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SYNTHESIS OF RAT APOLIPOPROTEIN E BY ESCHERICHIA COLI INFECTED WITH RECOMBINANT BACTERIOPHAGE

Felice A. de Jong, Geoffrey Howlett, Angela R. Aldred,
Noel Fidge*, and Gerhard Schreiber

The Russell Grimwade School of Biochemistry, University of Melbourne,
and *The Baker Medical Research Institute,
Melbourne, Australia

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A cDNA library was constructed from rat liver polyadenylated RNA using the expression vector λgt11-Amp3. Several clones expressing antigenic determinants for rat apolipoprotein E were identified. The cDNA insert in one clone was further characterized and found to have a sufficient length (1120 base pairs) to code for full length apolipoprotein E. Restriction mapping and nucleotide sequencing showed the clone to contain the coding region for apolipoprotein E flanked by about 120 nucleotides at the 3'-side and by about 64 nucleotides on the 5'-side. One of the proteins produced by the clone was found to be a prokaryotic/eukaryotic hybrid protein reacting with antibodies to both bacterial β-galactosidase and rat apolipoprotein E.

The synthesis of 3 mammalian plasma proteins, i.e. human serum albumin (1), rat α_1-acid glycoprotein (2) and rat major acute phase α_1-protein (3), by bacteria transformed with recombinant plasmids has been reported recently. In the following, we describe the synthesis of a protein possessing antigenic determinants of rat apolipoprotein E by E. coli BTA282 which had been infected with a recombinant bacteriophage derived from λgt11-Amp3.

METHODS

Reagents and Bacterial Strains

DNA polymerase I, restriction endonucleases EcoRI, TaqI, BglI, S1 nuclease, T4 ligase and alkaline phosphatase were from Boehringer Mannheim, EcoRI linkers, SstI, and other restriction nucleases from New England Biolabs, EcoRI methylase from Bethesda Research Laboratories, reverse transcriptase from J.W. Beard (National Institute of Health), oligo(dT)$_{12-18}$ and oligo(dT)-cellulose from PL Biochemicals, Bio-gel A-50m from BioRad, DEAE membrane filter (NA-45) and nitrocellulose sheets from Schleicher and Schüll, and [^{32}P]dATP from the Radiochemical Centre, Amersham. E. coli strains C600, LE 392, RY 1073, RY 1082 (4) and bacteriophage λgt10 were provided by R. Young, T. Huynh and R. Davis (Stanford University), the bacteriophage vector λgt11-Amp3 (5) and the lon$^-$ mutant BTA282, an R$^-$M$^+$ derivative of RY 1082 produced by transduction, by D. Kemp (Melbourne) and Biotechnology Australia,

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respectively, and antiserum against β-galactosidase by R. Anders (Melbourne). Antiserum against apolipoprotein E was prepared as described elsewhere (6).

Construction of cDNA Clones in λgt11-Amp3; Immunochemical Screening of Colonies

The λgt11-Amp3 libraries were constructed as described by Kemp et al. (5) except that cDNA methylated with EcoRI methylase was fractionated on a Bio-gel A-50m column prior to ligation with the EcoRI cleaved λgt10 DNA. The amplified cDNA inserts were then excised using endonuclease EcoRI and inserted into the λgt11-Amp3 vector. Colonies infected with λgt11-Amp3 were grown on nitrocellulose filters and immunochemically screened (5). Experiments were carried out under C1 containment conditions of the Committee on Recombinant DNA Molecules of the Australian Academy of Science.

Preparation of Bacteriophage λ and DNA Extracts

Large amounts of bacteriophage λ from one recombinant, named λAPE3, were prepared (7) and collected, after ultracentrifugation, at the interface of a step gradient of 3 M over 5 M cesium chloride. DNA was extracted with 1 volume formamide and precipitated with 2 volumes ethanol.

Isolation and Characterization of Apolipoprotein E Insert

Digestion of recombinant λDNA with EcoRI, separation of fragments by electrophoresis in polyacrylamide gel and restriction mapping of DNA followed conventional methods (7).

Western Blotting and Nucleotide Sequencing

Proteins separated by electrophoresis in a 7.5% polyacrylamide gel containing 0.1% sodium dodecylsulfate were transferred electrophoretically onto nitrocellulose sheets (8) and incubated with antiserum against β-galactosidase (1:2000 dilution) or against apolipoprotein E (1:15,000 dilution). After washing, the nitrocellulose sheets were treated with ^{125}I-labeled protein A (40-70 Ci/g), washed again, dried and exposed for 15 hours on XRP-film (Kodak) using an intensifying screen. DNA sequencing was performed according to Maxam and Gilbert (9).

RESULTS

Construction of Recombinant λgt10 and λgt11-Amp3 Libraries

In the preparation of recombinant λgt10, 1 μg polyadenylated RNA was copied into cDNA giving a total of 2.2×10^7 recombinants. The cDNA amplified in λgt10 was ligated into the EcoRI site of λgt11-Amp3. A λgt11-Amp3 library with 16,000 recombinants (of which 94% had inserts) was screened for the expression of apolipoprotein E.

Identification of Clones Expressing Apolipoprotein E

Initial screening of 9,000 λgt11-Amp3 cDNA colonies resulted in the detection of eight colonies which reacted strongly with antiserum against apolipoprotein E (Fig. 1A). Positive colonies were streaked out to achieve

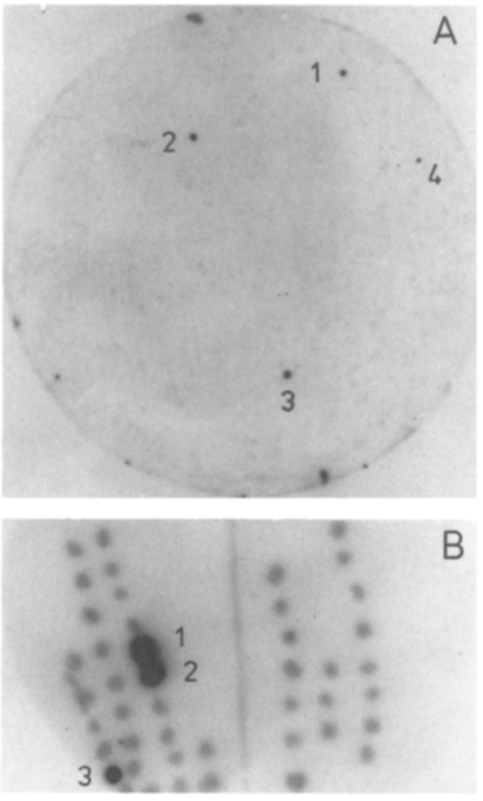

Figure 1. Example of immunological screening of E. coli colonies with antiserum against apolipoprotein E (dilution 1:15000). Colonies binding antibody were detected using ^{125}I-protein A followed by autoradiography as described by Kemp et al. (5).
A. Primary Screening: Numbers indicate areas from which colonies were subjected to a second round of screening. (Areas situated on the margin were not considered since it was found in preliminary experiments that ^{125}I-protein A tends to stick nonspecifically to creases or margins of nitrocellulose sheets.)
B. Secondary Screening: Apolipoprotein E expressing colonies 1, 2 and 3 were derived from areas 1,2 and 3 respectively from A.

colonies derived from single cells which were then screened again with antiserum. Five colonies were obtained in this way from the eight original colonies (Fig. 1B).

Characterization of Insert

From one colony, containing λAPE3, the cDNA insert was excised with endonuclease EcoRI. Its size was determined by comparing electrophoretic mobility in a 2% agarose gel with that of EcoRI/HindIII, and PstI fragments of wild type λDNA to be 1120 base pairs (Fig. 2). Digestion with restriction endonucleases SstI, TaqI, BglI, and EcoRI led to the construction of the

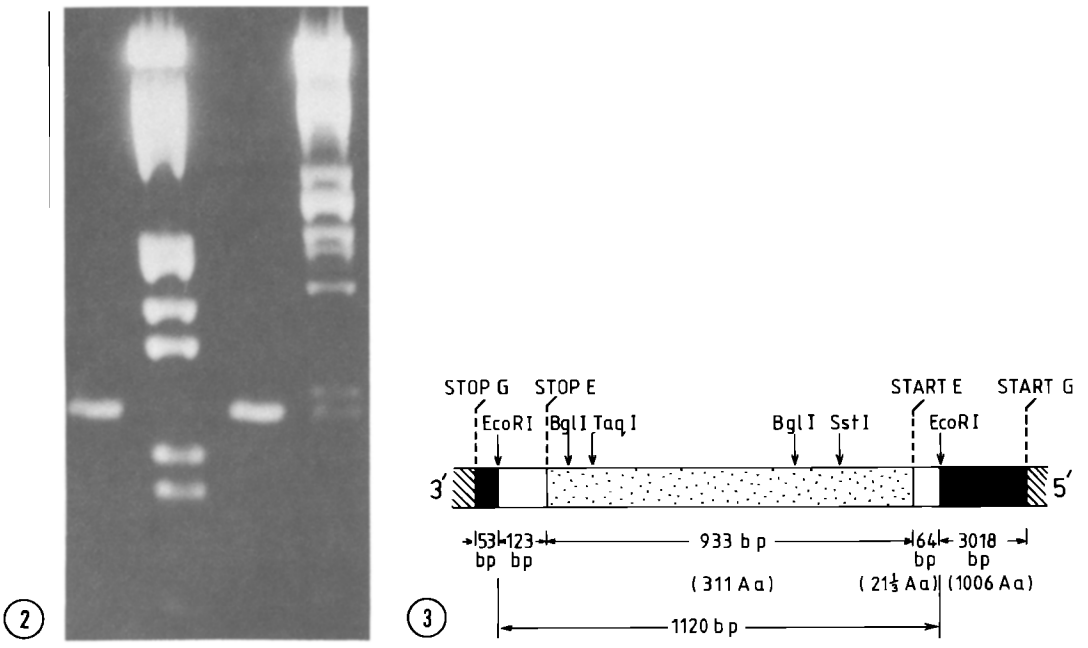

Figure 2. Determination of the size of the insert in λAPE3 by electrophoresis in a 2% agarose gel. Lanes 2 and 4 (counting from left to right), insert excised with restriction endonuclease EcoRI. Lanes 1 and 3, molecular weight marker fragments prepared from wild type λDNA by digestion with endonucleases EcoRI plus HindIII, and PstI, respectively. Direction of migration was from top to bottom.

Figure 3. Restriction map of λAPE3. Sites of cleavage by restriction endonucleases are designated by arrows. The section coding for apolipoprotein E in the liver is indicated by the dotted region (11). The clear area represents the section of nucleic acid not translated in the liver cell, but which is translated in the bacteria. The solid area (not drawn to scale) outlines the section of DNA coding for β-galactosidase, and the hatched area corresponds to other phage DNA. Start G and stop G indicate stop and start signals, respectively, for β-galactosidase. Start E and stop E represent start and stop signals for apolipoprotein E. bp, base pairs; Aa, amino acids.

restriction map shown in Fig. 3. The restriction map suggests that the insert in λAPE3 extends beyond the region coding for apolipoprotein E of 933 base pairs at both the 5' and 3' end.

Identification of Fused Polypeptides

Electrophoretic separation and transfer of λAPE3 proteins from gels to nitrocellulose sheets permitted the immunochemical detection of a polypeptide of molecular weight 150,000 which reacted strongly with antisera against both β-galactosidase and apolipoprotein E. Marker β-galactosidase

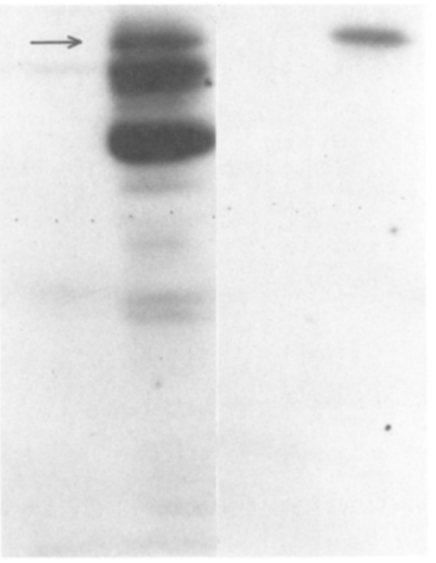

Figure 4. Analysis of synthesized protein by electrophoresis in a polyacrylamide gel, subsequent transfer to nitrocellulose sheets and binding of antibody and ^{125}I-protein A.
Lane 1 and 2 (from left to right): Protein from BTA282 infected with λgt11-Amp3 (lane 1) or λAPE3 (lane 2) reacting with antiserum against β-galactosidase. The band near the upper end of lane 1 indicates the position of β-galactosidase (Mr 116,000). The arrow marks the position in lane 2 of the protein produced by fusion of β-galactosidase and apolipoprotein E (Mr 150,000).
Lane 3 and 4: Protein from BTA282 infected with λgt11-Amp3 (lane 3) or λAPE3 (lane 4) reacting with antiserum against apolipoprotein E. A distinct band is obtained only for protein synthesized by BTA282 containing λAPE3. The position of this band is identical to that of the fused protein in lane 2.

from parental BTA282 infected with λgt11-Amp3 was observed as a major band of molecular weight 116,000 reacting with antiserum against β-galactosidase but not with antiserum against apolipoprotein E (Fig. 4). These results clearly demonstrate that λAPE3 codes for a chimeric polypeptide containing antigenic determinants from both apolipoprotein E and β-galactosidase.

Identification of the Insert in λAPE3 by Nucleotide Sequencing

The nucleotide sequence was determined for two sections of DNA, one coding for amino acids 3 to 54 and the other for amino acids 266 to 344 of apolipoprotein E. The sequence obtained was identical to that reported by McLean et al. (10).

DISCUSSION

From its site of synthesis, hepatocytes mainly, apolipoprotein E (Mr 34000) is secreted into the bloodstream to cooperate with other apolipo-

proteins in the transport of lipids. Interaction of apolipoprotein E with receptors on cell surfaces (11-14) is involved in the exchange of lipids, such as cholesterol, between tissues and blood lipoprotein particles (15). Altered apolipoprotein E, produced by expression in E. coli of in vitro mutated recombinant bacteriophage, could be useful as a probe to study this interaction.

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