ent with the binaural estimates for the same stimulus conditions.

Although our measurements represent only a limited investigation of higher-frequency combination tones, we feel confident in concluding that, at least for low primary frequencies, such combination tones exist 20 to 40 dB below the primary levels. As others have suggested (*2, 8*), the difficulty in identifying their presence appears to be largely due to the upward masking by the primary tones. Our observations indicate that the relative level of higher-frequency combination tones can be quite high. Thus, even though they are not clearly audible, they may be of some importance in identifying the nonlinear mechanism from which they arise. In this respect, the presence of higher-frequency combination tones brings the psychoacoustic results into closer agreement with physiological investigations of cochlear microphonic in which distortion components above and below the primary frequencies are routinely measured (*14*).

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13 March 1979

## Human Growth Hormone: Complementary DNA Cloning and Expression in Bacteria

Abstract. *The nucleotide sequence of a DNA complementary to human growth hormone messenger RNA was cloned; it contains 29 nucleotides in its 5′ untranslated region, the 651 nucleotides coding for the prehormone, and the entire 3′ untranslated region (108 nucleotides). The data reported predict the previously unknown sequence of the signal peptide of human growth hormone and, by comparison with the previously determined sequences of rat growth hormone and human chorionic somatomammotropin, strengthens the hypothesis that these genes evolved by gene duplication from a common ancestral sequence. The human growth hormone gene sequences have been linked in phase to a fragment of the* trp *D gene of* Escherichia coli *in a plasmid vehicle, and a fusion protein is synthesized at high level (approximately 3 percent of bacterial protein) under the control of the regulatory region of the* trp *operon. This fusion protein (70 percent of whose amino acids are coded for by the human growth hormone gene) reacts specifically with antibodies to human growth hormone and is stable in* E. coli.

Growth hormone, along with at least two other polypeptide hormones, chorionic somatomammotropin (placental lactogen) and prolactin, forms a set of proteins with amino acid sequence homology and to some extent overlapping biological activities (*1, 2*). Since the genes of this set of proteins probably have a common ancestral origin (*1*), they constitute an excellent model to study the evolution, structure, and differential regulation of related genes. In addition, since human growth hormone is of considerable medical importance and its supply is limited, the synthesis of growth hormone in bacteria might provide the required alternate source of this critical hormone.

We have previously isolated and analyzed bacterial clones containing copies of complementary DNA (cDNA) transcripts of messenger RNA's (mRNA's) for these hormones. The complete sequence of rat pregrowth hormone mRNA (*3*) has been reported; in addition, sequence data have been presented for fragments of about 550 bases complementary to part of the coding (amino acid residues 24 to 191) and 3′ untranslated portions of human chorionic somatomammotropin (hCS) (*3, 4*) and human growth hormone (hGH) mRNA's (*5*). A partial sequence of rat prolactin has been determined by Gubbins *et al.* (*6*). These sequence data showed that, whereas the growth hormone genes of the rat and man had significant homology, they also had diverged substantially, such that they differed more than the genes for the functionally distinct human hormones hCS and hGH.

We now report the synthesis, cloning, and sequence analysis of cDNA containing the entire coding and most of the noncoding portions of hGH mRNA. We also describe the insertion of these sequences into an "expression plasmid" containing part of the *Escherichia coli* tryptophan (*trp*) operon whose construction has been realized by Hallewell and Emtage (*7*). We describe the use of this plasmid to promote the inducible bacterial synthesis of high levels of a hybrid protein, 70 percent of which is composed of amino acids coded for by the hGH gene.

*Human growth hormone mRNA isolation.* Polyadenylated RNA was isolated (*8*) from human pituitary tumors removed by transphenoidal hypophysectomy. To obtain an indication of the integrity and the relative abundance of growth hormone mRNA in each sample, the individual mRNA preparations were translated in the wheat germ cell-free system, and the products were analyzed by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels (Fig. 1). Among the translation products of the five acromegalic tumor RNA's (Fig. 1, lanes 1 to 5), the most prominent band corresponds to a protein of approximate-


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ly 24,000 daltons. This protein is assumed to be human pregrowth hormone since it is similar in size to rat pregrowth hormone (Fig. 1, "rat") and is precipitated by antiserum to hGH (data not shown). This assumption is further justified by comparison with the translation products of polyadenylated RNA isolated from bovine pituitary (Fig. 1, "cow") and from a human prolactin-producing tumor (Fig. 1, lane 6). Both of these RNA's directed the synthesis of a protein similar in size to human and rat pregrowth hormone, but also directed the synthesis of a larger quantity of a protein of higher molecular weight, presumably preprolactin. The tumors show variation in the extent to which hGH mRNA is present, as measured by their translational activities. Nevertheless, hGH mRNA appears to be the most abundant mRNA species in the acromegalic tumors (Fig. 1, lanes 1 to 5). These results are consistent with and verify the clinical diagnoses made prior to surgery.

*Molecular cloning of hGH cDNA*. The polyadenylated RNA from the tumors that appeared to have the greatest abundance of hGH mRNA by the translational assay were pooled for synthesis of double-stranded cDNA (Fig. 2). Portions of the double-stranded cDNA were analyzed by restriction endonuclease digestion before and after treatment with S1 nuclease. A high proportion of the cDNA was about 1000 nucleotides long (Fig. 2, lanes a and b), the length expected for hGH mRNA, assuming analogy with rat pregrowth hormone mRNA (*3*). Endonuclease Hae III digestion of the DNA generated a 550-base pair (bp) fragment (Fig. 2, lanes h and j) previously reported to occur in hGH and hCS cDNA's (*4, 5*). The prominence of this band supports the idea that the cDNA is highly enriched in hGH gene sequences. This is further suggested by the finding of a fragment of about 400 bp generated by digestion with Hinf I and Sma I (Fig. 2, lane g). The fragment of about 500 bp generated by Pvu II (Fig. 2, lanes e and i) extends beyond the previously cloned 550 bp fragment, which contains only one Pvu II site. However, its presence is predictable from the sequence of rat growth hormone cDNA (*3*), and by the conservation between species of the amino acid sequence in this region (*9*). The fragments of about 350 bp and 150 bp generated by combined digestion with Pvu II and Bgl II (Fig. 2, lane f) would also be anticipated from the previously determined structure of the 550 bp hGH fragment and knowledge of the existence of the additional Pvu II site. Therefore, this cDNA preparation appears to be highly enriched in full-length copies of hGH mRNA.

The uncleaved cDNA was cloned in the plasmid pBR322 and *E. coli* $\chi$1776 in a P3 physical containment facility (*10*) by methods similar to those previously described (*3*). Briefly, the cDNA was first treated with S1 nuclease and subsequently with DNA polymerase I in the presence of the four deoxynucleoside triphosphates to generate blunt-ended cDNA molecules. Synthetic DNA containing the site for the restriction endonuclease Hind III was then added to

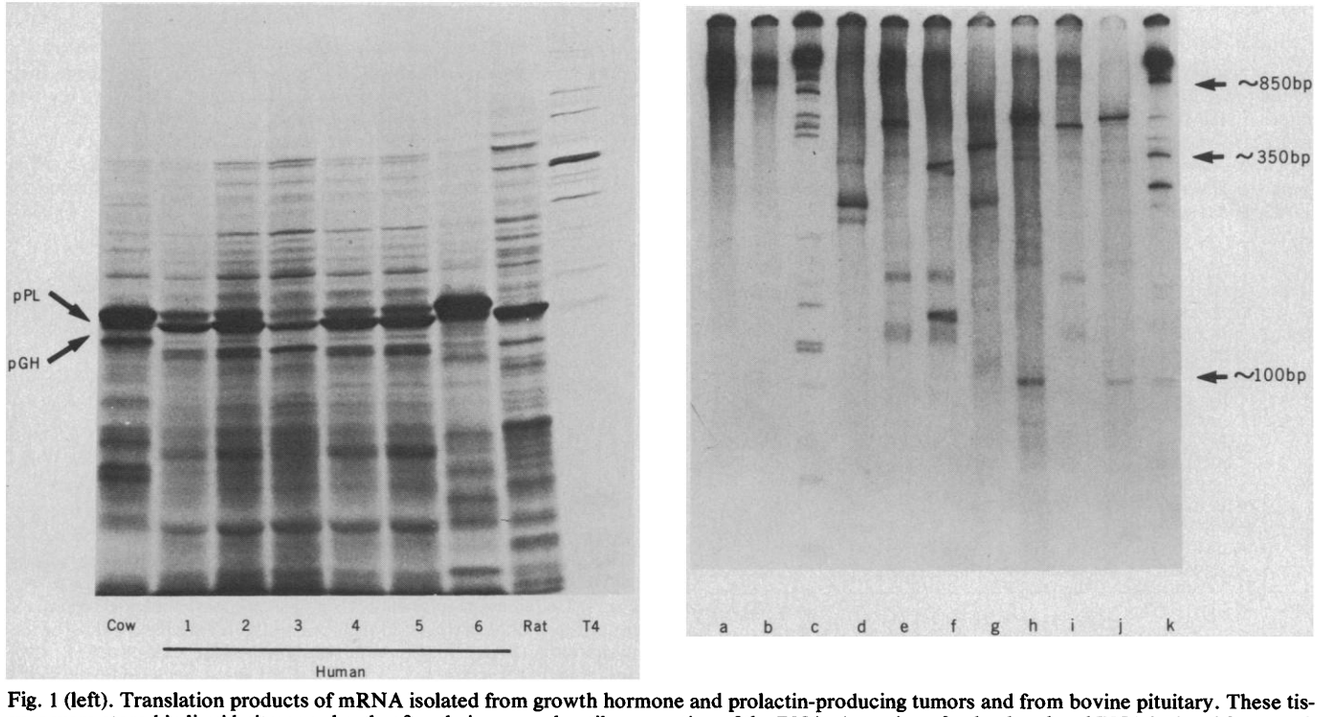


Fig. 1 (left). Translation products of mRNA isolated from growth hormone and prolactin-producing tumors and from bovine pituitary. These tissues were stored in liquid nitrogen shortly after their removal until preparation of the RNA. A portion of polyadenylated RNA isolated from each human tumor, the bovine pituitary, and the cultured rat pituitary tumor (GC) cells was used as a messenger in the wheat germ cell-free protein synthesis system (*20*). The $^{35}$S-labeled proteins were analyzed by electrophoresis and autoradiography on sodium dodecyl sulfate-polyacrylamide gels (12.5 percent) (*20*). The translation products from five acromegalic and one prolactin-secreting tumor are shown in lanes 1 to 5 and lane 6, respectively. The lanes labeled "cow" and "rat" show the translation products from bovine pituitary and rat GC cell RNA. Lane 7 shows the bacteriophage T4 proteins (*21*) used as molecular weight markers. The arrows indicate the bands corresponding to pregrowth hormone (pGH) and preprolactin (pPL). Fig. 2 (right). Analysis of cDNA synthesized from mRNA extracted from the growth hormone-producing pituitary tumors. The polyadenylated RNA from tumors two, four, and five were pooled (135 $\mu$g) and used as a template for synthesis of $^{32}$P-labeled double-stranded cDNA as described (*3*). Samples of this cDNA were cleaved with various restriction endonucleases before and after S1 nuclease digestion. The figure shows an autoradiogram of the resulting DNA fragments after electrophoresis on a 4.5 percent polyacrylamide gel (*3*). (Lane a) Uncleaved cDNA before S1 digestion; (lane b) uncleaved cDNA after S1 digestion; (lane c) bacteriophage fd DNA, Hpa II (molecular weight markers); (lane d) cDNA, Pst I + Bgl II; (lane e) cDNA, Pvu II; (lane f) cDNA, Bgl II + Pvu II; (lane g) cDNA, Hinf I + Sma I; (lane h) cDNA, Hae III; (lane i) cDNA, Pvu II; (lane j) cDNA, Hae III; (lane k) bacteriophage fd DNA, Hae III (molecular weight markers).

each end of the cDNA, and cohesive ends were generated by digestion with endonuclease Hind III. The resulting cDNA was purified on gel and ligated to Hind III-cut and bacterial alkaline phosphatase-treated pBR322 plasmid DNA (*8*). Bacteria were then transformed with this recombinant plasmid. Colonies with recombinant DNA containing plasmids, selected by antibiotic resistance ($tet^S amp^R$) were grown, and the plasmid DNA's were isolated. The DNA was digested with Hind III, treated with various other restriction endonucleases, and analyzed by gel electrophoresis. One clone contained an insert of about 800 bp whose digestion by Hae III, Pvu II, and Bgl II generated fragments similar in size

```
                                        -26                 -20                          -10
                                        met ala thr gly ser arg thr ser leu leu leu ala phe gly leu leu cys leu pro trp
 GC AUC CUG UGG ACA GCU CAC CUA CCU CCA AUG GCU ACA CGC UCC CGG ACC UCC CUG CUC CUG GCU UUU GGC CUG CUC UGC CUG CCC UGC

                          1                                 10                           20
leu gln glu gly ser ala phe pro thr ile pro leu ser arg leu phe asp asn ala met leu arg ala his arg leu his gln leu ala
CUU CAA CAG GGC AGU GCC UUC CCA ACC AUU CCC UUA UCC AGC CUU UUU CAC AAC GCU AUG CUC CGC GCC CAU CGU CUG CAC CAG CUG GCC

                         30                                 40                           50
phe asp thr tyr gln glu phe glu glu ala tyr ile pro lys glu gln lys tyr ser phe leu gln asn pro gln thr ser leu cys phe
UUU GAC ACC UAC CAG GAG UUU GAA GAA GCC UAU AUC CCA AAG GAA CAG AAG UAU UCA UUC CUG CAG AAC CCC CAG ACC UCC CUC UGU UUC

                         60                                 70                           80
ser glu ser ile pro thr pro ser asn arg glu glu thr gln gln lys ser asn leu glu leu leu arg ile ser leu leu leu ile gln
UCA GAG UCU AUU CCG ACA CCC UCC AAC AGG GAG GAA ACA CAA CAG AAA UCC AAC CUA GAG CUG CUC CGC AUC UCC CUG CUC CUC AUC CAG

                         90                                100                          110
ser trp leu glu pro val gln phe leu arg ser val phe ala asn ser leu val tyr gly ala ser asp ser asn val tyr asp leu leu
UCG UGG CUG GAG CCC GUG CAG UUC CUC AGG AGU GUC UUC CCC AAC ACC CUG GUC UAC GGC CCC UCU GAC AGC AAC GUC UAU GAC CUC CUA

                        120                                130                          140
lys asp leu glu glu gly ile gln thr leu met gly arg leu glu asp gly ser pro arg thr gly gln ile phe lys gln thr tyr ser
AAG GAC CUA GAG GAA GGC AUC CAA ACG CUG AUG GGG AGG CUG GAA GAU GGC AGC CCC CGG ACU GGG CAG AUC UUC AAG CAG ACC UAC AGC

                        150                                160                          170
lys phe asp thr asn ser his asn asp asp ala leu leu lys asn tyr gly leu leu tyr cys phe arg lys asp met asp lys val glu
AAG UUC GAC ACA AAC UCA CAC AAC GAU GAC GCA CUA CUC AAG AAC UAC GGG CUC CUC UAC UGC UUC AGG AAG GAC AUG GAC AAG GUC GAG

                        180                                190 191
thr phe leu arg ile val gln cys arg ser val glu gly ser cys gly phe AM
ACA UUC CUG CGC AUC GUC CAG UGC CGC UCU GUG GAG GCC AGC UGU GGC UUC UAG CUG CCC GGG UGG CAU CCU GUG ACC CCU CCC CAG UGC

CUC UCC UCG CCC UCG AAC UUG CCA CUC CAG UGC CCA CCA GCC UUG UCC UAA UAA AAU UAA GUU CCA UCA AAA AAA AAA
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Fig. 3. Nucleotide sequence of hGH mRNA and the amino acid sequence of human pregrowth hormone. The sequence was determined according to the procedure of Maxam and Gilbert (*11*) from 5′- or 3′-end-labeled restriction fragments of chGH800/pBR322. The sequence between the two internal Hae III sites was taken from our previous work (*5*). Most of it, and all of the sequence outside these two internal Hae III sites was resequenced by the chain-termination technique (*12*) as described in detail elsewhere (*22*) using as single-stranded templates the chGH800 Hind III fragment recloned in the vector M13 mp5 and as primers restriction fragments of chGH800/pBR322. The RNA sequence has been taken from the DNA sequence. The amino acid sequence has been deduced from the RNA sequence using the genetic code. The termination codon, UAG, is designated by the symbol AM for "amber."

| | | | |
|---|---|---|---|
| 4/UUU/phe | 3/UCU/ser | 3/UAU/tyr | 2/UGU/cys |
| 10/UUC/phe | 7/UCC/ser | 5/UAC/tyr | 3/UCC/cys |
| 1/UUA/leu | 3/UCA/ser | 0/UAA/OC | 0/UGA/OP |
| 0/UUG/leu | 1/UCG/ser | 1/UAG/AM | 2/UGG/trp |
| 2/CUU/leu | 0/CCU/pro | 1/CAU/his | 1/CGU/arg |
| 10/CUC/leu | 6/CCC/pro | 2/CAC/his | 4/CCC/arg |
| 4/CUA/leu | 2/CCA/pro | 3/CAA/gln | 0/CGA/arg |
| 16/CUG/leu | 1/CCG/pro | 11/CAG/gln | 2/CCC/arg |
| 2/AUU/ile | 1/ACU/thr | 0/AAU/asn | 2/AGU/ser |
| 6/AUC/ile | 4/ACC/thr | 9/AAC/asn | 5/AGC/ser |
| 0/AUA/ile | 5/ACA/thr | 1/AAA/lys | 0/AGA/arg |
| 4/AUG/met | 2/ACG/thr | 8/AAG/lys | 5/AGG/arg |
| 0/GUU/val | 3/GCU/ala | 2/GAU/asp | 0/GGU/gly |
| 3/GUC/val | 6/GCC/ala | 9/GAC/asp | 8/GGC/gly |
| 0/GUA/val | 1/GCA/ala | 6/GAA/glu | 0/GGA/gly |
| 4/GUG/val | 0/GCG/ala | 9/GAG/glu | 3/GGG/gly |

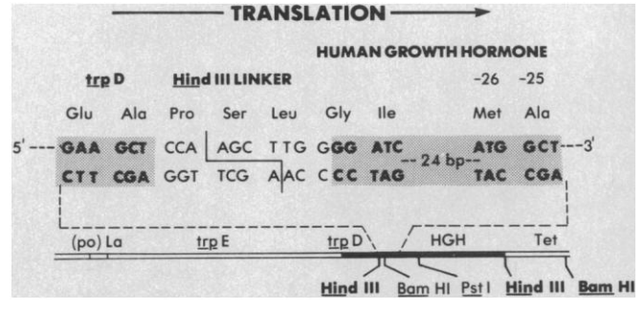


Fig. 4. Codon usage in hGH mRNA. The numbers indicate how many times the codons are used in the region of hGH mRNA coding for the prehormone; OC, OP, and AM designate the stop codons ochre, opal, and amber, respectively. Fig. 5. Postulated nucleotide sequence around the Hind III site in the hybrid gene of expression plasmid p*trp*ED50-chGH800. Plasmid p*trp*ED50 was constructed from p*trp*ED5-1 (*7*) by linearizing the plasmid with Hind III, filling in the protruding 5′ ends with the use of DNA polymerase I (Klenow fragment from Boehringer) and ligating synthetic decamers containing a Hind III site (collaborative research) to the blunt-ended material (*3*, *8*). After digestion with Hind III the plasmid was separated from residual linker molecules by chromatography on Sephadex G-200, recircularized with T4 DNA ligase, and used to transform *E. coli* W3110 trpoE∇1(*23*) by a standard procedure (*24*). Plasmid DNA isolated from one of these colonies was digested with Hind III and treated with alkaline phosphatase (*4*, *8*). A portion (5 $\mu$g) of this DNA was end-labeled with [$\gamma$-$^{32}$P]-ATP with the use of T4 DNA kinase (Boehringer) and cut by Hae III. The DNA sequence of the labeled fragments was determined by chemical cleavage (*11*), after they were isolated by polyacrylamide electrophoresis. The cloned chGH800 DNA was cleaved from pBR322 with Hind III and isolated by polyacrylamide gel electrophoresis. This DNA was ligated to similarly cleaved and alkaline phosphatase-treated p*trp*ED50. The ligation mixture was used to transform *E. coli* strains W3110 *trp*oE∇1 and RR1 (*25*) in a P3 facility, and transformants resistant to ampicillin were selected. Resistant colonies were examined for the presence of inserted chGH800 sequences by gel analysis of such plasmids after digestion with restriction endonucleases Bam HI and Pst I. Plasmids with the growth hormone initiator codon proximal to the *trp*D gene sequence showed bands of 250 and 900 bp, whereas plasmids with the inserted cDNA in the opposite orientation showed bands of 250 and 350 bp.

to those from the 550 bp hGH cDNA clone (data not shown) and to the digested uncloned cDNA (Fig. 2). This suggested that this clone did contain cDNA complementary to full-length or nearly full-length hGH mRNA. (This clone is designated chGH800/pBR322.)

*Sequence analysis of cloned DNA.* The nucleotide sequence of the cloned DNA was determined by the chemical cleavage method of Maxam and Gilbert (*11*) and the chain-termination technique of Sanger, Nicklen, and Coulson (*12*). The hGH mRNA sequence and the corresponding amino acid sequence of human pregrowth hormone can be derived from the DNA sequence (Fig. 3). The amino acid sequence determined from the DNA sequence is consistent with the known amino acid sequence of hGH (*13*) with the following exceptions: the DNA sequence predicts glutamine, asparagine, glutamine, glutamic acid, glutamine, aspartic acid, asparagine, and glutamine at amino acid positions 29, 47, 49, 74, 91, 107, 109, and 122, respectively, while the protein sequence indicates glutamic acid, aspartic acid, glutamic acid, glutamine, glutamic acid, asparagine, aspartic acid, and glutamic acid. It is likely that the DNA sequence is correct in this regard since it is sometimes difficult in protein sequence analysis to differentiate aspartic acid from asparagine and glutamic acid from glutamine. The amino acid sequence of the signal peptide portion of human pregrowth hormone had not been previously determined and is deduced from the mRNA sequence. If translation begins with the methionine codon "in phase," 26 codons proximal to the first amino acid of growth hormone (Fig. 3), then the primary translation product of hGH mRNA would be a protein of 24,851 daltons, a value in agreement with the cell-free translation data shown in Fig. 1.

A comparison of the amino acid and nucleic acid sequence homologies between rat growth hormone, hGH, and hCS and their respective mRNA's is shown in Table 1. In the coding regions, there is higher homology between the nucleic acid sequences than between the amino acid sequences. This difference is consistent with the already mentioned view that the genes of these related hormones evolved from a common evolutionary precursor gene, and is further supported by the marked homology in the 5′-noncoding portions of the mRNA's for rat and human growth hormone. (Data for the 5′-noncoding region of hCS are not yet available.) Human growth hormone has more homology with hCS than with rat growth hormone, especially for the 3′-noncoding portions. This finding supports the hypothesis developed earlier that the chorionic somatomammotropin and growth hormone genes probably evolved by a gene duplication mechanism (*1*) at some time after the separation of the human and rat species. In addition, the fact that both hormones exist in both species implies that the same hormones may have evolved independently more than once.

Table 1. Amino acid and nucleic acid sequence homology of growth hormone, chorionic somatomammotropin, and their mRNA's. Data for rat growth hormone (rGH) and human chorionic somatomammotropin (hCS) and their mRNA's are from (*3*. *4*). For hCS, only data for amino acids residues 24 to 191 (and the corresponding portion of the mRNA) and a portion of the noncoding 3′-region corresponding to the cloned 550 bp fragment are used for comparisons, since data for the other portions are not available. The amino acid sequence of the prepeptide portion of hCS was determined by Sherwood *et al.* (*27*).

| Source | Homology (percent) | |
|---|---|---|
| | hGH versus rGH | hGH versus hCS |
| Nucleic acid: | | |
| 5′-Noncoding | 73 | |
| Presequence | 76 | |
| Coding | 76 | 92 |
| 3′-Noncoding | 38* | 94 |
| Amino acid: | | |
| Presequence | 58 | 84 |
| Coding | 67 | 86 |

*The homology in this region can be increased to 55 percent by adding appropriate gaps in the sequences. This procedure also reveals that there is a homology of 27 out of 30 bases in the region of the AAUAAA (*28*). Similar conservation between species but with different sequences are found when human and rabbit β-globin (*29*) and human and rat insulin (*30*) are compared.

Figure 4 shows the codons used for hGH mRNA. As is the case with rat growth hormone (*3*) and hCS (*6*) mRNA's, there is a nonrandom selection of codons. This appears to be mostly due to the preference for G (guanine) or C (cytosine) over A (adenine) or U (uracil) for the third position of the triplet codon. This is also the case with most (*3*, *14*) but not all (*14*) eukaryotic mRNA's whose structures are known.

*Construction of a plasmid for growth hormone expression.* To see whether hGH gene sequences can be expressed in bacteria, we used the plasmid p*trp*ED5-1 (*7*), which contains the regulatory region [(po)La], the first gene (*trp*E), and 15 percent of the second gene (*trp*D) of the *E. coli trp* operon. Cells containing p*trp*ED5-1 normally synthesize small amounts of *trp* gene products. However, if *trp* operon transcription is derepressed by addition of 3β-indolylacrylic acid, synthesis of *trp* gene products increases, so that within 3 hours *trp* proteins account for about 30 percent of the total cellular proteins (*7*). We hoped that by placing the hGH gene sequence under control of the *trp* operon, not only would it be expressed, but a higher level of hGH production could be obtained than was previously achieved with rat growth hormone gene sequences under control of the β-lactamase gene (*15*).

The hGH sequences from chGH800 were inserted at the Hind III site of the *trp*D gene sequence as described in the legend to Fig. 5. In order to insert the hGH codons in phase with those of the *trp*D sequence, the Hind III site in the *trp*D gene was manipulated in such a way as to shift the reading frame of any DNA inserted through the Hind III site of the plasmid by one base. To do this, p*trp*ED5-1 was cleaved with Hind III, the protruding 5′-ends "filled in" with the use of DNA polymerase I (Klenow fragment) and synthetic DNA decamers containing the Hind III site ligated to the blunt-ended material. This DNA was then digested with Hind III to produce new cohesive Hind III ends, and plasmid molecules were recircularized with the use of DNA ligase after the residual Hind III linker molecules were removed on a Sephadex (G-200) column. This material was used to transform *E. coli* and, after selection for ampicillin-resistant colonies, the plasmid DNA was isolated from one of the transformants. The DNA sequence at the Hind III site of the newly constructed plasmid (designated p*trp*ED50) was determined and showed that the enzymatic reactions had altered the reading frame as predicted. In this way, when chGH800/pBR322 was cleaved with Hind III and the hGH sequences ligated to the Hind III site of the newly constructed plasmid, the codons of hGH would be in phase with those of the *trp*D gene, provided that the hGH gene sequences were inserted in the proper orientation. This was achieved by obtaining several clones, isolating plasmid DNA, and determining the orientation of the cloned segment by restriction endonuclease analysis (Fig. 5, legend).

As is indicated in Fig. 5, the construction of the hGH "expression" plasmid was such that the anticipated product would be a fusion protein containing the $NH_2$-terminal region of the *trp*D protein, amino acids coded by the 5′-untranslated portion of hGH mRNA, the 26 amino acids of the signal peptide, and all of the amino acids of hGH.

*Synthesis of growth hormone in bacteria.* To determine whether the newly

constructed gene can direct the synthesis of large amounts of a new fused polypeptide, and whether its expression is regulated by the *trp* promoter, cells containing the expression plasmid were derepressed for *trp* transcription, and proteins were labeled for 5 minutes with $^{14}C$-labeled amino acids. Figure 6 shows an autoradiogram of sodium dodecyl sulfate–polyacrylamide gel of such proteins labeled at various times from 0 to 4 hours after induction of the *trp* operon. Two proteins (53,000 and 32,000 daltons) seem to be specifically derepressed by the inducer. The higher molecular weight protein is the *trp*E gene product (*7*). The 32,000-dalton protein has approximately the anticipated size for the *trp*D–hGH fusion protein (34,000 daltons). It is immunoprecipitated by antiserum to hGH (Fig. 6, lane a) but not by the control antiserum (Fig. 6, lane b), and precipitation can be blocked by a large excess of hGH (Fig. 6, lanes c to h). Some of the *trp*E protein is immunoprecipitated by antiserum to hGH, but the amount is less when an excess of competitor hGH is added or control antiserum is used. This result might be expected for two reasons. Precipitation of *trp*E protein by control antiserum may be due to the high abundance of this protein. More interestingly, specific precipitation of *trp*E by hGH antiserum (Fig. 6, lane c) and blockage of precipitation by an excess of competitor hGH (Fig. 6, lane e) may be the result of association of the *trp*E protein and the *trp*D–hGH fused polypeptide. The *trp*E and *trp*D proteins are normally associated in *E. coli* as a tetramer containing two subunits of each protein (*16*); the resulting enzymatic activity (anthranilate synthetase) requires the *trp*E protein and the $NH_2$-terminal 30 percent of the *trp*D protein (*17*). Thus, the fused *trp*D–hGH protein may contain those *trp*D residues required for binding *trp*E. All of these lines of evidence suggest that the 32,000-dalton protein is a fused *trp*D–hGH polypeptide.

On the basis of the relative quantity of radioactivity incorporated into the *trp*D–hGH gene product, the fusion product appears to be a major protein made by the bacteria, constituting 3 percent of the total bacterial protein synthesis. Thus, the natural hGH gene sequence can be expressed at a high level in bacteria.

The *trp*E and *trp*D protein molecules ordinarily accumulate at a similar rate (molar ratio 1:1) when the *trp* operon is induced (*7*). However, the molar ratio of *trp*E to *trp*D-hGH is about 6:1, indicating that synthesis of *trp*D-hGH is only 17 percent of the expected level. The reduced level of synthesis is not due to instability of the fused polypeptide since a "pulse-chase" experiment has shown that no significant degradation of the fused polypeptide occurs during the 60-minute period after incorporation of the label (data not shown). There is some evidence that the chick ovalbumin protein is also synthesized in *E. coli* at lower levels than expected (*18*).

Hypopituitary dwarfism is a fairly common disease treatable only by replacement with hGH (*19*). Growth hormone may also be useful in the treatment of other disorders. However, the potential uses of this hormone have not been adequately investigated because its only source is pituitaries from human cadavers. In order to have an adequate supply of this hormone, it is necessary to find alternative means of producing it; the synthesis of hGH in bacteria may provide such a means.

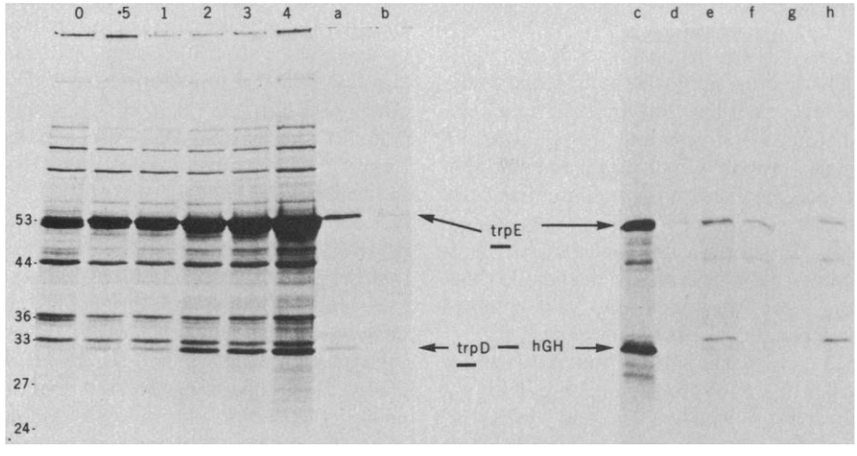


Fig. 6. Autoradiograms of sodium dodecyl sulfate–polyacrylamide gels (10 percent) of $^{14}C$- and $^{35}S$-labeled proteins from bacteria harboring the p*trp*ED50-chGH800 expression plasmid. Cultures of strains W3110 *trp*oE∇1 and RRI harboring this plasmid were induced with 3$\beta$-indolylacrylic acid and 3-ml samples labeled for 5 minutes with 2 $\mu$Ci of $^{14}C$-labeled amino acids (W3110 *trp*oE∇1) or 10 $\mu$Ci of $^{35}S$-labeled methionine (RRI) as described (*7*). Samples labeled at zero, 0.5, 1, 2, 3, and 4 hours were centrifuged and resuspended (50 $\mu$l) by sonication (*26*) prior to loading (5 $\mu$l per gel slot) in sodium dodecyl sulfate sample buffer (*20*). Samples were immunoprecipitated by means of the SAC technique (*20*) in order to collect antigen-antibody complexes. Immunoprecipitations contained 10 $\mu$l of sonicated cells, 390 $\mu$l of 0.5 percent NP40 (Particle Data Laboratory, Elmhurst, Ill.) in phosphate saline buffer (0.025*M* potassium phosphate, *p*H 7.4, 0.1*M* NaCl), 20 $\mu$l of rabbit antiserum to hGH (Antibodies Inc., 1000 unit/ml) or 20 $\mu$l of nonimmune rabbit antiserum each diluted 50-fold in phosphate saline buffer containing bovine serum albumin (2 mg/ml). Competitor hGH was added at 40 $\mu$g per reaction mixture. The *E. coli* proteins in lane 0 were used as molecular weight markers (*8*). (a and b) Immunoprecipitates of $^{14}C$-labeled proteins from the 4-hour time point with (a) antiserum against hGH and with (b) nonimmune serum. (c to e) Immunoprecipitates of $^{35}S$-labeled proteins from the 4-hour time point, with (c) antiserum against hGH, (d) nonimmune serum, and (e) antiserum against hGH together with an excess of competitor hGH. (f to h) Immunoprecipitates of $^{35}S$-labeled proteins from the zero time point with the use of (f) antiserum against hGH, (g) nonimmune serum, and (h) antiserum against hGH together with an excess of competitor hGH.


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## Changed Lyotropic Liquid Crystalline Structure Due to Polymerization of the Amphiphilic Component

Abstract. *Optical patterns in polarized light and x-ray reflections in the low-angle region were used to detect a shift from one liquid crystalline structure to another during polymerization. The polymerization took place in a lyotropic liquid crystal of water and sodium undecenoate, with a structure consisting of cylinders in a two-dimensional hexagonal close packing. After polymerization, a lamellar liquid crystalline structure was obtained.*

The comprehensive knowledge of liquid crystalline structures (*1–4*) that has been obtained through their use for display systems and the excellent properties of Kevlar fibers drawn from liquid crystals (*5*) have resulted in a recent focus on polymerization in systems of liquid crystalline character. The contributions so far have mainly been in the area of thermotropic liquid crystals (*6*); the equilibrium problems being more complex in lyotropic structures, which by definition are multicomponent systems.

Early attempts to polymerize in lyotropic liquid crystals (*7–10*) have recently (*11, 12*) been criticized as achieving retention of the long-range order by freezing the structure with cross-coupling agents rather than forming an equilibrium liquid crystal. The conditions for polymerization with retained liquid crystalline structure have been stated (*11*) by comparison with homotropic polymerization in crystalline structures (*12–15*). For a liquid crystalline structure the translational entropy component in the expression for the total free energy should also be included. Its magnitude is difficult to estimate; for microemulsions (*16*) the contribution of the entropic free energy is similar in magnitude to the contributions of other components of the free energy.

The fact that polymerization with retention of the liquid crystalline structure may be a rare phenomenon in lyotropic liquid crystals (*11*) encouraged us to choose an alternative route: to observe structural changes during polymerization in a lyotropic liquid crystalline matrix. We are now able to report a change from one liquid crystalline structure to another during polymerization of the amphiphilic component. To our knowledge, this is the first report of such a change. It is essential to realize that the structure obtained represents the stable conformation of the system; there is no "freezing in" of a structure—it forms spontaneously from an isotropic melt.

The components of the liquid crystal were distilled water (with 0.05*M* ammonium persulfate as an initiator) and sodium undecenoate. They formed a liquid crystal in the concentration range 47 to 59 percent sodium undecenoate (by

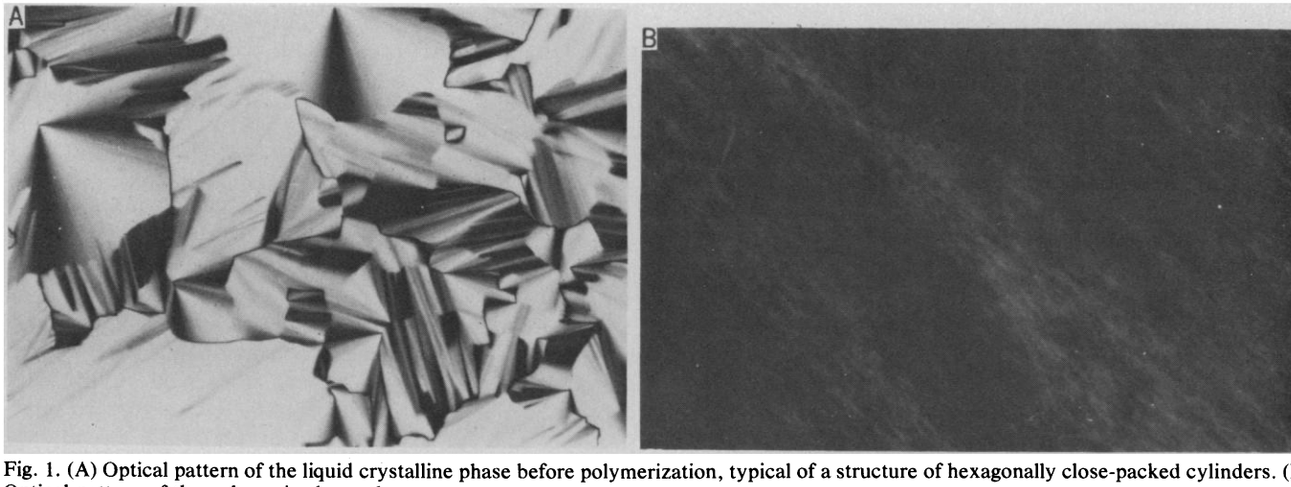


Fig. 1. (A) Optical pattern of the liquid crystalline phase before polymerization, typical of a structure of hexagonally close-packed cylinders. (B) Optical pattern of the polymerized sample.


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