five micrograms of the PTH recombinant plasmid was digested with *Pst* I in a 100-μl reaction mixture. After digestion, 5 units of DNA polymerase I (large fragment) was added and incubation was continued for 5 min at 37°C. The reaction mixture was then transferred to another tube containing 10 μCi of each of four [α-^{32}P]dNTPs (400 Ci/mmol) and the reaction was continued for 5 min at 37°C. Then the reaction mixture was adjusted to 40 μM of each of the four dNTPs and the reaction was continued for 10 min. The digest, now possessing molecules labeled with ^{32}P at the 3′ end, was precipitated with ethanol and then fractionated by electrophoresis on a 10% polyacrylamide slab gel in Tris/borate/EDTA buffer (17). The eukaryotic insert was then eluted from the gel (18). Aliquots of the insert were then digested with restriction enzymes and the procedures of 3′-end labeling and gel electrophoresis were repeated. For reactions involving enzymes that produce DNA with single-stranded 5′ extensions (e.g., *Hin*fI), the enzyme was removed by chloroform extraction before end-labeling.

DNA Sequence Analysis. Both the chemical (18) and enzymatic dideoxynucleotide (19) methods were used without modification. Restriction fragments were 5′-end labeled by using the polynucleotide kinase exchange reaction (20).

RESULTS

Construction of cDNA Clones and Translational Analysis. Earlier studies (7, 21) have established that the mRNA coding for PreproPTH is a predominant mRNA in the parathyroid gland and that use of formamide/sucrose gradients and oligo(dT)-cellulose chromatography can lead to partial purification of the mRNA. Furthermore, cDNA made from this mRNA can function in a linked transcription/translation system to produce the entire PreproPTH molecule (7) and thus contains all the structural information encoded in the mRNA. We made this cDNA double stranded, selected the largest DNA molecule on a sucrose gradient, and introduced the DNA into plasmid pBR322 by using the poly(dG)·poly(dC) homopolymer extension technique. From 15 ng of double-stranded parathyroid DNA, we obtained 49 transformed colonies with the pattern of tetracycline resistance and ampicillin sensitivity, the pattern expected from bacteria containing pBR322 with eukaryotic inserts at the *Pst* I site.

Six of these clones were grown and the plasmid DNA was isolated. We found it important to purify the plasmid DNA by two cycles of centrifugation through CsCl, because DNA after only one centrifugation gave erratic results in hybrid-arrested translation. After *Pst* I digestion, phenol extraction, and ethanol precipitation, each DNA, as well as control pBR322 DNA, was hybridized to 50 ng of parathyroid gland mRNA as indicated in Fig. 1. Vesicular stomatitis virus RNA was added to the mixture to provide control mRNAs that stimulated protein synthesis as well as did PreproPTH mRNA. After annealing,

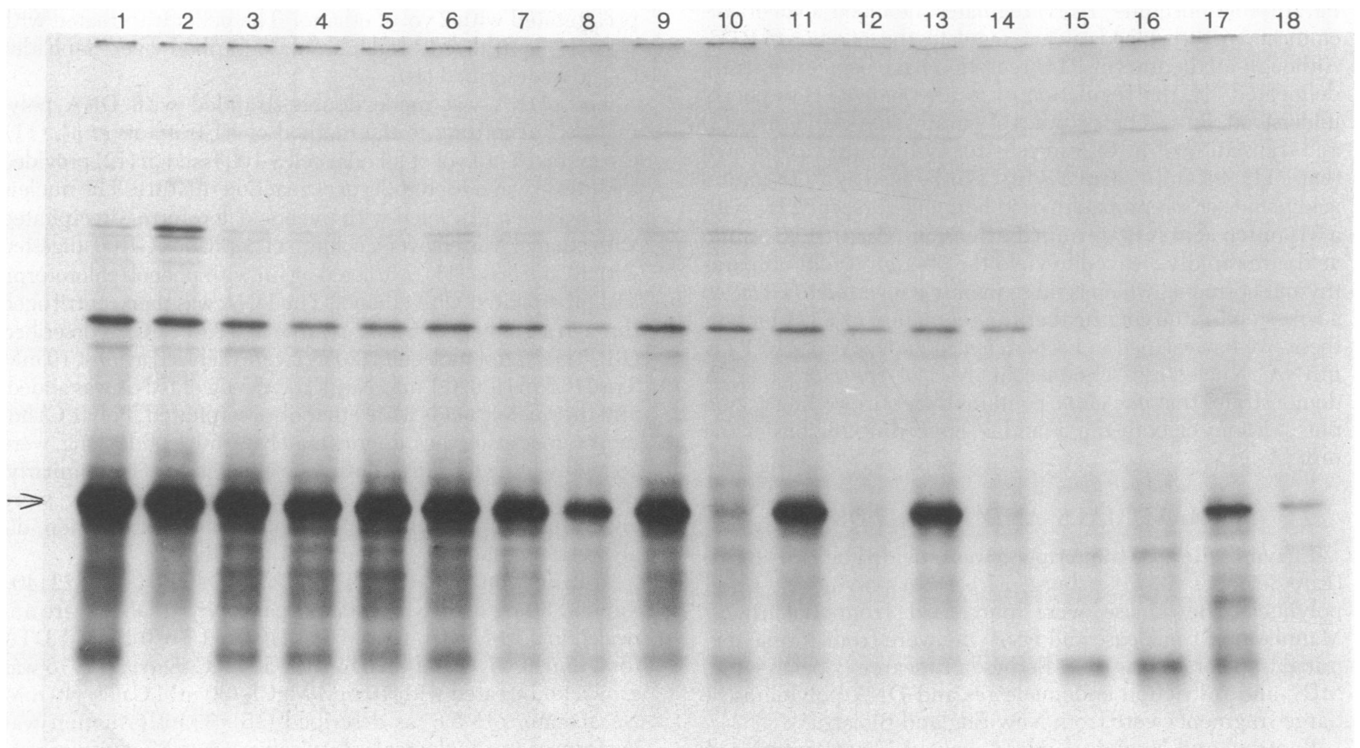

FIG. 1. Hybrid-arrested translation. Plasmid DNA was annealed with 50 ng of parathyroid gland mRNA that had been purified by oligo(dT)-cellulose chromatography and sucrose gradient centrifugation. A similar amount of VSV mRNA was added to reactions 1–16. One half of each sample was heated and cooled quickly after annealing. After ethanol precipitation along with 25 μg of yeast tRNA, each sample was added to a 50-μl wheat germ translation reaction mixture in the presence of [^{35}S]methionine. Five microliters of each sample was then electrophoresed through a 15–20% polyacrylamide gradient gel containing sodium dodecyl sulfate, and fluorography was performed on the dried gel. Arrow points to PreproPTH. Lanes: 1, no DNA was added and mRNA was mock-annealed, heated, and quick-cooled; 2, same as lane 1 but with no heating; 3, 1 μg of pBR322 DNA, heated; 4, same as lane 3 but with no heating; 5, 2 μg of pBR322 DNA, heated; 6, same as lane 5 but with no heating; 7, 4 μg of pBR322 DNA, heated; 8, same as lane 7 but with no heating; 9, 1 μg of pPTHm1 DNA, heated; 10, same as lane 9 but with no heating; 11, 2 μg of pPTHm1 DNA, heated; 12, same as lane 11 but with no heating; 13, no DNA or mRNA added, heated; 14, same as lane 13 but with no heating; 15, no DNA added, mRNA was not mock-annealed but was heated and added directly to translation mixture; 16, same as lane 15 but with no heating; 17, PTH mRNA mock-annealed without DNA or VSV mRNA; 18, same as lane 17 but with no heating.

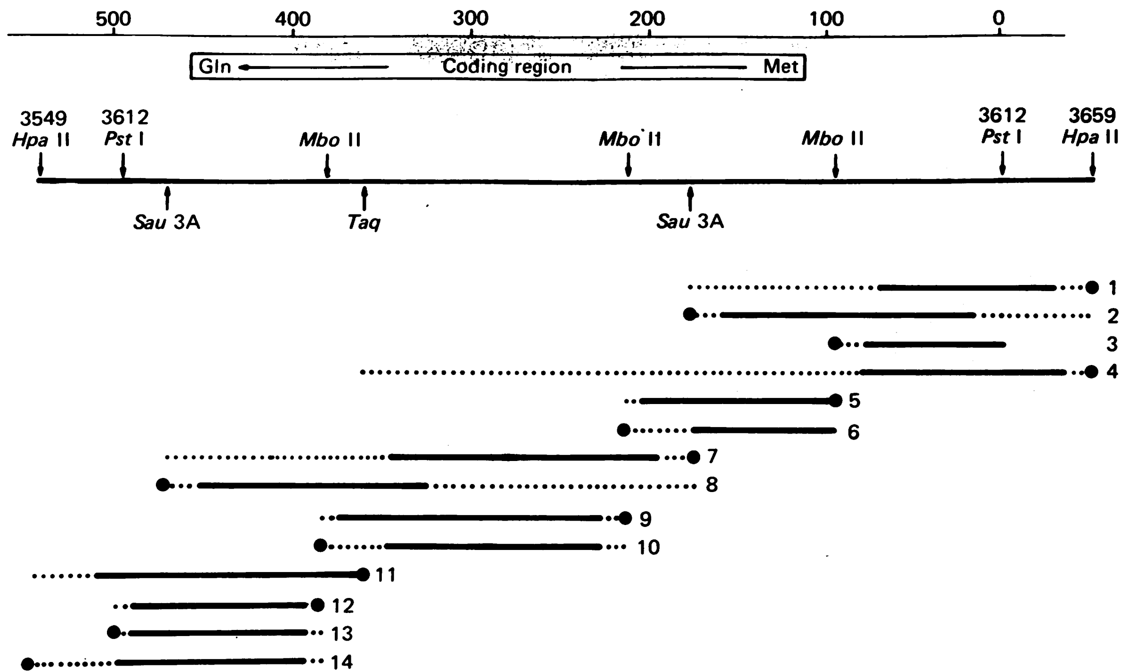

FIG. 2. Restriction enzyme map of pPTHm1. Numbers above the *Hpa* II and *Pst* I sites correspond to the cutting sites on pBR322 (32); numbers above the insert correspond to the numbers used in Fig. 3 to describe the eukaryotic insert. Lines below the map indicate the restriction fragments used in chemical sequence determinations. Solid parts of each line indicate areas of readable sequence. Fragments 5, 6, 9, and 10 were separated on a 5% polyacrylamide strand-separating gel (18); all other fragments were generated by using combinations of restriction enzyme digestions to produce molecules with only one 5′-labeled end. Dots represent the 5′ end so labeled. *Mbo* II-generated fragments 5, 6, 9, and 10 were also digested with exonuclease III and used as primers for dideoxynucleotide sequence analysis, using as template pPTHm1 DNA cut with *Eco*RI, and then were partially digested with exonuclease III or T7 exonuclease.

the DNA/RNA mixture was divided in half, and one half was heated and cooled quickly to break up the hybrids. After precipitation with ethanol, the nucleic acids were then added to a wheat germ cell-free protein-synthesizing system in the presence of [^{35}S]methionine. The proteins made were then electrophoresed, and autoradiography was performed. Fig. 1 shows that pPTHm1 DNA, in each of three concentrations, inhibited the translation of PreproPTH while not affecting the

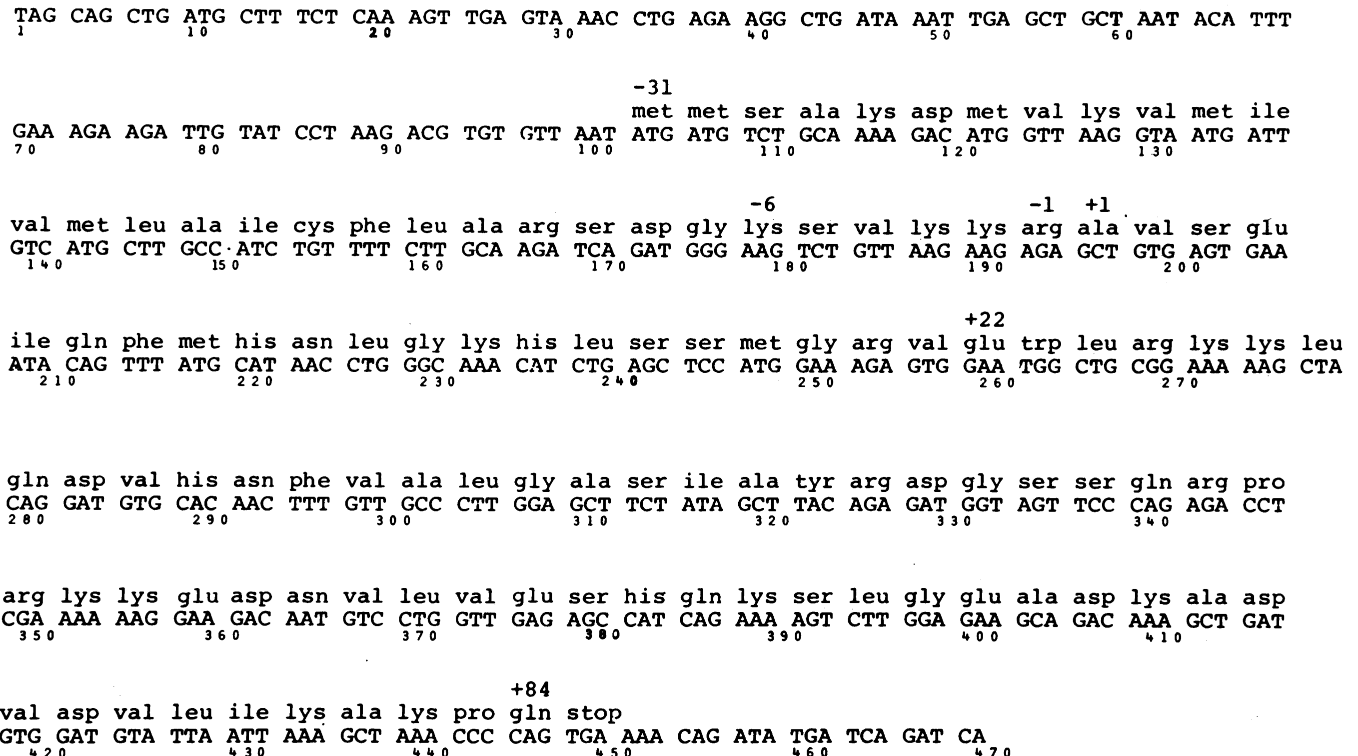

FIG. 3. Nucleotide sequence of sense strand of parathyroid insert in pPTHm1. Orientation of insert is indicated in Fig. 2. Numbers above the protein sequence refer to amino acid position; by convention −31 to −7 is "pre" sequence, −6 to −1 is "pro" sequence, and +1 to +84 is PTH sequence.

translation of other proteins (lanes 10, 12, and 14). Furthermore, heating and quick-cooling of the nucleic acid restored the translation of PreproPTH. In contrast, pBR322 DNA at similar concentrations had no effect on protein synthesis (lanes 3–8).

By using DNA from pPTHm1 as a probe, we screened our other 48 clones for PTH inserts larger than that of pPTHm1. A toothpick tip was used to mix a small portion of each individual colony taken from an agar plate in Tris/EDTA/sucrose/lysozyme as described (22). After precipitation of proteins with potassium acetate and sodium dodecyl sulfate and digestion of the nucleic acids with RNase and then *Pst* I, DNA from each colony was electrophoresed on a 1.4% agarose gel (23) and transferred to nitrocellulose (24). PTH inserts with intact *Pst* I sites were detected by hybridization with nick-translated pPTHm1 DNA. Seven of 48 colonies yielded bands of size similar to the insert in pPTHm1, but no larger bands were found (data not shown).

Restriction Enzyme Analysis and DNA Sequence Analysis. The eukaryotic insert of pPTHm1 was isolated, digested with a series of restriction endonucleases, labeled with ^{32}P at the 3′ end, and fractionated by electrophoresis on a 10% acrylamide gel (17). The enzymes *Bam*HI, *Hin*dIII, *Eco*RI, *Sal* I, *Hin*cII, *Hin*fI, *Hae* III, *Hpa* I, *Bgl* II, *Xho* I, *Hha* I, and *Msp* I did not cut this insert. *Mbo* II, *Alu* I, *Taq* I, and *Sau* 3A did cut the insert (Fig. 2). (*Alu* sites are omitted from the figure because fragments cut by *Alu* I were not used in the subsequent sequence analyses.)

The sequences of the restriction enzyme-generated fragments noted in the legend to Fig. 2 were determined by the chemical method. Furthermore, the *Mbo* II fragments were used as primers for dideoxynucleotide sequence analysis after either partial exonuclease III or T7 exonuclease digestion of *Eco*RI-cut pPTHm1 DNA.

We found that the 5′-end labeling of restriction fragments generated by the enzymes *Mbo* II and *Pst* I was inefficient, presumably because these fragments lacked 5′ single-stranded DNA extensions. Therefore, we added DNA polymerase (large fragment), 1 unit/μg of DNA, for 5 min at 37°C to the restriction enzyme digestion reaction mixtures at the end of the digestions and then extracted the DNA with phenol to terminate the reactions. These modified fragments were more efficiently end-labeled, presumably because the 3′ → 5′ exonuclease activity of the DNA polymerase I-generated 5′ single-stranded DNA extensions at the ends of the fragments.

Because we found that the nucleotides at the beginnings and ends of restriction fragments were usually difficult to resolve, we used a number of different restriction enzymes to generate overlapping fragments for analysis by chemical sequence analysis. Furthermore, the use of the dideoxynucleotide enzymatic sequence analysis strategy confirmed the sequence derived by the chemical approach. Because restriction fragments are used as primers of DNA synthesis in this technique, the DNA synthesized by using specific primers allowed additional independent data for ordering the sequence-analyzed fragments. Fig. 3 depicts the sequence thus established.

DISCUSSION

The parathyroid DNA in pPTHm1, 470 nucleotides in length, represents about 70% of the nucleotide sequence of PreproPTH mRNA, as estimated from the size of the mRNA determined by electrophoresis through 98% formamide/polyacrylamide gels (31). The sequence includes the entire coding region of the mRNA, 102 residues of the 5′ noncoding region, and 23 residues of the 3′ noncoding region. The sequence of pPTHm1 DNA confirms the amino acid sequence of bovine PreproPTH, as determined by a combination of traditional (25, 34) and radiomicrosequence analysis (3) approaches. Furthermore, the GAA codon coding for the glutamic acid at position 22, found when the sequences of DNA fragments 7, 8, and 9 were determined (see Fig. 2), supports our previous assignment, a finding that has been disputed by another group (25). Bovine genetic heterogeneity could still, of course, explain the disparate sequences found at position 22. We cannot, in addition, exclude the possibility that the sequence was modified by the process of cloning, but the accurate prediction of the rest of the PreproPTH amino acid sequence is reassuring, as are other examples of faithful cDNA cloning (26, 30). To minimize chances of error in determination of nucleotide sequences, every nucleotide in the coding sequence was ascertained at least twice (see Fig. 2).

Codon usage analysis (Table 1) demonstrates some uneven usage of codons, but, as noted by others (26, 30), comparisons with usage patterns in other eukaryotic mRNA show no consistent patterns. For instance, when one compares the codon usage patterns between bovine corticotropin-β-lipotropin precursor mRNA and bovine PreproPTH mRNA, the codons UUC, CUC, and AGG, unused in PreproPTH mRNA, are frequently used in corticotropin-β-lipotropin precursor mRNA. The glutamine codon CAA is unused in PreproPTH mRNA and used only 1 of 10 possible times in corticotropin-β-lipotropin precursor mRNA. Also, as noted by others (30), the dinucleotide CG is used infrequently—two times—in the coding region. In marked contrast to the pattern in several other mammalian sequences (26, 30), however, no preference is found for G or C in the third codon position—30% of codons terminate in T, 17% terminate in C, 27% terminate in A, and 26% terminate in G.

Table 1. Codon usage in bovine PreproPTH mRNA

Amino acid	Codon	Usage	Amino acid	Codon	Usage
Phe	UUU	3	Tyr	UAU	0
	UUC	0		UAC	1
Leu	UUA	1	Term	UAA	0
	UUG	0	Term	UAG	0
	CUU	4	His	CAU	3
	CUC	0		CAC	1
	CUA	1	Gln	CAA	0
	CUG	4		CAG	5
Ile	AUU	2	Asn	AAU	1
	AUC	1		AAC	2
	AUA	2	Lys	AAA	8
Met	AUG	7		AAG	6
Val	GUU	4	Asp	GAU	5
	GUC	2		GAC	3
	CUA	2	Glu	GAA	5
	GUG	4		GAG	1
Ser	UCU	3	Cys	UGU	1
	UCC	2		UGC	0
	UCA	1	Term	UGA	1
Pro	UCG	0	Trp	UGG	1
	CCU	1	Arg	CGU	0
	CCC	1		CGC	0
Thr	CCA	0		CGA	1
	CCG	0		CGG	1
	ACU	0	Ser	AGU	3
Ala	ACC	0		AGC	2
	ACC	0	Arg	AGA	5
	ACG	0		AGG	0
	GCU	4	Gly	GGU	1
	GCC	2		GGC	1
	GCA	3		GGA	2
	GCG	1		GGG	1

Based on a statistical analysis of peptide sequences, Cohn *et al.* (27) have proposed that PreproPTH originated after gene duplication. They noted nine amino acid matches when peptides −27 to +22 and +26 to +74 were aligned. They calculated $P = 0.0001$ for this being a chance occurrence. When the nucleotide sequences of these regions are similarly aligned, 53 of 147 nucleotides match. Thus, the nucleotide sequence, like the amino acid sequence, suggests similarities that are unlikely to have occurred by chance. Similar comparisons have been made between the sequences of rat growth hormone and human chorionic somatomammotropic hormone (30) and between four regions of the bovine corticotropin-β-lipotropin precursor (26). In both of these comparisons, there was little variation in the third nucleotide of triplets, even though that variation would not have resulted in a changed amino acid sequence. In contrast, in the PreproPTH comparison, only two of nine third bases remain invariant when the triplets coding for the nine invariant amino acids are compared—no more than expected by chance. Furthermore, when one excludes the nucleotides associated with the amino acid matches, only 32 of 120 nucleotides match, again no more than expected by chance. Thus, the mRNA sequence, although consistent with the hypothesis that the gene arose by duplication, does not add evidence beyond that provided already by the amino acid homologies. Perhaps the pattern of intervening sequences that may exist in the genomic DNA will shed light on the origin of the PreproPTH sequence. We might expect to find, for instance, an intervening sequence separating the duplicated structural regions if RNA splicing acts as a means of bringing together in one mRNA two sets of sequences that arose by tandem gene duplication.

The AUG at position 10–13 in the 5′ noncoding region is only the second example in eukaryotes of an AUG in the 5′ noncoding region of an mRNA, the first example being the mRNA coding for VP1 of simian virus 40. The sequences of all other mRNAs are consistent with the hypothesis of Kozak that ribosomes bind at a 5′-terminal cap and scan the mRNA until they initiate at the first available AUG (28). Kozak notes that the two AUGs in the 5′ noncoding region of VP1 mRNA may well be part of a 5′-terminal double-stranded RNA stem and therefore might be sequestered from the ribosome. It is of interest, then, that the AUG in the PreproPTH sequence may be part of a small interior loop in an extensive stem structure involving nucleotides 1–20 and 34–51 [$\Delta G = -15.6$ kcal, including eight consecutive nucleotide pairs at the base of the stem (29)]. Alternatively, the AUG at position 10–13 might serve as an initiator codon for a pentapeptide terminated by UGA at position 25–27.

The sequence AUCCU at position 83–87 may bind to the sequence UAGGA close to the 3′ end of 18S ribosomal RNA. Hagenbüchle and coworkers (33) have pointed out that most eukaryotic mRNAs contain sequences that could bind to this highly conserved purine-rich sequence in 18S RNA, thus favoring initiation of protein synthesis.

We thank Alfred Bothwell, Argiris Efstratiadis, Alan Maxam, Bryan Roberts, and Richard Roberts for their help and advice. We also thank J. Simpson for help in preparing the manuscript. This work was supported by National Institutes of Health Grants AM 21529-01 and AM 11794 and National Science Foundation Grant PCM 76-20603.

1. Sherwood, L. M., Hanley, D. A., Takatsuki, K., Birmbaumer, M. E., Schneider, A. B. & Wells, S. A., Jr. (1978) in *Endocrinology of Calcium Metabolism*, eds. Copp, D. H. & Talmage, R. V. (Excerpta Medica, Amsterdam), pp. 301–307.
2. Habener, J. F., Potts, J. R., Jr. & Rich, A. (1976) *J. Biol. Chem.* **251**, 3893–3899.
3. Habener, J. F., Rosenblatt, M., Kemper, B., Kronenberg, H. M., Rich, A. & Potts, J. T., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2616–2620.
4. Curtiss, R., III, Pereiva, D. A., Hsu, J. C., Hull, S. C., Clark, J. E., Maturin, L. J., Goldschmidt, R., Moody, R., Inoue, M. & Alexander, L. (1977) in *Recombinant Molecules: Impact on Science and Society*, eds. Beers, R. F., Jr. & Bassett, E. G. (Raven, New York), pp. 45–56.
5. Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heyneker, H. L., Boyer, H. W., Crosa, J. H. & Falkow, S. (1977) *Gene* **2**, 95–113.
6. Clewell, D. B. & Helsinki, D. R. (1970) *Biochemistry* **9**, 4428–4440.
7. Kronenberg, H. M., Roberts, B. E., Habener, J. F., Potts, J. T., Jr. & Rich, A. (1977) *Nature (London)* **267**, 804–807.
8. Rose, J. K. & Lodish, H. F. (1976) *Nature (London)* **262**, 32–37.
9. Friedman, F. Y. & Rosbash, M. (1977) *Nucleic Acids Res.* **4**, 3455–3471.
10. Efstratiadis, A., Maniatis, T., Kafatos, F. C., Jeffrey, A. & Vournakis, J. N. (1975) *Cell* **4**, 367–378.
11. Efstratiadis, A., Kafatos, F. C., Maxam, A. & Maniatis, T. (1976) *Cell* **7**, 279–288.
12. Rose, J. K., Lodish, H. F. & Brock, M. L. (1977) *J. Virol.* **21**, 683–693.
13. Humphries, P., Cochet, M., Krust, A., Gerlinger, P., Kourilsky, P. & Chambon, P. (1977) *Nucleic Acids. Res.* **4**, 2389–2406.
14. Roychoudhury, R., Jay, E. & Wu, R. (1976) *Nucleic Acids Res.* **3**, 101–116.
15. Villa-Komaroff, L., Efstratiadis, A., Broome, S., Lomedico, P., Tizard, R., Naber, S., Chick, W. L. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3727–3731.
16. Patterson, B. M., Roberts, B. E. & Kuff, E. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4370–4374.
17. Maniatis, T., Jeffrey, A. & van deSande, H. (1975) *Biochemistry* **14**, 3787–3794.
18. Maxam, A. M. & Gilbert, W. (1979) *Methods Enzymol.*, in press.
19. Sanger, F., Nicklen, S. & Coulson A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
20. Berkner, K. L. & Folk, W. R. (1977) *J. Biol. Chem.* **252**, 3176–3184.
21. Kemper, B., Habener, J. F., Ernst, M. D., Potts, J. T., Jr. & Rich, A. (1976) *Biochemistry* **15**, 20–25.
22. Davis, R. (1980) in *Methods Enzymol.*, in press.
23. Sharp, P., A., Sugden, B. & Sambrook, J. (1973) *Biochemistry* **12**, 3055–3063.
24. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
25. Brewer, H. B., Jr., Fairwell, T., Rittel, W., Littledike, T. & Arnaud, C. (1974) *Am. J. Med.* **56**, 759–766.
26. Nakanishi, S., Inoue, A., Kita, T., Nakamura, M., Chang, A. C. Y., Cohen, S. N. & Numa, S. (1979) *Nature (London)* **278**, 423–427.
27. Cohn, D. V., Smardo, F. L., Jr. & Morrissey, J. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1469–1471.
28. Kozak, M. (1978) *Cell* **15**, 1109–1124.
29. Tinoco, I., Borer, P. N., Dengler, B., Levine, M. D., Uhlenbeck, O. C., Crothers, D. M. & Gralla, J. (1973) *Nature (London) New Biol.* **246**, 41–42.
30. Shine, J., Seeburg, P. H., Martial, J. A., Baxter, J. D. & Goodman, H. M. (1977) *Nature (London)* **270**, 494–499.
31. Stolarsky, L. & Kemper, B. (1978) *J. Biol. Chem.* **253**, 7194–7201.
32. Sutcliffe, J. G. (1978) *Nucleic Acids Res.* **5**, 2721–2728.
33. Hagenbüchle, O., Santer, M., Steitz, J. A. & Mans, R. J. (1978) *Cell* **13**, 551–564.
34. Niall, H. B., Keutmann, H. T., Sauer, R., Hogan, M. L., Dawson, B. F., Auerbach, G. B. & Potts. J. T., Jr. (1970) *Hoppe-Seyler's Z. Physiol. Chem.* **351**, 1586–1588.