Construction of a Recombinant Bacterial Plasmid Containing Pro-α(I) Collagen DNA Sequences

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A partially purified mRNA preparation enriched for chick collagen messenger RNA activity was used as template for the synthesis of double stranded cDNA. The cDNA was ligated into the HindIII site of the plasmid vector pBR322 and used to transform Escherichia coli x3776. One plasmid with an 800-base pair insert was shown to contain DNA sequences corresponding to Type I pro-α(I) collagen.

The collagens are a group of proteins which play an important structural function in the extracellular matrix surrounding the cells of vertebrate tissues and also perform a central role in the morphogenesis of tissues during embryonic development (1). The synthesis of collagen in cultured cells can be influenced by various factors (2-4) including tumor viruses (5, 6). Studies on the regulation of the collagen genes and their arrangement in the genome will help elucidate the changes that occur in neoplastic transformation and differentiation. To perform such studies we have constructed and cloned hybrid bacterial plasmids containing cDNA sequences corresponding to some of the collagen genes. One hybrid plasmid containing a pro-α2 collagen cDNA sequence was described recently by us (7), and by Lehrach et al. (8). Here we report the isolation of a cDNA clone coding for chick pro-α(I) collagen.

EXPERIMENTAL PROCEDURES

Isolating cell strain, plasmid and growth conditions. E. coli K1277 were grown in aerobic conditions as described by K. Modrich (1).

Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Maryland 20014. Request Document 9-1546, cite author(s), and include a check or money order for $1.00 per set of photocopies. The abbreviations used in the miniprint are: SSC, 0.015 M sodium citrate, 0.15 M NaCl; SDS, sodium dodecyl sulfate.
Molecular Cloning of Chick Pro-α1(I) Collagen cDNA Sequences

FIG. 1. Endonuclease digestion products of pCOL3 and its cloned inserted DNA. pCOL3 DNA was digested by various restriction enzymes as described under "Experimental Procedures." Restriction fragments were fractionated by electrophoresis on a 4% (A), 4% (B), 7% (C), or 5% (D) polyacrylamide gel and identified after staining with ethidium bromide. Lanes A1, C1, and D1, φX174 DNA digested by HaeIII, providing molecular size markers. The arrow beside lanes C1 and D1 point to DNA fragments from top to bottom of 1342, 1078, 872, 606, 310, 278, 271, 234, 194, 118, and 72 base pairs. Lane A2, pCOL3 DNA digested by HindIII; lane B1, φH532 digested by ApuI; lane B2, pCOL3 DNA digested by ApuI; lanes C2 to C9 and D2 and D3 show the endonuclease digestion products of the 800-base pair cloned insert digested by HapII (lane C2); HaeIII (lane C3); ApuI (lane C4); Hinfl (lane C5); Hinfl and ApuI (lane C6); HaeIII and ApuI (lane C7); Hinfl and HapII (lane C8); HaeIII and HapII (lane C9); KpnI (lane D2); KpnI and HapII (lane D3).

RESULTS AND DISCUSSION

Recombinant plasmids containing cDNA sequences were constructed as described under "Experimental Procedures." The plasmids were screened for collagen DNA sequences with DNA restriction fragments which had been tentatively shown to contain collagen DNA sequences (9). Out of 124 colonies containing hybrid plasmids one gave a strong hybridization signal and several others gave weaker signals. Plasmid DNA was prepared from the colony giving a strong signal; this plasmid was designated pCOL3.

Characterization of pCOL3—Cleavage of pCOL3 by restriction endonucleases showed that this recombinant plasmid contained a DNA insert of 800 base pairs. (Fig. 1, lane A2). To verify that some of the restriction fragments which were used as probes were also found in the recombinant plasmid, we cleaved the 800-base pair insert by endonuclease HapII. Fig. 1 (lane C2) shows that such digestion generates four fragments with sizes 270, 220, 200, and 110 base pairs. Two of these subfragments (200 base pairs and 110 base pairs) are identical in size to two of the cDNA fragments (9) which were used as probes in the colony hybridization. The identity of these fragments was confirmed by direct comparison of the DNA sequences of the cloned fragments with the DNA sequence of the cDNA fragments which we had determined previously (9). Digestion of the 800-base pair insert by endonuclease HaeIII (Fig. 1, lane C3) also generated four fragments of 320, 190, 170, and 120 base pairs. The 170-base pair and 120-base pair fragments are probably identical with two of the fragments obtained when ds collagen cDNA is cleaved by HaeIII (9).

To construct a more complete restriction map, we first performed single digestions with the restriction enzymes ApuI, BstXI, and KpnI, and then performed double digestions with BstXI and ApuI, ApuI and HaeIII, BstXI and HaeIII, and HaeIII and KpnI (Fig. 1, lanes C4 to C9, and D2 and D3). The resulting restriction map is shown in Fig. 2.

Evidence that pCOL3 Contains pro-α1 Collagen DNA Sequences—To determine if the cloned insert was a collagen gene sequence, DNA from pCOL3 was made linear with PstI, denatured, and coupled to DBM-cellulose (10, 11). Total poly(A)-containing RNA from chick embryo calvaria and long bones was hybridized to cellulose-bound pCOL3 DNA. After washing extensively, the complemenary RNA was eluted and translated in a reticulocyte lysate (Fig. 3). The RNA which hybridized to pCOL3 directs the synthesis of one discrete protein.

The abbreviations used are: ds, double stranded; DBM-cellulose, diazobenzyloxymethyl-cellulose.

1 H. DiLauro and M. Sobel, manuscript in preparation.

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The data presented in Fig. 1 were used to deduce this restriction map.

Fig. 2. Restriction map of the cloned insert of pCOL3. The polypeptide with a Mr of 180,000 (lanes 3 and 4). This polypeptide was completely digested by purified bacterial collagenase, whereas other proteins in the extract were barely affected (lane 5). This indicates the peptide is some type of collagen. The translation product has a Mr of 180,000 which strongly suggests it is pro-α1 collagen, since pro-α2 collagen has a Mr of 150,000. These are the two major types of collagen species found in chick embryo calvaria and long bones.

To confirm the identity of the clone, limited DNA sequencing was performed and compared with the sequence of a cyanogen bromide fragment derived from the C peptide of pro-α1(1) collagen. The partial DNA sequence of the 170 base pair HaeIII fragment (see Fig. 2) is as follows:

ATGGACGTTGGCGCTCCGGAC. This corresponds to a protein sequence Met-Asp-Val-Gly-Ala-Pro-Asp which is found in the fragment. Therefore, we conclude pCOL3 contains DNA sequences corresponding to pro-α1(1) collagen.

We also performed an R-loop experiment (12) by hybridizing pCOL3 to collagen mRNA enriched to 30% purity on a sucrose gradient. The hybrid plasmid was treated with PstI, which cleaves about 770 base pairs from the HindIII site. Fig. 4 shows a representative R-loop. Measurements on 49 different hybrids indicated an average R-loop size of about 600 to 800 base pairs, which occurs about 750 base pairs from one end of the plasmid.

The hybrid clone pCOL3 as well as a hybrid cDNA clone corresponding to pro-α2 collagen (7) have been used to show that procollagen mRNA levels are markedly reduced in Rous sarcoma virus-transformed cells (11).

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