Pre-proparathyroid Hormone: Fidelity of the Translation of Parathyroid Messenger RNA by Extracts of Wheat Germ†

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ABSTRACT: Pre-proparathyroid hormone is the major protein synthesized in wheat-germ extracts in response to addition of an 8–15S fraction of parathyroid RNA. The accuracy of the translation of the mRNA from parathyroid tissue was examined by analysis of the carboxyl-terminal tryptic peptide and the amino-terminal amino acid of the protein, by analysis of the size distribution of the mRNA, and by translation of the mRNA in a second cell-free extract. When 8–15S RNA was fractionated on a sucrose gradient containing formamide, RNA that supported the synthesis of pre-proparathyroid hormone was present in a single symmetrical peak, suggesting that it was homogeneous. Analyses by paper chromatography and electrophoresis of the proline-containing tryptic peptides of pre-proparathyroid hormone indicate that they are identical with the corresponding proline-containing peptides of parathyroid hormone. Because the COOH-terminal tryptic peptide of parathyroid hormone contains proline, the data indicate that the COOH termini of pre-proparathyroid hormone and parathyroid hormone are identical. Methionine from initiator [^{35}S]Met-tRNA^{fMet} was rapidly incorporated into pre-proparathyroid hormone by the wheat-germ extract, and a single-step Edman degradation selectively removed almost all of the initiator [^{35}S]methionine present in pre-proparathyroid hormone. Translation of the 8–15S RNA in a cell-free extract from Krebs-II ascites cells resulted in a protein that comigrated with pre-proparathyroid hormone on sodium dodecyl sulfate–acrylamide gel electrophoresis. These data support the conclusion that the wheat-germ system accurately translates the mRNA for parathyroid hormone, and they strengthen the contention that pre-proparathyroid hormone is the initial biosynthetic product.

Pre-proparathyroid hormone (Pre-ProPTH)[1] was recently identified as the major translation product of bovine and human parathyroid RNA in extracts of wheat germ (Kemper et al., 1974; Habener et al., 1975). Pre-ProPTH contains tryptic peptides in common with both parathyroid hormone (PTH) and proparathyroid hormone (ProPTH) (Habener et al., 1975; Kemper et al., 1976) and contains 25 extra amino acids at the NH_2 terminus of ProPTH (Kemper et al., 1976). We suggested that Pre-ProPTH may be the initial product of the translation of PTH mRNA and is a precursor of both ProPTH and PTH (Kemper et al., 1974; Habener et al., 1975). The possibility, however, that Pre-ProPTH may be an artefactual product of the wheat-germ extract has not yet been eliminated. The fact that Pre-ProPTH has not been identified in intact cells of parathyroid tissue (Kemper et al., 1974; Habener et al., 1975), as well as reports of proteins larger than 25000 daltons that are immunoreactive to antisera against PTH (Wong and Lindall, 1973; Benson et al., 1974), could be interpreted to indicate that Pre-ProPTH may not be the initial precursor of PTH.

The synthesis of a protein in a cell-free system that is artefactually larger than the protein in vivo could occur either by initiation or by termination of chain growth at the wrong codon. The larger protein could result because the conditions of synthesis in vitro permit misreading of the initiator or terminator codons more easily than conditions in vivo do, or because the mRNA is partially degraded and lacks the proper initiator or terminator codons, thereby bringing about a false initiation or termination. For example, premature termination of chain growth was reported in mammalian cell-free systems (Boime and Leder, 1972; Kerr et al., 1972). In the present report, an examination of the COOH and NH_2 termini of Pre-ProPTH, the sedimentation behavior of denatured PTH mRNA, and the translation of PTH mRNA in Krebs-II ascites-cell extracts all suggest that the mRNA is being accurately translated in the wheat-germ extracts.

Methods

Isolation of RNA and Protein Synthesis. RNA sedimenting between 8 S and 15 S was isolated from the supernatant (centrifuged at 1000*g*) of bovine parathyroid tissue homogenates by extraction with phenol–chloroform–isoamyl alcohol as described previously (Kemper et al., 1974). The methods used for the isolation of wheat-germ extracts and translation of the mRNA in the wheat-germ extract were as described previously (Roberts and Paterson, 1973) with modifications (Kemper et al., 1974). The preparation of the Krebs-II ascites-cell extract and the conditions for the translation of mRNA were as described previously (Jacobs-Lorena and Baglioni, 1972), except that Tris-HCl was replaced with Hepes–HCl. The ascites-cell extract was a kind gift of Dr. Joan Ruderman. Radioactive amino acids and RNA added to the reactions are indicated in the figure legends. Trichloroacetic acid precipitates were prepared for

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[1] Abbreviations used are: PTH, parathyroid hormone; ProPTH, proparathyroid hormone; Pre-ProPTH, pre-proparathyroid hormone; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; [^{35}S]Met-tRNA^{fMet}, initiator transfer RNA from wheat germ charged with [^{35}S]methionine.

acrylamide gel electrophoresis as described previously (Kemper et al., 1974), except that residual Cl_3CCOOH was removed by two extractions with acetone–ether (1:1) instead of by lyophilization. The dried precipitate was dissolved directly in 0.1 *M* sodium phosphate (pH 7.2), 0.5% sodium dodecyl sulfate, 1% 2-mercaptoethanol, and 8 *M* urea, and heated to 100° for 1 min before analysis by sodium dodecyl sulfate–acrylamide gel electrophoresis, as described previously (Kemper et al., 1972), except that the gels contained 4 *M* urea instead of 8 *M* urea.

Isolation of PTH and Pre-ProPTH. Slices of parathyroid tissue were incubated at 37° as described previously (Kemper et al., 1972) with 8 μCi/ml of [^{14}C]proline (0.23 Ci/mmol) in Earle's Minimum Essential Medium (Grand Island Biological Co.). PTH was isolated after electrophoresis on acrylamide gels at pH 4 with 8 *M* urea as described previously (Kemper et al., 1972). Pre-ProPTH was isolated after electrophoresis on sodium dodecyl sulfate–acrylamide gels as described previously (Kemper et al., 1974) and was combined with the isolated PTH. Greater than 80% of the radioactive Pre-ProPTH isolated in this way migrated as a single peak upon acrylamide gel electrophoresis at pH 4 in 8 *M* urea (unpublished results, see Habener et al., 1975 for these data on human Pre-ProPTH). The proteins isolated from sodium dodecyl sulfate acrylamide gels were difficult to dissolve in aqueous solutions and apparently contained some residual sodium dodecyl sulfate. Therefore, the isolated proteins were dissolved in 0.15 *M* NH_4OH containing 4 *M* urea, adjusted to 0.30 *N* acetic acid, and chromatographed over a Bio-Gel-P-2 column preequilibrated with 0.15 *N* acetic acid. The samples eluting in the void volume were then lyophilized, the proteins were oxidized and digested with trypsin as described previously (Habener et al., 1975), and paper chromatography and electrophoresis were performed as described previously (Kemper et al., 1972).

Initiator Met-tRNA^{fMet}. The initiator [^{35}S]Met-tRNA^{fMet} was kindly provided by Ms. Joyce Heckman and Dr. U. L. Raj Bhandary. Initiator Met-tRNA^{fMet} was isolated from wheat germ as described previously (Ghosh et al., 1974) and charged with [^{35}S]methionine as described previously (Raj Bhandary and Ghosh, 1969) to a specific activity of 200 Ci/mmol. About 0.8 μCi of initiator [^{35}S]Met-tRNA^{fMet} was added to a 25-μl reaction mixture. To assay total trichloroacetic acid precipitable radioactivity, 1-μl aliquots were removed, and added to 0.1 ml of 0.2 N NaOH to deacylate the [^{35}S]Met-tRNA^{fMet}. Bovine serum albumin, 250 μg, was added and the sample was adjusted to 10% Cl_3CCOOH. The resulting precipitate was washed and analyzed for radioactivity as described previously (Kemper et al., 1974). For Edman degradation, a dried Cl_3CCOOH precipitate was prepared as described for acrylamide gel electrophoresis. The dried powder was dissolved in 0.3 *M* NH_4OH. An aliquot was removed and lyophilized. The lyophilized protein was then subjected to a single-step Edman reaction (Edman, 1960), and analyzed by sodium dodecyl sulfate–acrylamide gel electrophoresis.

Formamide Gradients. RNA was fractionated on sucrose gradients containing formamide as described previously (Anderson et al., 1974). Approximately 500 μg of 8S–15S RNA isolated from sucrose gradients without formamide, as described previously (Kemper et al., 1974), was precipitated with 2 vol of ethanol. The precipitate was dissolved in 0.2 ml of 1-X buffer (0.1 *M* LiCl, 0.05 *M* EDTA, 0.2% sodium dodecyl sulfate, and 0.01 *M* Tris-HCl (pH 7.4) containing 90% v/v formamide. The sample was heated to 37° for 5 min, and 0.2 ml of 1-X buffer without formamide was added. The sample was layered on a 12-ml, 5–20% sucrose gradient in 1-X buffer containing 50% formamide v/v and centrifuged for 40 hr in a Beckman SW41 rotor at 27000 rpm at 4°. Absorbance at 260 nm was recorded, and fractions of 0.5 ml were collected. The samples were adjusted to 0.45 *M* NaCl, 10 μg of yeast tRNA was added as carrier, and the RNA was precipitated at −20° overnight by adding 2 vol of ethanol. To wash the precipitated RNA, it was redissolved in 100 μl of H_2O, adjusted to 0.1 *M* NaCl, and reprecipitated with 2 vol of ethanol. The precipitate was washed twice with ethanol, dried, and redissolved in 50 μl of H_2O; 10 μl of each fraction was assayed in a wheat-germ reaction of 25 μl. The reactions were adjusted to 0.0625 *M* Tris-HCl (pH 6.8), 2.0% sodium dodecyl sulfate, 0.5% 2-mercaptoethanol, 0.001% bromophenol blue, and 10% glycerol. The samples were analyzed by electrophoresis on 15% polyacrylamide slab gels according to Laemmli (1970).

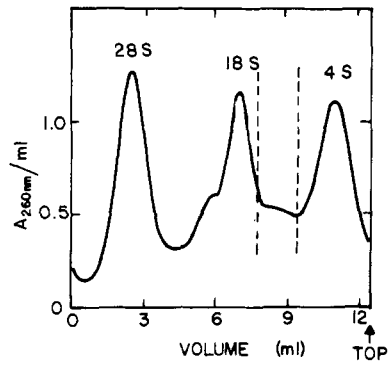

FIGURE 1: Analysis on sodium dodecyl sulfate–sucrose gradients of total parathyroid RNA. RNA was isolated from parathyroid tissue as described in Methods. The RNA was dissolved in 0.01 *M* Tris-HCl (pH 7.4), 0.5% sodium dodecyl sulfate, 0.1 *M* NaCl, and 1 m*M* EDTA, and layered over a gradient of 5–20% sucrose in the same buffer (Kemper et al., 1974). The samples were centrifuged in a Beckman SW41 rotor at 21000 rpm for 17 hr at 20°. The fraction of RNA between the dotted lines (approximately 8–15 S) was collected and concentrated by precipitation with two volumes of ethanol (Kemper et al., 1974).

Results

The analysis of a total parathyroid RNA preparation on a 5–20% sucrose gradient is shown in Figure 1. The RNA between the dotted lines, approximately 8S–15S, actively directs the synthesis of Pre-ProPTH (Kemper et al., 1974).

The distribution of PTH mRNA activity on sucrose gradients containing formamide is shown in Figure 2. The RNA analyzed on this gradient represents RNA sedimenting between 8 S and 15 S that had been fractionated previously (Figure 1) (Kemper et al., 1974). The RNA containing PTH mRNA activity was present in a single peak, with maximum activity in fractions 15 and 16. In addition, there was no major skewing of the PTH mRNA activity to the high molecular weight side, which might be expected if the synthesis of Pre-ProPTH was the result of the translation degradation products of an even larger mRNA. Several other protein bands with lesser amounts of incorporated radioactivity were observed migrating near Pre-ProPTH; the nature of these proteins is unknown. These data suggest that the mRNA activity for Pre-ProPTH resides in a discrete species of RNA that probably is not a degradation product of a larger in vivo mRNA.

[^{35}S]Methionine from initiator [^{35}S]Met-tRNA^{fMet} was rapidly incorporated into protein in wheat-germ extracts containing 8S–15S parathyroid mRNA (Figure 3). Essen-

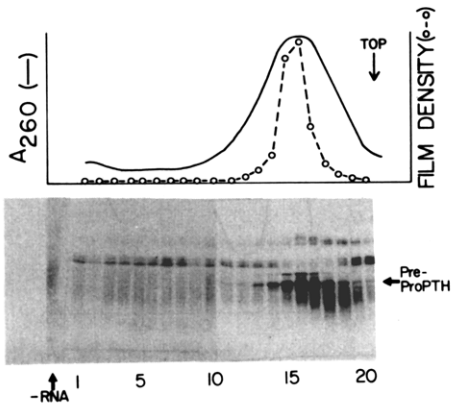

FIGURE 2: Fractionation of parathyroid RNA on sucrose gradients containing formamide and autoradiography of sodium dodecyl sulfate gels after electrophoresis of the [^{35}S]methionine-labeled translation products. An 8–15S fraction of RNA isolated on sucrose gradients without formamide was refractionated on a 5–20% sucrose gradient containing 50% formamide as described in Methods. The position of 18S rRNA (based on a parallel gradient) was approximately at fraction 10. The RNA in each fraction was assayed ([^{35}S]methionine incorporation) in the wheat-germ cell-free system, and the proteins synthesized were analyzed by electrophoresis in slab gels containing sodium dodecyl sulfate. An autoradiogram of the gel was prepared and is aligned with the gradient profile so that each gel sample corresponds to the appropriate fraction of RNA from the gradient. Background, or endogenous incorporation (−RNA), is unusually high in this experiment. The amount of Pre-ProPTH synthesized was estimated from a densitometry tracing of the autoradiograms of the gels. The autoradiogram shown is a 6-day exposure of Kodak RP Royal x-ray film. (—) A_{260} nm; (O) arbitrary units of radioactivity in Pre-ProPTH.

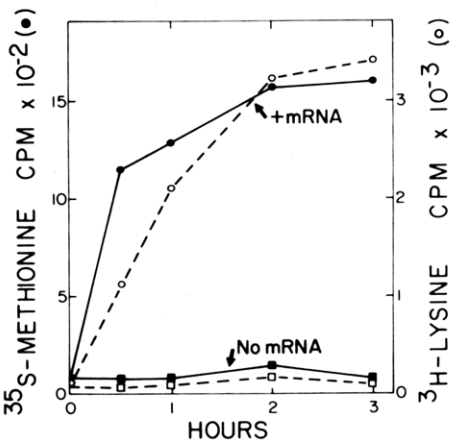

FIGURE 3: Incorporation of initiator [^{35}S]Met-tRNA^{fMet} and [^{3}H]lysine into protein. Wheat-germ reactions of 25 μl contained about 2 μg of parathyroid RNA, 0.8 μCi of initiator [^{35}S]Met-tRNA^{fMet}, and 10 μCi of [4,5-^{3}H]lysine (60 Ci/mmol). No parathyroid RNA was added to the RNA reaction. One-microliter aliquots were removed at the indicated times and assayed for trichloroacetic acid insoluble radioactivity, as described in Methods. The plotted values are the cpm present in 1 μl. (O - - - - O) [^{35}S]Lysine + RNA; (□ - - - - □) [^{3}H]lysine-RNA; (● — ●) [^{35}S]Met-tRNA^{fMet} + RNA; (■ — ■) [^{35}S]Met-tRNA^{fMet} − RNA.

tially no [^{35}S]methionine (<5%) was incorporated if RNA was not added to the reaction. Unlabeled methionine (40 μm) was added to prevent incorporation of [^{35}S]methionine released from tRNA into internal positions, and if [^{35}S]Met-tRNA^{fMet} was replaced by an equivalent amount of [^{35}S]methionine, incorporation was reduced by more than 90%. [^{3}H]Lysine was also added to the reaction to measure incorporation into internal amino acid postions. [^{35}S]Methionine is incorporated into protein more rapidly

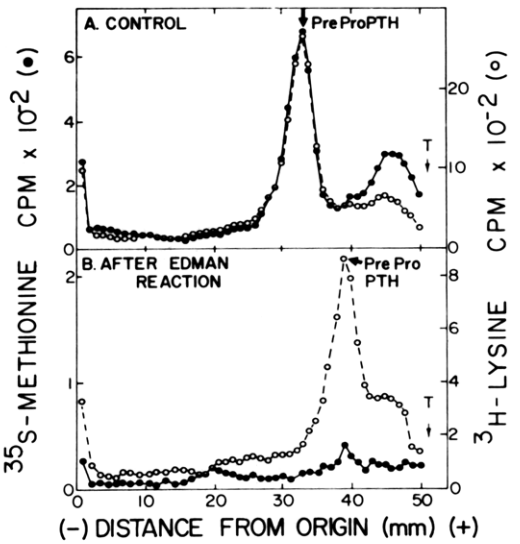

FIGURE 4: Sodium dodecyl sulfate gel electrophoresis of protein labeled with initiator [^{35}S]Met-tRNA^{fMet} and [^{3}H]lysine. A wheat-germ reaction was incubated for 3 hr as described in the legend to Figure 3, and the proteins were analyzed by sodium dodecyl sulfate–acrylamide gel electrophoresis. Panel A (control) shows the products of the reaction, whereas panel B shows protein subjected to a single-step Edman degradation. The [^{3}H]lysine-labeled material serves as an internal marker and a monitor of recovery. (● — ●) [^{35}S]Met-tRNA^{fMet}; (O - - - O) [^{3}H]lysine.

than [^{3}H]lysine is for the first 30 min of synthesis, but, after 30 min, [^{35}S]methionine is incorporated less rapidly than [^{3}H]lysine is. This is probably due to deacylation of the initiator [^{35}S]Met-tRNA^{fMet} (Marcus et al., 1970) and dilution of the specific activity of [^{35}S]Met-tRNA^{fMet} with endogenous initiator tRNA charged with unlabeled methionine.

Analysis of the proteins labeled with initiator [^{35}S]methionine and [^{3}H]lysine on sodium dodecyl sulfate–acrylamide gels shows that Pre-ProPTH clearly contains initiator [^{35}S]methionine (Figure 4A). In addition, some smaller proteins are present on the gel, that, when compared with the larger proteins, have an increased amount of ^{35}S radioactivity relative to ^{3}H radioactivity. This indicates that these proteins are shorter polypeptide chains with fewer internal lysines but they still have the proper initiator methionine incorporated. This is the expected result if these proteins are the product of premature chain termination.

To confirm that the [^{35}S]methionine incorporated into protein from initiator [^{35}S]Met-tRNA^{fMet} was not also incorporated into internal positions of the polypeptide chain, the NH_2-terminal amino acid was removed by a single-step Edman degradation. After removal of the NH_2-terminal amino acid, the proteins were analyzed by electrophoresis on sodium dodecyl sulfate–acrylamide gel electrophoresis (Figure 4B). The [^{3}H]lysine incorporation into internal residues indicates the position of Pre-ProPTH; almost all the [^{35}S]methionine incorporated into Pre-ProPTH was removed by the Edman degradation. Thus, methionine from initiator [^{35}S]Met-tRNA^{fMet} is accurately incorporated as the initiator methionine in Pre-ProPTH. Pre-ProPTH migrates more rapidly on the sodium dodecyl sulfate–acrylamide gels after the Edman degradation, presumably because the 14 positive charges normally contributed by the ϵ-NH_2 groups of lysine have been eliminated by reaction with phenyl isothiocyanate.

The COOH terminus of PTH may be examined by ana-

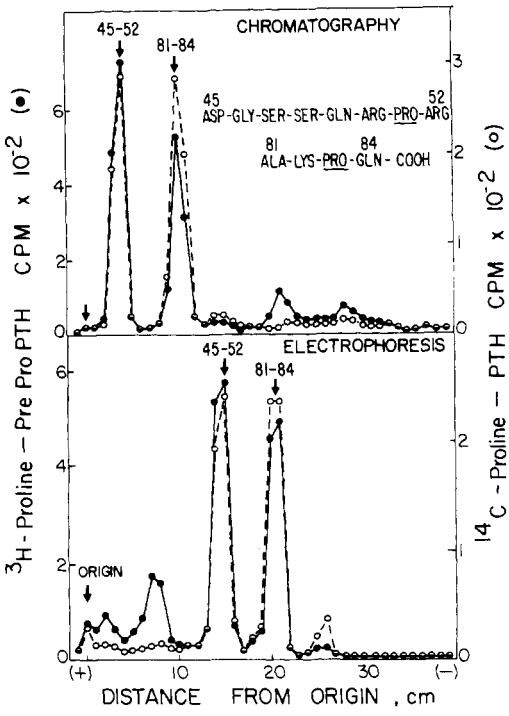

FIGURE 5: Paper electrophoresis and chromatography of [^{3}H]proline-labeled Pre-ProPTH and [^{14}C]proline-labeled PTH. PTH was isolated from parathyroid slices incubated for 60 min with [^{14}C]proline. Pre-ProPTH was isolated from a 50-μl wheat-germ reaction containing 20 μCi of [3,4-^{3}H]proline (34 Ci/mmol) and about 4 μg of 8–15S parathyroid RNA. The relative migrations of the internal peptide, 45–52, and the carboxyl-terminal peptide, 81–84, are in agreement with previous studies (Potts et al., 1968). Samples were electrophoresed for 2 hr at 3000 kV and chromatographed for 36 hr. (○ - - - - ○) [^{14}C]PTH; (● — ●) [^{3}H]Pre-ProPTH.

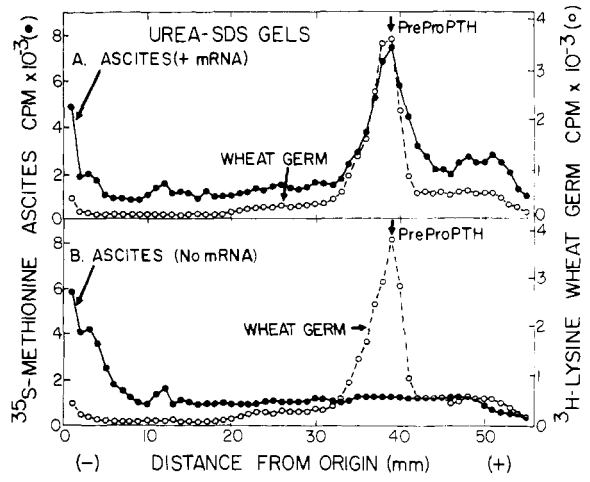

FIGURE 6: Analysis by sodium dodecyl sulfate gel electrophoresis of the cell-free products of protein synthesis directed by parathyroid RNA in Krebs-II ascites extracts. Cell-free protein syntheses in Krebs-II ascites and wheat-germ extracts were performed as described in Methods. In a 10-μl ascites reaction, 1 μg of parathyroid RNA and 3.8 μCi of [^{35}S]methionine (300 Ci/mmol) were present, and in a 25-μl wheat-germ reaction, 2 μg of parathyroid RNA, and 12.5 μCi of [^{3}H]lysine (60 Ci/mmol) were present. The ^{3}H-labeled proteins from about 2 μl of a wheat-germ reaction containing RNA were added to each of two samples of ^{35}S-labeled proteins from 5 μl of the ascites extracts. Analysis of proteins from an ascites reaction with parathyroid RNA added (A), and without parathyroid RNA (B). (● — ●) [^{35}S]methionine-labeled proteins from the Krebs-II ascites extract; (○ - - - - ○) [^{3}H]lysine-labeled proteins from the wheat-germ extract.

lyzing the proline-containing tryptic peptides of the hormone. The structure of the two tryptic peptides of PTH that contain proline are shown in Figure 5 (insert). One peptide, residues 45–52, is located internally in the sequence of PTH, and the second peptide, residues 81–84, is located at the COOH terminus. The COOH terminus of PTH is glutamine, and, because trypsin does not cleave on the COOH side of glutamine, an extra sequence on the COOH terminus of Pre-ProPTH will alter the proline-containing tryptic peptide 81–84. Such a peptide containing residues 81–84 plus an unknown number of additional amino acids would be expected to have chromatographic and electrophoretic mobilities different from those of the PTH peptide 81–84. The profiles of tryptic peptides of [^{3}H]proline-labeled Pre-ProPTH and [^{14}C]proline-labeled PTH after paper chromatography and electrophoresis demonstrate that the proline-containing peptides of PTH and Pre-ProPTH are not detectably different (Figure 5). These results indicate that there probably is no additional sequence of amino acids at the COOH terminus of Pre-ProPTH.

The principal proteins synthesized in an extract of Krebs-II ascites cells containing parathyroid RNA are similar in molecular weight to those synthesized in the wheat-germ extract. Coelectrophoresis on sodium dodecyl sulfate–acrylamide gels of [^{35}S]methionine-labeled ascites-cell products and [^{3}H]lysine-labeled wheat-germ products demonstrates that a protein that comigrates with Pre-ProPTH is made in the ascites-cell extract (Figure 6A). If parathyroid RNA is omitted from the ascites-cell extract, no peak of radioactivity corresponding to Pre-ProPTH is observed (Figure 6B). A peak corresponding to Pre-ProPTH was also observed in the ascites-cell extract when the proteins were analyzed on acrylamide gels, pH 4, containing 8 *M* urea (not shown). The increased radioactivity on the low molecular weight side of Pre-ProPTH in the ascites-cell products relative to the wheat-germ products suggests that additional smaller proteins are also being made in the ascites-cell extract. Preliminary experiments suggest that one of these proteins corresponds to ProPTH.

Discussion

In this paper we have provided evidence to strengthen our proposal that Pre-ProPTH synthesized in vitro in extracts of wheat germ is identical with the initial product translated from the mRNA for PTH in vivo. Analysis by formamide–sucrose gradient centrifugation of the mRNA that directs the synthesis of Pre-ProPTH indicates that the mRNA is relatively homogeneous and is not degraded, inasmuch as it sediments in a single, sharp band. In other studies we have provided evidence that selected tryptic peptides from the internal portions of Pre-ProPTH are not detectably different from those of PTH or ProPTH (Kemper et al., 1976; Habener et al., 1975). The focus of the present experiments was, therefore, to examine whether the size of Pre-ProPTH was accurate, that is, whether initiation and termination of polypeptide chain growth were correctly executed in the wheat-germ extracts.

In the accompanying paper (Kemper et al., 1976) we have shown that Pre-ProPTH contains a methionine at the NH_2 terminus. In the present paper, the NH_2-terminal methionine is shown to be derived from initiator $tRNA^{fMet}$. In most circumstances, the initiator methionine is cleaved from the newly synthesized proteins when the polypeptide chain is 15–30 amino acids in length in vivo (Jackson and Hunter, 1970; Yoshida et al., (1970) or 50–65 amino acids in length in vitro (Koffer-Gutmann and Arnstein, 1973), unless the amino group of the methionine has been blocked

by formlyation (Housman et al., 1970). Because the initiator methionine is not cleaved from Pre-ProPTH in the wheat-germ extract, either the peptidase that removes the methionine is missing in the wheat germ, or the structure of Pre-ProPTH at the NH_2 terminus, Met–Met (Kemper et al., 1976), is resistant to cleavage.

Proteins directed by tobacco mosaic-virus RNA in wheat-embryo cell-free systems also retain a methionine at the NH_2 terminus that is derived from initiator Met-tRNA (Marcus et al., 1970). On the other hand, completed proteins directed by satellite tobacco necrosis-virus RNA in the wheat-embryo system do not contain NH_2-terminal methionine (Lundquist et al., 1972), although NH_2-terminal methionine is found on short nascent polypeptide chains (Klein and Clark, 1973; Seal and Marcus, 1973). On the supposition that the wheat germ and wheat embryo are similar, this suggests that some methionine aminopeptidase activity is present that can cleave Met–Ala at the beginning of the satellite tobacco mosaic-virus protein but activity is not present to cleave the methionine from tobacco mosaic-virus proteins or the Met–Met of Pre-ProPTH. Some other eukaryotic proteins retain their initiator methionine in vivo. Duck hemoglobin has initiator methionine at the NH_2 terminus (Pemberton et al., 1972), and ox and sheep hemoglobin have methionines at the NH_2 terminus, although they have not been shown to be the initiator methionine (Boyer et al., 1967). In studies in which the initiator codon for yeast iso-1-cytochrome *c* was relocated by mutation, Met–Leu, Met–Ile, and Met–Ala bonds were not cleaved, whereas Met–Thr, Met–Ala, and, somewhat less efficiently, Met–Val were cleaved (Steward et al., 1971). These results suggest that multiple forms of methionine aminopeptidase exist with differing specificities or that some specificity for the cleavage is provided by the structure of the amino acid penultimate to the NH_2-terminal methionine. In this regard, an examination of the NH_2-terminal structure of Pre-ProPTH synthesized in other mammalian cell-free systems that normally remove the initiator methionine may help to distinguish whether Met–Met is resistant to cleavage per se or whether different tissues have methionine aminopeptidases of differing specificities.

Premature termination of chain growth was observed in the Krebs-II ascites cell-free system (Boime and Leder, 1972; Kerr et al., 1972) and possibly in the wheat-germ system (Roberts and Paterson, 1973; Roberts et al., 1973). The COOH-terminal tryptic peptides of Pre-ProPTH and PTH have the same electrophoretic and chromatographic mobilities and thus probably have the same amino acid sequence. These data indicate that termination of the growth of the polypeptide chain of Pre-ProPTH in the wheat-germ extract occurs normally at the proper codon unless, by unlikely coincidence, a post-translational modification or a specific premature termination occurs following the glutamine at the COOH terminus of PTH in the wheat-germ system.

Because Pre-ProPTH contains all peptides present in ProPTH (Kemper et al., 1976), ProPTH, like Pre-ProPTH, would not be expected to have additional amino acids at the COOH terminus. This agrees with our previous experiments in which the COOH-terminal tryptic peptides of PTH and ProPTH were found to have identical mobilities following electrophoresis and chromatography on paper (Habener et al., 1973) and in which the large COOH-terminal fragments of PTH, ProPTH, and Pre-ProPTH formed by cyanogen bromide cleavage had identical electrophoretic mobilities on sodium dodecyl sulfate–acrylamide gels (Kemper et al., 1974). In contrast, the amino acid composition of ProPTH (Cohn et al., 1972) indicated that amino acids were present in addition to those that have been identified at the NH_2 terminus (Hamilton et al., 1974; Cohn et al., 1974; Jacobs et al., 1974), suggesting that additional amino acids may be at the COOH terminus. Recently, a partial sequence of the COOH terminus of ProPTH was reported (Hamilton et al., 1975), but these studies have not shown that this sequence is contiguous to the structure of ProPTH and do not exclude the possibility that these results are due to a contaminant in the preparation of ProPTH. At present, therefore, the nature of the COOH terminus of ProPTH, and by implication, of Pre-ProPTH, remains uncertain, but, according to the criteria we have employed in this paper, both proteins have carboxyl termini that are the same as those of PTH.

Possible precursors of other proteins have been reported that have properties similar to those of Pre-ProPTH. Boime et al. (1975) reported that a protein larger than placental lactogen is synthesized when human placental RNA is translated in wheat-germ extracts, but lactogen of normal size is synthesized in Krebs-II ascites extracts. The translation of light-chain myeloma mRNA also produces a protein in reticulocyte extracts (Milstein et al., 1972) and in wheat-germ extracts (Honjo et al., 1974) that is larger than the protein in Krebs-II ascites extracts (Brownlee et al., 1972). Likewise, in our preliminary results, no ProPTH was observed when parathyroid RNA was translated in wheat-germ extracts, but some ProPTH was observed when RNA was translated in Krebs-II ascites extracts. These three presumed percursors also share the additional property of not being detectably present in intact cells.

Knowledge that the NH_2-terminal methionine of Pre-ProPTH is derived from initiator Met-tRNA^{fMet} and that the COOH-terminal of Pre-ProPTH is the same as that of PTH greatly strengthens the hypothesis that Pre-ProPTH represents all the codons of PTH mRNA that are translated in vivo. The possibility remains, however, that the in vitro conditions are such that an internal methionine codon is read as a normal initiator codon or that premature termination occurs by coincidence at the normal COOH terminus of PTH. These possibilities are obviated to a great extent by the discrete, apparently undegraded, nature of the PTH mRNA and by the synthesis of a protein of the same molecular weight as Pre-ProPTH in a second cell-free system, the Krebs-II ascites extract. In addition, RNA from human parathyroid tissue also directs the synthesis of an analogous human Pre-ProPTH (Habener et al., 1975). The final proof that Pre-ProPTH is synthesized in vivo will, however, require the demonstration of Pre-ProPTH in intact cells and its temporal conversion to ProPTH and PTH.

References

Anderson, C. W., Lewis, J. B., Atkins, J. F., and Gesteland, R. F. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71*, 2756.

Benson, R. C., Jr., Riggs, B. L., Packard, B. M., and Arnaud, C. D. (1974), *J. Clin. Invest. 54*, 175.

Boime, I., Boguslawski, S., and Caine, J. (1975), *Biochem. Biophys. Res. Commun. 62*, 103.

Boime, O., and Leder P. (1972), *Arch Biochem. Biophys. 153*, 706.

Boyer, S. H., Hathaway, P., Pascasio, F., Bordley, J., Orton, C., and Naughton, M. A. (1967), *J. Biol. Chem.*

242, 2211.
Brownlee, G. G., Harrison, T. M., Mathews, M. B., and Milstein, C. (1972), *FEBS Lett. 23,* 244.
Cohn, D. V., MacGregor, R. R., Chu, L. L. H., Huang, J., Anast, C. T., and Hamilton, J. W. (1974), *Am. J. Med. 56,* 767.
Cohn, D. V., MacGregor, R. R., Chu, L. L. H., Kimmel, J. R., and Hamilton, J. W. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 1521.
Edman, P. (1960), *Ann. N.Y. Acad. Sci. 88,* 602.
Ghosh, K., Ghosh, H. P., Simsek, M., and Raj Bhandary, U. L. (1974), *J. Biol. Chem. 249,* 4720.
Habener, J. F., Kemper, B., Potts, J. T., Jr., and Rich, A. (1973), *Endocrinology 92,* 219.
Habener, J. F., Kemper, B., Potts, J. T., Jr., and Rich, A. (1975), *J. Clin. Invest. 56,* 1328.
Hamilton, J. W., Huang, D. W. Y., Chu, L. L. H., MacGregor, R. R., and Cohn, D. V. (1975), in Calcium-Regulating Hormones, Talmage, R. V., Owen, M., and Parson, J. A., Ed., Excerpta Medica, Amsterdam, American Elsevier Publishing Co., p 40.
Hamilton, J. W., Niall, H. D., Jacobs, J. W., Keutmann, H. J., Potts, J. T., Jr., and Cohn, D. V. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 653.
Honjo, T., Packman, S., Swan, D., Nau, M., and Leder, P. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3659.
Housman, D., Jacobs-Lorena, M., Raj Bhandary, U. L., and Lodish, H. F. (1970), *Nature (London) 227,* 913.
Jackson, R., and Hunter, T. (1970), *Nature (London) 227,* 672.
Jacobs, J. W., Kemper, B., Niall, H. D., Habener, J. F., and Potts, J. T., Jr. (1974), *Nature (London) 249,* 155.
Jacobs-Lorena, M., and Baglioni, C. (1972), *Biochemistry 11,* 4970.
Kemper, B., Habener, J. F., Ernst, M., Potts, J. T., Jr., and Rich, A. (1976), *Biochemistry,* preceding paper in this issue.
Kemper, B., Habener, J. F., Mulligan, R. C., Potts, J. T., Jr., and Rich, A. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3731.
Kemper, B., Habener, J. F., Potts, J. T., Jr., and Rich, A. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 643.
Kerr, I. M., Brown, R. E., and Tovell, D. R. (1972), *J. Virol. 10,* 73.
Klein, W. H., and Clark, J. N., Jr. (1973), *Biochemistry 12,* 1528.
Koffer-Gutmann, A., and Arnstein, A. R. V. (1973), *Biochem. J. 134,* 969.
Laemmli, U. K., (1970), *Nature (London) 227,* 680.
Lundquist, R. E., Lazar, J. M., Klein, W. H., and Clark, J. M., Jr. (1972), *Biochemistry 11,* 2014.
Marcus, A., Weeks, D. P., Leis, J. P., and Keller, E. B. (1970), *Proc. Natl. Acad. Sci. U.S.A. 67,* 1681.
Milstein, C., Brownlee, G. G., Harrison, T. M., and Mathews, M. B. (1972), *Nature (London), New Biol. 239,* 117.
Pemberton, R. E., Housman, D., Lodish, H. F., and Baglioni, C. (1972), *Nature (London), New Biol. 235,* 99.
Potts, J. T., Jr., Keutmann, H. T., Niall, H. D., Deftos, L., Brewer, H. B., Jr., and Aurbach, G. D. (1968), *Parathyroid Horm. Thyrocalcitonin (Calcitonin), Proc. Parathyroid Cont., 3rd, 1967,*44.
Raj Bhandary, U. L., and Ghosh, H. P. (1969), *J. Biol. Chem. 244,* 1104.
Roberts, B. E., Mathews, M. B., and Bruton, C. J. (1973), *J. Mol Biol. 80,* 733.
Roberts, B. E., and Paterson, B. M. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2330.
Seal, S. N., and Marcus, A. (1973), *J. Biol. Chem. 248,* 6577.
Steward, J. W., Sherman, F., Shipman, N. A., and Jackson, M. (1971), *J. Biol. Chem. 246,* 7429.
Wong, E. T., and Lindall, A. W. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2291.
Yoshida, A., Watanabe, S., and Morris, J. (1970), *Proc. Natl. Acad. Sci. U.S.A. 67,* 1600.