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CLONING AND REGULATION OF RAT APOLIPOPROTEIN B mRNA

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Recombinant cDNA clones that code for apolipoprotein B(apoB) were isolated from a rat liver cDNA library, using synthetic oligonucleotide probe derived from the sequence of human apoB cDNA. The nucleotide and deduced amino acid sequences of the rat apoB clone pRB5, 1.2 kb in length, showed 83% and 84% homology to those of human apoB. Northern blot analysis revealed that rat apoB cDNA probe cross-reacts with human and rabbit apoB mRNA sequences and the size of those mRNAs, approximately 15 kb long, were not discernibly different. In addition, apoB mRNA was abundant only in the liver and intestine. Finally, cholesterol feeding to rats for six weeks resulted in a several-fold increase in the level of apoB mRNA in the liver.

Apolipoprotein B (apoB) is the principal apolipoprotein of chylomicrons, very low density lipoprotein (VLDL), and low density lipoprotein (LDL)[1-4]. In rat plasma, as well as in human plasma, apoB is heterogeneous and exists in two major forms, apo B-100 and B-48. ApoB-100 is synthesized by the liver and is secreted on triglyceride rich VLDL, while apoB-48 is synthesized by the intestine and liver in the rat[5]. The structure and physicochemical properties of apoB have been studied for nearly a decade. It has been difficult to analyze apo B because delipidated apoB is insoluble in aqueous solution and sensitive to protease cleavage[2, 6-10]. Only a limited number of works has been performed, as to the regulation of apoB synthesis in vivo. Studies of intestinal and hepatic apoB synthesis in response to fat administration have produced conflicting results[11-13].

Recently, several groups reported the isolation of partial cDNA clones for rat and human apoB[14-17]. Yet, the regulation of expression of apoB mRNA is unclear. To determine the primary amino acid sequence and to elucidate the biosynthesis and processing of apoB, we have isolated and charac-

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terized the cDNA of rat liver apoB from a rat liver cDNA library. Using the cDNA, we also examined the distribution of rat apoB mRNA in various tissues and the effects of cholesterol feeding on the expression of the apo B protein.

EXPERIMENTAL PROCEDURES

Animals---All rats used were of the Sprague-Dawley strain, obtained from Nisseizai Laboratories(Tokyo). Male rats weighing 180-200 g were maintained on a standard diet of laboratory rat chow. For studying the effect of cholesterol feeding to apo B synthesis, rats were fed milled rat chow supplemented with 2 % cholesterol, 0.5 % cholic acid, and 10 % coconut oil for 6 weeks. All animals were allowed to feed ad libitum.

RNA preparation---Tissues were excised, rinsed with cold saline, and quickly frozen in liquid nitrogen. Total RNA was extracted from pulverized frozen tissue by the guanidine thiocyanate method of Chirgwin et al. [18] Poly(A^+)RNA were enriched by oligo(dT)-cellulose chromatography.

Construction and screening of the rat liver cDNA library---Rat liver mRNA was used for cDNA synthesis by the method of Gubler and Hoffman[19] and double stranded cDNA was cloned into lambda gt 10 vectors essentially as described by Huynh et al[20] and Young and Davis[21]. After packaging the DNA in vitro the phage were amplified on Escherichia coli C600hfl$^-$.

Rat apoB cDNA was identified from the rat liver cDNA library by the technique of oligonucleotide hybridization. The oligonucleotide probe used to screen the cDNA library was DNAs 26 nucleotides long, custom synthesized by YAMASA biochemicals(Choushi, Chiba): 5'-d[ATGCCCATATTTGTCACAAACTCCAC]-3'. This sequence was derived from that of human apoB cDNA recently reported by Deeb, et al[15], and corresponded to the amino acid sequence -Val-Glu-Phe-Val-Thr-Asn-Met-Gly-Ile- belonging to peptide R3-1 [22]. The oligonucleotide was radiolabeled at the 5' end to a specific activity of approximately 2×10^8 cpm per microgram by transfer of [gamma-^{32}P]ATP by using bacteriophage T4 polynucleotide kinase[23].

Phage were plated at a density of 5×10^3 phage per 150 mm plate, and plaques were transferred to "Plaque Screen" filters (New England Nuclear). Approximately 2×10^4 plaques were screened with the ^{32}P labeled oligonucleotide probe.

DNA sequence analysis---Phage DNA was prepared and cloned DNA fragments were subcloned into the plasmid vectors, pUC18 or 19, before sequencing. DNA sequencing was performed by the dideoxynucleotide-chain-termination method of Sanger et al[24].

Hybridization analysis of apoB mRNA---Total or poly(A^+) RNA were denatured with glyoxal (1h at 50^o), fractionated by electrophoresis through 1.1 % agarose gels containing 10 mM sodium phosphate, pH 7.0, and transferred to nitrocellulose filters. Filters were baked at 80^{o}C for 2h, prehybridized, and hybridized at 42^{o}C with [^{32}P]cDNA labeled by "nick translation". Prehybridization was performed in a solution containing 5x SSPE buffer, 5x Denhardt's solution, 1% SDS, and 100ug/ml of heat-denatured salmon sperm DNA for 6-12h at 42^{o}C. Hybridization was performed in the same solution containing 10% dextran sulfate with the addition of 5×10^5 cpm/ml of ^{32}P-labeled cDNA probe (specific activity about 10^8 cpm /ug) for 24-36h at 42^{o}C. Filters were washed twice at 50^{o}C with 2x SSPE and 1% SDS, and twice at 50^{o}C with 0.1x SSPE and 0.1% SDS, air dried, and exposed to X-ray film (Kodak XAR-5).

RESULTS

Isolation of apoBcDNA clones---26 base-long oligonucleotide was used to probe a rat liver cDNA library for apolipoprotein B specific sequences. Four positive clones were identified from amongst about 2×10^4 recombinant phages through two or three rounds of plaque hybridization. The EcoRI-digested fragment from these clones were subcloned into EcoRI restriction site of plasmid pUC19. One clone, designated pRB5 was subjected to further restriction mapping and then sequencing.

Restriction mapping and sequence analysis of pRB5 clone---A restriction endonuclease cleavage map of the pRB5 clone is illustrated in Fig. 1. The overall length of the sequence is 1159 nucleotides(Fig. 2). The oligonucleotide probe sequence is detected at nucleotide position 778 and identical to the sequence of rat apoB cDNA, except for nucleotide 778 (G in the human apoB cDNA is substituted to T in the rat). The correct reading frame of the nucleotide sequence of pRB5 was ascertained by the identification of the homology with the nucleotide and derived amino-acid sequence of the R3-1 peptide present in human apoB100. This clone represented an uninterrupted reading frame coding 386 amino acids, probably an internal segment of the rat apoB mRNA because it contained neither untranslated flanking sequences nor a 3' terminal poly(dA).

A partial cDNA sequence for rat apoB, isolated by antibody screening by Lusis et al[14], does not overlap any portion of ours. On the other hand, a comparison of our sequence with several human apoB cDNAs shows a homology with a sequence reported by Law et al.[17]. The extensive homology occurs between human apoB mRNAs and their corresponding proteins[Fig.3]. 83% of

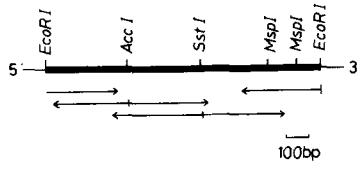

Fig. 1. Partial restriction map and sequencing strategy for the insert of plasmid pRB5. The extent and direction of sequencing are indicated by the arrows. Sequence was determined by the dideoxy methods. The 5' and 3' termini indicate the orientation of the coding strand. bp, base pairs.

```
1                                                                                                   90
TCT CGA CTT CCA ACG ATC ATG GAC TTC AGA AAA TTT TCC CGA AAC TAT CAA ATT TCC AAA TCT GTT TCT ATT CCA CTG TTT GAC CCA GTC
Ser-Arg-Leu-Pro-Thr-Ile-Met-Asp-Phe-Arg-Lys-Phe-Ser-Arg-Asn-Tyr-Gln-Ile-Ser-Lys-Ser-Val-Ser-Ile-Pro-Leu-Phe-Asp-Pro-Val
                                                                                                   180
TCA GCC AAA ATC GAA GGG AAT CTT GTA TTT GAT CCA AGC AGT TAT CTT CCC AAA GAA AGC ATG CTG AAA ACA ACC CTC ACG GTC TTT GGA
Ser-Ala-Lys-Ile-Glu-Gly-Asn-Leu-Val-Phe-Asp-Pro-Ser-Ser-Tyr-Leu-Pro-Lys-Glu-Ser-Met-Leu-Lys-Thr-Thr-Leu-Thr-Val-Phe-Gly
                                                                                                   270
ATT GCT TCA CTT GAT CTC TTT GAG ATT GGT TTA GAA GGA AAG GGC TTT GAG CCA ACA CTG GAA GCT CTT TTT GGT AAG CAA GGA TTC TTC
Ile-Ala-Ser-Leu-Asp-Leu-Phe-Glu-Ile-Gly-Leu-Glu-Gly-Lys-Gly-Phe-Glu-Pro-Thr-Leu-Glu-Ala-Leu-Phe-Gly-Lys-Gln-Gly-Phe-Phe
                                                                                                   360
CCA GAC AGT GTC AAC AAG GCT TTG TAT TGG GTC AAT GGT CAA GTT CCA GAT CGT GTC TCC AAG GTC TTG GTA GAC CAC TTT GGC TAC ACT
Pro-Asp-Ser-Val-Asn-Lys-Ala-Leu-Tyr-Trp-Val-Asn-Gly-Gln-Val-Pro-Asp-Arg-Val-Ser-Lys-Val-Leu-Val-Asp-His-Phe-Gly-Tyr-Thr
                                                                                                   450
AAG GAT GAC AAA CAT GAA CAG GAC ATG GTG AAT GGA ATC ATG CCC ATT GTG GAC AAG TTG ATC AAA GAA CTG AAA TCT AAA GAA ATT CCT
Lys-Asp-Asp-Lys-His-Glu-Gln-Asp-Met-Val-Asn-Gly-Ile-Met-Pro-Ile-Val-Asp-Lys-Leu-Ile-Lys-Glu-Leu-Lys-Ser-Lys-Glu-Ile-Pro
                                                                                                   540
GAA GCC AGG GCC TAT CTC CGC ATC CTA GGA AAA GAG CTT GGC TTT GTC AGG CTC CAA GAC CTC CAA GTC CTG GGA AAG TTA CTG CTG AAT
Glu-Ala-Arg-Ala-Tyr-Leu-Arg-Ile-Leu-Gly-Lys-Glu-Leu-Gly-Phe-Val-Arg-Leu-Gln-Asp-Leu-Gln-Val-Leu-Gly-Lys-Leu-Leu-Leu-Asn
                                                                                                   630
GGT GCG CAA ACT TTT CGG GGA GTG CCC CAG ATG ATT GTA CAG GCC ATC AGA GAA GGA TCA AAG GAT GAC TTG TTT CTC CAC TAC ATC TTC
Gly-Ala-Gln-Thr-Phe-Arg-Gly-Val-Pro-Gln-Met-Ile-Val-Gln-Ala-Ile-Arg-Glu-Gly-Ser-Lys-Asp-Asp-Leu-Phe-Leu-His-Tyr-Ile-Phe
                                                                                                   720
ATG GAG AAT GCC TTT GAG CTC CCC ACT GGA GTA GGG CTA CAG CTG CAA GTG TCC TCA TCT GGA GTC TTC ACC CCT GGG ATC AAG GCT GGT
Met-Glu-Asn-Ala-Phe-Glu-Leu-Pro-Thr-Gly-Val-Gly-Leu-Gln-Leu-Gln-Val-Ser-Ser-Ser-Gly-Val-Phe-Thr-Pro-Gly-Ile-Lys-Ala-Gly
                                                                                                   810
GTG AGA CTG GAG TTA GCC AAT ATT CAG GCA GAG CTA GTG GCA AAA GCC TCT GTG TCT TTG GAG TTT GTG ACA AAT ATG GGC ATC ATC ATC
Val-Arg-Leu-Glu-Leu-Ala-Asn-Ile-Gln-Ala-Glu-Leu-Val-Ala-Lys-Ala-Ser-Val-Ser-Leu-Glu-Phe-Val-Thr-Asn-Met-Gly-Ile-Ile-Ile
                                                                                                   900
CCA GAC TTC GCT AAG AGC GGT GTT CAG ATG AAT ACA AAC TTC TTC CAC GAG TCA GGC CTG GAG GCT CGA GTG GCC CTG AAG GCT GGG CAG
Pro-Asp-Phe-Ala-Lys-Ser-Gly-Val-Gln-Met-Asn-Thr-Asn-Phe-Phe-His-Glu-Ser-Gly-Leu-Glu-Ala-Arg-Val-Ala-Leu-Lys-Ala-Gly-Gln
                                                                                                   990
CTG AAG GTC ATC ATT CCT TCT CCG AAG AGG CCG GTC AAG CTG TTC AGT GGC ACG AAC ACG CTG CAC CTG GTC TCT ACC ACC AAA ACG GAA
Leu-Lys-Val-Ile-Ile-Pro-Ser-Pro-Lys-Arg-Pro-Val-Lys-Leu-Phe-Ser-Gly-Thr-Asn-Thr-Leu-His-Leu-Val-Ser-Thr-Thr-Lys-Thr-Glu
                                                                                                  1080
GTG ATC CCA CCT TTG ATT GAG AAC AGG AAG TCC TGG TCA ACT TGC AAG CCT TTC TTC ACC GGC ATG AAC TAC TGT ACC ACA GGG GCT TAC
Val-Ile-Pro-Pro-Leu-Ile-Glu-Asn-Arg-Lys-Ser-Trp-Ser-Thr-Cys-Lys-Pro-Phe-Phe-Thr-Gly-Met-Asn-Tyr-Cys-Thr-Thr-Gly-Ala-Tyr
                                                                                                  1155
TCC AAT GCC AGC TCC ACA GAG TCT GCC TCT TAC TAC CCA CTG ACA GGA GAC ACA AGG TAT GAG CTG GAG TTG AAG
Ser-Asn-Ala-Ser-Ser-Thr-Glu-Ser-Ala-Ser-Tyr-Tyr-Pro-Leu-Thr-Gly-Asp-Thr-Arg-Tyr-Glu-Leu-Glu-Leu-Lys
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Fig. 2. Nucleotide sequence and deduced amino acid sequence of clone pRB5. The nucleic acids are numbered beginning at the first triplet of the cloned apoB sequence.

the nucleotide positions were identical and the corresponding amino acid positions for this region of the protein showed 84% homology. To achieve an alignment with maximum homology between human and rat apoB sequences, neither deletion nor insertion was introduced anywhere.

RNA blot hybridization analysis of rat and human apoB mRNA---ApoB mRNA in the various rat tissues were evaluated by RNA blot hybridization analysis using a 1159-bp EcoRI-digested insert of pRB5 as a probe. The labeled apoB cDNA clone hybridized to a single major RNA species corresponding in electrophoretic mobility to about 15 kb in the liver. Diffuse hybridization in lower molecular weight regions was observed, but it probably represents partial degradation of this very large mRNA species. ApoB mRNA was detected not only in the liver, but also in the small intestine [Fig. 4].

```
          1                                                                  80
Rat       SRLPTIMDFRKFSRNYQISKSVSIPLFDPVSAKIEGNLVFDPSSYLPKESMLKTTLTVFGIASLDLFEIGLEGKGFEPTL
          • ••• •••••••••••  •••• •  •• ••••••••• •••  ••••••••••••• •• •• •• ••••••••••••
Human     SQLPTVMDFRKFSRNYQLYKSVSLPSLDPASAKIEGNLIFDPNNYLPKESMLKTTLTAFGFASADLIEIGLEGKGFEPTL
        138                                                                 217
                                                                            160
Rat       EALFGKQGFFPDSVNKALYWVNGQVPDRVSKVLVDHFGYTKDDKHEQDMVNGIMPIVDKLIKELKSKEIPEARAYLRILG
          •••••••••••••••••••••••••• ••••••••••••••••••••••••  • •••• ••••• ••••••••••
Human     EALFGKQGFFPDSVNKALYWVNGQVPDGVSKVLVDHFGYTKDDKHEQDMVNGIMLSVEKLIKDLKSKEVPEARAYLRILG
                                                                            297
                                                                            240
Rat       KELGFVRLQDLQVLGKLLLNGAQTFRGVPQMIVQAIREGCSKDDLFLHYIFMENAFELPTGVGLQLQVSSSGVFTPGIKAG
           ••••  • ••• •••••• •• •  • ••••   •• ••• • ••••••••••••••• ••••• •••••  •• •••
Human     EELGFASLHDLQLLGKLLLMGARTLQGIPQMIGEVIRKGSKNDFFLHYIFMENAFELPTGAGLQLQISSSGVIAPGAKAG
                                                                            377
                                                                            320
Rat       VRLELANIQAELVAKASVSLEFVTNMGIIIPDFAKSGVQMNTNFFHESGLEARVALKAGQLKVIIPSPKRPVKLFSGTNT
          • •• •• ••••••• ••• •••••••••••••• ••••••••••••••••••• •••••• •• •••••••••• •• ••
Human     VKLEVANMQAELVAKPSVSVEFVTNMGIIIPDFARSGVQMNTNFFHESGLEAHVALKAGKLKFIIPSPKRPVKLLSGGNT
                                                                            457
                                                                      385
Rat       LHLVSTTKTEVIPPLIENRKSWSTCKPFFTGMNYCTTGAYSNASSTESASYYPLTGDTRYELELK
          ••••••••••••••••••••• ••• ••  • • •••• •••••••••• •••••••••••• ••••
Human     LHLVSTTKTEVIPPLIENRQSWSVCKQVFPGLNYCTSGAYSNASSTDSASYYPLTGDTRLELELR
                                                                      522
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Fig. 3. Comparison of rat and human apoB amino acid sequence. The human apoB protein sequence is taken from Ref. 21. The corresponding rat sequences are those shown in Fig. 2. The standard one-letter amino acid abbreviations are used. Neither insertion nor deletion of amino acid was introduced anywhere to achieve an alignment with maximum homology to human and rat apoB sequences.

The rat organs other than the liver and intestine produce no detectable apoB mRNA. Our probe cross-hybridized with human and rabbit liver apoB mRNA sequences and the size of those mRNAs were not discernibly different as judged by mobility on agarose gels(data not shown). This result is in agreement with recently published data[14-17].

Effect of cholesterol feeding on apoB mRNA---Six weeks later, serum cholesterol concentrations in cholesterol-fed rats were approximately six-

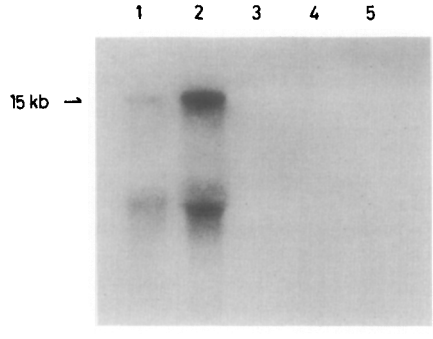

Fig. 4. Tissue distribution of apoB mRNA. Northern blot hybridization of electrophoretically fractionated Poly(A)$^+$RNA. Each line contained 5ug of Poly(A)$^+$RNA isolated from (1)rat liver, (2)intestine, (3)kidney, (4)spleen, (5)brain. The filter were exposed to x-ray film(Kodak XAR-5) with an intensifying screen at -70^{0}C. The size of 15-kb mRNA was estimated by comparison to HindIII-digested lambda DNA fragments.

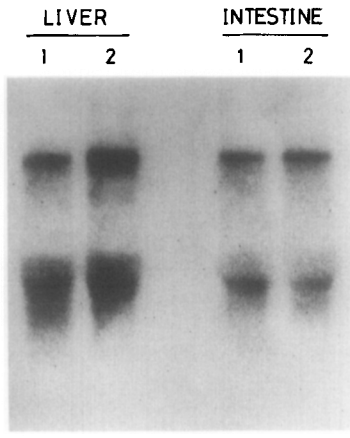

Fig. 5. Effect of cholesterol feeding on apoB mRNA. Male SD rats, 180-200g, were maintained on normal rat chow or, for a period of six weeks, on a cholesterol-rich diet(described under "Materials and Methods"). RNA was then isolated from the liver and intestine, fractionated electrophoretically, transferred to a nylon filter and hybridized with ^{32}P-labeled apoB probe. Each sample contained 25ug of total RNA.

fold higher than those in control rats. We measured the relative amount of apoB mRNA in the two major tissues involved in apolipoprotein B synthesis, liver and intestine. The amount of apoB mRNA in the liver was apparently higher in rats maintained in a high cholesterol diet than in rats maintained on a normal diet. On the other hand, there was no discernible change of the abundance of apoB mRNA in the intestine[Fig. 5].

DISCUSSION

Several important conclusions have emerged from these studies. First, we have presented the sequence of a 1.2-kilobase fragment of rat apoB cDNA cloned in the phage vector lambda gt10. Second, the expression of apoB mRNA showed a high degree of organ specificity. It is synthesized only in the liver and intestine. Finally, apoB production in the liver increased at the level of mRNA in response to cholesterol feeding, in the rat.

The rat apoB cDNA clone pRB5 represented an uninterrupted reading frame coding 386 amino acids. On the basis of limited sequencing and cross-hybridization analysis, rat apoB appears to be highly homologous to human apoB. The overall nucleotide and deduced amino acid homology between rat and human[17] is 83% and 84%, respectively. RNA blot analysis showed that rat

apoB cDNA probe cross-reacts with human and rabbit apoB mRNA sequences and the size of those mRNAs was not discernibly different.

The tissue distribution of apoB mRNA with abundant expression in the liver and intestine, is consistent with that expected on the basis of protein production and secretion. Blue et al[25] reported that a protein immunologically related to apoB was found in rooster kidney, as well as in the liver and intestine. There might be some difference in apo B metabolism between mammalians and rooster.

We have studied the effect of cholesterol feeding on apoB synthesis at the level of mRNA in rats. The data indicated that in the liver there was a significant increase of apoB mRNA in response to cholesterol feeding. However, Davis et al[26] reported that dietary cholesterol does not affect the synthesis of apoB by rat cultured hepatocytes. Schonfeld et al[11] demonstrated an increases of apoB protein in rat intestinal mucosa after administration of fat. However, apoB mRNA level in the small intestine was not elevated in our study. The explanation of these discrepancies is unclear and requires further investigation.

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REFERENCES

1. Havel RJ, Goldstein JL, Brown MS: in Metabolic Control and Disease, Bondy PK, and Rosenberg LE, Eds. 8th ed.(Saunders, Philadelphia, 1980), pp.393-494
2. Kane JP: Annu Rev Physiol 45, 637-650 (1983)
3. Mahley RW and Innerarity TL: Biochim Biophys Acta 737,197 (1983)
4. Mahley RW, Innerarity TL, Rall SC Jr., and Weisgraber KH: J Lipid Res 25,1277-1294 (1984)
5. Krishnaiah KV, Walker LF, Borensztajn G, Schonfeld G and Getz GS: Proc Natl Acad Sci USA 77, 3806-3810 (1980)
6. Smith R, Dawson JR and Tanford C: J Biol Chem 247, 3376-3381 (1977)
7. Steele JCH Jr. and Reynolds JA: J Biol Chem 254, 1639-1643 (1979)
8. Kane JP, Hardman DA and Paulus HE: Proc Natl Acad Sci USA 77, 2465-2469 (1980)
9. Elovson J, Huang YO, Baker N and Kannan R: Proc Natl Acad Sci USA 77, 151-161 (1981)
10. Aburatani H, Kodama T, Ikai A, Itakura H, Akanuma Y and Takaku F: J Biochem 94, 1241-1245 (1893)

11. Schonfeld G, Bell E and Alpers DH: J Clin Invest 61, 1539-1550 (1978)
12. Davidson NO, Kollmer ME and Glickman RM: J Lipid Res 27, 30-39 (1986)
13. Windmueller HG and Wu AL: J Biol Chem 256, 3012-3016 (1981)
14. Lusis AJ, West R, Mehrabian M, Reuben MA, LeBoeuf RC, Kaptein JS, Johnson DF, Schumaker VN, Yuhasz MP, Schotz MC and Elovson J: Proc Natl Acad Sci USA 82, 4597-4601 (1985)
15. Deeb, SS Motulsky AG and Albers JJ: Proc Natl Acad Sci USA 82, 4983-4986 (1985)
16. Knott TJ, Rall SC Jr., Innerarity TL, Jacobson SF, Urdea MS, Levy-Wilson B, Powell LM, Pease RJ, Eddy R, Nakai H, Byers M, Priestley LM, Robertson E, Rall LB, Betzholtz C, Shows TB, Mahley RW and Scott J: Science 230, 37-43 (1985)
17. Law SW, Lackner KJ, Hospattankar AV, Anchors JM, Sakaguchi AY, Naylor SL and Brewer HB Jr.: Proc Natl Acad Sci USA 82, 8340-8344 (1985)
18. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ: Biochemistry 18, 5294-5299 (1979)
19. Gubler U and Hoffman BJ: Gene 25, 263-269 (1983)
20. Huynh TV, Young RA, Davis RW: in DNA Cloning Techniques: A practical approach, Glover D Ed. Vol. I, pp.49-78, IRL Press, Oxford (1985)
21. Young RA and Davis RW: Proc Natl Acad Sci USA 80, 1194-1198 (1983)
22. LeBouef RC, Miller C, Shively JE, Schumaker VN, Balla MA, Lusis AJ: FEBS Lett 170, 105-108 (1984)
23. Wallace RB, Shaffer J, Murphy RF, Bonner J, Hirose T, Itakura K: Nucleic Acids Res 6, 3543-3557 (1979)
24. Sanger F, Nicklen S, Coulson AR: Proc Natl Acad SCi USA 74, 5463-5467 (1977)
25. Blue M-L, Protter AA, Williams DL: J Biol Chem 255, 10048-10051 (1980)
26. Davis RA and Malone-McNeal M: Biochem J. 227, 29-35 (1985)