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Construction and identification by positive hybridization-translation of a bacterial plasmid containing a rat growth hormone structural gene sequence

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ABSTRACT

The construction, identification, and use of a recombinant DNA clone containing a growth hormone structural gene sequence is described. A cDNA copy of partially purified pregrowth hormone mRNA from cultured rat pituitary tumor (GC) cells was employed in the construction of a hybrid plasmid, designated pBR322-GH1. The cloned DNA sequence was positively identified by a hybridization-translation procedure which should be applicable to any cloned structural gene sequence. This procedure involved hybridization of cytoplasmic poly(A)-containing RNA from GC cells to the cloned DNA immobilized on nitrocellulose filters, followed by elution of the hybridized RNA and translation in a mRNA-depleted rabbit reticulocyte lysate system. Physical and immunological criteria were employed to show that the translation products were enriched for pregrowth hormone. Hybridization to excess plasmid DNA of [^{3}H]uridine-labeled, size fractionated GC cell cytoplasmic RNA was used to show that all growth hormone-specific RNA sequences are the same size as functional pregrowth hormone mRNA.

INTRODUCTION

The GH cells are related clonal strains of rat pituitary tumor cells which produce one or both of the specialized proteins growth hormone and prolactin (1). Recent studies from a number of laboratories have shown that glucocorticoid (2, 3, 4) and thyroid (3, 5, 4) hormones increase the amount of growth hormone mRNA in these cells, while thyrotropin-releasing hormone increases the amount of prolactin mRNA (6, 7).

The availability of homogeneous DNA probes would permit molecular hybridization studies to be performed, to investigate both the characteristics (size, etc.) of the initial transcripts of the growth hormone and prolactin genes, and the step(s) in the synthesis and processing of the transcripts of these genes at which the hormones listed above exert their actions. It would thus be desirable to use recombinant DNA technology to obtain DNA probes which are complementery to the mRNAs for growth hormone and prolactin.

When a selection procedure for a particular genetic marker is unavailable, as is almost invariably the case when eukaryotic DNA is being

cloned, the conceptual approach of Grunstein and Hogness is invaluable for preliminary screening for cloned DNAs complementary to particular RNAs (8). However, it is the exceptional RNA which can be purified to the homogeneity required for positive identification of a cloned DNA by this procedure (8-12). We have devised a hybridization-translation procedure which positively identifies plasmids which contain DNA complementary to a specific mRNA. In this procedure cytoplasmic poly(A)-containing mRNA is first hybridized to plasmid DNA immobilized on nitrocellulose filters. Following this selection step, hybridized RNA is eluted and translated in a cell-free system. Characterization of the translation product then provides specific positive identification of the recombinant DNA clone. This procedure has been employed to identify a clone containing growth hormone structural gene sequences. We have used this clone to demonstrate that all cytoplasmic growth hormone-specific RNA sequences are the same size as functional pregrowth hormone mRNA.

EXPERIMENTAL PROCEDURES

Growth and fractionation of GC cells. The GH_3 cells are a clonal line of rat pituitary tumor cells which produce both growth hormone and prolactin (1). The GC cells, a subclone of the GH_3 cells which synthesize little or no prolactin and contain little or no preprolactin mRNA (Sussman-Berger, Sehgal and Bancroft, manuscript in preparation) were grown in suspension culture as described (13). In some experiments, cytoplasmic levels of pregrowth hormone mRNA were stimulated by growth of the cells in the presence of the synthetic glucocorticoid dexamethasone (5×10^{-7} M) (2, 15). Preparation of a cytoplasmic lysate (13), or of a post-nuclear membrane fraction (14) which is enriched for pregrowth hormone mRNA (15, 16), were as described.

RNA isolation and fractionation. Precipitation and deproteinization of RNA from a cytoplasmic lysate or the membrane fraction with LiCl-urea (2), chromatography on oligo(dT)-cellulose to yield poly(A)-containing RNA, and fractionation on sucrose gradients were performed as described (15).

Cell-free translation and product analysis. Translation of poly(A)-containing RNA in the Krebs II ascites translation system, followed by direct immunoprecipitation with baboon antiserum to growth hormone plus carrier rat growth hormone was performed as described (16, 15).

Translation of poly(A)-containing RNA in the mRNA-depleted rabbit reticulocyte translation system (17) was as described (15). Following translation, aliquots of the reaction mixture were either: 1. subjected to indirect immunoprecipitation with antiserum to growth hormone plus fixed Staphylococcus aureus as described (15), followed either by treatment of the

precipitates with KOH and Cl_3CCOOH and determination of total acid-insoluble radioactivity (18), or by analysis of the precipitated proteins by SDS-acrylamide gel electrophoresis on slab gels containing 15% acrylamide (19); 2. treated with KOH and Cl_3CCOOH, followed by determination of total acid-insoluble radioactivity as above; or 3. diluted 20-fold with SDS sample buffer (19), boiled for 2 min, then analyzed by SDS-acrylamide gel electrophoresis as above. Following electrophoresis, dried gels were subjected to fluorography using x-ray film which had been pre-fogged to an A_{540} of 0.1-0.2, thus yielding a linear relationship between radioactivity in the gel and absorbance of the film image (20). The developed films were then scanned in a Quick Scan Jr. TLC densitometer equipped with a peak area integrator (Helena Laboratories, Beaumont, Texas).

Enzymatic synthesis of cDNA and construction of hybrid pBR322 DNA-cDNA molecules. Complementary DNA molecules were synthesized from partially purified pregrowth hormone mRNA using the AMV reverse transcriptase enzyme (generously supplied by Dr. J.W. Beard, Life Sciences, Inc.) according to the procedure developed by Monahan et al (21) except that the final concentration of all four deoxyribonucleoside triphosphates was 1 mM, and synthesis was for 60 min. The cDNA was prepared for cloning by synthesis of the second strand of cDNA using E. coli DNA polymerase I, followed by S_1 nuclease treatment and the addition of approximately 100 dA residues to each 3'-OH terminus using calf thymus terminal transferase in the presence of $CoCl_2$ as described by Higuchi et al (11).

The NIH approved EK2 vector plasmid pBR322 (22) was prepared for the insertion of the poly(dA)-tailed cDNA by cleavage with the restriction endonuclease Bam H1 (23) at the single site within the gene encoding tetracycline resistance. Poly(dT), approximately 130 dT residues in length, was synthesized onto each 3'-OH terminus using the terminal transferase enzyme as previously described (11). Poly(dA)-tailed cDNA (25-50 ng) was annealed with 550 ng of poly(dT)-tailed pBR322 DNA in 0.01 M Tris-HCl pH 7.6, 0.1 M NaCl, 0.001 M EDTA for 10 minutes at 65°C followed by incubation for 2 hours at 45°C. The hybrid DNA molecules were used to transform the NIH approved EK2 E. coli strain χ1776. A 50 ml aliquot of χ1776 culture (A_{600} = 0.25) was centrifuged and resuspended in 15 ml of transformation buffer (0.01 M Tris-HCl, 0.14 M NaCl, 0.075 M NaCl, 0.075 M $CaCl_2$, final pH 8.0) and allowed to stand at room temperature 20 minutes. The suspension was centrifuged and the cells resuspended in 3 ml of transformation buffer. A 600 µl aliquot of this χ1776 suspension was added to 300 µl of hybrid DNA molecules in the

reannealing buffer, and the mixture was placed on ice for 20 minutes. The transformed bacteria were spread directly on L-Broth agar plates containing 15 μg/ml ampicillin and incubated at 37°C. Preliminary screening of the resultant colonies for tetracycline sensitivity was performed on L-Broth agar plates containing 10 μg/ml tetracycline.

All cloning procedures were conducted in a P3 biocontainment facility as specified by the NIH Guidelines for Research Involving Recombinant DNA Molecules issued in June, 1976.

Hybridization of ^{3}H-cDNA to RNA. ^{3}H-cDNA synthesized as described above was hybridized to RNA in one to 25 μl of 0.18 M NaCl, 0.01 M Tris-HCl pH 7.4, 0.001 M EDTA, 0.1% SDS in sealed capillaries at 65°C. Two RNA concentrations, 5 μg/ml and 1 mg/ml, were employed. Each reaction was assayed by S1 nuclease digestion as described (24).

Hybridization-translation procedure for identification of cloned cDNA. pBR322-GH1 DNA was alkali-denatured and applied to 25 mm Millipore HA filters under alkaline conditions as described by Melli et al (25). RNA to be hybridized was resuspended in hybridization buffer: 50% formamide, 0.75 M NaCl, 0.1 M Tris-HCl pH 7.6, 0.008 M EDTA, 0.5% SDS. RNA hybridization to pBR322-GH1 DNA filters was carried out at 37°C for 18 hours. The filters were then washed with three successive aliquots of the hybridization buffer at 37°C for 30 minutes. These washes were pooled with the hybridization mixture in which the filters had been incubated to yield the RNA fraction which did not hybridize. The RNA was then precipitated by the addition of 2 vols of ethanol and storage overnight at -20°C. The hybridized RNA was eluted from the filters by three consecutive incubations in 90% formamide, 0.1 M Tris-HCl pH 7.6, 0.008 M EDTA, 0.5% SDS at 60°C for 2 minutes (28). The hybridized RNA was also recovered by ethanol precipitation as above.

The ethanol precipitates were washed twice with 95% ethanol, dried in vacuo, and dissolved in 50 μl of H_2O. Each RNA fraction (total, RNA which did not hybridize, and hybridized RNA) was then assayed by translation in the mRNA-depleted rabbit reticulocyte system as described above.

Hybridization of [^{3}H]uridine-labeled GC cell RNA to pBR322-GH1 DNA. The RNA was hybridized to filter-bound pBR322-GH1 DNA, prepared as described above, in 0.3 M NaCl, 0.01 M Tris-HCl pH 7.4, 0.01 M EDTA, 0.2% SDS at 65°C for 18 hours. The filters were then treated with 2.5 μg/ml pancreatic RNAse A in 2X SSC at 37°C for 0.5 hours, followed by extensive washing with 2X SSC. The filters were then dried and counted in a toluene-based scintillation solution. Non-specific hybridization was corrected for by subtraction of

radioactivity obtained with blank filters without DNA.

Materials. Materials were obtained from the indicated sources. [4,5-^{3}H]leucine (60 Ci/mmole) and [5,6-^{3}H]uridine (40 Ci/mmole), New England Nuclear; [^{35}S]methionine (580 Ci/mmole), Amersham-Searle; oligo(dT)-cellulose (type T3), Collaborative Research (Waltham, Mass.); X-ray film (XR-5, formerly RP-R XOMAT), Eastman-Kodak.

RESULTS

Characterization of mRNA and cDNA

Previous studies have shown that sucrose gradient fractionation of poly(A)-containing RNA from the membrane fraction of GC cells grown in the presence of dexamethasone yields a peak fraction in which pregrowth hormone mRNA represents about 70% of the total mRNA translational activity (15). Following the sucrose gradient centrifugation step of this procedure, we located pregrowth hormone mRNA by cell-free translation of an aliquot of the RNA in each fraction, followed by immunoprecipitation employing specific antiserum to rat growth hormone (Fig. 1). It is seen that, in agreement with our previous results (15), functional pregrowth hormone mRNA has a sedimentation coefficient of about 12S.

The RNA obtained from the 12S region of the sucrose gradient shown in Fig. 1 was pooled and used as a template for the synthesis of a ^{3}H-cDNA probe. This probe was then hybridized with excess poly(A)-containing RNA prepared from the membrane fraction of GC cells which had been grown in the presence of dexamethasone (Fig. 2). Approximately 50-60% of the cDNA sequences are complementary to an abundant component of the mRNA which exhibits a $C_{Rot_{\frac{1}{2}}} \simeq 10^{-2}$. This value of 50-60% corresponds well to the fraction of mRNA which codes for pregrowth hormone. Hence it seemed likely that at least half of our cDNA preparation represented growth hormone-specific sequences. This preparation was used to clone a pregrowth hormone cDNA sequence.

Cloning of pregrowth hormone cDNA

The basic strategy for cloning this cDNA preparation into a bacterial plasmid has been described by Higuchi et al (11). Recombinant DNA molecules were constructed by insertion of cDNA sequences within the gene encoding tetracycline resistance of the plasmid pBR322 as described in Experimental Procedures. These hybrid DNA molecules were then used to transform *E. coli* strain χ1776 as described in Experimental Procedures. To select for transformants, the transformed χ1776 were spread directly on L-Broth agar plates containing ampicillin and incubated at 37°C for 2-4 days. This

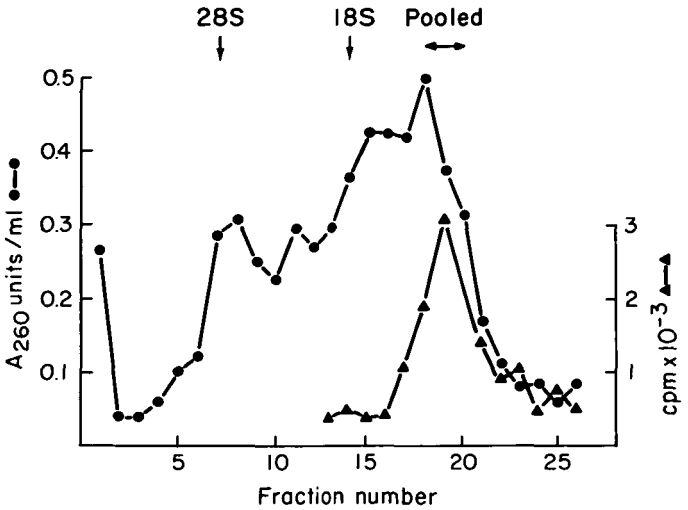

Figure 1. Preparative sucrose gradient ultracentrifugation of GC cell membrane fraction poly(A)-containing RNA.

Membrane fraction poly(A)-containing RNA (120 µg) was isolated from GC cells which had been grown in the presence of dexamethasone (5×10^{-7} M), and analyzed on a 15-30% sucrose gradient, as described (15). The direction of sedimentation was from right to left. Following measurement of the absorbance at 260 nm of each fraction, 10 µg/ml carrier E. coli tRNA was added, and the RNA was ethanol precipitated. Aliquots (16%) of the RNA in each fraction were assayed for pregrowth hormone mRNA activity by translation in the Krebs II ascites cell-free system containing [^{3}H]leucine, followed by immunoprecipitation with antiserum to rat growth hormone as described (18). The RNA in fractions 18-20 was pooled and used as described in the text.
(—●—●—), A_{260}
(—▲—▲—), pregrowth hormone mRNA activity

procedure yielded 14 transformants.

Plasmids containing inserts at the Bam H1 restriction site should lose resistance to tetracycline. Any unrestricted plasmids carried through this procedure should retain tetracycline resistance. None of the 14 transformants grew on L-Broth agar plates containing tetracycline. However, after two weeks only two of the original 14 colonies could be propagated. These colonies were grown on nitrocellulose filters and screened for inserts using the colony-hybridization procedure described by Grunstein and Hogness (8). ^{32}P-cRNA for the hybridization was made from the cDNA preparation employed in the cloning procedure. By autoradiography one colony, designated pBR322-GH1, was positive. This colony was further characterized.

Characterization of plasmid pBR322-GH1

In order to carry out a rapid and convenient characterization of the cloned DNA sequence in plasmid pBR322-GH1, we employed a cell-free translation system to assay the ability of the cloned DNA to selectively hybridize a

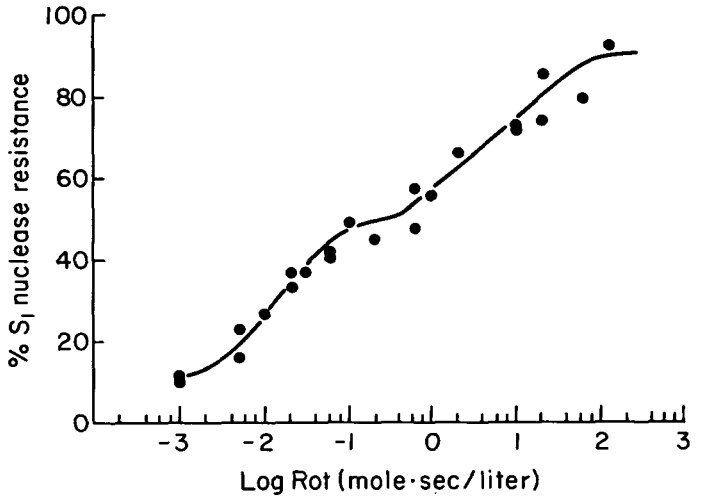

Figure 2. Kinetics of hybridization of cDNA with excess poly(A)-containing RNA from the membrane fraction of GC cells.
^{3}H-cDNA (2000-4000 cpm), synthesized as described in Experimental Procedures from the RNA gradient fractions indicated in Figure 1, was hybridized with an excess of membrane fraction poly(A)-containing RNA isolated from GC cells which had been grown in the presence of dexamethasone, as described in Experimenal Procedures. The percentage of S_1 nuclease resistance (hybridization) is plotted against log R_0t, the product of concentration of RNA in moles of nucleotides per liter X time in seconds.

specific mRNA from a heterogenous population of mRNAs. Plasmid pBR322-GH1 DNA was isolated and bound to nitrocellulose filters, and hybridized to total cytoplasmic poly(A)-containing RNA from GC cells. In order to minimize degradation of RNA, hybridization was carried out for 18 hr at 37°C in a high salt buffer containing 50% formamide. Following extensive washing of the filters, hybridized RNA was eluted from the filters as described in Experimental Procedures. Each RNA fraction (total, hybridized, and RNA which did not hybridize) was assayed by cell-free translation for potential enrichment or depletion of pregrowth hormone mRNA activity. Translation products were analyzed both for radioactivity in total protein and in pregrowth hormone (Table 1) and on SDS-polyacrylamide gels (Fig. 3 and Table 1). Fig. 3A demonstrates that total poly(A)-containing RNA isolated from GC cell cytoplasm codes for a heterogeneous population of proteins, including a prominent peak of pregrowth hormone (19) at 5.9 cm (corresponding to about 24,000 daltons). Gel analysis of an immunoprecipitate of these products (Fig. 3B) shows that only pregrowth hormone [plus a small amount of a product of premature termination of translation of pregrowth hormone mRNA at 7.4 cm, which is occasionally observed in this translation system (15)] is immunoprecipitated.

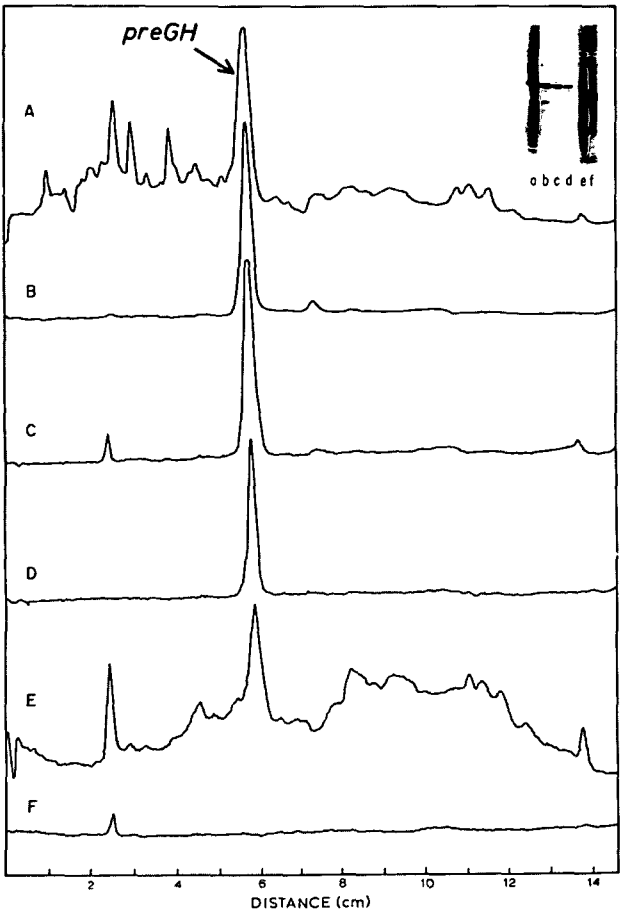

Figure 3. Hybridization of GC cell RNA to pBR322-GH1 DNA and recovery of hybridized pregrowth hormone mRNA.

Total cytoplasmic poly(A)-containing RNA was isolated from GC cells not treated with dexamethasone. An aliquot (57 µg) of this RNA was hybridized to 150 µg of filter-bound pBR322-GH1 DNA, and the hybridized RNA eluted, as described in Experimental Procesures. Under these hybridization conditions, poly(rA)·poly(dT) hybrids are not stable. An aliquot of each fraction (total, hybridized, and unhybridized RNA) was assayed by translation in the mRNA-depleted rabbit reticulocyte lysate system. Immunoprecipitation of aliquots of the reaction mixtures and the analysis of dissociated immunoprecipitates on an SDS-polyacrylamide gel, followed by fluorography (see insert) and scanning, was performed as described in Experimental Procedures. In each case, either 2 µl of reaction product, or the immunoprecipitate of 2 µl of reaction product, was applied to one of the lanes of the gel.

A. Translation products of total RNA.
B. Immunoprecipitate of translation products of total RNA.
C. Translation products of hybridized RNA.
D. Immunoprecipitate of translation products of hybridized RNA.
E. Translation products of RNA which did not hybridize.
F. Translation products in the absence of exogenous RNA.

Table 1. Quantitative analysis of the translation products of RNA hybridized by pBR322-GH1 DNA

RNA translated	Fraction preGH (gel scan)[a]	Fraction preGH (immunoprecipitation)[b]	preGH/globin (gel scan)[a]
	(%)	(%)	
Experiment 1 (Figure 3). Hybridization of GC cell cytoplasmic poly(A)-containing RNA.			
Total	16	16	
Hybridized	62	63	
Unhybridized	8.2	10	
Experiment 2 (Figure 4). Hybridization of a mixture of preGH mRNA and globin mRNA.			
Total	28	27	0.46
Hybridized	75	70	3.00
Unhybridized	12	12	0.17

[a]The area under the pregrowth hormone (preGH) peak divided by either the area of the total fluorogram ("fraction preGH") or by the area under the globin peak ("preGH/globin") was determined for the fluorograms depicted in Figures 3 and 4, respectively.

[b]Following translation of the RNA in the mRNA-depleted reticulocyte lysate system containing [^{35}S]methionine, the ratio of the radioactivity specifically precipitated by indirect immunoprecipitation with antiserum to rat growth hormone plus fixed S. aureus to the total stimulated acid-insoluble radioactivity was determined.

Upon gel analysis of the translation products of the RNA which hybridized to pBR322-GH1 DNA (Fig. 3C), only pregrowth hormone was observed, identified by its position and also by its precipitation by antiserum to growth hormone (Fig. 3D). In addition, a small amount of the product of an endogenous mRNA (2.5 cm; Fig. 3F) was detected. Gel analysis of the translation products of the RNA which did not hybridize (Fig. 3E) again yielded a heterogeneous population of proteins.

A quantitative analysis of this experiment is shown in Table 1 (Experiment 1). The fraction of the total translation products represented by pregrowth hormone, assayed either by quantitation of the gels of total products or by immunoprecipitation of the total products, are in excellent agreement. The hybridized fraction of GC cell cytoplasmic poly(A)-containing RNA was enriched for pregrowth hormone mRNA activity, and a depletion of pregrowth hormone mRNA was observed in the RNA which did not hybridize.

The experiment described above demonstrates that hybridization of GC

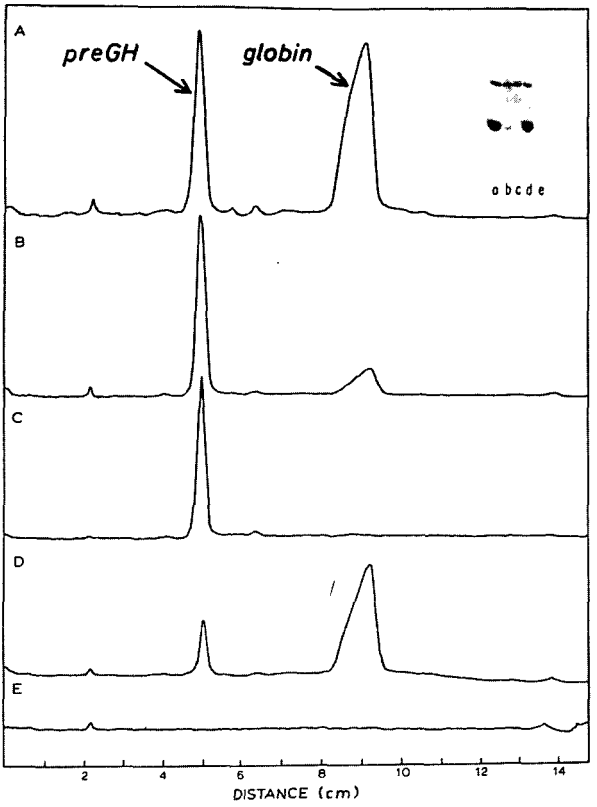

Figure 4. Hybridization of pregrowth hormone mRNA by pBR322-GH1 DNA in a reconstruction experiment.

Equal quantities of poly(A)-containing RNA prepared from the membrane fraction of GC cells treated with dexamethasone, and of poly(A)-containing RNA prepared from rabbit reticulocyte polysomes were mixed. An aliquot (89 μg) of this mixture was hybridized to 150 μg of filter-bound pBR322-GH1 DNA and processed as described in Fig. 3. Translation of the resulting RNA fractions, immunoprecipitation, and analysis by SDS-polyacrylamide gel electrophoresis was as in Fig. 3, except that the volumes of the reaction products applied to the gel or immunoprecipitated were 3 μl. A fluorograph of the gel is shown in the insert.

A. Translation products of total RNA.
B. Translation products of hybridized RNA.
C. Immunoprecipitate of translation products of hybridized RNA.
D. Translation products of RNA which did not hybridize.

cell mRNA with pBR322-GH1 DNA enriches for pregrowth hormone mRNA translational activity. However, it could be argued that this result was due to increased translation efficiency of pregrowth hormone mRNA relative to other GC cell mRNA's following hybridization. To test this possibility, a reconstruction experiment was performed, in which a mixture of equal amounts of partially purified pregrowth hormone mRNA and globin mRNA was hybridized

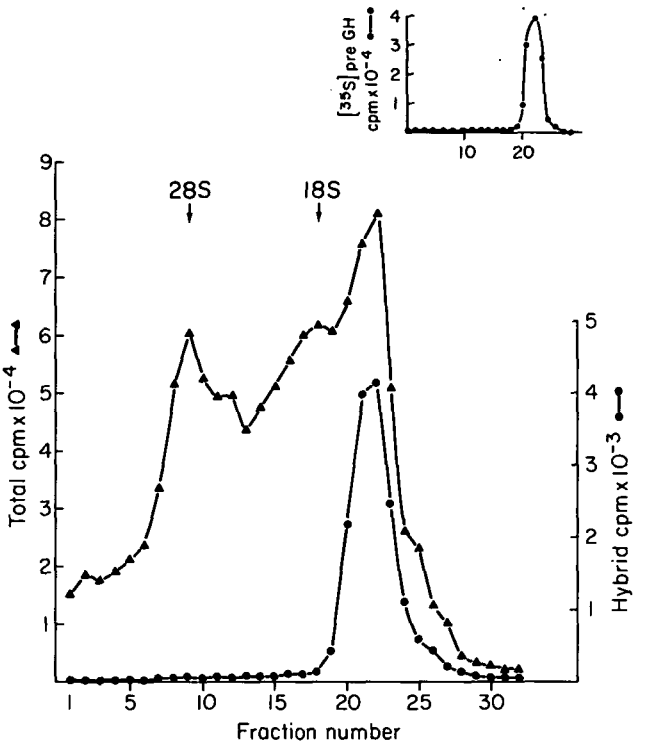

Figure 5. Hybridization of [^{3}H]uridine-labeled GC cell RNA to pBR322-GH1 DNA.
GC cells were incubated with dexamethasone plus 100 μCi/ml [^{3}H]uridine for 48 hrs. The poly(A)-containing RNA was then isolated from the membrane fraction and analyzed on a sucrose gradient as in Fig. 1. Aliquots (400 μl) of each fraction were assayed for total acid-insoluble radioactivity. Other aliquots (150 μl) were hybridized to 5 μg of filter-bound pBR322-GH1 DNA as described in Experimental Procedures. Inset: Cold GC cell RNA was prepared, and analyzed on a parallel gradient. Each fraction was assayed for pregrowth hormone mRNA activity in the mRNA-depleted reticulocyte lysate system.
(—▲—▲—), total acid-insoluble [^{3}H]uridine-labeled RNA in 400 μl.
(—●—●—), hybridized [^{3}H]uridine-labeled RNA in 150 μl.
(—●—●—), pregrowth hormone mRNA activity. (Inset).

to pBR322-GH1 DNA. Globin mRNA was chosen for this experiment because it is translated in the mRNA-depleted reticulocyte lysate system twice as efficiently as GC cell mRNA (15). Analysis on gels of the translation products of total RNA, hybridized RNA, and RNA which did not hybridize is shown in Figure 4, and the quantitation of the gels is shown in Table 1 (Experiment 2). The ratio of pregrowth hormone to globin synthesized in response to the total reconstruction RNA mixture was about 0.5 (Fig. 4A and Table 1). This ratio was increased about 6-fold when the RNA which hybridized to pBR322-GH1 DNA was translated (Fig. 4B and Table 1), and was decreased

about 3-fold when the RNA which did not hybridize was translated (Fig. 4D and Table 1). (The large width of the globin peaks in Fig. 4 is due to the high concentration of globin in the translation system employed). Definitive identification of the protein migrating at 5.0 cm in Fig. 4B as pregrowth hormone was provided by its position, by its precipitation by antiserum to growth hormone (Fig. 4C), and by the observation that the products of digestion by Staphylococcus aureus V8 protease (26) of either total translation products of the hybridized RNA in the present experiments or of the translation products of purified pregrowth hormone mRNA (15) yielded the same four major peptides upon SDS-polyacrylamide gel electrophoresis (Bancroft, unpublished observations).

Use of pBR322-GH1 DNA as a tool for the analysis of labeled pregrowth hormone mRNA

A primary purpose for cloning a DNA sequence complementary to pregrowth hormone mRNA was to provide a DNA probe to analyze the stages in the production of this mRNA by pituitary cells. Figure 5 shows the size distribution of [^{3}H]uridine-labeled poly(A)-containing RNA from cells grown in the presence of dexamethasone. When this RNA was hybridized to nitrocellulose filters containing pBR322-GH1 DNA, a single peak in the 12S region of the gradient was observed (Fig. 5). This peak coincides with the position of pregrowth hormone mRNA activity, determined in a gradient run in parallel (Fig. 5, insert). This result provides additional evidence for the selectivity of pBR322-GH1 DNA in hybridizing pregrowth hormone mRNA. Furthermore, it demonstrates that all pregrowth hormone mRNA sequences in the cytoplasm of the GH cells are the same size as pregrowth hormone mRNA translational activity.

DISCUSSION

We have constructed a hybrid bacterial plasmid containing a structural gene sequence for rat growth hormone. The basic strategy involved the cloning of a cDNA sequence, copied from partially pure (50-60%) rat pregrowth hormone mRNA, according to procedures developed for purified mRNA (11). The use of the plasmid pBR322 allowed a rapid identification of bacterial colonies containing recombinant plasmids. The colony hybridization procedure then provided identification of a clone, designated pBR322-GH1, containing a cDNA sequence which was an abundant component of the original cDNA preparation. However, definitive identification of the cloned DNA sequence required further characterization.

To identify the cDNA sequence in pBR322-GH1, we have developed an assay which allows the rapid and accurate identification of such a sequence. Total GC cell poly(A)-containing RNA was hybridized to pBR322-GH1 DNA immobilized on nitrocellulose filters, under conditions which would minimize RNA degradation. The RNA which hybridized or did not hybridize to the cloned DNA sequence was recovered and analyzed in the mRNA-depleted rabbit reticulocyte cell-free translation system. A further analysis of the specificity of the hybridization procedure was obtained in a reconstruction experiment using a mixture of globin mRNA and partially purified pregrowth hormone mRNA. Analysis of the *in vitro* translation products by size, indirect immunoprecipitation, and proteolytic cleavage provided conclusive evidence that pBR322-GH1 DNA selectively hybridizes pregrowth hormone mRNA (Figs.3 and 4; Table 1).

This hybridization-translation procedure should be generally applicable to the rapid identification of any cloned DNA sequence complementary to a mRNA for which the translation product can be identified. This procedure should be applicable to mRNAs of low abundance, since the product of as little as 50 pg of mRNA can be detected in this translation system (Soreq, Evans and Harpold, unpublished observations). In addition, this procedure provides a simple technique for the purification of a specific mRNA from a heterogeneous RNA preparation. Thus we have shown that at least a 4-fold purification of pregrowth hormone mRNA can be obtained in a single hybridization step (Table 1).

Our primary purpose for constructing a hybrid plasmid containing a rat pregrowth hormone cDNA sequence was to provide essentially unlimited amounts of this cDNA sequence for use as a specific probe for investigations of both growth hormone gene structure and of the various steps involved in the production of pregrowth hormone mRNA. Investigations of pregrowth hormone mRNA production require the demonstration that this DNA will specifically hybridize labeled growth hormone RNA sequences.

Molecular hybridization of size fractionated labeled GC cell poly(A)-containing RNA to an excess of pBR322-GH1 DNA showed that the plasmid DNA specifically hybridized a discrete cytoplasmic 12S growth hormone-specific species of RNA (Fig. 5). The observation that the profiles of hybridization and of pregrowth hormone mRNA activity were coincident shows that all cytoplasmic growth hormone-specific RNA is the same size as functional pregrowth hormone mRNA. These results are consistent with earlier observations that the size of pregrowth hormone mRNA (including the poly(A) tract) is

approximately 1000 nucleotides (2, 15). Restriction endonuclease mapping of pBR322-GH1 DNA indicates that the size of the insert is 880 b.p. (data not shown). Therefore, excluding the poly(dA·dT) tails, there are approximately 680 b.p. of cDNA complementary to pregrowth hormone mRNA within pBR322-GH1.

In conclusion, we have reported here the cloning of a growth hormone structural gene sequence in a bacterial plasmid, and have presented evidence of its application in various preparative and analytical procedures. This hybrid plasmid should be useful for defining the structure of the growth hormone gene and its expression.

While this manuscript was in preparation, a report appeared describing the construction and sequence analysis of a bacterial plasmid containing a rat growth hormone structural gene sequence (27).

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