Expression of Low Density Lipoprotein Receptor Gene in Human Placenta during Pregnancy

Madoka Furuhashi, Hisao Seo, Shigehiko Mizutani, Osamu Narita, Yutaka Tomoda, and Nobuo Matsui

Department of Endocrinology and Metabolism
The Research Institute of Environmental Medicine (M.F., H.S., N.M.)
and Department of Obstetrics and Gynecology
School of Medicine (M.F., S.M., O.N., Y.T.),
Nagoya University
Furo-cho, Chikusa-ku, Nagoya 464-01 Japan

Mammalian cells require cholesterol as a structural component of plasma membranes. It is also required for placental steroid synthesis. *De novo* synthesis of cholesterol is limited in human placenta and cholesterol is obtained mainly from plasma low density lipoprotein (LDL). Cholesterol delivery from LDL is mediated by receptor-mediated uptake and the receptor amount is the most important factor for cellular delivery. Thus, the regulation of receptor synthesis is important for placental development and function. Since the regulation of LDL receptor gene expression has not been studied in human placenta, LDL receptor mRNA was measured in placentae of 5–40 weeks of gestation by hybridization of RNA with ^{32}P-labeled cDNA for human LDL receptor. Two mRNA species for LDL receptor were demonstrated by Northern blot analysis. The longer mRNA [5.3 kilobases (kb)] was much more abundant than the shorter mRNA (3.7 kb). The amount of 5.3 kb mRNA was highest early in gestation and decreased during pregnancy. However, the amount of 3.7 kb mRNA did not change appreciably during gestation. Dot blot analysis of 26 placental mRNAs obtained from various stages of gestation revealed a negative correlation between LDL receptor mRNA and gestation ($r = -0.76$, $P < 0.001$). Considering the rapid growth of the trophoblast during gestation, especially in the first and the second trimester, increased expression of the LDL receptor gene and subsequent translation are expected for efficient cholesterol uptake to provide a sufficient substrate for cell growth. Possible mechanisms for the appearance of two mRNA species for LDL receptor are also discussed. (Molecular Endocrinology 3: 1252–1256, 1989)

INTRODUCTION

Cholesterol is an important component of plasma membranes (1). Plasma low density lipoprotein (LDL) is the major cholesterol carrier protein in humans, although plasma high density lipoprotein is important for cholesterol delivery in rodents (2–4). LDL enters cells by a receptor-mediated endocytotic pathway, initially described by Goldstein and Brown (5). The receptor number is regulated by the cellular demand for cholesterol (6). Thus, cells that synthesize steroid hormones and grow rapidly have high levels of LDL receptors (6, 7).

The human placenta grows rapidly during gestation and possesses endocrine activity for steroid synthesis (8, 9). The development of the trophoblast and synthesis of steroid hormones require a large amount of cholesterol as a constituent of plasma membrane and as a precursor for progesterone. However, *de novo* production of cholesterol by the placental trophoblast is limited (10, 11). Winkel *et al.* (2) reported that placental biosynthesis of progesterone is principally dependent upon cholesterol derived from maternal plasma LDL.

LDL receptor in placenta has been characterized by several investigators (12, 13). It was shown that the receptors are located on the microvillous membranes and are present as early as the sixth week of gestation (13). The affinity for LDL is constant during pregnancy and at any gestational age, is similar to that in other human tissues such as fibroblast, adrenal gland, and corpus luteum (4, 13). The changes in the amount of the receptor in placenta could be the most important factor for cholesterol delivery during pregnancy. However, quantitative estimates of the receptor number seem to be difficult due to the presence of endogenous LDL in microvillous preparations (13). On the other hand, the changes in LDL receptor mRNA level was shown to correlate with the changes in the receptor synthesis (14). This, to study the regulation of LDL receptor synthesis in human placenta, the changes in the steady state levels of LDL mRNA in human placenta at various gestational stages were analyzed.

0888-8809/89/1252-1256$02.00/0
Molecular Endocrinology

RESULTS

As shown in Fig. 1, a negative correlation between total RNA recovery from placental villi and gestational weeks was observed ($r = -0.68$, $P < 0.001$).

Northern blots of poly(A)$^+$ RNAs from placentae of 8, 20, and 37 weeks' gestation were hybridized with ^{32}P-labeled LDL receptor cDNA (Fig. 2A). In the autoradiogram, two bands were visualized, corresponding to 5.3 kilobases (kb) and 3.7 kb long. Northern blot analysis of β-actin mRNA showed a single band whose size was 2.1 kb (Fig. 2B). Densitometric analysis revealed the amount of 5.3 kb LDL receptor mRNA decreased 50% from 8 to 37 weeks of gestation (Table 1). The levels of 3.7 kb mRNA remained unchanged during gestation. These data indicated that the change of LDL receptor gene expression during gestation was primarily due to a decrease in the amount of 5.3 kb mRNA. β-Actin mRNA also decreased with gestation.

Although Northern blot analysis is accepted as a tool for the measurement of mRNAs, dot blot analysis is thought to be more accurate because RNA loss at blotting step can be eliminated and the method allows the measurement of many samples at a time. Thus, LDL receptor mRNA levels in 26 individual placentae of 5–40 weeks gestation were determined by dot blot analysis. The mRNA levels were most abundant in the first trimester placentae and decreased to approximately one quarter of the level in the third trimester placentae (Fig. 3). The mRNA levels were negatively correlated with gestational weeks ($r = -0.76$, $P < 0.001$).

The amount of β-actin was similarly determined. As shown in Fig. 4, β-actin mRNA levels also negatively correlated with gestational weeks ($r = -0.72$, $P < 0.001$).

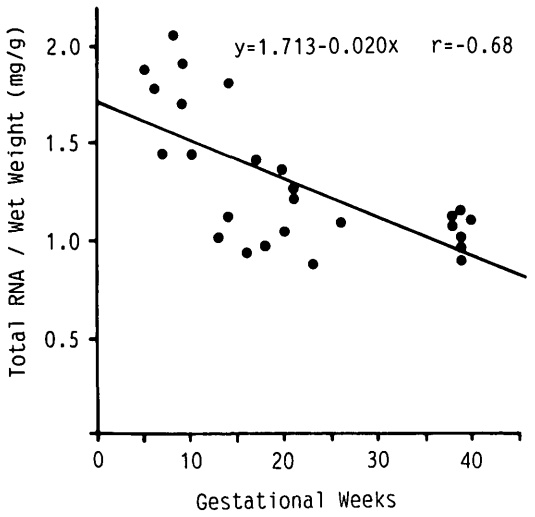

Fig. 1. Total RNA Recovery from Placental Villi as a Funciton of Gestational Weeks

The amounts of total RNA were determined by A at 260 nm. Correlation between the amounts of total RNA per wet weight (mg/g) and gestational weeks was assessed by linear regression analysis. A significant negative correlation ($r = -0.68$, $P < 0.001$) was observed.

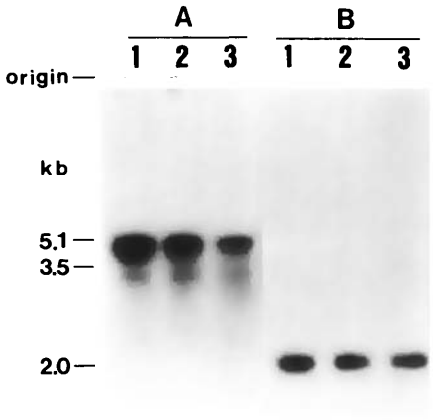

Fig. 2. Northern Blot Analysis of LDL Receptor mRNA and β-Actin mRNA in Human Placentae from Various Gestational Stages

Five micrograms of placental poly(A)$^+$ RNA from 8 weeks (lane 1), 20 weeks (lane 2), and 37 weeks of gestation (lane 3) were fractionated by electrophoresis through 0.8% agarose gel and transferred onto nylon membrane (Gene Screen Plus membrane). The probes were LDL receptor cDNA (A) and β-actin cDNA (B), respectively. The *numbers at the left* indicate molecular size in thousands of bases, determined from the migration of molecular weight marker (*Eco*RI and *Hin*dIII-cut bacteriophage λ).

Table 1. Amounts of LDL Receptor mRNA and β-Actin mRNA in Placentae from Various Gestational Weeks

Gestational Weeks	LDL Receptor mRNA		β-Actin mRNA
	5.3 kb	3.7 kb	
8	100	2.9	100
20	74.1	3.1	82.0
37	51.4	3.3	65.1

The amounts of mRNA for LDL receptor and β-actin were determined by densitometric analysis of corresponding bands in Northern blot (Fig. 2). Data were expressed as the percentage of the densities of the band at 5.3 kb at 8 weeks of gestation for both 5.3 kb and 3.7 kb LDL receptor mRNAs. For β-actin mRNA, densities of the bands at 2.1 kb were also expressed as the percentage of the densities at 8 weeks of gestation.

Decreased LDL receptor and β-actin mRNAs in the second and the third trimester placentae may not be due to general RNA degradation. Since the relative ratio of human PL (hPL) mRNA against total RNA in placenta increases with gestation (15), we measured the hPL mRNA by hybridization with human GH (hGH) cDNA which shows a high degree of homology (16) and cross-hybridizes with hPL mRNA. The cDNA hybridized with only 0.9 kb mRNA in Northern blot analysis of poly(A)$^+$ RNAs from placentae (data not shown). This size is consistent with that of hPL mRNA. The best fit line between the amounts of hPL mRNA and gestational

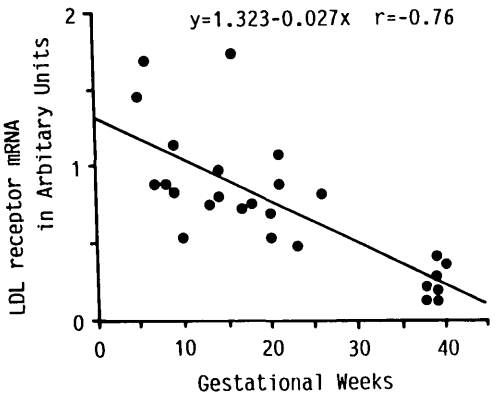

Fig. 3. LDL Receptor mRNA as a Function of Gestational Weeks

The amounts of LDL receptor mRNA were determined by dot blot analysis and were expressed as arbitrary units. Correlation between the amounts of LDL receptor mRNA and gestational weeks was assessed by linear regression analysis. A significant negative correlation ($r = -0.76$, $P < 0.001$) was observed.

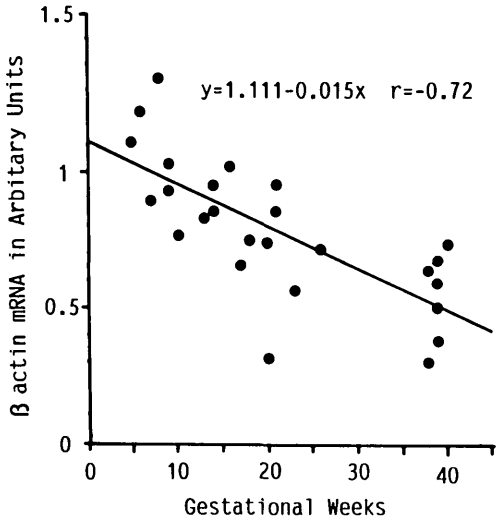

Fig. 4. β-Actin mRNA as a Function of Gestational Weeks

The amounts of β-actin mRNA were determined by dot blot analysis and were expressed as arbitrary units. Correlation between the amounts of β-actin mRNA and gestational weeks was assessed by linear regression analysis. A significant negative correlation ($r = -0.72$, $P < 0.001$) was observed.

weeks is shown in Fig. 5. Although data for 27–37 weeks were missing, the amounts of hPL mRNA increased from first trimester, reaching plateau at second trimester ($r = 0.52$, $P < 0.05$).

DISCUSSION

Yamamoto *et al.* (17) reported that a single and discrete LDL receptor mRNA (5.3 kb) was detected when poly(A)$^+$ mRNA was analyzed from a variety of human

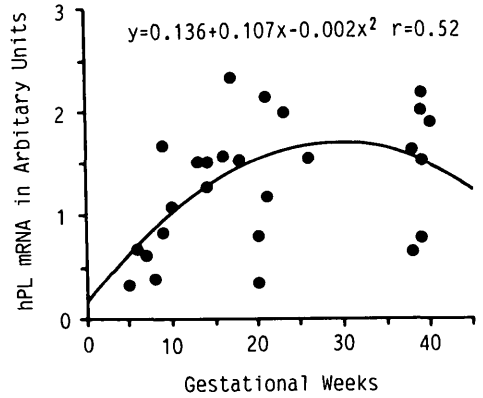

Fig. 5. Human PL mRNA as a Function of Gestational Weeks

The amounts of hPL mRNA were determined by dot blot analysis and were expressed as arbitrary units. The best fit line between the amounts of hPL mRNA and gestational weeks were drawn by polynominal regression analysis.

tissues such as fibroblast, fetal adrenal gland, adult adrenal gland, fetal liver and A-431 carcinoma cells. Our data show that two distinct mRNA species (5.3 kb and 3.7 kb) hybridizing with LDL receptor cDNA are present in human placenta. Considering the high stringency of washing, it is unlikely that 3.7 kb mRNA is nonspecific.

According to the nucleotide sequence of LDL receptor cDNA reported by Yamamoto *et al.* (17), it contains Alu sequences which are frequently present in intervening sequences and removed by splicing during mRNA processing (18). The Alu repeats are flanked by sequences similar, but not identical, to the consensus sequence for the splicing donor site 5′-$^{A}_{C}$AGGT$^{A}_{G}$AGT-3′ and for the acceptor site 5′-PyPyPyPyPyPy-XCAGG$^{G}_{T}$-3′ (19). There are four potential 5′-donor sites before the Alu repeats and four potential 3′-acceptor sites after the Alu repeats. Thus, the differential usage of splicing sites may give rise to the smaller LDL receptor mRNA. Also, there are three candidate regions for a polyadenylation signal according to the cDNA sequence. The first AATAAA is at 4486-4491; it is followed by an overlapping polyadenylation signal at position 4490. The third AATTAAA is at 5065-5071, which is considered to function as a polyadenylation signal for human LDL receptor mRNA reported by Yamamoto *et al.* (17). Thus, the first or the second AATAAA may serve as a polyadenylation signal. This could generate an mRNA species of about 4.7 kb but not the 3.7 kb mRNA. Thus, alternative splicing is the most probable cause for the production of 3.7 kb mRNA. Further studies are required to elucidate the mechanisms that give rise to two kinds of LDL receptor mRNAs in human placenta and their functional significance.

The amount of 5.3 kb mRNA was much greater than that of 3.7 kb mRNA throughout pregnancy and the amount of the latter remained unchanged (Table 1). Thus, the changes in the amount of LDL receptor mRNA observed in dot blot analysis primarily reflect

changes in the amount of 5.3 kb mRNA. Although we did not assess the translatable levels of mRNA or determine the receptor protein, Ma *et al.* (14) reported that the estradiol-mediated increase in LDL receptor mRNA correlated with an increase in receptor protein synthesis in rabbit liver. Thus, the change in the level of LDL receptor mRNA we observe here may reflect a change in the synthesis of LDL receptor protein in human placenta.

Expression of LDL receptor gene was highest in the first trimester. Cholesterol is a structural component of the plasma membranes in all mammalian cells and it is absolutely essential for cell growth and survival (20). Certain malignant cells have been shown to have greater LDL receptor activity than the corresponding normal cells (21, 22). The growth rate of placenta is extremely high. It seems likely that the high level of LDL receptor gene expression enables the trophoblast in the first trimester to acquire cholesterol for cell growth.

Expression of β-actin gene was also highest in the first trimester. It is known that the mammalian actin mRNA levels increase during cell growth and differentiation *in vitro* (23, 24). The human placenta continues to grow and differentiate during pregnancy. Progeny of mitotically active mononucleated cytotrophoblasts fuse to form the mitotically inactive syncytiotrophoblasts (25–27). The ratio of cytotrophoblast to syncytiotrophoblast decreases progressively until the syncytial layer is the dominant trophoblastic component at term. It is likely that the high level of LDL receptor and β-actin gene expression in early gestation are related to the growth and the differentiation of the trophoblast.

Since both LDL receptor and β-actin mRNA decreased during pregnancy, the total RNA recovery per gram of tissue was calculated. It also decreased linearly during pregnancy. The recovery from the term placentae was two thirds of that from placentae in early gestation (Fig. 1). These results may suggest that 1) mitotically inactive syncytiotrophoblasts may have nonspecifically less RNA synthesis or more degradation than cytotrophoblasts or 2) RNA recovery from the placentae of late gestation may be poorer because of the increased connective tissues. However, the increase in hPL mRNA from first to second trimester indicates that the decrease in LDL receptor and β-actin mRNA may be regulated in a specific manner. The mechanism involved in the regulation of the expression of these genes remains to be elucidated.

In summary, Northern blot analysis showed that two mRNA species in human placenta hybridized with the LDL receptor cDNA corresponding to a size of approximately 5.3 kb and 3.7 kb. The expression of the longer mRNA was greater than that of the shorter mRNA. The amount of LDL receptor mRNA in the total RNA in placenta was most abundant in the first trimester and decreased during pregnancy. Our data suggest that large amount of LDL receptor mRNA in early stage of gestation is related to the requirement of much cholesterol as a component of cell membranes for trophoblastic growth.

MATERIALS AND METHODS

Tissues

Placental tissues of 5–40 weeks gestation were obtained by therapeutic abortion, elective cesarean section, or spontaneous vaginal delivery. Decidual tissues and vessels were carefully removed by scissors. Villous portion was extensively rinsed in saline to remove the blood, immediately frozen in liquid nitrogen, and kept at −80 C until RNA extraction.

Preparation of RNA

Frozen tissues (approximately 2 g) were pulverized and homogenized in a solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol using a glass-Teflon homogenizer. RNA was precipitated by isopropanol and washed with 75% ethanol (28). Poly(A)$^+$ RNA was purified by affinity chromatography using oligo(dT) cellulose (29). Amounts of RNA were determined by optical density at 260 nm. Recovery of poly(A)$^+$ RNA from total RNA was approximately 2%.

Northern Blot Analysis

Five micrograms of poly(A)$^+$ RNA were denatured in 0.01 M phosphate buffer (pH 7.0) with 1 M glyoxal and fractionated by electrophoresis through 0.8% agarose gel. Then poly(A)$^+$ RNA was transferred onto nylon membranes (Gene Screen Plus, New England Nuclear, Boston, MA). The blots were immersed in a 50 mM NaOH solution for 15 sec to reverse the glyoxal reaction, placed into a solution of 1× SSC (150 mM NaCl and 15 mM sodium citrate), 0.2 M HCl, pH 7.5, and incubated for 30 sec.

The blots were prehybridized in a solution containing 5× SSPE (900 mM NaCl, 5 mM EDTA, 50 mM sodium phosphate, pH 8.3), 5× Denhardt's solution (0.1% Ficoll (400,000 mol wt), 0.1% polyvinylpyrrolidone (360,000 mol wt), 0.1% BSA), 1.0% sodium dodecyl sulfate (SDS), herring sperm DNA (0.1 μg/mL) (Boehringer Mannheim Yamanouchi, Tokyo, Japan), and 50% formamide.

Human LDL receptor cDNA corresponding to nucleotides 718-2544 was used as a probe. The cDNA was a part of coding region which did not contain Alu sequence. The probe was labeled with [^{32}P]dCTP (New England Nuclear, Boston, MA) using random primed DNA labeling kit (Boehringer Mannheim Yamanouchi, Tokyo, Japan) (30). The hybridization was performed at 42 C for 20 h in a solution containing 5× SSPE, 5× Denhardt's solution, 1% SDS, herring sperm DNA (0.1 μg/ml), 50% formamide, and the labeled probe. The blots were washed at room temperature twice for 5 min in 2× SSC, then at 65 C twice for 30 min in 2× SSC, 1% SDS, and at room temperature twice for 30 min in 0.1× SSC.

The membranes were exposed to Kodak X-AR film at −80 C. After the LDL receptor probe was washed off, the blot was autoradiographed to show that no radioactivity remained before probing with β-actin. The washing was repeated and the blot was reprobed with ^{32}P-labeled hGH cDNA.

Densitometric analysis of the autoradiogram was performed by using image analyzer Model: TIB-100 (Immunomedica, Shizuoka, Japan).

RNA Dot Analysis

RNA samples (50 μg total RNA) were denatured in 20% formaldehyde and 6× SSC at 50 C for 15 min and serially diluted with 10× SSC. The diluted RNAs were spotted onto Gene Screen Plus membranes using a 96-well Minifold device (Schleicher & Schuell, Keene, NH). The membranes were baked *in vacuo* for 2 h at 80 C. Hybridization, autoradiography, and densitometric analysis were performed in the same manner as described above.

Acknowledgments

We are grateful to Dr. Tokuo Yamamoto and Dr. Joseph L. Goldstein for generously providing us with the human LDL receptor cDNA and Dr. Joseph A. Martial for the hGH cDNA. We also thank Dr. Irving Boime for his critical comments.

Received January 5, 1989. Revision received May 1, 1989. Accepted May 4, 1989.

Address requests for reprints to: Hisao Seo, M.D., Department of Endocrinology and Metabolism, The Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-01, Japan.

REFERENCES

1. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD 1983 Molecular biology of the cell. Garland Publishing, Inc, New York, p 259
2. Winkel CA, Snyder JM, MacDonald PC, Simpson ER 1980 Regulation of cholesterol and progesterone synthesis in human placental cells in culture by serum lipoproteins. Endocrinology 106:1054–1060
3. Simpson ER, MacDonald PC 1981 Endocrine physiology of the placenta. Annu Rev Physiol 43:168–188
4. Gwynne JT, Strauss III JF 1982 The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. Endocr Rev 3:299–329
5. Goldstein JL, Brown MS 1974 Binding and degradation of low density lipoproteins by cultured human fibroblasts. J Biol Chem 249:5153–5162
6. Goldstein JL, Brown MS 1977 The low-density lipoprotein pathway and its relation to atherosclerosis. Annu Rev Biochem 46:897–930
7. Brown MS, Kovanen PT, Goldstein JL 1979 Receptor-mediated uptake of lipoprotein-cholesterol and its utilization for steroid synthesis in the adrenal cortex. Recent Prog Horm Res 35:215–257
8. Pearlman WH 1957 [16-^{3}H]Progesterone metabolism in advanced pregnancy and in oophorectomized-hysterectomized women. Biochem J 67:1–5
9. Lin TJ, Lin SC, Erlenmeyer F, Kline IT, Underwood R, Billiar RB, Little B 1972 Progesterone production rates during the third trimester of pregnancy in normal women, diabetic women, and women with abnormal glucose tolerance. J Clin Endocrinol Metab 34:287–297
10. Van Leusden H, Villee CA 1965 The de novo synthesis of sterols and steroids from acetate by preparations of human term placenta. Steroids 6:31–45
11. Zelewski L, Villee CA 1966 The biosynthesis of squalene, lanosterol, and cholesterol by minced human placenta. Biochemistry 5:1805–1814
12. Cummings SW, Hatley W, Simpson ER, Ohashi M 1982 The binding of high and low density lipoproteins to human placental membrane fractions. J Clin Endocrinol Metab 54:903–908
13. Alsat E, Bouali Y, Goldstein S, Malassiné A, Berthelier M, Mondon F, Cedard L 1984 Low-density lipoprotein binding sites in the microvillous membranes of human placenta at different stages of gestation. Mol Cell Endocrinol 38:197–203
14. Ma PTS, Yamamoto T, Goldstein JL, Brown MS 1986 Increased mRNA for low density lipoprotein receptor in livers of rabbits treated with 17 α-ethinyl estradiol. Proc Natl Acad Sci USA 83:792–796
15. McWilliams D, Callahan RC, Boime I 1977 Human placental lactogen mRNA and its structural genes during pregnancy: Quantitation with a complementary DNA. Proc Natl Acad Sci USA 74:1024–1027
16. Fiddes JC, Seeburg PH, DeNoto FM, Hallewell RA, Baxter JD, Goodman HM 1979 Structure of genes for human growth hormone and chorionic somatomammotropin. Proc Natl Acad Sci USA 76:4294–4298
17. Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russell DW 1984 The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA. Cell 39:27–38
18. Sharp PA 1983 Conversion of RNA to DNA in mammals: Alu-like elements and pseudogenes. Nature 301:471–472
19. Breathnach R, Chambon P 1981 Organization and expression of eucaryotic split genes coding for proteins. Annu Rev Biochem 50:349–383
20. Goldstein JL, Brown MS 1985 The LDL receptor and the regulation of cellular cholesterol metabolism. J Cell Sci [Suppl] 3:131–137
21. Gal D, Ohashi M, MacDonald PC, Buchsbaum HJ, Simpson ER 1981 Low-density lipoprotein as a potential vehicle for chemotherapeutic agents and radionucleotides in the management of gynecologic neoplasms. Am J Obstet Gynecol 139:877–885
22. Vitols S, Gahrton G, Öst Å, Peterson C 1984 Elevated low density lipoprotein receptor activity in leukemic cells with monocytic differentiation. Blood 63:1186–1193
23. Elder PK, Schmidt LJ, Ono T, Getz MJ 1984 Specific stimulation of actin gene transcription by epidermal growth factor and cycloheximide. Proc Natl Acad Sci USA 81:7476–7480
24. Greenberg ME, Greene LA, Ziff EB 1985 Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. J Biol Chem 260:14101–14110
25. Pierce GB, Midgley AR 1963 The origin and function of human syncytiotrophoblastic giant cell. Am J Pathol 43:153–173
26. Enders AC 1965 Formation of syncytium from cytotrophoblast in the human placenta. Obstet Gynecol 25:378–386
27. Wynn RM 1972 Cytotrophoblastic specialization: an ultrastructural study of the human placenta. Am J Obstet Gynecol 114:339–355
28. Chomczynski P, Sacchi N 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159
29. Aviv H, Leder P 1972 Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA 69:1408–1412
30. Feinberg AP, Vogelstein B 1983 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13