Proc. Natl. Acad. Sci. USA
Vol. 76, No. 8, pp. 3819–3823, August 1979
Biochemistry

Primary structure of the NH_2-terminal extra piece of the precursor to human placental lactogen

(placental lactogen mRNA/cell-free translation of mRNA/amino acid sequence of cell-free product/sequence homology of pre-hormones/secretory proteins)

LOUIS M. SHERWOOD*†, YIGAL BURSTEIN‡, AND ISRAEL SCHECHTER*

Departments of *Chemical Immunology and ‡Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel

Communicated by Donald F. Steiner, May 31, 1979

ABSTRACT **The cell-free translation product of human placental lactogen mRNA is a precursor molecule larger than the mature hormone that circulates in plasma. To determine the structure of pre-placental lactogen, the poly(A)-rich RNA fraction of term placenta was isolated and translated in a wheat germ cell-free system. The mRNA programmed the synthesis of a major protein, 3000 daltons larger than placental lactogen, that was specifically precipitated by hormone antibodies. The immunoprecipitated protein was labeled separately with 20 radioactive amino acids and subjected to sequence analysis. The results showed the synthesis of pre-placental lactogen in which an extra piece 25 residues long preceded the NH_2 terminus of the mature protein. The structure of the extra piece is as follows: Met-Pro-Gly-Ser-Arg-Thr-Ser-Leu-Leu-Leu-Ala-Phe-Ala-Leu-Leu-Cys-Leu-Pro-Trp-Leu-Gln-Glu-Ala-Gly-Ala-. Met^1 is the initiator residue because only initiator [^{35}S]Met-$tRNA_1^{Met}$, but not internal [^{35}S]Met-$tRNA_2^{Met}$, donated NH_2-terminal methionine. The structure of the extra piece showed little homology with that of unrelated hormones but striking homology (64%) with the extra piece of rat pre-growth hormone. Most amino acid substitutions involved a single base change in the codon. Mature human placental lactogen and rat growth hormone have 59% homology in sequence. Thus, our findings provide additional evidence to support the common evolutionary origin of these hormones, not only of the mature proteins but also of the extra piece segments.**

Human placental lactogen (hPL), a single-chain polypeptide hormone of 191 amino acids, is derived from the syncytiotrophoblast layer of the placenta, where it is synthesized and released in increasing amounts throughout gestation (1, 2). There is specific enrichment of the mRNA for hPL by 4- to 5-fold during the growth and development of the placenta from first trimester to term (3). The biologic function of hPL has not been defined clearly, but it is thought to have important effects on lactation and maternal metabolism during pregnancy (2). There are similarities in lactogenic and immunologic activity of hPL and human growth hormone (hGH) (1, 4), and there is 84% homology in the amino acid sequences of the two hormones (5–7).

Placental lactogen, like other polypeptide hormones, is synthesized through a precursor molecule (pre-hPL) somewhat larger than mature hPL (8). Messenger RNA isolated from term placenta directs the cell-free synthesis of pre-hPL, which can be converted during synthesis to the mature hormone by addition to the cell-free extract of endoplasmic membranes (8). We have now determined the complete amino acid sequence of the NH_2-terminal extra piece (Xp) of pre-hPL and compared its structure with the Xp segments of related hormones. The structure of the Xp we determined had important differences from the partial sequence reported by Birken *et al.* (9).

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.

MATERIALS AND METHODS

Materials. Highly purified hPL was obtained through the courtesy of Lederle Laboratories (Pearl River, NY). [^{35}S]Met (800 Ci/mmol), [^{14}C]Gly (114 mCi/mmol), [^{3}H]Ala (35 Ci/mmol), [^{3}H]Arg (8.5 Ci/mmol), [^{3}H]Asp (12 Ci/mmol), [^{3}H]Gln (29 Ci/mmol), [^{3}H]Glu (28 Ci/mmol),[^{3}H]His (10 Ci/mmol), [^{3}H]Ile (17 Ci/mmol), [^{3}H]Leu (38 Ci/mmol), [^{3}H]Lys (18 Ci/mmol), [^{3}H]Phe (15.6 Ci/mmol), [^{3}H]Pro (117 Ci/mmol), [^{3}H]Ser (15 Ci/mmol), [^{3}H]Thr (12.1 Ci/mmol), [^{3}H]Tyr (22 Ci/mmol), and [^{3}H]Val (15.3 Ci/mmol) were obtained from The Radiochemical Centre (Amersham, England); [^{35}S]Cys (522 Ci/mmol) and [^{3}H]Trp (18.6 Ci/mmol) were from New England Nuclear. [^{3}H]Asn (22 Ci/mmol) was from Schwarz/Mann. The [^{35}S]cystine was reduced to [^{35}S]cysteine prior to translation (10).

Preparation of mRNA. Sterile term placentas, obtained from Caesarian section, were kept on ice and transported to the laboratory within minutes after delivery. Trophoblastic tissue was washed free of blood with 0.15 M sodium chloride/10 mM sodium phosphate, pH 7.4, dissected free of surrounding membranes, and immediately frozen in liquid N_2 to be stored at −80°C prior to extraction. Total cellular RNA was extracted from about 30 g of term placental tissue by a modification (11) of the method of Kirby (12). About 0.5 mg of RNA ($A_{260/280}$ = 2) was obtained from 1 g of term placental tissue. Total cellular RNA was purified further on oligo(dT)-cellulose to yield poly(A)-rich RNA that served as the mRNA (13). The mRNA comprised 1–2% of the total RNA.

[^{35}S]Met-$tRNA^{Met}$. The [^{35}S]Met-$tRNA^{Met}$ species of wheat germ that transfer methionine to the NH_2-terminal (initiator Met-$tRNA_1^{Met}$) and internal (internal Met-$tRNA_2^{Met}$) positions in proteins were prepared as described (14). Cross contamination of one $tRNA^{Met}$ species by the other was undetectable when tested by translation of natural mRNAs and sequence analyses of the cell-free products (14).

Cell-Free Translation of mRNA. Translation of placental mRNA was performed with wheat germ extract (15) as described (16). Typically, 1–2.5 μg of mRNA was translated in a 40-μl volume of the translation system. When [^{35}S]Met-$tRNA^{Met}$ was used as the sole source of labeling, the reaction mixture was supplemented to 50 μM of unlabeled methionine to prevent incorporation of [^{35}S]Met released from the charged $tRNA^{Met}$ species (14).

Immunoprecipitation of Translation Products. Antibodies to highly purified hPL were prepared by serial immunization

Abbreviations: hPL, human placental lactogen; pre-hPL, pre-placental lactogen; hGH, human growth hormone; pre-GH, pre-growth hormone; Xp, extra piece; $NaDodSO_4$, sodium dodecyl sulfate.

† Present address and address for reprint requests: Department of Medicine, Michael Reese Hospital, Chicago, IL 60616.

of goats by using Freund's adjuvant. Anti-goat Ig was prepared in rabbits. Purified antibodies to hPL and goat Ig were prepared by means of specific immune adsorbents (17).

To the translation products from three reaction mixtures (120 μl) were added unlabeled amino acid (to 3 μM) and 1.3 ml of a solution that contained 10 mM Tris·HCl (pH 7.4), 0.15 M NaCl, 10 mM EDTA, 0.5% Triton X-100, 0.5% sodium desoxycholate, and 3 mg of gelatin per ml. After centrifugation at 10,000 × *g* for 15 min, 63 μg of goat anti-hPL or normal goat Ig was added to the clear supernatant. After 1 hr at 4°C, 750 μg of rabbit antibody to goat Ig was added, and the mixture was kept overnight at 4°C. The immunoprecipitated material was collected by centrifugation, washed twice with phosphate-buffered saline and once with water, and dissolved in 0.5 ml of 40% acetic acid for sequence analyses. The immune precipitate of cell-free products labeled with [^{35}S]Cys was completely reduced and alkylated in 6 M guanidine·HCl as described (10).

Amino Acid Sequence Analysis. The total cell-free products labeled with one radioactive amino acid at a time were analyzed in the Beckman model 890 C automatic sequencer as described (17). The radioactive samples were supplemented with 5 mg of sperm whale apomyoglobin carrier. Repetitive yields of the protein carrier and of the radioactively labeled protein ranged between 90 and 93%. The sequences of all samples were determined twice. In duplicate analyses, the pattern of radioactive peaks was identical.

RESULTS

Placental mRNA purified by oligo(dT) chromatography caused a 6- to 12-fold stimulation of [^{35}S]Met incorporation in a wheat germ cell-free system. Analysis of the translation products by sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis showed a number of radioactive proteins, but there was a dominant band of about 25,000 daltons (Fig. 1 lane A). When the cell-free products were reacted with goat antibodies to hPL, 25% of the radioactive proteins precipitated. Control incubation with normal goat Ig showed only 3–4% precipitation. $NaDodSO_4$ gel analysis of the products precipitated by anti-hPL showed a single radioactive band (Fig. 1 lane B) in the same region as the dominant band (Fig. 1 lane A). The protein recognized by specific antibodies to hPL was about 3000 daltons larger than authentic hPL marker run on the same gel. The sequence data shown below indicated that the specifically precipitated protein was the precursor of hPL (pre-hPL).

Results from sequence analyses of immunoprecipitated cell-free products showed radioactive peaks (Fig. 2) that correspond to the following amino acids at the positions indicated: methionine at position 1, proline at positions 2 and 18, glycine at positions 3 and 24, serine at positions 4 and 7, arginine at position 5, threonine at position 6, leucine at positions 8, 9, 10, 14, 15, 17, and 20, alanine at positions 11, 13, 23, and 25, phenylalanine at position 12, cysteine at position 16, tryptophan at position 19, glutamine at position 21, and glutamic acid at position 22 (Fig. 2). The primary structure derived from the radioactive sequence data is given in Fig. 3. After the first 25 residues (from Met^{-25} to Ala^{-1}), all 12 residues identified in the cell-free product (Val^{1} to Leu^{9} and His^{12}, Met^{14}, and Leu^{15}) match the corresponding residues at the NH_2 terminus of mature hPL (from Val^{1} to Leu^{15}) (5). These findings established the cell-free synthesis of pre-hPL in which an Xp, 25 residues long, preceded the NH_2 terminus of the mature protein. Flat and low backgrounds of radioactivity were obtained from sequencer runs (of 28 degradative cycles) of protein products labeled with aspartic acid, asparagine, histidine, isoleucine, lysine, and tyrosine, thus showing that these amino acids are not present in the Xp of pre-hPL. Valine was not identified in the Xp, but was at sequence positions 26 and 29, corresponding to residues 1 and 4 of the mature hormone.

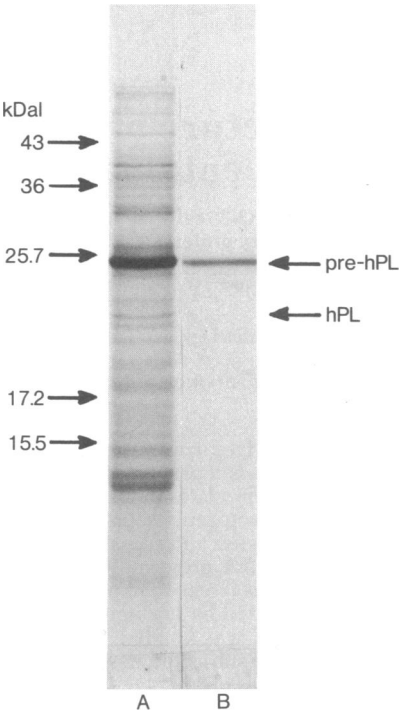

FIG. 1. Autoradiograph of $NaDodSO_4$/polyacrylamide gel (13%) electrophoresis (18) of proteins translated in a cell-free system by mRNA from term placenta in the absence (lane A) and presence (lane B) of specific antibodies to hPL. Marker proteins from 43,000 to 15,500 daltons were ovalbumin, glyceraldehyde phosphate dehydrogenase, chymotrypsinogen, myoglobin, and hemoglobin, respectively. kDal, kilodalton.

To ascertain the size of the Xp (i.e., to rule out the possibility that Met^{1} was preceded by another methionine or a peptide that was rapidly cleaved), we demonstrated that Met^{1} was the initiator residue. Experiments summarized in Fig. 4 showed that insertion of [^{35}S]Met into the NH_2-terminal position occurred with the initiator [^{35}S]Met-$tRNA_1^{Met}$, but not with the internal [^{35}S]Met-$tRNA_2^{Met}$.

DISCUSSION

The complete amino acid sequence of the NH_2-terminal Xp segment of pre-hPL was determined by radiosequencing. The preparation of mRNA used programmed radioactive pre-hPL as the major product of translation. With specific antiserum to hPL, a single radioactive band was identified by $NaDodSO_4$/polyacrylamide gel electrophoresis that comprised at least 25% of the total translation products. Because immunoprecipitation resulted in a single labeled protein, more highly purified mRNA was not required for amino acid sequence determination.

In order to establish the authenticity of pre-hPL in the immune precipitate that was subjected to sequence analysis, the following measures were used: (*i*) Translation studies were performed with only a single radioactive amino acid at a time, and each of the 20 amino acids was analyzed at least twice. The sequence data from analyses of labeled precursor were unambiguously interpreted as a distinct sequence without double occupancy at any position. (*ii*) Residues 1–9, 12, 14, and 15 in the mature hPL sequence, which were analogous to pre-hPL residues 26–35, 37, 39, and 40, were correctly identified in prolonged sequencer runs (5). (*iii*) In each analysis in which

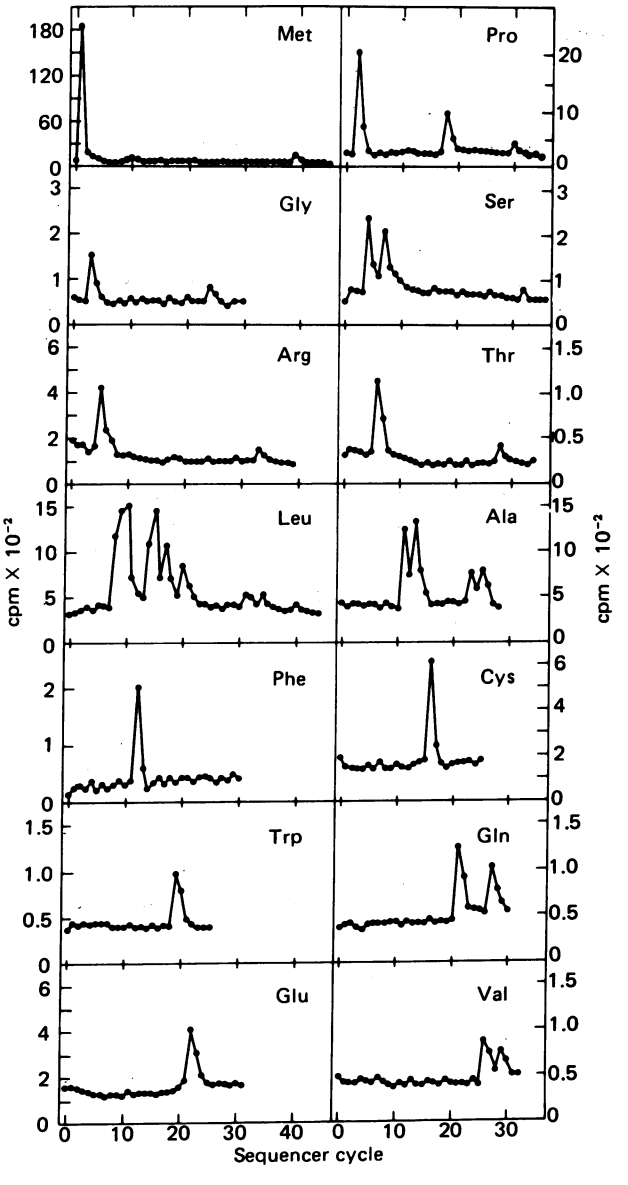

FIG. 2. Radioactivity recovered at each sequencer cycle from the cell-free products programmed by term placental mRNA after specific immune precipitation with anti-hPL antibodies. The product was labeled with the following radioactive amino acids (numbers in parentheses represent cpm in the sample analyzed): [^{35}S]Met (212,500), [^{3}H]Pro (72,700), [^{14}C]Gly (7000), [^{3}H]Ser (12,100), [^{3}H]Arg (37,400), [^{3}H]Thr (8200), [^{3}H]Leu (165,800), [^{3}H]Ala (49,000), [^{3}H]Phe (43,800), [^{35}S]Cys (10,000), [^{3}H]Trp (10,000), [^{3}H]Gln (12,300), [^{3}H]Glu (35,400), and [^{3}H]Val (47,800). Background radioactivity obtained from sequencer run of the control sample was subtracted. The control sample consisted of wheat germ extract containing each of the labeled amino acids, but without added mRNA (17). Cycle zero represents a blank cycle (without phenylisothiocyanate) which was used to wash out potential radioactive contaminants.

labeled residues were identified in the mature hormone sequence, the length of the Xp was identified unambiguously as 25 amino acids. (*iv*) Labeling with initiator Met-tRNA$_1^{Met}$ and internal Met-tRNA$_2^{Met}$ confirmed the length of 25 amino acids and eliminated the possibility of an NH_2-terminal Met-Met sequence as in pre-proparathyroid hormone (19). (*v*) Semilogarithmic graphs for residues that were present more than once in the identified sequence confirmed the analysis of a single protein (20).

The complete sequence we determined had several important differences from the partial sequence reported earlier by Birken *et al.* (9). A total of seven leucine residues were identified in the Xp at positions 8, 9, 10, 14, 15, 17, and 20, while the leucine at position 20 was not identified in the earlier report. Furthermore, proline was readily identified by us at positions 2, 18, and 30, whereas they noted proline at residues 2, 19, and 31. Thus, Birken *et al.* (9) suggested a possible length for the Xp of 26 residues, while our data unequivocally established the peptide as 25 residues in length.

Ten of the 13 amino acid residues from positions 8–20 in the

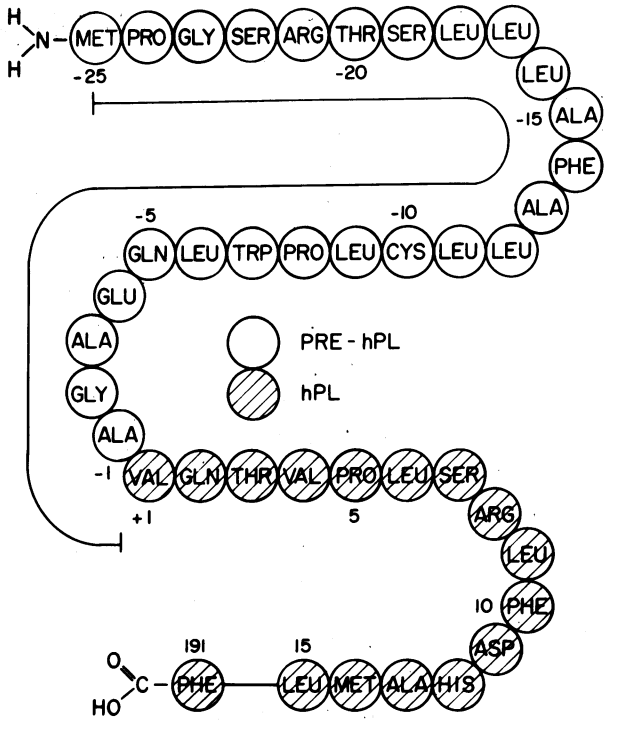

FIG. 3. Amino acid sequence of the NH_2-terminal Xp of human pre-hPL determined by radioactive sequencing of the immune precipitate of translated term placental mRNA. Open residues indicate the sequence of the Xp determined and hatched residues, the hPL sequence (residues 1–9, 12, 14, and 15 in hPL were confirmed by prolonged sequencer runs).

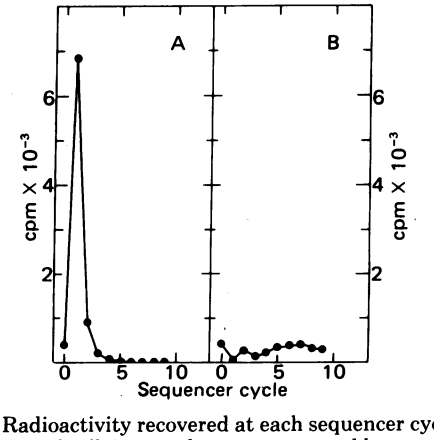

FIG. 4. Radioactivity recovered at each sequencer cycle for immune-precipitated cell-free products programmed by term placental mRNA. The protein products were labeled with (*A*) initiator [^{35}S]Met-tRNA$_1^{Met}$ (30,000 cpm) and (*B*) internal [^{35}S]Met-tRNA$_2^{Met}$ (228,000 cpm). Background radioactivity obtained from sequencer run of the control sample was subtracted. Cycle zero represents a blank cycle (without added phenylisothiocyanate) which was used to wash out potential radioactive contaminants.

Xp could be classified as hydrophobic, making this Xp analogous to that of similar sequences in polypeptide hormones and a variety of other secretory proteins (19, 21–29). From the criteria developed by Segrist and Feldman (30), this region would have a calculated hydrophobicity index of 3.04. The precise function of the Xp is not completely clear, although current theory favors the close association of this hydrophobic peptide region with endoplasmic reticular membranes (31). The Xp is believed to be involved in translocating the secretory protein into the subcellular cisternae, during the course of which the Xp is cleaved off (32).

The earlier studies of Szczesna and Boime (8) established clearly that hPL was synthesized as a higher molecular weight precursor molecule that was cleaved in the presence of membranes to the mature hormone. Similar biosynthetic precursors have been identified for a number of polypeptide hormones, such as insulin (21), growth hormone (22), prolactin (23), adrenocorticotrophin (24), and parathyroid hormone (19). In general, there has been little or no sequence homology between the Xp of one hormone and that of another, except for the general characteristics of hydrophobicity and approximate length common to all Xp segments.

Earlier studies of the structure of hPL by our own group (5) and others (6, 7) showed 84% homology between the sequence of hPL and that of hGH. The two molecules share immunologic crossreactivity and identical lactogenic activity, although hPL is much less potent as a growth-promoting agent (2). Human prolactin has only 16% structural homology with hGH and 13% with hPL (33), although all three hormones share lactogenic activity in similar bioassay or membrane receptor systems (34). Although the overall homology of prolactin with the other two hormones is modest, there are homologous internal sequences that suggest that the three hormones may have resulted from duplication of a common ancestral gene (35).

It was therefore of considerable interest to compare the amino acid sequence of the Xp of pre-hPL with that of GH and prolactin. No data have yet been published on the structure of the Xp of hGH or the human prolactin precursor. However, the Xp of both rat pre-GH (36) and pre-prolactin (23) is known. The structure of rat GH precursor (pre-GH) was deduced by determining the nucleotide sequence of cloned DNA complementary to the mRNA of rat GH (36). The Xp of rat pre-GH consists of 26 amino acids, beginning with methionine and ending with alanine (see Fig. 5). When this sequence was compared with that of pre-hPL, striking homology was noted, with 16 of 25 amino acids (64%) being identical, assuming a codon deletion in pre-hPL corresponding to position of 2 of rat GH Xp. The nine amino acid substitutions did not significantly change the hydrophobic or charge characteristics of the Xp,

Table 1. Comparison of the known mRNA codons of rat pre-GH Xp (36) with theoretical codons for amino acid substitutions in pre-hPL involving least number of changes (38)

Residue no., pre-hPL	Amino acid		Codon, pre-GH	Theoretical codon for pre-hPL with least changes*
	Pre-GH	Pre-hPL		
2	Ala	Pro	GCA	CCA (1)
3	Asp	Gly	GAC	GGC (1)
5	Gln	Arg	CAG	CGG (1)
7	Pro	Ser	CCC	UCC (1)
8	Trp	Leu	UGG	UUG (1)
11	Thr	Ala	ACC	GCC (1)
13	Ser	Ala	AGC	GCC (2)
18	Leu	Pro	CUG	CCG (1)
20	Pro	Leu	CCU	CUU (1)

Amino acid and codon substitutions are shown for pre-hPL and rat pre-GH.
* Numbers in parentheses refer to number of base changes.

except for the additional negative charge in pre-GH associated with aspartic acid at position 3 instead of glycine. The hydrophobicity index for the analogous segment of rat pre-GH was 3.12. In contrast, the sequence of rat pre-prolactin contained an Xp of 29 amino acids which had little homology with either rat GH precursor (23%) or pre-hPL (20%). The homologous alignment with the Xp of pre-hPL was not significantly different from the degree of homology found for totally unrelated proteins (19, 25).

Although Shine *et al.* (37) determined the nucleotide sequence of most of the complementary DNA from hPL mRNA, their synthetic DNA was not long enough to include the coding region for the Xp. When the codons known to be present in the RNA sequence of rat pre-GH Xp (36) were compared with the codons needed to produce the amino acids present in pre-hPL, all but one could be accounted for by a single base change (Table 1) (38). When the nine substitutions were evaluated by their likelihood of occurrence, four were highly favored (positions 2, 5, 11, and 13), one was moderately favored (position 7), one was slightly favored (position 3), and three were not favored (positions 8, 18, and 20) (38).

The above findings can be interpreted in terms of the proposed evolutionary origin of the three hormones from a common gene (35). Prolactin, on the basis of large sequence differences, was thought to have diverged from GH approximately 350 million years ago, well before mammalian radiation (38). For example, prolactin is clearly identified as a separate and important hormone in fish (39). The genes for GH and PL diverged much more recently, at an unknown point after mammalian radiation 75 million years ago. The homology present

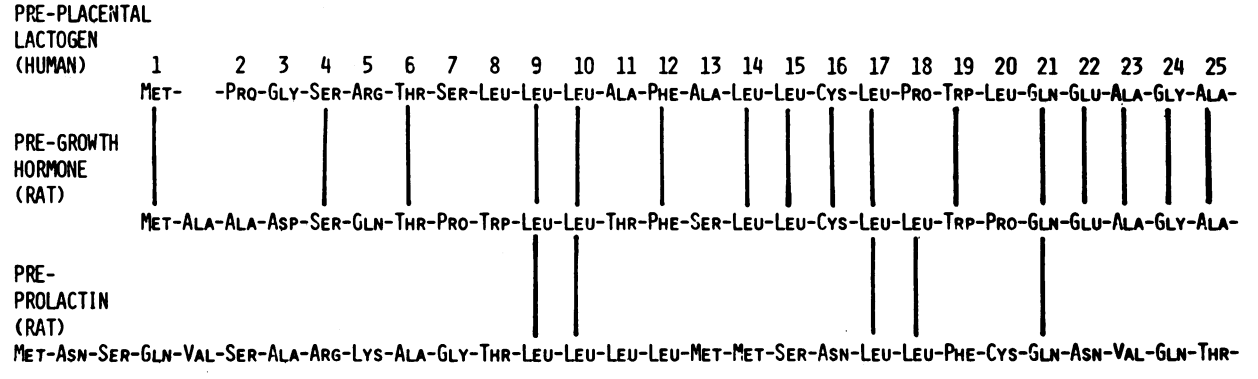

FIG. 5. Comparison of the amino acid sequences of the Xp segments of pre-hPL, rat pre-GH (36), and rat pre-prolactin (23). Vertical bars refer to homologous residues. Numbered residues refer to pre-hPL sequence.

for the Xp in the three hormones is consistent with the degree of homology for the mature hormones. Unfortunately, data on the precursors of hGH and human prolactin are not available, and the current comparisons were performed on a human hormone compared with rat hormones. hPL shows 59% structural homology with rat GH and 64% with its Xp as opposed to 12% homology with rat prolactin and 20% with its Xp. Further data on the structure of the Xp for higher mammalian precursors for GH, PL, and prolactin are needed to amplify these observations. It is of interest, however, that partial sequence data for the Xp of bovine growth hormone precursor shows homology at 9 of the 11 positions so far determined with our structure of pre-hPL (40). These correlations strongly indicate a coevolutionary origin for the DNA coding for the Xp and the mature portions of the hormone precursor.

We thank Mrs. Ida Oren for excellent assistance and Mrs. Zillah Cohen for excellent typing. L.M.S. was on sabbatical leave in the Department of Chemical Immunology at the Weizmann Institute of Science. The research was supported by Grants HD08225 and CA20817 from the U.S. Public Health Service. L.M.S. was a faculty scholar of the Josiah Macy, Jr., Foundation, New York.

1. Josimovich, J. B. & MacLaren, J. A. (1962) *Endocrinology* **71,** 209–220.
2. Grumbach, M. M., Kaplan, S. L., Sciarra, J. J. & Burr, I. M. (1968) *Ann. N. Y. Acad. Sci. USA* **148,** 501–506.
3. Boime, I., McWilliams, D., Szczesna, E. & Camel, M. (1976) *J. Biol. Chem.* **251,** 820–825.
4. Friesen, H. G. (1965) *Endocrinology* **76,** 369–381.
5. Sherwood, L. M., Handwerger, S., McLaurin, W. D. & Lanner, M. (1971) *Nature (London) New Biol.* **233,** 59–61.
6. Li, C. H., Dixon, S. & Chung, D. (1971) *Science* **173,** 56–59.
7. Niall, H. D., Hogan, M. L., Tregear, G. W., Segre, G. V., Huang, P. & Friesen, H. (1973) *Recent Prog. Horm. Res.* **29,** 387–416.
8. Szczesna, E. & Boime, I. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1179–1183.
9. Birken, S., Smith, V. L., Canfield, R. E. & Boime, I. (1977) *Biochem. Biophys. Res. Commun.* **74,** 106–112.
10. Schechter, I. & Burstein, Y. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3273–3277.
11. Gorecki, M. & Zeelon, E. P. (1979) *J. Biol. Chem.* **254,** 525–529.
12. Kirby, K. S. (1968) *Methods Enzymol.* **123,** 87–99.
13. Schechter, I. (1974) *Biochemistry* **13,** 1875–1885.
14. Zemell, R., Burstein, Y. & Schechter, I. (1978) *Eur. J. Biochem.* **89,** 187–193.
15. Roberts, B. & Paterson, B. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2330–2334.
16. Burstein, Y. & Schechter, I. (1976) *Biochem. J.* **157,** 145–151.
17. Schechter, I. & Burstein, Y. (1976) *Biochem. J.* **153,** 545–550.
18. Maizel, J. V. (1972) in *Methods in Virology,* eds. Maramorosch, K. & Koprowski, H. (Academic, New York), Vol. 5, pp. 179–246.
19. Habener, J. F., Rosenblatt, M., Kemper, D., Kronenberg, H. M., Rich, A. & Potts, J. T., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2616–2620.
20. Smithies, O., Gibson, D., Fanning, E. M., Goodfliesh, R. M., Gilman, J. G. & Ballantyne, D. L. (1971) *Biochemistry* **10,** 4912–4921.
21. Chan, S. J., Keim, P. & Steiner, D. F. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1964–1968.
22. Sussman, P. M., Tushinski, R. J. & Bancroft, F. C. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 29–33.
23. McKean, D. J. & Maurer, R. A. (1978) *Biochemistry* **17,** 5215–5219.
24. Mains, R. E., Eipper, B. A. & Ling, N. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3014–3018.
25. Burstein, Y. & Schechter, I. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 716–720.
26. Devillers-Thiery, A., Kindt, T., Schele, G. & Blobel, G. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 5016–5020.
27. Mercier, J. C., Haze, G., Gaye, P. & Hue, D. (1978) *Biochem. Biophys. Res. Commun.* **82,** 1236–1245.
28. Thibodeau, S. N., Palmiter, R. D. & Walsh, K. A. (1978) *J. Biol. Chem.* **253,** 9018–9023.
29. Strauss, A. W., Bennett, C. D., Donohue, A. M., Rodkey, J. A. & Alberts, A. W. (1977) *J. Biol. Chem.* **252,** 6846–6855.
30. Segrist, J. T. & Feldman, R. J. (1974) *J. Mol. Biol.* **87,** 853–858.
31. Blobel, G. & Sabatini, D. D. (1971) *Biomembranes* **2,** 193–195.
32. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67,** 835–851.
33. Shome, B. & Parlow, A. F. (1977) *J. Clin. Endocrinol. Metab.* **45,** 1112–1115.
34. Sherwood, L. M., Handwerger, S., McLaurin, W. D. & Pang, E. C. (1971) in *Growth and Growth Hormone,* eds. Pecile, A. & Müller, E. E. (Excerpta Med., Amsterdam), pp. 209–223.
35. Niall, H. D., Hogan, M. L., Sauer, R., Rosenblum, I. Y. & Greenwood, F. C. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 866–869.
36. Seeburg, P. H., Shine, J., Martial, J. A., Baxter, J. D. & Goodman, H. M. (1977) *Nature (London)* **270,** 486–494.
37. Shine, J., Seeburg, P. H., Martial, J. A., Baxter, J. D. & Goodman, H. M. (1977) *Nature (London)* **270,** 494–499.
38. Dayhoff, M. O. (1972) *Atlas of Protein Sequence and Structure,* Vol. 5, (Natl. Biomed. Res. Found., Washington, DC), pp. 89–99.
39. Sherwood, L. M. (1971) *N. Engl. J. Med.* **284,** 774–779.
40. Lingappa, V. R., Devillers-Theiry, A. & Blobel, G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2432–2436.