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Construction and Screening of a cDNA Library to Isolate Chicken Pituitary Hormone Genes

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ABSTRACT. A chicken pituitary cDNA expression library has been constructed in the bacteriophage vector λgt 11 (methods for the bacteriophage DNA propagation, modification, ligation of the double-stranded cDNA insert and in vitro packaging are included.) The chicken pituitary cDNA library was screened with heterogeneous pituitary cDNA clones, and several putative chicken specific pituitary cDNA clones were isolated. These putative chicken specific cDNAs should prove most useful in determining the structural organization of the various pituitary hormone genes and help to better understand the molecular mechanisms of how these sequences affect growth, reproduction, and metabolism in the chicken. For educational purposes, the techniques of cDNA library construction and screening should allow advanced undergraduate students the opportunity to isolate specific cDNA clones of interest. After learning such methodologies, students should be in an advantageous position in the promising job market for recombinant DNA technology and for admission to the best graduate schools in the areas of cellular and molecular biology.

INTRODUCTION

Complementary DNA (cDNA) synthesized from poly A+ -containing, messenger RNA (mRNA) transcripts can be used to construct cDNA libraries in plasmid or bacteriophage vectors. Such cDNA libraries are useful for isolating and cloning specific cDNA sequences using homologous as well as heterologous probes. Since cDNA libraries contain only sequences that are actively transcribed (as opposed to genomic libraries that theoretically contain all the sequences of a particular genome), the relative abundance levels of the various mRNAs will be reflected in the representative number of recombinant cDNA molecules present in the library. Less than half the mass of the mRNA in a cell consists of a large number of mRNA sequences (10,000 or more), each of which is represented by a small number of mRNA copies per cell (Lewin 1980). These low abundance mRNA molecules may only be present in 10 copies per cell and constitute about 0.005% or less of the mass of total cellular mRNA. In order to isolate the desired low abundance cDNA clone, it would be necessary to screen 10^7 members (i.e., bacteriophage plaques containing specific cDNA inserts) of a recombinant cDNA library. For isolating middle abundant mRNA molecules (approximately 10^4-10^5 copies per cell or 0.05% to 5% of the mass of total mRNA), 10^3-10^5 recombinant members would have to be screened. On the other hand, high abundance mRNA molecules (i.e., 10^5-10^6 copies per cell or 5-50% of the mass of total mRNA mass) would only require screening 10^2-10^4 recombinant members. Due to their increased representation in a cDNA library, a number of high abundance class mRNA molecules have been isolated and cloned. There is no correlation between the abundance level of a particular mRNA species in a cell and the copy number of that corresponding gene. Examples of single copy genes that are highly transcribed include the ovalbumin gene (Har-
and their role in growth, reproduction, and metabolism in vivo.

The approaches outlined can be used as workable methodologies for educating undergraduates in the cellular molecular biology area. The technologies are not merely limited to the larger research laboratories since many of the procedures actually have been simplified and optimized as molecular biology reaction kits for the various parts of the cDNA library construction. By introducing the techniques and terminology of recombinant DNA molecular biology, undergraduates will be better prepared for the expanding job market in biotechnology or acceptance into the better cellular and molecular biology graduate school programs.

MATERIALS AND METHODS

VECTOR PREPARATION. The bacteriophage vector Agt 11 was grown in Escherichia coli BNN97 in T broth (10 g tryptone, 5 g NaCl, 2 g maltose, 10 mM MgSO4 per L) at 32°C until reaching 0.4 OD units (A260). The phage preparation was heat-shocked for 15 min at 42°C to induce lysis, and was then grown overnight at 37°C shaking. The phage were purified essentially as described in Maniatis et al. (1982). The purified Agt 11 DNA was cut with restriction endonuclease Eco R1 (Boehringer Mannheim Biochemicals, Indianapolis, IN) followed by phosphatase treatment. This last step prevents self-reigation of the vector and greatly increases the number of recombinant molecules. (For best results we use 2-3 units of Boehringer Mannheim, molecular biology-grade, calf intestine, alkaline phosphatase per 100 µg DNA per 30-min reaction at 37°C, followed by heat inactivation at 68°C for 15 min, phenol extraction twice, and a final chloroform extraction before ethanol precipitation of the Agt 11 DNA.)

cDNA INSERT SYNTHESIS AND MODIFICATION. Approximately 300 pituitaries from adult layer hens were isolated and immediately frozen on dry ice. Tissues (approximately 2.1 g) were dispersed by growing in radiolabeled nucleotide using essentially the procedure of Buell et al. mRNA were primed with +PdCTP in a 500: 1 ratio of unlabeled to +PdCTP in the presence of Eco R1 and phosphatase treatment. This indicates that full length, cDNA represents full length ovalbumin cDNA can be seen in the second lane of the Agt 11 vector that had been cleaved with Eco R1, then ligated to Agt 11 vector, and phosphatased. The recombinant molecules were packaged in vitro packaging kits (Amersham Corp., Arlington Heights, IL) and grown in E. coli Y1088 to generate a cDNA library. The 3 X 10⁶ plaque-forming units in the library contained 7 X 10⁶ individual members of which 76% were recombinant (as scored by white or blue plaques with the chromogenic indicator X gal and isopropyl β-D-thiogalactopyranoside).

RESULTS

To screen the library, approximately 10⁶ members of the cDNA library (enough members to detect a RNA abundance level of 50-100 transcripts) were adsorbed to E. coli Y1090. After a 15-min incubation, the mixture was added to 7 ml of melted, 0.7%, low-gelling, temperature agarose in T broth and plated onto 20 T broth plates and incubated overnight at 37°C. After allowing the top agarose to harden for 1 h at 4°C, two replica nitrocellulose filters were made per plate with modifications of the Benton and Davis (1977) technique. Filters were denatured in NaOH, neutralized, then baked and prepared for hybridization with essentially the procedures of Jeffries and Flavell (1977). The filters were probed with a cloned, heterologous, bovine TSHβ cDNA (clone 24-7). The bTSHβ cloned DNA was first transfected into recA⁺ HB 101 cells under antibiotic selection and then grown in mass. The cloned plasmid DNA was digested with Pst I and electrophoresed on a 1.0% agarose gel. The 531-bp bTSH insert was cut out of the gel. The DNA was removed from agarose by electroelution and recovered after ethanol pre-
Hybridization conditions as in Fig. 2. Plaques hybridize, suggesting sequences have been plaque-purified. Clones from the chicken pituitary cDNA expression library. All clones were plaque-purified (i.e., every plaque present was plaque-purified). A single plaque from one of the four clones was selected and grown in mass in T broth. The phage were sedimented after DNase 1, RNase A, and RNase T1 digestion and then lysed after heat inactivation of the DNase. The cloned DNA was digested with proteinase K, extracted twice with phenol, and precipitated with ethanol. Cloned DNA was digested with EcoRI, electrophoresed on a 1% agarose gel, and stained with ethidium bromide. A single EcoRI band (approx. 380 bp) was observed. The DNA was transferred to nitrocellulose paper according to Jeffries and Flavell (1977), baked and hybridized for 24 h to the nick-translated bTSHβ probe according to Johnson et al. (1984). Following washing at 55°C in .2 x SSC, the filter was dried and exposed to X-ray film. Audioradiography (data not shown) revealed that the digested, putative chicken TSHβ cDNA-cloned insert hybridized to the heterologous, bTSHβ probe.

**DISCUSSION**

One of the goals of this laboratory is to better understand what regulates the growth, reproduction, and metabolism of the chicken. Toward this goal we have begun isolating a number of the chicken pituitary hormone genes in order to define their organization and to determine by what mechanisms the gene products of these sequences regulate a number of physiological processes in the bird. Also, we are interested in learning more about the regulation of pituitary hormone gene expression in vitro and in the animal. The construction of a cDNA library from chicken pituitary poly A+ mRNA has served as a first step for isolating and cloning a number of the pituitary hormone genes. We have isolated putative chicken pituitary hormone cDNA clones from the recently constructed library and will be proving their identity by sequence analysis. Once the various chicken pituitary hormone genes and their cDNAs have been isolated and characterized, we hope to produce these hormones in large amounts by recombinant DNA methodologies. For the first time we should have enough purified, biologically active material to assess the effects of these molecules in the chicken. It is hoped that through such techniques as site-directed mutagenesis, more potent pituitary hormones may be produced, and that a more efficient animal in terms of growth and metabolism might result.

**RELEVANCE OF TECHNIQUES TO UNDERGRADUATE EDUCATION**

The recombinant DNA methodologies that have been outlined are also well suited for use in a number of advanced undergraduate courses and senior honors projects. These techniques at one time were appropriate only for graduate and post-doctoral investigators, but now in many instances undergraduates are expected to have a working knowledge of such procedures before entering graduate school or the technical job market. The principles of cDNA library construction and the isolation of specific cDNA sequences using antibody or nucleic acid probes can be taught in undergraduate courses in genetics and molecular biology, or in a seminar on laboratory techniques. In addition, the "hands-on" laboratory techniques outlined can be most beneficial for students that are pursuing a senior honors project involving laboratory work. The techniques lend themselves well to usage by the individual student or groups of students. There are also many "stopping points" along the way, so that if one group of undergraduates can complete only a certain portion of the library construction or screening, another group can continue on toward the completion of the project at a later time. During the course of working with these recombinant DNA methodologies, students are exposed to a variety of basic molecular techniques. When a solid undergraduate...
biology and chemistry background is combined with a knowledge of some or all of the techniques involved with cDNA library construction and gene cloning, students will also have a distinct advantage over those devoid of such training for the very competitive positions in molecular genetics and biochemistry departments in our best graduate schools. If these students do not wish to pursue post-graduate education, their training will allow them to compete effectively in the technical job market. It has been estimated that by the year 1990 some 37,000 jobs involving some aspect of recombinant DNA technology will be available. This number will undoubtedly increase greatly by the year 2000 (U.S. Dept. of Health and Human Services 1985), both in industrial and academic settings. There is already a demand for well trained technicians in industry and academic institutions. The employment future for such individuals is indeed bright.

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LITERATURE CITED


