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J Exp Med Apr 1 1980, 151 (4) p975-9, ISSN 0022-1007 Journal Code: I2V

32/3/171
04072481 80183481
Serological and immunochemical studies of H-2 allospecificities on K36, a syngeneic tumour of AKR.
Schmidt W; Festenstein H
J Immunogenet Feb 1980, 7 (1) p7-17, ISSN 0305-1811 Journal Code: IF2

32/3/172
03935703 80046703
Synthesis and characterization of a DNA complementary to pre-uteroglobin mRNA.
Arnemann J; Heins B; Beato M
Eur J Biochem Sep 1979, 99 (2) p361-7, ISSN 0014-2956 Journal Code: EMZ

32/3/173
03901840 80012840
Modulation of thyroglobulin messenger RNA level by thyrotropin in cultured thyroid cells.
Chebath J; Chabaud O; Mauchamp J
Nucleic Acids Res Jul 25 1979, 6 (10) p3353-67, ISSN 0301-5610 Journal Code: 08L

32/3/174
03818734 79195734
Expression of xenotropic murine leukemia viruses as cell-surface gp70 in genetic crosses between strains DBA/2 and C57BL/6.
Morse HC 3d; Chused TM; Hartley JW; Mathieson BJ; Sharrow SO; Taylor BA
J Exp Med May 1 1979, 149 (5) p1183-96, ISSN 0022-1007 Journal Code: I2V

32/3/175
03818210 79195210
T100: a new murine cell surface glycoprotein detected by anti-Lyt-2.1 serum.
Durda PJ; Boos SC; Gottlieb PD
J Immunol Apr 1979, 122 (4) p1407-12, ISSN 0022-1767 Journal Code: IFB

32/3/176
03476626 78110626
Genetic control of endotoxic responses in mice.
Watson J; Largen M; McAdam KP
J Exp Med Jan 1 1978, 147 (1) p39-49, ISSN 0022-1007 Journal Code: I2V

32/3/177
03389884 78023884
Reverse transcription of thyroglobulin 33-S mRNA.
Dinsart C; Van Voorthuizen F; Vassart G
Eur J Biochem Aug 15 1977, 78 (1) p175-81, ISSN 0014-2956 Journal Code: EMZ

32/3/178
03165782 77067782
The ia antigens.
Sachs DH
Contemp Top Mol Immunol 1976, 5 p1-33, ISSN 0090-8800 Journal Code: DQO
?

Set	Items	Description
S1	66204	RECOMBINAN? OR RDNA OR CDNA OR GENETIC?(2N)ENGINEER??? OR CLON??? OR CLONING()MOLECULAR OR MRNA OR BIOTECHNOLOG?
S2	7	REPO OR RERYTHROPOIETIN
S3	1	R()ERYTHROPOIETIN
S4	135002	DC=D12.776.543? OR DC=D12.776.395?
S5	7853	GLYCOSYLAT???? OR CARBOHYDRATE? ?(2N)STRUCTUR?? OR CARBOHYDRATE? ?(N)CONFORMATION? ?
S6	564	S4 AND PROTEIN()PROCESSING()POST()TRANSLATIONAL/DE
S7	5693	(S1 OR S3) AND (S4 OR S5 OR S6)
S8	341	CHO()CELL? ?/TI
S9	3333	CHO OR CHINESE()HAMSTER()OVARY
S10	1541	CV1 OR BSC1 OR BHK
S11	228	COS AND (CELL()LINE OR CELLS()CULTURED)
S12	149	S7 AND (S8 OR S9 OR S10 OR S11)
S13	2480	RECOMBINANT()PROTEINS
S14	2	DNA()BI
S15	133	DNA()BIOSYNTHESIS
S16	0	S15 AND (S4 OR S5 OR S6) AND (S8 OR S9 OR S10 OR S11)
S17	87	S12/1985:1988
S18	62	S12 NOT S17
S19	0	S12 AND (UD=8501 OR UD=8502 OR UD=8503)
S20	22375	JA="8501"
S21	62	(S12 AND S20) OR S18
S22	62	(S12 AND JA=8502) OR S18

?

AM 27 014723

22/7,JA,LA/1
05456238 85072238
Phosphorylation of membrane proteins in monensin-resistant mutant of Chinese hamster ovary cell with altered insulin-receptor activity.
Ono M; Takahashi K; Sato Y; Kuwano M
Biochem Int Sep 1984, 9 (3) p369-77, ISSN 0158-5231 Journal Code: 9Y9
Languages: ENGLISH
Journal Announcement: 8503
A Chinese hamster ovary (CHO) cell mutant resistant to the Na+/K+ ionophoric antibiotic, monensin, is partly defective in insulin-receptor activity. Growth of the monensin-resistant mutant required higher dose of insulin in defined medium than the parental CHO cell. Membrane proteins of CHO and a monensin-resistant clone, MonR-31, were compared for their capacity to be phosphorylated by endogenous phosphorylation enzyme in vitro in the absence or presence of insulin. Several membrane proteins of CHO were more highly phosphorylated as compared with MonR-31 in either the absence or presence of insulin.

22/7,JA,LA/2
05440508 85056508
Purification of recombinant glycosylated human gamma interferon expressed in transformed Chinese hamster ovary cells.
Devos R; Opsomer C; Scahill SJ; Van der Heyden J; Fiers W
J Interferon Res Fall 1984, 4 (4) p461-8, ISSN 0197-2[illegible]
Journal Code: IJI
Languages: ENGLISH
Journal Announcement: 8503
Human IFN-gamma was produced in cultures of a Chinese hamster ovary (CHO) cell line transformed with a combination of plasmids encoding HuIFN-gamma cDNA and mouse DHFR cDNA and subsequently selected for growth in the presence of methotrexate. Confluent monolayers of these cells constitutively secrete HuIFN-gamma into the medium reaching a concentration of 2-5 micrograms/ml; the supernatant of the monolayer could be harvested daily for a period of more than 10 days. IFN-gamma was purified by passing the filtered CHO cell culture medium directly through a phosphocellulose column followed by elution and adsorption on a Con A-Sepharose column. Further concentration on an AMICON PM 10 filter and removal of high mw contaminating proteins with DEAE-Sephacel resulted in a IFN-gamma preparation of more than 99% purity (specific activity of about 10(8) International units per mg of protein). Each liter of CHO conditioned culture medium yielded 1-2 mg pure HuIFN-gamma. Its molecular weight, as determined by gel filtration, is about 50 kD and corresponds to a dimer structure. SDS-polyacrylamide gel electrophoresis indicated the presence of a 21 kD and a 25 kD polypeptide as compared with 17 kD for unglycosylated, bacterially made HuIFN-gamma and consistent with the two glycosylated forms of HuIFN-gamma produced in mitogen-stimulated human lymphocyte cultures.

22/7,JA,LA/3
05432951 85048951
Reconstitution of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of N-acetylglucosamine.
Balch WE; Dunphy WG; Braell WA; Rothman JE

Cell Dec 1984, 39 (2 Pt 1) p405-16, ISSN 0092-8674 Journal Code: CQ4
Contract/Grant No.: AM 27044
Languages: ENGLISH
Journal Announcement: 8503

Transport of the VSV-encoded glycoprotein (G protein) between successive compartments of the Golgi has been reconstituted in a cell-free system and is measured, in a rapid and sensitive new assay, by the coupled incorporation of 3H-N-acetylglucosamine (GlcNAc). This glycosylation occurs when G protein is transported during mixed incubations from the "donor" compartment in Golgi from VSV-infected CHO clone 15B cells (missing a key Golgi GlcNAc transferase) to the next, successive "acceptor" compartment (containing the GlcNAc transferase) in Golgi from wild-type CHO cells. Golgi fractions used in this assay have been extensively purified, and account for all of the donor and acceptor activity in the cells. Together with several other lines of evidence, this indicates that the cell-free system is highly specific, measuring only transport between sequential compartments in the Golgi stack. Transport in vitro is almost as efficient as in the cell, and requires ATP and the cytosol fraction in addition to protein components on the cytoplasmic surface of the Golgi membranes.

22/7,JA,LA/4
05432939 85048939

Translocation across Golgi vesicle membranes: a CHO glycosylation mutant deficient in CMP-sialic acid transport.

Deutscher SL; Nuwayhid N; Stanley P; Briles EI; Hirschberg CB

Cell Dec 1984, 39 (2 Pt 1) p295-9, ISSN 0092-8674 Journal Code: CQ4
Contract/Grant No.: CA-30345; GM 30365
Languages: ENGLISH
Journal Announcement: 8503

Golgi vesicle membranes from the Lec2 CHO glycosylation mutant translocate CMP-sialic acid at only 2% the rate of vesicles from wild-type CHO cells. The deficiency is specific, because vesicles from Lec2 cells can translocate UDP-N-acetylglucosamine, adenosine 3'-phosphate 5'-phosphosulfate, and UDP-galactose at rates comparable to those of vesicles from wild-type cells. Complementation analyses show that Lec2 mutants belong to the same genetic complementation group as clone 1021, a CHO mutant of similar phenotype. Both mutants have previously been shown to have a 90% reduction in the sialylation of glycoproteins and gangliosides compared with wild-type cells. However, 1021 cells appear to have normal levels of CMP-sialic acid, sialyltransferase activity, and endogenous acceptors for sialylation. It seems likely that the primary defect in Lec2 and 1021 cells is their inability to translocate CMP-sialic acid across Golgi vesicle membranes.

22/7,JA,LA/5
05415790 85031790

Immunologic differentiation between E. coli and CHO cell-derived recombinant and natural human beta-interferons.

Colby CB; Inoue M; Thompson M; Tan YH

J Immunol Dec 1984, 133 (6) p3091-5, ISSN 0022-1767 Journal Code: IFB
Languages: ENGLISH
Journal Announcement: 8502

The products of the human IFN-beta gene expressed in E. coli, Chinese hamster ovary (CHO) cells, and human fibroblasts appear similar when purified on a monoclonal antibody column and analyzed by reverse-phase HPLC, indicating little difference in their hydrophobic nature. SDS-PAGE

differentiates E. coli-rHuIFN-beta ser (Mr = 17,000) from CHO-rHuIFN-beta and HuIFN-beta (Mr = 23,000), with glycosylation accounting for 26% of the apparent m.w. of the latter two proteins. CHO-rHuIFN-beta is preferentially neutralized by mouse monoclonal and monospecific rabbit polyclonal anti-HuIFN-beta antibodies, whereas E. coli-rHuIFN-beta ser is preferentially neutralized by goat polyclonal anti-E. coli-rHuIFN-beta antibodies. Adsorption measurements by a sensitive radioimmunoassay indicate that the binding of the three proteins to anti-HuIFN-beta antibodies is similar. The results show that all three molecules can be differentiated by the heteroclitic cross-reactivities of anti-HuIFN-beta and anti-E. coli-rHuIFN-beta antibodies to the antigens.

22/7,JA,LA/6
05408898 85024898

The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA.

Yamamoto T; Davis CG; Brown MS; Schneider WJ; Casey ML; Goldstein JL; Russell DW

Cell Nov 1984, 39 (1) p27-38, ISSN 0092-8674 Journal Code: CQ4
Contract/Grant No.: HL20948; HL31346; HD11149; +
Languages: ENGLISH
Journal Announcement: 8502

The nucleotide sequence of a cloned 5.3 kilobase cDNA for the human low density lipoprotein receptor revealed five domains in the 839 amino acid protein: 322 NH2-terminal amino acids, extremely rich in disulfide-bonded cysteine residues (15%) and including an 8-fold repeat of 40 residues that may contain the LDL binding site; 350 residues homologous to the precursor of mouse epidermal growth factor; a region immediately outside the plasma membrane, rich in serine and threonine and the site of O-linked glycosylation; 22 hydrophobic amino acids, spanning the plasma membrane; and 50 COOH-terminal amino acids, projecting into the cytoplasm. The mRNA for the receptor contains a 3' untranslated region of 2.5 kilobases that includes multiple copies of the Alu family of repetitive DNA . Transfection of simian COS cells with the human LDL receptor cDNA linked to the SV40 early promoter resulted in expression of functional cell surface receptors.

22/7,JA,LA/7
05393844 85009844

Mapping and expression of a human cytomegalovirus major viral protein.

Davis MG; Mar EC; Wu YM; Huang ES

J Virol Oct 1984, 52 (1) p129-35, ISSN 0022-538X Journal Code: KCV
Languages: ENGLISH
Journal Announcement: 8501

We constructed a DNA fragment map of low-passage Towne strain cytomegalovirus by analyzing cross-blot hybridization and hybridizations of isolated recombinant clones. The abundant late transcripts were located on this map by hybridization of labeled total RNA of virus-infected cells to blotted DNA fragments. The most abundant late transcript, carried by the 11.7-kilobase EcoRI fragment (EcoRI-G), was precisely mapped. The EcoRI fragment was fragmented and subcloned in a plasmid carrying simian virus 40 sequences (pSV-OH, constructed by Chi-Bom Chae, Department of Biochemistry, University of North Carolina, Chapel Hill). One resulting recombinant plasmid, pHD713SV2, was transferred to simian virus 40-transformed monkey kidney cells (COS-1) by DNA transfection. Synthesis of a cytomegalovirus-specific 67-kilodalton protein was detected in these cells by reaction of blotted proteins with virus-specific monoclonal antibody. The 67-kilodalton protein is a major phosphorylated protein found in virions; it is not glycosylated. The location of the gene for this

67-kilodalton protein is therefore assigned to the center of the L-unique region of human cytomegalovirus, at 0.37 to 0.39 map units.

22/7,JA,LA/8
05288790 84212790

Mouse myeloma cells that make short immunoglobulin heavy chains: pleiotropic effects on glycosylation and chain assembly.

Kenter AL; Warren T; Shields D; Birshtein BK

J Cell Biol Jun 1984, 98 (6) p2215-21, ISSN 0021-9525

Journal Code: HMV

Contract/Grant No.: AI 13509; AI 10702

Languages: ENGLISH

Journal Announcement: 8409

Two variants in immunoglobulin heavy chain production, derived from the MPC 11 mouse myeloma cell line, make short heavy (H) chains with identical precise deletions of the CH3 domain. The CH3 domain is expressed in the H chain mRNA from both variants. Although in vitro translation of this mRNA produces one H chain species, deleted heavy chains are secreted as heavy-light (HL) and H2L2 moieties in contrast to MPC 11, which secretes only H2L2 . The heavy chains of HL apparently contain more carbohydrate (CHO+) than do the H chains of H2L2 , and inhibition of N-linked glycosylation results in the secretion of relatively more H2L2 . Here we present evidence suggesting that (a) the absence of the CH3 domain has led to conformational changes in these molecules, (b) these changes permit posttranslational glycosylation, and (c) unrestrained glycosylation can frequently yield unusual CHO+ structures that make complete assembly unlikely.

22/7,JA,LA/9
05270003 84194003

NH2-terminal hydrophobic region of influenza virus neuraminidase provides the signal function in translocation.

Bos TJ; Davis AR; Nayak DP

Proc Natl Acad Sci USA Apr 1984, 81 (8) p2327-31, ISSN 0027-8424

Journal Code: PV3

Contract/Grant No.: ROI AI-16348; ROI AI-12749; GM-07104

Languages: ENGLISH

Journal Announcement: 8408

Influenza virus neuraminidase (NA), unlike the majority of integral membrane proteins, does not contain a cleavable signal sequence. It contains an NH2-terminal hydrophobic domain that functions as an anchor. We have investigated the signal function for translocation of this NH2-terminal hydrophobic domain of NA by constructing chimeric cDNA clones in which the DNA coding for the first 40 NH2-terminal hydrophobic amino acids of NA was joined to the DNA coding for the signal-minus hemagglutinin (HA) of influenza virus. The chimeric HA (N40H) containing the NH2 terminus of NA was expressed in CV1 cells by using a simian virus 40 late-expression vector. The chimeric HA is synthesized, translocated into the rough endoplasmic reticulum, and glycosylated, whereas HA lacking the signal sequence is present only in small amounts and is unglycosylated. These results clearly show that the NH2 terminus of NA, in addition to its anchor function, also provides the signal function in translocation. However, the acquisition of complex oligosaccharides and the transport of N40H to the cell surface are greatly retarded. To determine if the presence of two anchor sequences, one provided by NA at the NH2 terminus and the other provided by HA at the COOH terminus of N40H, was responsible for the slow transport, the NH2 terminus of NA was fused to an "anchorless" HA. The resulting chimeric HA (N40H482) contains the hydrophobic domain of NA at

the NH2 terminus but lacks the HA anchor at the COOH terminus. N40H482 was synthesized and glycosylated; however, as with N40H, the acquisition of complex oligosaccharides and the migration to the cell surface are greatly retarded. Immunofluorescence data also support that, compared to the native HA, only a small amount of chimeric HA proteins is transported to the cell surface. Thus, the hydrophobic NH2 terminus of NA, although capable of providing the signal function in translocation across the rough endoplasmic reticulum, interferes with the tra

22/7,JA,LA/10
05261856 84185856
Chinese hamster ovary cell variants resistant to monensin, an ionophoric antibiotic. II. Growth requirement for insulin and altered insulin-receptor activity.
Sato Y; Ono M; Takaki R; Kuwano M
J Cell Physiol May 1984, 119 (2) p204-10, ISSN 0021-9541
Journal Code: HNB
Languages: ENGLISH
Journal Announcement: 8408
From the Chinese hamster ovary (CHO) cell, genetic variants (MonR-31 and MonR-32) relatively resistant to monensin, an ionophoric antibiotic, have been isolated. Growth of both MonR-31 and MonR-32 clones required higher doses of serum than CHO. Addition of insulin to media containing a low dose of serum restored full colony formation, but growth of MonR-31 or MonR-32 cells required more insulin than CHO cells. Specific binding of [125I]insulin was observed in these cell lines. The two MonR clones bound about one-half or less the [125I]insulin bound by CHO cells. Scatchard analysis for [125I]insulin binding at 4 degrees C and 37 degrees C showed altered number of binding sites, but not insulin affinity: The number of binding sites in the MonR cell was about a half or less that of the parental CHO cell. Down-regulation of insulin receptor was assayed when both CHO and MonR cells were incubated with 1 microgram/ml insulin. A 50-60% decrease in levels of insulin surface binding capacities was observed in CHO after exposure to insulin, whereas there was no decrease in MonR cell. The cellular uptake of 2-[3H]deoxyglucose into CHO cells was significantly enhanced in the presence of insulin, but only slight, if any, increase was observed in MonR cells.

22/7,JA,LA/11
05256761 84180761
BHK cell variant with defective fibronectin receptor function.
Oppenheimer-Marks N; Border B; Grinnell F
Cell Biol Int Rep Feb 1984, 8 (2) p171-8, ISSN 0309-1651
Journal Code: CRC
Contract/Grant No.: CA14609
Languages: ENGLISH
Journal Announcement: 8408
Baby hamster kidney cells were mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine and selected to obtain a population of non-attaching cells. The cell variant FN-1 was cloned from the non-attaching cell population, recloned, and tested for cell adhesive interactions using four different assays of fibronectin (pFN) receptor function: cell attachment and spreading on culture dishes and cell binding and phagocytosis of latex beads. On pFN-coated culture dishes, FN-1 cells had decreased attachment compared to parental cells and were unable to spread. With pFN-coated beads, only one third as many pFN-bead binding sites could be detected on FN-1 cells as on the parental cells, and the FN-1 cells were unable to phagocytose the pFN-coated beads. In other

studies, the variant cells were able to attach normally and spread partially on substrata coated with polycationic ferritin, concanavalin A, or anti-BHK cell surface antibody. The results suggest that the pFN-receptor function of FN-1 cells is defective.

22/7,JA,LA/12
05232538 84156538
Microinjection of mRNA coding for an anti-Golgi antibody inhibits intracellular transport of a viral membrane protein.
Burke B; Warren G
Cell Apr 1984, 36 (4) p847-56, ISSN 0092-8674 Journal Code: C04
Languages: ENGLISH
Journal Announcement: 8407
Messenger RNA was prepared from a hybridoma cell line secreting a monoclonal antibody (53FC3) directed against a luminal epitope of a Golgi membrane protein (Mr = 135 kd) found in rodent cells. When this mRNA was microinjected into the cytoplasm of BHK cells, mouse IgG was seen to accumulate in the Golgi complex after 5-6 hr of incubation. No accumulation was seen in 3T3 cells which lack the epitope recognized by 53FC3. When microinjected BHK cells were infected with vesicular stomatitis virus, surface expression of the viral G protein was considerably reduced when compared with neighboring noninjected cells.

22/7,JA,LA/13
05218230 84142230
Expression and regulation of human low-density lipoprotein receptors in Chinese hamster ovary cells.
Sege RD; Kozarsky K; Nelson DL; Krieger M
Nature Feb 23-29 1984, 307 (5953) p742-5, ISSN 0028-0836
Journal Code: NSC
Contract/Grant No.: GM07753
Languages: ENGLISH
Journal Announcement: 8406
Low-density lipoprotein (LDL), the major cholesterol transport component of human plasma, delivers cholesterol to mammalian cells via the LDL pathway of receptor-mediated endocytosis. LDL receptor activity and cholesterol biosynthesis are coordinately regulated by cholesterol-mediated feedback suppression. We have developed methods for the isolation of mutant and revertant (M.K., unpublished data) Chinese hamster ovary (CHO) cells having alterations in cholesterol biosynthesis and in the receptor-mediated endocytosis of LDL. The defective locus of one LDL receptor-negative CHO mutant (clone 7a-1) is apparently the structural gene for the LDL receptor (D. Kingsley, M. Segal and M.K., unpublished data). Here we have transfected 7a-1 cells with human DNA and selected colonies which synthesize functional human LDL receptors whose expression is regulated normally. This selection may prove useful for cloning genes required for the receptor-mediated endocytosis of LDL and other ligands and thus for elucidating the molecular defects responsible for familial hypercholesterolaemia, one of the most common genetic diseases in humans.

22/7,JA,LA/14
05217852 84141852
Inducible expression of amplified human beta interferon genes in CHO cells.
McCormick F; Trahey M; Innis M; Dieckmann B; Ringold G
Mol Cell Biol Jan 1984, 4 (1) p166-72, ISSN 0270-7306
Journal Code: NGY

Languages: ENGLISH
Journal Announcement: 8406

Plasmid DNA containing the human beta-interferon (IFN-beta) gene and mouse dihydrofolate reductase cDNA was transfected into dihydrofolate reductase-negative Chinese hamster ovary cells. Dihydrofolate reductase-positive transformants were obtained, and cells containing amplified copies of mouse dihydrofolate reductase were selected by exposure to increasing methotrexate concentrations. These cells were found to express high levels of human IFN-beta after polyriboinosinic acid-polyribocytidylic acid superinduction or NDV infection; this was a result of coamplification of the IFN-beta gene. Levels of expression of 1 U/cell per day were achieved on superinduction, giving corresponding titers of up to 10(10) U/liter medium in culture supernatants. Constitutive production of IFN-beta rates of about 0.5% of superinduced rates was observed; cells producing these levels of IFN-beta had acquired resistance to cytotoxic antiviral effects of IFN-beta. Two forms of human IFN-beta were produced; a major glycosylated 23,000-dalton form and an unglycosylated 18,500-dalton form. The latter had greatly reduced antiviral activity. IFN-beta production was very sensitive to cellular growth rate; the highest levels were produced by density-arrested cultures. Regulation of IFN-beta production by polyriboinosinic acid-polyribocytidylic acid or by cell density effects required the presence of DNA sequences 5' to the IFN-beta-coding sequences; replacement of these sequences with the simian virus 40 early promoter resulted in uninducible, density-independent production of IFN-beta.

22/7,JA,LA/15
05195494 84119494

Chimeric influenza virus hemagglutinin containing either the NH2 terminus or the COOH terminus of G protein of vesicular stomatitis virus is defective in transport to the cell surface.

McQueen NL; Nayak DP; Jones LV; Compans RW
Proc Natl Acad Sci USA Jan 1984, 81 (2) p395-9, ISSN 0027-8424
Journal Code: PV3
Contract/Grant No.: RO1 AI2749; RO1 AI16348; RO1 AI12680; +
Languages: ENGLISH
Journal Announcement: 8405

Chimeric cDNA clones of influenza virus hemagglutinin (HA) were constructed in which the DNA encoding either the NH2 terminus or the COOH terminus of HA was replaced with that of a vesicular stomatitis virus G protein. The chimeric cDNAs (GHA or HAG) were expressed in CV1 cells using the simian virus 40 late replacement promoter. Both chimeric proteins are synthesized, glycosylated, and transported to the rough endoplasmic reticulum. These results show that the NH2-terminal sequences of vesicular stomatitis virus G protein can provide a signal function for translocation and the COOH-terminal sequences can provide the anchor function for the influenza virus HA, when substituted for similar sequences. However, the chimeric glycoproteins were not transported to the Golgi complex or the plasma membrane. The implication of these results in translocation, sorting, and transport processes is discussed.

22/7,JA,LA/16
05163936 84087936

Biosynthesis of N- and O-linked oligosaccharides of the low density lipoprotein receptor.

Cummings RD; Kornfeld S; Schneider WJ; Hobgood KK; Tolleshaug H; Brown MS; Goldstein JL
J Biol Chem Dec 25 1983, 258 (24) p15261-73, ISSN 0021-9258

Journal Code: HIV
Contract/Grant No.: RO1 CA08759; HL 20948; 5 T32 HD07103
Languages: ENGLISH
Journal Announcement: 8404

In human fibroblasts, the receptor for low density lipoprotein (LDL) is synthesized as a precursor of apparent Mr = 120,000 which is converted to a mature form of apparent Mr = 160,000, as determined by migration in sodium dodecyl sulfate (SDS)-polyacrylamide gels (Tolleshaug, H., Goldstein, J. L., Schneider, W. J., and Brown, M. S. (1982) Cell 30, 715-724). The current paper describes the relationship of N- and O-glycosylation to this post-translational modification. Oligosaccharides were analyzed from precursor and mature forms of LDL receptors that had been immunoprecipitated from cells grown in media containing radioactive sugars. In human epidermoid carcinoma A-431 cells, the receptor precursor appears to contain one N-linked high mannose oligosaccharide and approximately 6-9 N-acetylgalactosamine residues linked O-glycosidically to Ser/Thr residues. In the mature receptor, the O-linked oligosaccharides are mono- and disialylated species having the core structure of galactose leads to N-acetylgalactosamine leads to Ser/Thr. The single N-linked oligosaccharide of the mature receptor can either be a tri- or tetraantennary complex-type species. Similar results were obtained with normal human fibroblast receptor except that the O-linked oligosaccharides on the precursor are neutral disaccharides, of which one component is GalNAc and the N-linked complex type unit on the mature receptor is less branched. Since the addition of GalNAc residues to Ser/Thr residues precedes the conversion of N-linked high mannose-type oligosaccharides to complex-type structures, the transfer of N-acetylgalactosamine must occur prior to the entry of glycoproteins into the region of the Golgi containing the processing enzyme alpha-mannosidase I. We also studied the receptor from tunicamycin-treated cells and after treatment with neuraminidase. In addition, we analyzed the receptor synthesized by a lectin-resistant clone of Chinese hamster ovary cells that is deficient in adding galactose

22/7,JA,LA/17
05106747 84030747

Supplementary factors required for serum-free culture of rat kidney cells of line NRK-49F.

Bradshaw GL; Dubes GR

In Vitro Oct 1983, 19 (10) p735-42, ISSN 0073-5655 Journal Code: GHD
Languages: ENGLISH
Journal Announcement: 8402

Factors required as supplements to basal tissue culture medium for the multiplication of cells of the cloned rat fibroblast line called normal rat kidney 49F (NRK-49F) were identified as epidermal growth factor, fibronectin, insulin, and retinoic acid. The requirement for fibronectin was manifested on a clean glass surface but not on the polystyrene plastic surface tested. This set of required factors differs substantially from the factor sets required by the Madin-Darby canine kidney (MDCK) and LLC-PK1 pig kidney lines of epithelial cells and the baby hamster kidney 21 (BHK-21) line of fibroblasts. The serum-free medium supplemented with the four factors supported rapid growth of NRK-49F cells when the initial cell population density was about 8,000 cells/cm2 or greater. At lower initial densities, cell multiplication was markedly increased by adding serum-free medium that had been conditioned by NRK-49F cells. Cell growth rate in the defined serum-free medium stayed high through two serial passages but declined in the third serial passage unless the cell-conditioned medium was added.

22/7,JA,LA/18
05102233 84026233
Hybrid, sialylated N-glycans accumulate in a ricin-resistant mutant of baby hamster kidney BHK cells.
Hughes RC; Mills G; Stojanovic D
Carbohydr Res Aug 16 1983, 120 p215-34, ISSN 0008-6215
Journal Code: CNY
Languages: ENGLISH
Journal Announcement: 8402
Glycoproteins synthesized in a ricin-resistant mutant of BHK cells, clone RICR21, were labelled by growth of the cells in radioactive D-mannose, D-glucosamine, or L-fucose. Glycopeptides obtained from disrupted cells by exhaustive digestion with Pronase were fractionated into components binding to concanavalin A-Sepharose and nonbinding components. The binding components eluted with methyl alpha-D-mannopyranoside were separated by gel filtration on Bio-Gel P-4 into two main subfractions: an oligomannosidic fraction that was susceptible to Jack bean alpha-D-mannosidase and a fraction that became totally degraded only in the additional presence of neuraminidase, beta-D-galactosidase, and N-acetyl-beta-D-glucosaminidase. Further analysis of the latter fraction by exoglycosidase digestion together with consideration of the known pathways for the biosynthesis of asparagine-linked sugar chains of glycoproteins was consistent with a "hybrid" structure containing a NeuAc leads to Gal leads to GlcNAc sequence linked to the alpha-D-mannosyl-(1 leads to 3) residue of the core sequence, and a terminal alpha-D-mannosyl group linked to the alpha-(1 leads to 6) branch of the core sequence. The hybrid fraction was labelled after growth of the cells in radioactive L-fucose and was adsorbed to a lentil lectin-Sepharose column indicating the presence of core fucosylation. The novel structure represented about 30-35% of the total cellular glycopeptides of RICR21 cells and was not present in the glycopeptides of normal, ricin-sensitive BHK cells. Conversely, double-branched (biantennary) complex N-glycans, a prominent constituent of BHK cell glycoproteins, were absent in RICR21 cells, and analysis of the nonbinding fraction obtained from concanavalin A-Sepharose indicated that triple- and quadruple-branched (tri- and tetra-antennary), complex N-glycans present in normal BHK cell glycoproteins were also absent.

22/7,JA,LA/19
05067018 83300018
Amphotericin B selection of mutant Chinese hamster cells with defects in the receptor-mediated endocytosis of low density lipoprotein and cholesterol biosynthesis.
Krieger M; Martin J; Segal M; Kingsley D
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This paper describes a rapid and efficient two-step procedure for the isolation of mutant cells with defects in receptor-mediated endocytosis. The procedure takes advantage of two fungal metabolites, compactin (ML236B), a potent inhibitor of cholesterol biosynthesis, and amphotericin B, a polyene antibiotic that forms toxic complexes with sterols in membranes. Mutagen-treated Chinese hamster ovary cells were preincubated overnight in a medium containing mevalonate, low density lipoprotein (LDL), and compactin (Mev/LDL/Com). At the end of the preincubation period, wild-type cells were cholesterol replete while mutant cells that could not utilize the cholesterol in LDL were cholesterol deficient. Subsequent

incubation with amphotericin B for 6 hr killed most of the wild-type cells. After a second round of Mev/LDL/Com-amphotericin B selection, endocytosis-defective clones appeared at a frequency of approximately equal to 2.6 X 10(-5). Some of these clones expressed LDL receptor-defective phenotypes and fell into one of two previously defined classes of mutation. Sensitivity of the mutants to infection by vesicular stomatitis virus suggested that the mutations do not disrupt the coated pit-coated vesicle pathway of endocytosis. Minor modifications in the Mev/LDL/Com-amphotericin B selection permit the isolation of cholesterol auxotrophs and might allow the isolation of conditional-lethal mutations. Because LDL can be coupled to ligands that bind to receptors other than the LDL receptor, Mev/LDL/Com-amphotericin B selection may permit the isolation of mutant cells with defects that specifically disrupt other endocytic pathways.

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Synthesis of hepatitis B surface antigen in mammalian cells: expression of the entire gene and the coding region.

Laub O; Rall LB; Truett M; Shaul Y; Standring DN; Valenzuela P; Rutter WJ
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We have constructed two simian virus 40 early replacement recombinants that have the coding sequences for hepatitis B virus surface antigen (HBsAg). One construction, LSV-HBsAg, has the coding region for HBsAg but not the portion encoding the putative pre-surface antigen leader. Transformed monkey kidney cells (COS) infected with this recombinant express large quantities of the characteristic partially glycosylated HBsAg molecule, which are assembled into 22-nm particles that appear similar to those produced by human liver cells infected with hepatitis B virus. This result indicates that the pre-surface antigen sequences are not required for the synthesis of HBsAg or its assembly into particulate structures. The second recombinant, LSV-HBpresAg, has the entire surface antigen gene, including the putative promoter and pre-surface antigen region. COS cells infected with this recombinant plasmid produce 40- to 50-fold less HBsAg than those infected with the LSV-HBsAg recombinant plasmid. RNA mapping studies suggest that the transcription of the HBsAg gene is initiated at more than one site, or alternatively, that RNA splicing of transcripts occurs in the pre-surface antigen region.

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Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells.

Scahill SJ; Devos R; Van der Heyden J; Fiers W
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Cotransformation with two plasmids, one [pSV2-IFN-gamma] encoding human immune interferon (Hu IFN-gamma) and the other [pAdD26SV(A)-3] encoding mouse dihydrofolate reductase, has been used to establish Chinese hamster ovary (CHO) cell lines that secrete high levels of Hu IFN-gamma. Hu IFN-gamma production by the transformed CHO cell lines E-10B and E-10C reached approximately 50,000 units/ml of culture medium, which compares favorably with that of stimulated lymphocytes. Furthermore, as the Hu IFN-gamma cDNA gene used in these studies is under the transcriptional control of the simian virus 40 early promoter, Hu IFN-gamma production is