constitutive and thus does not require induction. CHO-produced Hu IFN-gamma migrates as two bands corresponding to molecular weights of 25,000 and 21,000 on NaDodSO4/polyacrylamide gels. These two species are shown to be the products of a single gene. As the molecular weight of native Hu IFN-gamma is around 55,000, it is likely to be a dimer. We have shown that the subunits of such a dimer cannot be linked by a disulfide bridge(s). Hu IFN-gamma from CHO cells is likely to be glycosylated and this should now permit comparison of the biological activities of glycosylated and nonglycosylated (bacterially produced) Hu IFN-gamma in animal studies.

22/7,JA,LA/22
05031556 83264556
Fibronectin and anchorage-independent and anchorage-dependent growth of benign and malignant cell lines.
Dodson MG; Gelder FB; Slota J; Lange C
Int J Cancer Aug 15 1983, 32 (2) p211-7, ISSN 0020-7136
Journal Code: GQU
Contract/Grant No.: RR-00350
Languages: ENGLISH
Journal Announcement: 8311
The presence of fibronectin in three "malignant" (AU-471, AU-436, LT-2) and two "benign" (BHK-21, WI-38) cell lines was demonstrated with a fluorescent antibody technique; two malignant (AU-471, AU-436) cell lines were fibronectin-negative and one (LT-2) retained fibronectin expression. One "benign" cell line (WI-38) expressed fibronectin, the other (BHK-21) did not. Anchorage-independent soft agar (AISA) growth correlated better with loss of fibronectin than with malignant potential. All three fibronectin-negative cell lines (benign and malignant) grew anchorage-independently (AU-471, AU-436, BHK-21), and both fibronectin-positive cell lines were anchorage-dependent (LT-2, WI-38). Surprisingly, the addition of C1g to anchorage-independent cells increased their anchorage-independent soft-agar cloning efficiency, but had no effect on anchorage-dependent cell lines. Anti-C1g antibodies decreased AISA growth. The effect of C1g on anchorage-independent growth varied with the concentration, and also between cell lines, and a variation in effect was noted between anchorage-independent (AISA) and anchorage-dependent (in flasks) growth even in the same cell line.

22/7,JA,LA/23
05026031 83259031
Daunorubicin-resistant Chinese hamster ovary cells expressing multidrug resistance and a cell-surface P-glycoprotein.
Kartner N; Shales M; Riordan JR; Ling V
Cancer Res Sep 1983, 43 (9) p4413-9, ISSN 0008-5472 Journal Code: CNF
Languages: ENGLISH
Journal Announcement: 8311
Independent lines of Chinese hamster ovary cells resistant to the antineoplastic drug, daunorubicin, were obtained by clonal isolation in increasing drug concentrations. A single daunorubicin-resistant phenotype typified by reduced cellular drug accumulation was observed. These mutants displayed a complex phenotype of resistance to a variety of unrelated drugs. Such properties are similar to those of membrane-altered colchicine-resistant lines (V. Ling and L.H. Thompson, J. Cell. Physiol., 83: 103-116, 1974). Analysis of the plasma membrane components of the daunorubicin-resistant clones by gel electrophoresis revealed a prominent cell surface glycoprotein with a molecular weight of about 170,000. This component was immunologically cross-reactive with the cell surface

P-glycoprotein of about the same molecular weight, previously identified in colchicine-resistant cells. Thus, it appears that the mechanism of resistance characterized by P-glycoprotein expression could be the basis of many drug-resistant phenotypes.

22/7,JA,LA/24
04999877 83232877

Complementation of mutations in the LDL pathway of receptor-mediated endocytosis by cocultivation of LDL receptor-defective hamster cell mutants.

Krieger M

Cell Jun 1983, 33 (2) p413-22, ISSN 0092-8674 Journal Code: C04

Contract/Grant No.: K04-HL00960; R01-GM30243

Languages: ENGLISH

Journal Announcement: 8310

We have previously isolated Chinese hamster ovary (CHO) cell mutants that do not express low density lipoprotein (LDL) receptors. When one mutant clone was cocultivated with other receptor-defective clones, it was induced to express receptors that could mediate normal endocytosis. These LDL receptor-defective clones defined two classes of mutations: cbc (complemented by cocultivation) and icc (inducer cells in cocultivation). The induction and short-term (18 hr) stability of LDL receptors in cbc cells did not require protein synthesis by icc cells. Receptor activity could not be induced by DMSO, 5-azacytidine, phosphatidylcholine liposomes, dibutyryl cAMP, compactin, soybean trypsin inhibitor, low temperature (30 degrees C), or conditioned medium, but could be induced by cocultivation with parental CHO cells and normal and LDL receptor-negative human fibroblasts. Complementation by cocultivation only occurred when the cbc and inducing cells were in close proximity, suggesting that an unstable diffusible factor or intimate cell-to-cell association was required for complementation.

22/7,JA,LA/25
04935895 83168895

Constitutive, long-term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene.

Haynes J; Weissmann C

Nucleic Acids Res Feb 11 1983, 11 (3) p687-706, ISSN 0301-5610 Journal Code: O8L

Languages: ENGLISH

Journal Announcement: 8307

Hybrid plasmids containing the mouse dihydrofolate reductase (dhfr) and a human interferon (either IFN-alpha 5 or IFN-gamma) coding sequence under the control of viral promoters were transfected into dhfr- Chinese hamster ovary (CHO) cells. dhfr+ colonies produced IFN at 10-1000 units X ml-1 X day-1. Clones selected in methotrexate had a 20-50-fold increase in the IFN-alpha 5 and dhfr DNA and mRNA content and secreted IFN at 20,000-100,000 units X ml-1 X day-1. SDS-polyacrylamide gel electrophoresis of partially purified 35S-HuIFN-gamma from CHO cells showed a multiple of labeled bands with a mobility corresponding to 22,400 to 23,400 daltons which was absent in the supernatants of non-transformed CHO cells. The higher apparent molecular weight of human IFN-gamma from CHO cells as compared to that of human IFN-gamma from E. coli (about 18,800) suggests that the former was glycosylated.

22/7,JA,LA/26
04927745 83160745

AM 27 014735

The human fibroblast interferon gene(s) and their expression in heterologous cells.

Fiers W; Degrave W; Derynck R; Devos R; Gheysen D; Remaut E; Stanssens P; Tavernier J; Content J; De Clercq E

Int Symp Princess Takamatsu Cancer Res Fund 1982, 12 p227-36,

Journal Code: HHI

Languages: ENGLISH

Journal Announcement: 8307

A clone has been identified which contains the genetic information specifying human fibroblast interferon (beta 1) and which was derived from a messenger RNA population. On the basis of the nucleotide sequence the complete amino acid sequence (166 residues) of human fibroblast interferon was deduced. It is preceded by a 21 amino acids long signal sequence. Also, a genomic clone was isolated and characterized in detail; on this basis we can conclude that the interferon beta 1 gene does not contain introns. The nucleotide sequence of the putative promoter region was deduced and compared with other eukaryotic promoters. So far, no evidence has been obtained for other human interferons of the fibroblast type. The interferon beta 1 was expressed in the mature form of Escherichia coli without glycosylation. Fully processed interferon was obtained by transfection of monkey CV1-cells with an SV40-derivative, in which the interferon gene replaced the major structural protein VP1.

22/7,JA,LA/27

04820132 83053132

The ovary: a target organ for 1,25-dihydroxyvitamin D3.

Dokoh S; Donaldson CA; Marion SL; Pike JW; Haussler MR

Endocrinology Jan 1983, 112 (1) p200-6, ISSN 0013-7227

Journal Code: EGZ

Contract/Grant No.: AM-15781

Languages: ENGLISH

Journal Announcement: 8303

Using both clonal Chinese hamster ovary (CHO) cells in culture and the hen ovary, we have searched for the presence of specific 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3] receptors. Receptor analyses were carried out on high salt cytosols of CHO cells and high salt extracts of hen ovarian nuclei that were originally isolated in low salt buffer. Both CHO and hen ovary contain a specific high affinity 1,25-(OH)2D3 receptor (Kd = 10(-10) - 10(-11) M), which sediments (3.3S) in high salt sucrose gradients identically to the chick intestinal receptor for 1,25-(OH)2D3. This 3.3S macromolecule from both sources absorbed to DNA-cellulose at 0.1 M KCl and eluted during a linear salt gradient at 0.2-0.22 M KCl, a property characteristic of the 1,25-(OH)2D3 receptor. Saturation analysis indicated that there are approximately 2000 copies of the receptor molecule per CHO cell. We also investigated the effect of 1,25-(OH)2D3 on ovarian cell growth in monolayer culture of CHO cells. Significant inhibition of CHO cell growth (up to 60%) was observed in the presence of physiological (100 pM) levels of 1,25-(OH)2D3 in the culture medium. This inhibition of growth was dose dependent and was accompanied by a parallel decrease in total cell protein. Concentrations of 25-hydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 as high as 1 nM did not affect CHO cell growth, indicating that, like receptor binding, cell proliferation is selectively influenced by 1,25-(OH)2D3 over other vitamin D metabolites. These data demonstrate that ovarian cells in mammals and birds possess the 1,25-(OH)2D3 receptor which may play a role in the effect of 1,25-(OH)2D3 on the growth of these cells in culture.

22/7,JA,LA/28

04728815 82271815

Selection of mutant Chinese hamster ovary cells altered glycoproteins by means of tritiated fucose suicide.

Hirschberg CB; Baker RM; Perez M; Spencer LA; Watson D

Mol Cell Biol Oct 1981, 1 (10) p902-9, ISSN 0270-7306
Journal Code: NGY
Contract/Grant No.: GM 30365; GM 21665; CA 26712; +
Languages: ENGLISH
Journal Announcement: 8212

Mutant Chinese hamster ovary cells altered in glycoproteins have been isolated by selecting for ability to survive exposure to [6-3H]fucose. Mutagenized wild-type cells were permitted to incorporate [3H]fucose to approximately 1 cpm of trichloroacetic acid-insoluble radioactivity per cell and then frozen for several days to accumulate radiation damage. The overall viability of the population was reduced by 5- to 50-fold. Four consecutive selection cycles were carried out. The surviving cells were screened by replica plating-fluorography for clones showing decreased incorporation of fucose into trichloroacetic acid-insoluble macromolecules. Considerable enrichment for cells deficient in fucose uptake or incorporation into proteins (or both) was found in populations surviving the later selection cycles. Two mutant clones isolated after the fourth selection cycle had the same doubling time as the wild type, but contained only 30 to 40% as much fucose bound to proteins as the wild type. Sialic acid contents of the mutants and the wild type were similar. The mutants differed quantitatively and qualitatively from the wild type and from each other with respect to total glycoprotein profiles as visualized by sodium dodecyl sulfate gel electrophoresis. Differences were also found in resistances to cytotoxicity of lectins such as concanavalin A and wheat germ agglutinin.

22/7,JA,LA/29

04721222 82264222

Attachment of gonococcal pili to lectin-resistant clones of Chinese hamster ovary cells.

Gubish ER Jr; Chen KC; Buchanan TM

Infect Immun Jul 1982, 37 (1) p189-94, ISSN 0019-9567
Journal Code: G07
Contract/Grant No.: 1-AI-52535; 1-AI-13149
Languages: ENGLISH
Journal Announcement: 8212

Pili facilitate the attachment of virulent Neisseria gonorrhoeae to host cells. Isolated pili and peptides from pili obtained by cyanogen bromide cleavage were used in attachment assays to Chinese hamster ovary cells and their lectin-resistant clones. Pili and the largest cyanogen bromide fragment (CNBrI) from the amino-terminal portion of the pilin molecule attached to a greater degree to the parent cell and showed 40 to 75% reduced attachment to clones deficient in cell surface oligosaccharides. The CNBrI fragment, with a molecular weight of approximately 10,000, bound specifically to host proteins with subunit molecular weights of 14,000 to 16,000 that were electrophoretically transferred onto nitrocellulose sheets from polyacrylamide gel patterns of host cells. Periodate or galactosidase treatment of pili or the CNBrI fragment markedly reduced attachment, suggesting the importance of galactose residues on pili for their attachment function. Similarly, highly purified exoglycosidase or trypsin treatment of the parent cell reduced attachment, suggesting that oligosaccharide moieties of cell surface components (glycoproteins or glycolipids or both) were receptors for pili attachment. This study indicated that the portion of the pilin molecule involved in attachment resides on the CNBrI fragment and that sugar moieties, both on pili and on

the host cell, were required for optimal attachment.

22/7,JA,LA/30
04696563 82239563
Autoradiographic detection and characterization of a Chinese hamster ovary cell mutant deficient in fucoproteins.
Hirschberg CB; Perez M; Snider M; Hanneman WL; Esko J; Raetz CR
J Cell Physiol Jun 1982, 111 (3) p255-63, ISSN 0021-9541
Journal Code: HNB
Contract/Grant No.: CRIC 77015; GM 30365; AM 21722; +
Languages: ENGLISH
Journal Announcement: 8211
Autoradiography of colony replicas immobilized on filter paper was used to isolate a Chinese hamster ovary cell line deficient in incorporation of radiolabeled fucose into a trichloroacetic acid-insoluble fraction. This cell line, called 62.1, has the same growth rate at 37 degrees C as wild-type cells, but incorporates five times less fucose into acid-insoluble radioactivity. Chemical analysis of fucose bound to macromolecules also showed a fivefold reduction in the mutant. The fucoproteins of the mutant cell line differ qualitatively from those of wild-type cells as visualized by SDS gel electrophoresis fluorography; no differences were detected between total proteins as visualized by coomassie blue staining. The macromolecular sialic acid content of the mutant was somewhat higher than the wild type (20%). Studies of the synthesis of the glycoprotein of vesicular stomatitis virus in mutant and wild-type cells showed that the mutant is unable to synthesize complex-type N-linked oligosaccharides. Enzyme assays show that ths defect in the mutant is due to reduction in UDP-N-acetylglucosamine-glycoprotein N-acetyl-glucosaminylt ransferase, a key enzyme in the assembly of complex glycopeptides. Hybridization studies have shown that mutant 62.1 has common mutations belonging to the same complementation group as mutant PhaR1-1. This latter mutant was previously isolated using lectin resistance by Stanley et al. (1975) and was also deficient in the above N-acetyl-glucosaminyltransferase.
?t22/7,la/31-62

22/7,LA/31
04696408 82239408
Increase of a surface glycoprotein by cyclic AMP in Chinese hamster ovary cells. Dependence on cell-cell interaction.
Imada S; Imada M
J Biol Chem Aug 10 1982, 257 (15) p9108-13, ISSN 0021-9258
Journal Code: HIV
Contract/Grant No.: CA-15823; CA-22096
Languages: ENGLISH
Cell surface proteins and glycoproteins of the CHO-K1 clone of Chinese hamster ovary cells were radiolabeled by surface-specific reactions as well as by metabolic incorporation of glycoprotein precursors. After the proteins were separated by two-dimensional gel electrophoresis, they were quantified according to the amount of radioactivity which was associated with individual proteins spots. A glycoprotein of 133,000 daltons (P133-11) wa found to increase severalfold by the elevation of the cytoplasmic level of adenosine 3':5'-cyclic monophosphate. In this paper, we report that the increase of P133-11 was more efficient in dense cultures than in sparse cultures. The use of conditioned medium or frequent medium changes during the treatment with the cyclic nucleotide did not influence the efficiency of the increase of the glycoprotein, indicating that the enrichment or

exhaustion of diffusible materials was not responsible for this density-dependent phenomenon. The same conclusion was drawn from an experiment in which the amount of P133-11 was compared between homogeneously plated sparse cultures and cultures containing the same number of cells in the form of colonies with well contacted cells at an average density of 400 cells/colony. The increase of the protein was higher in the latter type of cell cultures. We, therefore, propose that the interaction of surface components upon cell-cell contact plays a role in the expression of P133-11 surface glycoprotein.

22/7,LA/32
04601418 82144418
Sulphated glycoproteins induced by herpes simplex virus.
Hope RG; Palfreyman J; Suh M; Marsden HS
J Gen Virol Feb 1982, 58 (Pt 2) p399-415, ISSN 0022-1317
Journal Code: I98
Languages: ENGLISH
BHK cells infected with strain 17 syn+ (HSV-1) or HG52 (HSC-2) incorporated inorganic sulphate into polypeptides which co-migrated on SDS-polyacrylamide gels with virus-induced glycoproteins. The major sulphated glycoprotein was glycoprotein E. In addition, less-intense sulphated bands co-migrated with glycoprotein D and HSV-1 glycoprotein A/B/C. Sulphate label co-migrating with HSV-2 glycoprotein A/B/C was occasionally observed. We have investigated which sulphated polypeptides are excreted from infected cells. Major ones of apparent mol. wt. 32000, 34000 and 35000 were excreted from cells infected with syn+. In addition, polypeptides which migrated in the vicinity of glycoprotein D were often excreted from cells infected with either 17 syn+ or HG52. The 32K, 34K and 35K polypeptides were antigenically related to glycoprotein D and over 95% of the total amount synthesized was excreted. Analysis of intracellular sulphated polypeptides using intertypic recombinants mapped glycoprotein E to between 0.832 and 0.950 units of the HSV genome.

22/7,LA/33
04488059 82031059
Transient activity of Golgi-like membranes as donors of vesicular stomatitis viral glycoprotein in vitro.
Fries E; Rothman JE
J Cell Biol Sep 1981, 90 (3) p697-704, ISSN 0021-9525
Journal Code: HMV
Contract/Grant No.: AM-27044
Languages: ENGLISH
Previous reports demonstrated that the vesicular stomatitis viral glycoprotein (G protein), initially present in membranes of a Chinese hamster ovary mutant cell line (clone 15B) that is incapable of terminal glycosylation, can be transferred in vitro to exogenous Golgi membranes and there glycosylated (E. Fries and J. E. Rothman, 1980, Proc. Natl. Acad. Sci. U. S. A. 77:3870-3874; and J. E. Rothman and E. Fries, 1981, J. Cell Biol. 89:162-168). Here we present evidence that Golgi-like membranes serve as donors of G protein in this process. Pulse-chase experiments revealed that the donor activity of membranes is greatest at approximately 10 min of chase, a time when G protein has been shown to have arrived in Golgi stacks (J. E. Bergmann, K. T. Tokuyasu, and S. J. Singer, 1981, Proc. Natl. Acad. Sci. U. S. A. 78:1746-1750). Additional evidence that the G protein that is transferred to exogenous Golgi membranes in vitro had already entered the Golgi membranes in vivo was provided by observations that its oligosaccharides had already been trimmed, and that its distribution in a sucrose density gradient was coincident with that of enzymatic markers of

Golgi membranes. The capacity of this Golgi-like membrane to serve as donor is transient, declining within 5 min after "trimming" in vivo as the G protein enters a "nontransferable" pool. The rapidity of the process suggests that both the "transferable" and "nontransferable" pools of G protein reside in Golgi-like membranes.

22/7,LA/34
04387419 81215419
Selective stimulation of the synthesis of an 80,000-dalton protein by calcium ionophores.
Wu FS; Park YC; Roufa D; Martonosi A
J Biol Chem Jun 10 1981, 256 (11) p5309-12, ISSN 0021-9258
Journal Code: HIV
Contract/Grant No.: AM 18117; AM 26545
Languages: ENGLISH
Brief exposure of cultured chicken pectoralis muscle cells to ionomycin or A23187 selectively increases the rate of incorporation of [35S]methionine into an 80,000-dalton protein was also observed upon cell-free translation of poly(A)-enriched RNA isolated from ionomycin-treated, as compared with control, cultures. These observations suggest that ionomycin selectively increases the cellular concentration of mRNA, which codes for the 80,000-dalton protein. The effect is probably mediated through an increase in cytoplasmic [Ca2+] caused by the ionophore. A similar effect of ionomycin was observed in cultured fibroblasts, HeLa cells, mouse LSP cells, and monkey kidney CV1 cells.

22/7,LA/35
04320812 81148812
Two distinct mechanisms of fibroblast adhesion.
Harper PA; Juliano RL
Nature Mar 12 1981, 290 (5802) p136-8, ISSN 0028-0836
Journal Code: NSC
Languages: ENGLISH
The adhesion of cells to the connective tissue matrix is commonly thought to be governed by fibronectin, a pericellular glycoprotein with binding sites for cell surfaces, collagen and glycosaminoglycans. Here we report evidence that Chinese hamster ovary (CHO) cells possess an alternative mechanism for adhesion which is independent of fibronectin. Cells of a variant CHO clone called ADvF11 are defective in their ability to adhere to fibronectin-coated substrata, but can adhere to a substratum coated with SAM (substrate-attached material), a pericellular material produced by fibroblasts. The adhesion of wild-type CHO cells to fibronectin-coated substrate and adhesion of ADvF11 cells to SAM-coated substrata are differentially sensitive to proteolytic treatment. This suggests that there are two distinct adhesion mechanisms for CHO cells, only one of which is dependent on fibronectin.

22/7,LA/36
04289490 81117490
Isolation and characterization of Chinese hamster ovary cell variants defective in adhesion to fibronectin-coated collagen.
Harper PA; Juliano RL
J Cell Biol Dec 1980, 87 (3 Pt 1) p755-63, ISSN 0021-9525
Journal Code: HMV
Languages: ENGLISH
Variant clones of Chinese hamster ovary (CHO) cells were selected for reduced adhesion to serum-coated tissue culture plates. These clones also

displayed reduced adhesion to substrata composed of collagen layers coated with bovine serum or with fibronectin (cold-insoluble globulin). Wild-type (WT) and adhesion variant (ADv) cells grew at comparable rates in suspension culture, but the adhesion variants could not be grown in monolayer culture because of their inability to attach to the substratum. The adhesion deficit in these cells was not corrected by raising the concentration of divalent cations or of serum to levels 10-fold greater than those normally utilized in cell culture. However, both WT and ADv clones could adhere, spread, and attain a normal CHO morphology on substrata coated with concanavalin A or poly-L-lysine. In addition, the adhesion variants could attach to substrata coated with "footpad" material (substratum-attached material) derived from monolayers of human diploid fibroblasts or WT CHO cells. These observations suggest that the variant clones may have a cell surface defect that prevents them from utilizing exogeneous fibronectin as an adhesion-promoting ligand; however the variants seem to have normal cytoskeletal and metabolic capacities that allow them to attach and spread on substrata coated with alternative ligands. These variants should be extremely useful in studying the molecular basis of cell adhesion.

22/7,LA/37
04242014 81070014

Inhibition of fibronectin-mediated adhesion of hamster fibroblasts to substratum: effects of tunicamycin and some cell surface modifying reagents.

Butters TD; Devalia V; Aplin JD; Hughes RC

J Cell Sci Aug 1980, 44 p33-58, ISSN 0021-9533 Journal Code: HNK

Languages: ENGLISH

Using baby hamster kidney (BHK) fibroblasts we have studied the effect of tunicamycin, a specific inhibitor of protein glycosylation, on the ability of trypsinized cells to attach and spread onto fibronectin. Tunicamycin inhibited mannose incorporation into total acid-precipitable glycoproteins by at least 95% while glucosamine and leucine incorporation were less or hardly inhibited. Hydrolysis and analysis of [3H]glucosamine-labelled glycoproteins showed that radioactivity incorporated into cells exposed to tunicamycin was present predominantly as galactosamine, presumably present in O-glycosidically linked glycan chains whose assembly is insensitive to the drug. Treated cells exhibit reduced amounts of surface-associated fibronectin and adhere relatively poorly to plastic or collagen surfaces pre-coated with plasma or BHK cell-derived fibronectins at the minimum concentrations required to induce nearly quantitative attachment and spreading of untreated cells. Drug-treated cells do adhere and spread into a bipolar configuration on surfaces saturated with fibronectin. Cells treated with tunicamycin and then grown in the absence of the drug revert to a more normal behaviour, indicating that under certain conditions the effects of the drug are reversible. Fibronectin-mediated spreading of trypsinized BHK cells is also inhibited by pre-treatment of cells with several non-penetrating reagents reactive with cell surface amino groups, namely pyridoxal phosphate, trinitrobenzene sulphonate and fluorescein 5-isothiocyanate. Analysis of surface substitution indicates a strong correlation between the extent of amino group substitution and inability of treated cells to interact with a fibronectin lattice. While the extent of attachment under these conditions is normal, cells pretreated with a specific non-penetrating thiol reagent, p-chloromercuribenzenesulphonate fail to attach to fibronectin-coated culture dishes in a dose-dependent fashion, indicating that a biochemical di

22/7,LA/38

04241863 81069863

Synthesis of the N-linked oligosaccharides of glycoproteins. Assembly of the lipid-linked precursor oligosaccharide and its relation to protein synthesis in vivo.

Hubbard SC; Robbins PW

J Biol Chem Dec 25 1980, 255 (24) p11782-93, ISSN 0021-9258

Journal Code: HIV

Contract/Grant No.: CA14142; CA14051; 1-F32-CA06114-01

Languages: ENGLISH

The asparagine-linked oligosaccharides of chick embryo fibroblast glycoproteins were previously shown to derive from a common lipid-linked precursor, Glc3Man9GlcNAc2. The formation of this precursor oligosaccharide was examined in intact chick embryo fibroblasts, NIL-8 cells, and Chinese hamster ovary cells. The labeling kinetics and compositions of the lipid-linked oligosaccharides were examined, and the results indicate that lipid-linked Man5GlcNAc2 is rapidly assembled ((1.5 min) and then extended ((2.5 min) to Glc3Man9GlcNAc2 via the intermediate Man8GlcNAc2. Chain elongation from Man5GlcNAc2- to Man8GlcNAc2-lipid probably occurs by addition of single mannose residues. The pool of lipid-linked Glc3Man9GlcNAc2 turns over with a half-time of 3.5 to 6 min; since there is little if any degradation (the mannose residues do not turn over), this reflects the rate at which completed chains are transferred to acceptor proteins. The same intermediates and similar kinetics were observed in all three cell types. Oligosaccharide-lipid assembly was also examined in cells in which protein synthesis was decreased (using actinomycin D to depress levels of mRNA) or abolished (using cycloheximide). The results indicate that the rate of oligosaccharide-lipid synthesis is proportional to the rate of protein synthesis. The regulated step is prior to the Man5GlcNAc2 stage, and we suggest that the most likely control mechanism is limitation of available oligosaccharide carrier lipid.

22/7,LA/39

04193772 81021772

Induction of surface glycoprotein expression by cyclic AMP in Chinese hamster ovary cells.

Imada M; Imada S; Weiss D

Biochim Biophys Acta Sep 17 1980, 632 (1) p47-57, ISSN 0006-3002

Journal Code: AOW

Contract/Grant No.: CA-22096; CA-15823

Languages: ENGLISH

Surface expression of a membrane glycoprotein of 135,000 molecular weight (P135) was inducible by adenosine 3',5' -cyclic monophosphate in Chinese hamster ovary cells, CHO-K1 clone. Cells were cultured in the presence or absence of cyclic AMP derivatives, chemicals influencing cytoplasmic cyclic AMP levels, or inhibitors of protein or RNA synthesis. Surface proteins wre radiolabeled by a lactoperoxidase-catalyzed iodination reaction and analyzed by two-dimensional polyacrylamide gel electrophoresis. Surface expression of P135 increased 3---5-fold inthe presence of N6,02' -dibutyryl cyclic AMP or 8-parachlorophenylthio cyclic AMP. Induction was also observed after treatment with prostaglandins E1 and F2 alpha, but not with sodium butyrate. Phosphodiesterase inhibitor, Roche compound Ro20-1724, enhanced the effect of N6,02' -dibutyryl cyclic AMP. Metabolic incorporation of [35S]methionine into P135 was increased by N6,02' -dibutyryl cyclic AMP. The induction was sensitive to inhibitors of protein and RNA biosynthesis. These results are consistent with a proposal that cyclic AMP controls the synthesis of this protein. Metabolic incorporation of a radioactive precursor suggested that P135 was a glucos-amine-containing glycoprotein. P135 appeared to be strongly associated with cell membrane because it was resistant to extraction of

plasma membrane by cole 0.1 N NaOH.

22/7,LA/40
04111946 80222946
Hormonal requirements of baby hamster kidney cells in culture.
Maciag T; Kelley B; Cerundolo J; Ilsley S; Kelley PR; Gaudreau J; Forand R
Cell Biol Int Rep Jan 1980, 4 (1) p43-50, Journal Code: CRC
Languages: ENGLISH
Baby hamster kidney cells (BHK) were able to proliferate in the complete absence of serum in synthetic medium supplemented with insulin, transferrin, fibroblast growth factor and epidermal growth factor on cell culture dishes coated with fibronectin. Although the addition of individual supplements had little or no effect on cell growth, the combination of the supplements resulted in a significant synergistic effect. The defined medium was also capable of supporting the clonal growth of BHK cells in the absence of serum. Studies on the effect of hormonal supplementation in the presence of low concentrations of fetal calf serum suggest that the function of serum is to supply hormones and growth factors for cell growth.

22/7,LA/41
04075102 80186102
Production of monoclonal antibodies against a cell surface concanavalin A binding glycoprotein.
Starling JJ; Simrell CR; Klein PA; Noonan KD
J Supramol Struct 1979, 11 (4) p563-77, ISSN 0091-7419
Journal Code: K75
Languages: ENGLISH
Concanavalin A-binding (Con-A)-binding cell surface glycoproteins were isolated, via Con A-affinity chromatography, from Triton X-100-solubilized Chinese hamster ovary (CHO) cell plasma membranes. The Con A binding glycoproteins isolated in this manner displayed a significantly different profile on sodium dodecyl sulfate--polyacrylamide gels than did the Triton-soluble surface components, which were not retarded by the Con-A-Sepharose column. [125I]-Con A overlays of the pooled column fractions displayed on sodium dodecyl sulfate--polyacrylamide gel electro-phoresis (SDS-PAGE) demonstrated that there were virtually no Con A receptors associated with the unretarded peak released by the Con A-Sepharose column, whereas the material which was bound and specifically eluted from the Con A-Sepharose column with the sugar hapten alpha-methyl-D-mannopyranoside contained at least 15 prominent bands which bound [125I]-Con A. In order to produce monoclonal antibodies against various cell surface Con A receptors, Balb/c mice were immunized with the pooled Con A receptor fraction. Following immunization spleens were excised from the animals and single spleen cell suspensions were fused with mouse myeloma P3/X63-Ag8 cells. Numerous hybridoma clones were subsequently picked on the basis of their ability to secrete antibody which could bind to both live and glutaraldehyde-fixed CHO cells as well as to the Triton-soluble fraction isolated from the CHO plasma membrane fraction. Antibody from two of these clones was able to precipitate a single [125I]-labeled CHO surface component of approximately 265,000 daltons.

22/7,LA/42
04049173 80160173
Isolation and characterization of tunicamycin resistant mutants from Chinese hamster ovary cells.
Sudo T; Onodera K

J Cell Physiol Oct 1979, 101 (1) p149-56, ISSN 0021-9541
Journal Code: HNB
Languages: ENGLISH

Stable clones selected for resistance to tunicamycin (TM) have been isolated from Chinese Hamster Ovary (CHO) cells. The TMR phenotype is stable for more than nine months in the absence of the drug. The morphology of TMR mutant varies from epitheloid to abnormally elongate. The mutants do not display cross-resistance for ConA but are slightly cross-resistant to PHA. Biochemically labeled membrane proteins and glycoprotein of Vesicular stomatitis virus (VSV) grown in the TMR mutants revealed that the incorporation of radioactive glucosamine was markedly reduced in the mutants. The results indicate that TMR cells are a novel type of membrane mutant.

22/7,LA/43
03862609 79239609

Characteristics of concanavalin A-resistant Chinese hamster ovary cells and certain revertants.

Cifone MA; Hynes RO; Baker RM

J Cell Physiol Jul 1979, 100 (1) p39-54, ISSN 0021-9541
Journal Code: HNB
Languages: ENGLISH

Clones of Chinese hamster ovary (CHO) cells were isolated by single-step selection for resistance to killing Concanavalin A (ConA) and certain cellular and membrane properties were examined. The ConA-resistant isolates were only about 2-fold more resistant than wild type cells to the selecting lectin, but exhibited pleiotropic temperature-sensitivity for growth, markedly altered morphology and adherence, and significant difference in susceptibility to other agents such as colchicine. Two revertants to full temperature-resistance were isolated from different ConA-resistant mutants. One revertant clone had reacquired wild type sensitivity to ConA while the other revertant remained ConA-resistant. The two series of wild typed, ConA-resistant, and temperature revertant clones were analyzed for altered mobility of cell surface glycoproteins using lactoperoxidase/125I and galactose oxidase/(3H) borohydride labelling procedures. The ConA-resistant clones showed increased mobility on polyacrylamide gels of three classes of labelled proteins, in the molecular weight ranges 225,000, 200,000, and 130,000 daltons. These changes persisted in the temperature-revertant that remained ConA-resistant, while two of the altered protein closses were restored to wild type mobility in the revertant that regained ConA-sensitivity. Cell hybridization experiments indicated that the temperature-sensitivity phenotypes of different ConA-resistant isolates are recessive and noncomplementing, implying that the same gene is affected in each case. The reversions to temperature resistance appear to be recessive suppressor mutation in different genes.

22/7,LA/44
03768482 79145482

An analysis of concanavalin A-mediated agglutination in two Chinese hamster ovary subclones whose surface phenotypes respond to maintenance in medium supplemented with dibutyryl cyclic AMP. V. Biochemical composition of the plasma membrane.

Noonan KD

Biochim Biophys Acta Feb 20 1979, 551 (1) p22-43, ISSN 0006-3002
Journal Code: AOW
Languages: ENGLISH

We have used two Chinese hamster ovary subclones whose surface phenotype has been extensively investigated with regard concanavalin A-mediated

cell-cell agglutination and concanavalin A-induced receptor site clustering to investigate what changes in membrane composition, if any, can be correlated with the concanavalin A-detected changes in surface phenotype. These cell clones are uniquely disposed for this purpose since maintenance of the cells under different growth conditions produces changes in agglutinability and receptor site mobility in one cell clone (H-7W) but not the other (K-1). After extensive characterization of the surface membranes of these two subclones we have been unable to identify any change in the membrane peptides, glycopeptide, cholesterol, or fatty acid composition which can be directly correlated with the concanavalin A-detected surface phenotypes. It is of particular interest to note that we have been unable to correlate the presence or absence of the large external transformation-sensitive glycoprotein with the relative mobility of the lectin receptors or with the degree of concanavalin A-mediated cell agglutination. Furthermore we have been unable, in this system, to corroborate earlier data suggesting a role for cholesterol in determining the relative mobility of the lectin receptors. Thus using a cell system consisting of genetically matched cell clones, we have been unable to identify any changes in the biochemical composition of the plasma membrane which might be associated with the surface phenotypes detected by concanavalin A.

22/7,LA/45
03747757 79124757
Studies of a large transformation-increased membrane protein in the BHK21 cell system.
Lage-Davila A; Montagnier L
Biochim Biophys Acta Feb 2 1979, 550 (3) p435-59, ISSN 0006-3002 Journal Code: AOW
Languages: ENGLISH
We have recently described in BHK cells a plasma membrane protein of molecular weight 177,000, which is significantly increased in Hamster Sarcoma Virus-transformed cells (Lage-Davila, A. and Montagnier, L. (1977) Biochem. Biophys. Res. Commun. 79, 577--584). We present now a study of proteins from purified plasma membrane fractions in the same pair of clones. Solubilization conditions, cross-linking experiments, metabolic labelling and enzymatic radioiodination allow to characterize this 177,000 transformation-increased protein as an integral membrane glycoprotein partially exposed at the outer cell surface. Additional information on other membrane proteins in this system is also given.

22/7,LA/46
03726882 79103882
Two-dimensional electrophoresis of surface glycoproteins of normal BHK cells and ricin resistant mutants.
Pena SD; Mills G; Hughes RC
Biochim Biophys Acta Jan 5 1979, 550 (1) p100-9, ISSN 0006-3002 Journal Code: AOW
Languages: ENGLISH
The surface glycoproteins of baby hamster kidney (BHK) cells were iodinated by lactoperoxidase and submitted to a two-dimensional electrophoresis procedure involving isoelectric focusing in the first dimension and SDS gel electrophoresis in the second dimension. After autoradiography a complex but reproducible pattern was obtained. The technique was then applied to the study of three ricin-resistant mutant clones with reduced rates of cell-cell and/or cell-substratum adhesion. Abnormal patterns were observed in all three mutant clones indicating different mechanisms of ricin resistance and identifying glycoproteins

which may be involved in cellular interactions.

22/7,LA/47
03608347 78242347
Structure of the altered oligosaccharide present in glycoproteins from a clone of Chinese hamster ovary cells deficient in N-acetylglucosaminyltransferase activity.
Li E; Kornfeld S
J Biol Chem Sep 25 1978, 253 (18) p6426-31, ISSN 0021-9258
Journal Code: HIV
Languages: ENGLISH
Clone 15B cells, derived from Chinese hamster ovary cells and deficient in a specific UDP-N-acetylglucosamine:glycoprotein N-acetylglucosaminyltransferase activity, synthesize glycoproteins with altered oligosaccharide units. Glycopeptides prepared from these glycoproteins contain large quantities of a glycopeptide with the composition (Man)5(GlcNAc)2-Asn whereas parent cells have only small amounts of this glycopeptide. The structure of the glycopeptide was determined by the combination of methylation analysis, acetolysis, Smith periodate degradation, and alpha- and beta-mannosidase digestion. Its complete structure is Manalpha 1 leads to 6[Manalpha1 leads to 3]-Manalpha1 leads to 6[Manalpha 1 leads to 3]-Manbeta1 leads to 4 GlcNAcbeta1 leads to 4 GlcNAc leads to Asn-peptide. The structures of two other glycopeptides found in smaller quantities in clone 15B but not detected in the parent cells were determined and are Manalpha 1 leads to 6 [Manalpha 1 leads to 3]-Manalpha1 leads to 6Manbeta 1 leads to 4 GlcNAcbeta 1 leads to 4GlcNAc-Asn-peptide and Manalpha 1 leads to 3 Manalpha 1 leads to 6[Manalpha 1 leads to 3] Manbeta 1 leads to 4GlcNAcbeta 1 leads to 4GlcNAc-Asn-peptide. It is proposed that the (Man)5(GlcNAc)2-Asn unit is the physiologic acceptor for the particular N-acetylglucosaminyltransferase which is deficient in clone 15B cells and that this reaction is necessary for complex oligosacchari-e biosynthesis.

22/7,LA/48
03570567 78204567
Dominance of colchicine resistance in hybrid CHO cells.
Ling V; Baker RM
Somatic Cell Genet Mar 1978, 4 (2) p193-200, ISSN 0098-0366
Journal Code: VAJ
Languages: ENGLISH
Intraspecific hybrids of colchicine-sensitive with colchicine-resistant (CHR) Chinese hamster ovary cells were constructed, using six different colchicine-resistant clones from two independent series. In each instance, colchicine resistance was expressed in an incompletely dominant manner. Some hybrid clones were examined further for the expression of the pleiotropic CHR phenotype and for the cell surface P glycoprotein. These features of the colchicine-resistant phenotype were also expressed coordinately.

22/7,LA/49
03419248 78053248
The role of plasma membrane and intracellular microskeletal elements in determining the lectin agglutinability of two CHO sub-clones.
Noonan KD; van Veen J; Roberts RM
Prog Clin Biol Res 1977, 17 p521-30, ISSN 0361-7742 Journal Code: PZ5
Languages: ENGLISH

22/7,LA/50
03416502 78050502
Intracellular development of membrane protein of influenza virus.
Maeno K; Yoshii S; Yoshida T; Iinuma M; Kawamoto Y
Microbiol Immunol 1977, 21 (8) p427-38, ISSN 0385-0000
Journal Code: MX7
Languages: ENGLISH
The intracellular development of membrane protein (MP) of influenza A virus was investigated by immunofluorescent staining. Monospecific antiserum was prepared by immunizing rabbits with MP eluted from SDS-polyacrylamide gels of SDS-disrupted NWS virions. In the productive infection in clone 1-5C-4 cells, MP antigen was first detected over the whole cell at 4 hr after infection, concomitantly with the appearance of hemagglutinin (HA) antigen in the cytoplasm, and bright nuclear fluorescence was then observed. Nucleoprotein (NP) antigen was detected in the nucleus prior to the appearance of fluorescence of MP antigen and thereafter the cytoplasmic fluorescence developed. Late in infection, all of these three antigens were observed predominantly in the cytoplasm with stronger fluorescence at the cell surface. Essentially similar findings were obtained in the abortive infections in L cells and BHK cells. The above results suggest that the membrane protein of influenza A virus is present in the nucleus as well as in the cytoplasm of infected cells.

22/7,LA/51
03213768 77115768
Properties of a baby-hamster-kidney cell line with increased resistance of 2-deoxy-D-glucose.
Meager A; Nairin R; Hughes RC
Eur J Biochem Jan 1977, 72 (2) p275-81, ISSN 0014-2956
Journal Code: EMZ
Languages: ENGLISH
A cultured cell line with increased resistance to 2-deoxy-D-glucose was obtained from cloned baby hamster kidney fibroblasts, BHK 21/C13, after repeated exposure to high concentrations of 2-deoxyglucose. The increased resistance could not be attributed to a decreased permeability of deoxysugar. The resistant cell line incorporated radioactive 2-deoxy-Dglucose in glycoproteins at a similar rate as parental BHK 21C13 cells. Incorporation of radioactive glucosamine, galactose and to lesser extent mannose into cellular glycoproteins was inhibited by 2-deoxyglucose to similar extents in the resistant cells and parental BHK 21/C13 cells. Changes induct cells were detected by altered to toxic plant lectins and by surface labelling as described for parental cells in the preceding paper. It is suggested that the toxicity of 2-deoxy-D-glucose to normal fibroblasts is not mediated through effects on glycosylation of cellular glycoproteins.

22/7,LA/52
03143648 77045648
A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants.
Juliano RL; Ling V
Biochim Biophys Acta Nov 11 1976, 455 (1) p152-62, ISSN 0006-3002
Journal Code: AOW
Languages: ENGLISH
Chinese hamster ovary cells selected for resistance to colchicine display pleiotropic cross-resistance to a wide range of amphiphilic drugs. The drug-resistant phenotype is due to a membrane alteration which reduces the

rate of drug permeation. Surface labelling studies reveal that drug-resistant Chinese hamster ovary cell membranes possess a carbohydrate-containing component of 170 000 daltons apparent molecular weight which is not observed in wild type cells. Through studies of the metabolic incorporation of carbohydrate and protein precursors, and through the use of selective proteolysis, this component is shown to be a cell surface glycoprotein. Since this glycoprotein appears unique to mutant cells displaying altered drug permeability, we have designated it the P glycoprotein. The relative amount of surface labelled P glycoprotein correlates with the degree of drug resistance in a number of independent mutant and revertant clones. A similar high molecular weight glycoprotein is also present in drug-resistant mutants from another hamster cell line. Observations on the molecular basis of pleiotropic drug resistance are interpreted in terms of a model wherein certain surface glycoproteins control drug permeation by modulating the properties of hydrophobic membrane regions...

22/7,LA/53
03032463 76213463
An analysis of lectin-initiated cell agglutination in a series of CHO subclones which respond morphologically to growth in dibutyryl cyclic AMP.
van Veen J; Roberts RM; Noonan KD
J Cell Biol Jul 1976, 70 (1) p204-16, ISSN 0021-9525 Journal Code: HMV
Languages: ENGLISH
We have investigated the molecular basis of the agglutinability of CHO subclones which respond differentially in terms of morphology and surface architecture in the presence of dB-cAMP in the medium. We have demonstrated that the agglutinability of these subclones with both wheat germ agglutinin (WGA) and concanavalin A (Con A) probably depends on the free lateral mobility of the lectin receptor sites in the plane of the membrane. The nonagglutinable surface architecture seems to depend on the presence in the membrane of a protease-labile peptide(s), which appears to be distinct from the lectin receptors, as well as on continuous protein and RNA synthesis. This dependence on continuous transcription and translation may be related to the maintenance of the protease-labile peptide(s) in such a state as to restrict mobility of the lectin receptors. The surface architecture defined as nonagglutinable also depends on the state of polymerization of the intracellular microtubules and microfilaments. It is suggested that these microskeletal elements serve to anchor the lectin receptors in such a manner as to restrict their mobility and thereby reduce the relative agglutinability of a cell line. We suggest that control of the free mobility of both the Con A and WGA receptor sites is dependent on two constraints, one applied by protease-labile ("surface") membrane components and the other by components of the intracellular microskeletal system.

22/7,LA/54
02985578 76166578
Selection and characterization of Chinese hamster ovary cells resistant to the cytotoxicity of lectins.
Stanley P; Siminovitch L
In Vitro Mar 1976, 12 (3) p208-15, ISSN 0073-5655 Journal Code: GHD
Languages: ENGLISH
Chinese hamster ovary (CHO) cells selected in a single step for resistance to the cytotoxicity of the lectin from red kidney beans (PHA) behave as authentic somatic cell mutants. The PHA-resistant (Phar) phenotype is stable in the absence of selection; its frequency in a sensitive-population is increased several-fold by mutagenesis; and it

behaves recessively in somatic cell hybrids. The activity of a specific glycosyl transferase which transfers N-acetylglucosamine (GlcNAc) to terminal alpha-mannose residues is dramatically reduced (less than or equal to 5% of the activity detected in wild-type CHO cells) in several independent PhaR clones. These clones also exhibit (a) a decreased ability to bind [125I]-PHA; (b) a marked resistance to the cytotoxicity of wheat germ agglutinin (WGA), Ricin (RIC) and Lens culinaris agglutinin (LCA); (c) a 4- to 5-fold increased sensitivity to the cytoxocity of concanavalin A (Con A); (d) an increased ability to bind 125I-Con A; and (e) decreased surface galactose residues - all properties consistent with the specific loss of the GlcNAc transferase activity. The lectins WGA, RIC, LCA and Con A have also been used to select, in a single step, resistance closes from each of two complementary CHO auxitrophic lines. These lectin-resistant clones have been characterized by their ability to survive cytotoxic doses of PHA, Con A, WGA, RIC, or LCA, and 4-5 "lectin-resistance" phenotypes have been demonstrated. Complementation data is being sought by somatic cell hybridization. Preliminary results show that two phenotypically-distinct Con AR mutants are complementary in that hybrid cells formed between them exhibit wild-type sensitivity to Con A.

22/7,LA/55
02909027 76090027
Drug resistance and membrane alteration in mutants of mammalian cells.
Ling V
Can J Genet Cytol Dec 1975, 17 (4) p503-15, ISSN 0008-4093
Journal Code: CID
Languages: ENGLISH

Independent colchicine-resistant (CHR) mutants of Chinese hamster ovary cells displaying reduced permeability to colchicine have been isolated. A distinguishing feature of these membrane-altered mutants is their pleiotropic cross-resistance to a variety of unrelated compounds. Genetic characterization of the CHR lines indicate that colchicine resistance and cross-resistance to other drugs are of a dominant nature in somatic cell hybrids. Revertants of CHR have been isolated which display decreased resistance to colchicine and a corresponding decrease in resistance to other drugs. These results strongly suggest that colchicine resistance and the pleiotropic cross-resistance are the result of the same mutation(s). Biochemical studies indicate that although colchicine is transported into our cells by passive diffusion, no major alterations in the membrane lipids could be detected in mutant cells. However, there appears to be an energy-dependent process in these cells which actively maintains a permeability barrier against colchicine and other drugs. The CHR cells might be altered in this process. A new glycoprotein has been identified in mutant cell membranes which is not present in parental cells, and is greatly reduced in revertant cells. A model for colchicine-resistance is proposed, which suggests that certain membrane proteins such as the new glycoprotein of CHR cells, are modulators of membrane fluidity (mmf proteins) whose molecular conformation regulates membrane permeability to a variety of compounds and that the CHR mutants are altered in their mmf proteins. The possible importance of the CHR cells as models for investigating aspects of chemotherapy related to drug resistance is discussed.

22/7,LA/56
02872113 76053113
Chinese hamster ovary cells selected for resistance to the cytotoxicity of phytohemagglutinin are deficient in a UDP-N-acetylglucosamine--glycoprotein N-acetylglucosaminyltransferase activity.

Stanley P; Narasimhan S; Siminovitch L; Schachter H
Proc Natl Acad Sci USA Sep 1975, 72 (9) p3323-7, ISSN 0027-8424
Journal Code: PV3
Languages: ENGLISH

Several clones of Chinese hamster ovary cells resistant to the cytotoxicity of the phytohemagglutinin from Phaseolus vulgaris show decreased binding of 125I-labeled phytohemagglutinin and contain decreased levels of a UDP-N-acetylglucosamine--glycoprotein N-acetylglucosaminyltransferase (EC 2.4.1.51; UDP-2-acetamido-2-deoxy-D-glucose:glycoprotein 2-acetamido-2-deoxy-D-glucosyltransferase) activity when compared to wild-type cells. The decrease in transferase activity varies from 45% to 96%, depending on the exogenous acceptor used in the enzyme assay. No differences between lectin-resistant and wild-type cells were noted for several other glycosyltransferases. The absence of a particular N-acetylglucosaminyltransferase in the lectin-resistant cells apparently results in defective glycosylation of lectin-binding glycoproteins on the cell surface. A phytohemagglutinin-resistant clone which shows decreased binding of 125I-labeled phytohemagglutinin but does not exhibit the enzyme deficiency has also been isolated.

22/7,LA/57
02860743 76041743

Selection and characterization of eight phenotypically distinct lines of lectin-resistant Chinese hamster ovary cell.

Stanley P; Caillibot V; Siminovitch L
Cell Oct 1975, 6 (2) p121-8, ISSN 0092-8674 Journal Code: CQ4
Languages: ENGLISH

Clones resistant to the lectins phytohemagglutinin (PHA), wheat germ agglutinin (WGA), the agglutinin(s) from Lens culinaris (LCA), and ricin (RIC) have been selected from parental auxotrophic Chinese hamster ovary (CHO) cells. The sensitivity to other lectins of these cells and of CHO cells resistant to concanavalin A (ConA) has been determined, and their activity of UDP-N-acetyl-glucosamine glycoprotein N-acetyl-glucosaminyltransferase (GlcNAc-T) has been measured. At least 8 different phenotypes have been identified on the basis of this analysis, and complementation between 2 of them demonstrated.

22/7,LA/58
02824589 76005589

Site of synthesis of membrane and nonmembrane proteins of vesicular stomatitis virus.

Morrison TG
J Biol Chem Sep 10 1975, 250 (17) p6955-62, ISSN 0021-9258
Journal Code: HIV
Languages: ENGLISH

Upon infection of Chinese hamster ovary cells (CHO), vesicular stomatitis (VSV) virus synthesizes two membrane proteins (the VSV glycoprotein and the VSV matrix or membrane (M) protein) and three nonmembrane proteins (the VSV nucleocapsid, the viral transcriptase, and an NS protein). We have used the VSV-infected cell as a model system for the study of the site of synthesis of these membrane and nonmembrane proteins. We have isolated VSV mRNA from free polyribosomes, membrane-bound polyribosomes, and the postribosomal supernatant, and identified the individual species of VSV mRNA present in each fraction. The mRNA which encodes the VSV glycoprotein is found exclusively on membrane-bound polyribosomes, while the mRNAs which encode the VSV, M, N, and NS proteins are found in free polyribosomes, in the membrane fraction of the cell, and in the postribosomal supernatant. Our results suggest that the VSV glycoprotein is synthesized exclusively on

membrane polyribosomes, while at least some of the M, N, and NS proteins are made on free polyribosomes.

22/7,LA/59
02726570 75133570
Deficient uridine diphosphate-N-acetylglucosamine:glycoprotein N-acetylglucosaminyltransferase activity in a clone of Chinese hamster ovary cells with altered surface glycoproteins.
Gottlieb C; Baenziger J; Kornfeld S
J Biol Chem May 10 1975, 250 (9) p3303-9, ISSN 0021-9258
Journal Code: HIV
Languages: ENGLISH
We have reported the isolation of a clone (termi(f@PZ)NO CARRIER

22/7,LA/59
02726570 75133570
Deficient uridine diphosphate-N-acetylglucosamine:glycoprotein N-acetylglucosaminyltransferase activity in a clone of Chinese hamster ovary cells with altered surface glycoproteins.
Gottlieb C; Baenziger J; Kornfeld S
J Biol Chem May 10 1975, 250 (9) p3303-9, ISSN 0021-9258
Journal Code: HIV
Languages: ENGLISH
We have reported the isolation of a clone (termed 15B) of Chinese hamster ovary (CHO) cells which are deficient in certain plant lectin-binding sites and have decreased amounts of sialic acid, galactose, and N-acetylglucosamine in its membranes (Gottlieb et al. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 1078-1082). This study demonstrates that extracts of 15B cells, in contrast to the parent cell line, do not transfer N-acetylglucosamine residues from UDP-GlcNAc to certain glycopeptide and glycoprotein acceptors containing terminal nonreducing alpha-linked mannose residues. The decreased enzyme activity could not be accounted for by the presence of inhibitors, altered pH, or Mn2+ requirements of the glycosyltransferase or increased N-acetylglucosaminidase activity in the extracts. The finding that the 15B cell extracts have significant but reduced N-acetylglucosaminyltransferase activity toward a degraded orosomucoid acceptor suggests that these cells have a selective loss of one of several specific N-acetylglucosaminyltransferases which are present in the parent CHO cells. The sialyl- and galactosyltransferase activities of 15B and parent CHO cells are comparable. Parent CHO and 15B cells were grown in radioactive glucosamine to label the membrane glycoproteins. Solubilization of these glycoproteins and passage over a Ricinus communis agglutinin I (RCA I) Sepharose affinity column revealed that no labeled 15B glycoprotein material bound, whereas 50 percent of the CHO membrane glycoproteins bound and could be eluted with the haptene lactose, demonstrating that 15B cells are virtually devoid of membrane oligosacharides capable of binding to the RCA I lectin. The 15B membrane glycoproteins exhibited a marked shift toward glycoprotein species of lower molecular weight when examined by gel electrophoresis in sodium dodecyl sulfate. It is proposed that this shift in the mobility of the 15B membrane glycoproteins results from a decreased glycosylation of a number of membra

22/7,LA/60
02709807 75116807
Translation and identification of the viral mRNA species isolated from subcellular fractions of vesicular stomatitis virus-infected cells.
Both GW; Moyer SA; Banerjee AK
J Virol Apr 1975, 15 (4) p1012-9, ISSN 0022-538X Journal Code: KCV
Languages: ENGLISH
The cytoplasm of vesicular stomatitis virus (VSV)-infected BHK cells has been separated into a fraction containing the membrane-bound polysomes and the remaining supernatant fraction. Total poly(A)-containing RNA was isolated from each fraction and purified. A 17S class of VSV mRNA was found associated almost exclusively with the membrane-bound polysomes, whereas 14,5S and 12S RNAs were found mostly in the postmembrane cytoplasmic supernatant. Poly(A)-containing VSV RNA synthesized in vitro by purified virus was resolved into the same size classes. The individual RNA fractions isolated from VSV-infected cells or synthesized in vitro were translated in cell-free extracts of wheat germ, and their polypeptide products were

compared by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. The corresponding in vivo and in vitro RNA fractions qualitatively direct the synthesis of the same viral polypeptides and therefore appear to contain the same mRNA species. By tryptic peptide analysis of their translation products, the in vivo VSV mRNA species have been identified. The 17S RNA, which is compartmentalized on membrane-bound polysomes, codes for a protein of molecular weight 63,000 (P-63) which is most probably a nonglycosylated form of the viral glycoprotein, G. Of the viral RNA species present in the remaining cytoplasmic supernatant, the 14.5S RNA codes almost exclusively for the N protein, whereas the 12S RNA codes predominantly for both the NS and M proteins of the virion.

22/7,LA/61
02382513 74100513
Reduced permeability in CHO cells as a mechanism of resistance to colchicine.
Ling V; Thompson LH
J Cell Physiol (United States) Feb 1974, 83 (1) p103-16, ISSN 0021-9541 Journal Code: HNB
Languages: ENGLISH

22/7,LA/62
01388623 70233623
Effect of collagen and acid polysaccharides on the growth of BHK-21 cells in semi-solid media.
Sanders FK; Smith JD
Nature (England) Aug 1 1970, 227 (257) p513-5, ISSN 0028-0836 Journal Code: NSC
Languages: ENGLISH
?

Chem Abs 1963 - 1984

"recombinant" x "glycosylation/membrane proteins" x mammals

L25 ANSWER 1 OF 49

AN CA101(25):223801s
TI Characterization of a cDNA coding for human protein C
AU Foster, Donald; Davie, Earl W.
CS Dep. Biochem., Univ. Washington
LO Seattle, WA 98195, USA
SO Proc. Natl. Acad. Sci. U. S. A., 81(15), 4766-70
SC 3-3 (Biochemical Genetics)
SX 13
DT J
CO PNASA6
IS 0027-8424
PY 1984
LA Eng
AN CA101(25):223801s
AB Protein C is a precursor to a serine protease that is present in mammalian plasma. In its activated form (blood coagulation factor XIVa [42617-41-4]) it readily inactivates factor Va and factor VIIIa, 2 proteins that participate as cofactors in the blood coagulation cascade. A .lambda.gt11 library contg. cDNA inserts prepd. from human liver mRNA was screened with an antibody to human protein C. Seven pos. clones were isolated from 2 .times. 106 phage and were plaque-purified. The cDNA inserts of 2 of these phage were sequenced and shown to code for human protein C. Each cDNA insert coded for a portion of the light chain of the mol., a connecting region, the heavy chain, a stop codon, a 3'-noncoding region, and a poly(A) tail. The length of the noncoding sequence on the 3' end differed in the 2 clones, but each contained a processing or polyadenylylation signal that was followed by a poly(A) tail. The amino acid sequence, as detd. from the cDNA, indicates that protein C is synthesized as a single-chain polypeptide contg. the light chain and the heavy chain connected by a dipeptide of Lys-Arg. The single-chain mol. is then converted to the light and heavy chains by cleavage of .gtoreq.2 internal peptide bonds. In plasma, the heavy and light chains of protein C are linked together by a disulfide bond. The amino acid sequence of human protein C shows a high degree of homol. with that of the bovine mol. The DNA sequence coding for the catalytic region near the active site serine in human protein C also showed a high degree of DNA and amino acid sequence identity with prothrombin, factor IX, and factor X, 3 of the other vitamin K-dependent serine proteases that are present in plasma.

L25 ANSWER 2 OF 49

AN CA101(21):185170a
TI Comparison of different eukaryotic vectors for the expression of hemagglutinin glycoprotein of influenza virus
AU Gething, Mary Jane; Sambrook, Joseph F.; Braciale, Thomas J.; Brand, Colin M.
CS Cold Spring Harbor Lab.
LO Cold Spring Harbor, NY 11724, USA
SO Mod. Approaches Vaccines: Mol. Chem. Basis Virus Virulence Immunogenicity, [Pap. Conf.], Meeting Date 1983, 263-8. Edited by: Chanock, Robert M.; Lerner, Richard Alan. Cold Spring Harbor Lab.: Cold Spring Harbor, N. Y.
SC 3-4 (Biochemical Genetics)

AM 27 014754

DT C
CO 52CRA4
PY 1984
LA Eng
AN CA101(21):185170a
AB Several vectors can be used to express the hemagglutinin (HA) gene of influenza virus in eukaryotic cells: (1) HA-carrying plasmids that become integrated into the chromosomes of the cells; (2) bovine papilloma virus-HA recombinants that are maintained as episomes in the cells; and (3) SV40 virus-HA recombinants that express HA during lytic infection of the cells. A cDNA copy of the RNA gene coding for HA from the A/Japan/305/57 strain of influenza virus was expressed in eukaryotic cells by using different vector systems. In all cases, the HA produced was identical in its antigenic and immunogenic properties to that produced in cells infected with influenza virus.

L25 ANSWER 3 OF 49

AN CA101(21):185100c
TI Rat major acute-phase protein: biosynthesis and characterization of a cDNA clone
AU Anderson, Kathleen P.; Martin, Anna D.; Heath, Edward C.
CS Coll. Med., Univ. Iowa
LO Iowa City, IA 52242, USA
SO Arch. Biochem. Biophys., 233(2), 624-35
SC 3-3 (Biochemical Genetics)
SX 13, 14
DT J
CO ABBIA4
IS 0003-9861
PY 1984
LA Eng
AN CA101(21):185100c
AB A cDNA clone specific for the major acute-phase protein of rat serum (.alpha.1-MAP) was isolated. The recombinant was pos. identified by hybrid selection procedures and contained a 1.55-kilobase insert. Partial sequence anal. of the primary translation product indicated the distribution of leucine, isoleucine, cysteine, and methionine in the N-terminal region of this protein. To relate the location of these amino acids to the nucleotide sequence, the cDNA was analyzed by the method of Maxam and Gilbert. Apparently, the cDNA insert contained the 3' poly(A) tail, and alignment of the 5' end of the cDNA with the available amino acid sequence of the primary translation product corroborated that the insert encodes the entire .alpha.1-MAP protein, except for the 1st 4 amino acids of the signal peptide.

L25 ANSWER 4 OF 49

AN CA101(21):184919q
TI Eukaryotic expression of cloned cDNA coding for influenza viral glycoproteins using an SV40 vector: use of recombinant DNA mutants to study structure-function relationships
AU Bos, Timothy J.; McQueen, Nancy L.; Davis, Alan R.; Nayak, Debi P.
CS Sch. Med., UCLA
LO Los Angeles, CA 90024, USA
SO Segmented Negat. Strand Viruses: Arenaviruses, Bunyaviruses, Orthomyxoviruses, [Proc. Symp. Mol. Biol. Negat. Strand Viruses], Meeting Date 1983, 125-30. Edited by: Compans, Richard W.; Bishop, David H. L. Academic: Orlando, Fla.

SC 3-0 (Biochemical Genetics)
DT C
CO 52EJA0
PY 1984
LA Eng
AN CA101(21):184919q
AB A review and discussion with 13 refs.

L25 ANSWER 5 OF 49

AN CA101(19):164715s
TI Cloning and physical mapping of a gene fragment coding for a 64-kilodalton major late antigen of human cytomegalovirus
AU Pande, Hema; Baak, Steven W.; Riggs, Arthur D.; Clark, Brian R.; Shively, John E.; Zaia, John A.
CS Div. Immunol., Beckman Res. Inst. City of Hope
LO Duarte, CA 91010, USA
SO Proc. Natl. Acad. Sci. U. S. A., 81(15), 4965-9
SC 3-4 (Biochemical Genetics)
SX 15
DT J
CO PNASA6
IS 0027-8424
PY 1984
LA Eng
AN CA101(19):164715s
AB A clone was isolated that contains a gene fragment for a 64-kildalton (kd) glycoprotein that is the major late antigen of human cytomegalovirus (HCMV). On the basis of the amino acid sequence of a tryptic peptide of this glycoprotein (HCMV gp64), 2 sets of mixed-sequence probes, 1 that consisted of a mixt. of 16 heptadecadeoxyribonucleotides, and the other, a mixt. of 32 icosadeoxyribonucleotides, were synthesized. A subgenomic library of HCMV (Towne strain) DNA was constructed in plasmid pBR327, and transformants were screened with 32P-labeled aliquots of these synthetic oligodeoxyribonucleotide probes. Two clones of 15,000 gave strong pos. signals. Plasmid DNA was isolated from the pos. clones and characterized by restriction mapping and Southern blot anal. with both probes. The plasmid DNA contained a 2.3-kilobase insert, which yielded an 800-base-pair (bp) and a 1500-bp fragment after Sau3A digestion. Only the 800-bp fragment hybridized to the mixed probes, and DNA sequence anal. revealed that it contains nucleotide sequences compatible with amino acid sequences of tryptic peptides of HCMV gp64. Restriction mapping studies of HCMV DNA using this 32P-labeled, 800-bp cloned DNA allowed the localization of this gene fragment in the long unique region of HCMV (Towne strain) genome at .apprx.0.5-0.51 map unit.

L25 ANSWER 6 OF 49

AN CA101(19):164631m
TI Nucleotide sequence of rat haptoglobin cDNA. Characterization of the .alpha..beta.-subunit junction region of prohaptoglobin
AU Goldstein, Leslie A.; Heath, Edward C.
CS Coll. Med., Univ. Iowa
LO Iowa City, IA 52242, USA
SO J. Biol. Chem., 259(14), 9212-17
SC 3-3 (Biochemical Genetics)
SX 13
DT J

CO JBCHA3
IS 0021-9258
PY 1984
LA Eng
AN CA101(19):164631m
AB The biosynthesis of rat haptoglobin, a hetrotetrameric glycoprotein (.alpha.2.beta.2), requires the post-translational cleavage of its glycosylated primary translation product (prohaptoglobin) into .alpha.- and .beta.-subunits. To elucidate the site(s) at which proteolytic cleavage occurs in prohaptoglobin, a recombinant plasmid was isolated whose cDNA insert encodes for the carboxyl terminus of the .alpha.-subunit, the .alpha..beta.-subunit junction, and the .beta.-subunit region, and also the entire 3'-untranslated region [142 base pairs (bp)] and poly(A) tail (55 bp) of the mRNA. A single arginine residue was found at the .alpha..beta. subunit junction region -Val-Gln-Arg-Ile-Ile-Gly-Gly- of prohaptoglobin. The sequence homol. of this region with serine protease precursors suggests that post-translational processing of prohaptoglobin involves cleavage of the Arg-Ile bond and extn. of the Arg residue. The rat .beta.-subunit shows a high degree (.apprx.80%) of sequence homol. with its human counterpart, although it possesses only 2 of the 4 N-glycosylation sites present in human haptoglobin .beta.-subunit.

L25 ANSWER 7 OF 49

AN CA101(17):145013v
TI Molecular cloning of cDNA encoding a murine hematopoietic growth regulator, granulocyte-macrophage colony stimulating factor
AU Gough, Nicholas M.; Gough, Jill; Metcalf, Donald; Kelso, Anne; Grail, Dianne; Nicola, Nicos A.; Burgess, Antony W.; Dunn, Ashley R.
CS Melbourne Tumour Biol. Branch, Ludwig Inst. Cancer Res.
LO Melbourne 3050, Australia
SO Nature (London), 309(5971), 763-7
SC 3-3 (Biochemical Genetics)
SX 13, 15
DT J
CO NATUAS
IS 0028-0836
PY 1984
LA Eng
AN CA101(17):145013v
AB CDNA clones specifying the murine granulocyte-macrophage colony-inducing factor [62683-29-8] have been isolated. This hematopoietic growth factor is encoded by a unique gene specifying a mRNA of 1200 nucleotides and a polypeptide of 118 amino acids. It bears no structural similarity to the functionally related factor, interleukin-3, described recently.

L25 ANSWER 8 OF 49

AN CA101(15):128828x
TI Production of an HSV subunit vaccine by genetically engineered mammalian cell lines
AU Lasky, Laurence A.; Dowbenko, Donald; Simonsen, Christian; Berman, Phillip W.
CS Dep. Vaccine Dev., Genentech, Inc.
LO South San Francisco, CA 94080, USA
SO Mod. Approaches Vaccines: Mol. Chem. Basis Virus Virulence Immunogenicity, [Pap. Conf.], Meeting Date 1983, 189-94. Edited by: Chanock, Robert M.; Lerner, Richard Alan. Cold Spring Harbor Lab.:

AM 27 014757

Cold Spring Harbor, N. Y.
SC 16-6 (Fermentation and Bioindustrial Chemistry)
SX 3, 63
DT C
CO 52CRA4
PY 1984
LA Eng
AN CA101(15):128828x
AB A subunit vaccine against herpes simplex virus infection was produced using genetically engineered mammalian cells. A truncated gene of glycoprotein D was cloned in Chinese hamster ovary cells using a plasmid vector derived from pJ2-9. These cell lines produced a glycoprotein D that is glycosylated and transported to the extracellular medium and is antigenically similar to the native glycoprotein D of herpes simplex viruses.

L25 ANSWER 9 OF 49

AN CA101(15):124052s
TI Evolution of haptoglobin: comparison of complementary DNA encoding Hp.alpha.1S and Hp.alpha.2FS
AU Brune, Jill L.; Yang, Funmei; Barnett, Don R.; Bowman, Barbara H.
CS Health Sci. Cent., Univ. Texas
LO San Antonio, TX 78284, USA
SO Nucleic Acids Res., 12(11), 4531-8
SC 3-3 (Biochemical Genetics)
SX 13
DT J
CO NARHAD
IS 0305-1048
PY 1984
LA Eng
AN CA101(15):124052s
AB Haptoglobn is a transport glycoprotein which removes free Hb from the circulation of vertebrates. In human populations, haptoglobin is polymorphic as a result of 3 alleles: Hp.alpha.1F, Hp.alpha.1S, and Hp.alpha.2. The Hp.alpha.2 allele is roughly twice the length of the Hp.alpha.1 alleles and is the product of a partial gene duplication that possibly results from an unequal crossover event in a heterozygous genotype Hp.alpha.1F/Hp.alpha.1S. The cDNA encoding Hpa1S is compared to that encoding Hp.alpha.2FS. Both have a leader sequence that is followed by the genotypic .alpha. chain sequence, a .beta. sequence, and an untranslated sequence in the 3' end. The cDNA encoding Hp.alpha.2FS is composed of .alpha.1F and .alpha.1S domains that differ by 4 nucleotide replacements. Hp.alpha.1S cDNA contains the same replacement site mutations found in the .alpha.1S domain of Hp.alpha.2FS, which indicate that this coding region has sustained few, if any, mutations since its incorporation into the Hp.alpha.2FS gene.

L25 ANSWER 10 OF 49

AN CA101(13):104538b
TI Conversion of a secretory protein into a transmembrane protein results in its transport to the Golgi complex but not to the cell surface
AU Guan, Jun Lin; Rose, John K.
CS Mol. Biol. Virol. Lab., Salk Inst.
LO San Diego, CA 92138, USA
SO Cell (Cambridge, Mass.), 37(3), 779-87

SC 2-5 (Mammalian Hormones)
SX 3, 6
DT J
CO CELLB5
IS 0092-8674
PY 1984
LA Eng
AN CA101(13):104538b
AB The possibility of converting a secretory protein into an integral membrane protein by appending the membrane spanning domain of an integral membrane protein to its carboxy terminus was studied. First, expression of a cDNA clone encoding rat growth hormone (GH) [9002-72-6] in eukaryotic cells was obtained; this protein was secreted. Then, a hybrid gene encoding GH fused to the membrane spanning and cytoplasmic domains of the vesicular stomatitis virus glycoprotein was constructed and expressed. This fusion protein was anchored in microsomal membranes in the expected transmembrane configuration. The fusion protein was transported to the Golgi app., and was esterified to palmitic acid [57-10-3], but it was not transported to the cell surface. Thus, the sorting signal which allows rapid secretion of sol. GH does not function when the protein is bound to the membrane.

L25 ANSWER 11 OF 49

AN CA101(11):87038u
TI Acquisition of host cell genetic information by avian sarcoma virus rescued from rat cells transformed by B77 virus
AU Kotler, M.; Salmon, Sarah; Olshevsky, U.
CS Hadassah Med. Sch., Hebrew Univ.
LO Jerusalem, Israel
SO Arch. Virol., 80(4), 249-64
SC 10-1 (Microbial Biochemistry)
DT J
CO ARVIDF
IS 0304-8608
PY 1984
LA Eng
AN CA101(11):87038u
AB The properties of avian sarcoma virus B77 grown in chick cells were compared with those of virus obtained by fusion of rat cells transformed by B77 virus (RB77 cells) with chick cells (FB77 virus). Differences in the B77 and FB77 viral genomes were demonstrated by the fingerprint technique. The rescued FB77 virus contained sequences homologous to the normal cell genome. Apparently, the rescued FB77 virus is a recombinant between B77 virus and rat genomic elements. The RNAs from B7m and FB77 viruses had similar mol. wts. The migration rates of the structural proteins and the large glycoprotein (gp85) in polyacrylamide gels were the same for the B77 and FB77 viruses, but the small glycoprotein of the FB77 virus was found to be slightly larger than the gp37 of the B77 virus.

L25 ANSWER 12 OF 49

AN CA101(11):84730c
TI Sequence homology between human and animal rotavirus serotype-specific glycoproteins
AU Dyall-Smith, Michael L.; Holmes, Ian H.
CS Dep. Microbiol., Univ. Melbourne
LO Parkville 3052, Australia

SO Nucleic Acids Res., 12(9), 3973-82
SC 3-2 (Biochemical Genetics)
DT J
CO NARHAD
IS 0305-1048
PY 1984
LA Eng
AN CA101(11):84730c
AB The double-stranded RNA gene segment coding for the major outer shell glycoprotein of a human rotavirus (Hu/Australia/5/77, serotype 2) was reverse transcribed into DNA and cloned into the PstI site of the plasmid pBR322. The cloned gene was sequenced and was of 1062 base pairs with 1 long open reading frame capable of coding for a protein of 326 amino acids. When this gene sequence was compared to the published sequences of the corresponding genes of 2 animal rotaviruses, SA11 (simian) and UK (bovine), all 3 were closely related (74-78%). The predicted amino acid sequences of the 3 genes were also highly conserved (75-86%), despite the fact that the 3 viruses belong to different serotypes.

L25 ANSWER 13 OF 49

AN CA101(5):34033g
TI Duplication within the haptoglobin Hp2 gene
AU Maeda, Nobuyo; Yang, Funmei; Barnett, Don R.; Bowman, Barbara H.; Smithies, Oliver
CS Lab. Genet., Univ. Wisconsin
LO Madison, WI 53706, USA
SO Nature (London), 309(5964), 131-5
SC 3-3 (Biochemical Genetics)
SX 13
DT J
CO NATUAS
IS 0028-0836
PY 1984
LA Eng
AN CA101(5):34033g
AB DNA sequencing shows that the intragenic duplication within the human haptoglobin Hp2 allele was formed by a nonhomologous, probably random, crossing-over within different introns of 2 Hp1 genes, probably in an Hp1F/Hp1S heterozygote.

L25 ANSWER 14 OF 49

AN CA101(3):18358m
TI Characterization of human haptoglobin cDNAs coding for .alpha.2FS.beta. and .alpha.1S.beta. variants
AU Van der Straten, Ariane; Herzog, Albert; Cabezon, Teresa; Bollen, Alex
CS Lab. Genet., Univ. Brussels
LO Rhode-St-Genese B-1640, Belg.
SO FEBS Lett., 168(1), 103-7
SC 3-3 (Biochemical Genetics)
SX 13
DT J
CO FEBLAL
IS 0014-5793
PY 1984
LA Eng
AN CA101(3):18358m

A human liver library, derived from a heterozygous (Hp2-1) donor, was used to isolate cDNA clones coding for the haptoglobin (Hp) .alpha.1S.beta. and .alpha.2FS.beta. variants. DNA sequencing showed that the 2 variants are identical except for the .alpha.F duplicated segment in Hp .alpha.2FS.beta.. Four nucleotide changes were found between the phenotypically different F and S regions of the Hp .alpha.2 gene, resulting in an Asp, Lys/Asn, Glu substitution.

ANSWER 15 OF 49

CA100(23):186671f
Production of a glycosylated human protein by recombinant DNA technology
Haynes, Joel; Weissmann, Charles
Inst. Molekularbiol. I, Univ. Zurich
Zurich CH-8093, Switz.
Humoral Factors Host Def., [Proc. Takeda Sci. Found. Symp. Biosci.], 1st, Meeting Date 1982, 111-29. Edited by: Yamamura, Yuichi.
Academic: Tokyo, Japan.
3-4 (Biochemical Genetics)
X 13, 15
T C
O 51INAF
Y 1983
A Eng
N CA100(23):186671f
AB Hybrid plasmids contg. the mouse dihydrofolate reductase (dhfr) and a human interferon (either IFN-.alpha.5 or IFN-.gamma.) coding sequence under the control of viral promoters were transfected into dhfr- CHO cells. The dhfr+ colonies produced IFN at 10-1000 units/mL/day. Clones selected in methotrexate had a 20-50-fold increase in the IFN-.alpha.5 and dhfr DNA and mRNA content and secreted IFN at 20,000-100,000 units/mL/day. SDS-polyacrylamide gel electrophoresis of partially purified 35S-HuIFN-.gamma. from CHO cells showed a multiplet of labeled bands with a mobility corresponding to 22,400-23,400 daltons that was absent in the supernatants of nontransformed CHO cells. The higher apparent mol. wt. of human IFN-.gamma. from CHO cells as compared to that of human IFN-.gamma. from Escherichia coli (.apprx.18,800) suggests that the former is glycosylated.

L25 ANSWER 16 OF 49

AN CA100(23):186661c
TI Expression of viral membrane proteins from cloned cDNA by microinjection into eukaryotic cell nuclei
AU Timm, Beate; Kondor-Koch, Claudia; Lehrach, Hans; Riedel, Heimo; Edstroem, Jan Erik; Garoff, Henrik
CS Eur. Mol. Biol. Lab.
LO Heidelberg D-6900, Fed. Rep. Ger.
SO Methods Enzymol., 96(Biomembranes, Pt. J), 496-511
SC 3-4 (Biochemical Genetics)
DT J
CO MENZAU
IS 0076-6879
PY 1983
LA Eng
AN CA100(23):186661c
AB A discussion is given on a cDNA expression system for viral membrane proteins of viruses such as Semliki Forest virus (SFV). The system

AM 27 014761

involves (1) insertion of the cDNA into a eukaryotic expression vector; (2) cloning of the recombinant DNA in Escherichia coli; (3) extn. of the plasmid mols. from the bacteria; (4) microinjection of the DNA into the cell nucleus; and (5) anal. for viral proteins using indirect immunofluorescence. For the SFV cDNA injected into BHK cells, the capsid protein was seen as a diffuse cytoplasmic stain in the cell cytoplasm, whereas the membrane proteins were assocd. with the endoplasmic reticulum, Golgi complex, and the plasma membrane.

L25 ANSWER 17 OF 49

AN CA100(23):186650y
TI An approach to the cloning of cell surface protein genes. Selection by cell sorting of mouse L-cells that express HLA or 4F2 antigens after transformation with total human DNA
AU Kuehn, Lukas C.; Barbosa, James A.; Kamarck, Michael E.; Ruddle, Frank H.
CS Dep. Biol., Yale Univ.
LO New Haven, CT 06511, USA
SO Mol. Biol. Med., 1(3), 335-52
SC 3-4 (Biochemical Genetics)
SX 13, 15
DT J
CO MBIMDG
IS 0735-1313
PY 1983
LA Eng
AN CA100(23):186650y
AB An approach to the cloning of cell surface proteins is described that is independent of mRNA isolation. Mouse LtK- cells are cotransformed with the thymidine kinase gene from herpes simplex virus and total human DNA. Transformants expressing the human surface antigens of interest are isolated by 2 selection steps, which consist of treatment with hypoxanthine, aminopterin, and thymidine and fluorescence-activated cell sorting. By this procedure, 7 transformants were isolated that express HLA-A,B,C antigens and 12 transformants that express the 4F2 antigen. No OKT-10 antigen-expressing L-cell transformants were found. Three independent secondary 4F2 transformants were obtained after identical cotransformation of fresh Ltk- cells with DNA from primary transformants. Hybridization of their genomes with human DNA revealed a shared set of human restriction fragments in all 3 cell lines. This 32 .times. 103-base-pair segment of DNA codes for the human 4F2 antigen; this offers the opportunity to clone the gene. The 7 HLA-expressing cell lines were examd. and all of them had acquired an HLA-coding sequence concomitant to its expression.

L25 ANSWER 18 OF 49

AN CA100(23):186500z
TI Expression of proteins on the cell surface using mammalian vectors
AU Sambrook, Joe; Gething, Mary Jane
CS Cold Spring Harbor Lab.
LO Cold Spring Harbor, NY, USA
SO Exp. Manipulation Gene Expression, 225-46. Edited by: Inouye, Masayori. Academic: New York, N. Y.
SC 3-0 (Biochemical Genetics)
SX 13
DT C
CO 51IOAI

PY 1983
LA Eng
AN CA100(23):186500z
AB A review with 105 refs., including a discussion of the hemagglutinin gene of influenza virus and its expression in animal cells transformed with recombinant virus vectors.

L25 ANSWER 19 OF 49

AN CA100(15):115813t
TI Construction and expression of a recombinant DNA gene encoding a polyomavirus middle-size tumor antigen with the carboxyl terminus of the vesicular stomatitis virus glycoprotein G
AU Templeton, Dennis; Voronova, Anna; Eckhart, Walter
CS Mol. Biol. Virol. Lab., Salk Inst.
LO San Diego, CA 92138, USA
SO Mol. Cell. Biol., 4(2), 282-9
SC 3-4 (Biochemical Genetics)
DT J
CO MCEBD4
IS 0270-7306
PY 1984
LA Eng
AN CA100(15):115813t
AB A mol. clone encoding the N-terminal 379 amino acids of the polyomavirus middle-size tumor antigen, followed by the C-terminal 60 amino acids of the vesicular stomatitis virus glycoprotein G was constructed. This hybrid gene contained the coding region for the C-terminal hydrophobic membrane-spanning domain of the G protein in place of the C-terminal hydrophobic domain of the middle-size tumor antigen. The hybrid gene was expressed in COS-1 cells under the control of the SV40 virus late promoter. The hybrid protein was located in cell membranes and was assocd. with a tyrosine-specific protein kinase activity, as was the middle-size tumor antigen. Plasmids encoding the hybrid protein failed to transform mouse NIH 3T3 or rat F2408 cells.

L25 ANSWER 20 OF 49

AN CA100(13):97556e
TI Human fibronectin: molecular cloning evidence for two mRNA species differing by an internal segment coding for a structural domain
AU Kornblihtt, A. R.; Vibe-Pedersen, K.; Baralle, F. E.
CS Sir William Dunn Sch. Pathol., Univ. Oxford
LO Oxford OX1 3RE, UK
SO EMBO J., 3(1), 221-6
SC 3-3 (Biochemical Genetics)
SX 13
DT J
CO EMJODG
IS 0261-4189
PY 1984
LA Eng
AN CA100(13):97556e
AB Two different fibronectin (FN) mRNA species were detected in the human cell line Hs578T. One species, mRNA I, contains an addnl. 270-nucleotide insert (ED) that encodes exactly 1 of the internally repeated structural domains of the protein. The 90-amino acid extra domain belongs to the so-called type III homol., and it is located in the C-terminal half of FN, between the cell attachment and the

heparin-binding sites of the protein. The evidence of 2 mRNAs is provided by the isolation and characterization of 4 independent cDNA clones from a library prepd. with a synthetic oligonucleotide primer, and it was confirmed by S1 nuclease anal. of cDNA-mRNA hybrids. This kind of anal. also showed that in the human cell line, mRNA I is present at a lower level than mRNA II (the mRNA species without the ED), whereas in human liver, mRNA I is virtually undetectable. Since liver tissue is reported to be the source of plasma FN, the presence of the ED insert could be a particular feature of cellular FN.

L25 ANSWER 21 OF 49

AN CA99(25):207279n
TI Identification and characterization of human haptoglobin cDNA
AU Yang, Funmei; Brune, Jill L.; Baldwin, W. David; Barnett, Don R.; Bowman, Barbara H.
CS Health Sci. Cent., Univ. Texas
LO San Antonio, TX 78284, USA
SO Proc. Natl. Acad. Sci. U. S. A., 80(19), 5875-9
SC 3-3 (Biochemical Genetics)
SX 13
DT J
CO PNASA6
IS 0027-8424
PY 1983
LA Eng
AN CA99(25):207279n
AB Recombinant plasmids contg. human cDNA encoding haptoglobin, a plasma protein that binds free Hb, were isolated by screening an adult human liver library with a mixed oligonucleotide probe. Four cDNA clones contg. inserts were obtained that span 1218 nucleotides of the haptoglobin coding sequence, including the 3' end of the haptoglobin cDNA. The cDNA sequence included a leader sequence followed by .alpha.2-chain and .beta.-chain sequences. An arginine residue was deduced between the human .alpha.- and .beta.-chain sequences. This is a probable site of limited proteolysis leading to the formation of the .alpha. and .beta. polypeptides in mature haptoglobin. A comparison of the haptoglobin .alpha.-.beta. junction region and the heavy-light-chain junction of tissue-type plasminogen activator strengthens the evolutionary homol. found in haptoglobin and the serine proteases. The Hp.alpha.2 gene, which is a partial duplication produced by unequal crossing over between Hp.alpha.1 genes, was impossible to align by protein characterization. The cDNA sequence establishes the alignment of Hp.alpha.2FS in the Hp.alpha.2 gene studied.

L25 ANSWER 22 OF 49

AN CA99(25):207277k
TI Sequence of human haptoglobin cDNA: evidence that the .alpha. and .beta. subunits are coded by the same mRNA
AU Raugei, G.; Bensi, G.; Colantuoni, V.; Romano, V.; Santoro, C.; Costanzo, F.; Cortese, R.
CS Eur. Mol. Biol. Lab.
LO Heidelberg 6900, Fed. Rep. Ger.
SO Nucleic Acids Res., 11(17), 5811-19
SC 3-3 (Biochemical Genetics)
SX 13
DT J
CO NARHAD

IS 0305-1048
PY 1983
LA Eng
AN CA99(25):207277k
AB A cDNA clone coding for human haptoglobin was isolated and sequenced. Haptoglobin is probably synthesized as a single polypeptide chain which is then cleaved at an arginine residue to generate its 2 characteristic .alpha. and .beta. subunits. Southern blot anal. suggests that there are .gtoreq.2 copies of the haptoglobin gene/haploid genome.

L25 ANSWER 23 OF 49

AN CA99(21):170553u
TI Identification of glucocorticoid-induced genes in rat hepatoma cells by isolation of cloned cDNA sequences
AU Feinberg, Ronald F.; Sun, Lee Hwei; Ordahl, Charles P.; Frankel, Fred R.
CS Sch. Med., Univ. Pennsylvania
LO PA 19104, USA
SO Proc. Natl. Acad. Sci. U. S. A., 80(16), 5042-6
SC 3-3 (Biochemical Genetics)
SX 2, 13
DT J
CO PNASA6
IS 0027-8424
PY 1983
LA Eng
AN CA99(21):170553u
AB The expression of specific cellular genes in M1.19 rat hepatoma cells involves glucocorticoid regulation by mechanisms that are not well understood. To approach this problem, cDNA prepd. from dexamethasone [50-02-2]-induced poly(A)-RNA was cloned and a comparative colony hybridization method was used to identify recombinant clones contg. hormone-regulated sequences. Two cDNA clones, p1394 and p255, hybridize to a homogeneous RNA species of 900 nucleotides that is present in high abundance in 24-h-induced cells but is undetectable in uninduced cells. This RNA can be seen as early as 1 h after dexamethasone stimulation. Inhibition of protein synthesis with cycloheximide significantly reduces the accumulation of the RNA but does not abolish the induction response. In normal adult rat liver, the RNA is abundant, and this RNA is induced by dexamethasone in adrenalectomized rats. Plasmids p1394 and p255 contain sequences that are homologous to the mRNA coding for the acute-phase reactant protein .alpha.1-acid glycoprotein. Two other cDNA clones, p655 and p333, hybridize to a more heterogeneous RNA species of 200-400 nucleotides with a lower induction response to dexamethasone. Southern blot anal. of M1.19 genomic DNA indicates that p1394 and p255 are complementary to a single DNA fragment, whereas p655 and p333 are complementary to repetitive sequences in the M1.19 genome. Apparently, the genetic domain of glucocorticoid control in M1.19 rat hepatoma cells involves low copy no. genes such as .alpha.1-acid glycoprotein as well as repetitive sequence elements.

L25 ANSWER 24 OF 49

AN CA99(21):170484x
TI Nucleotide sequencing of an apparent proviral copy of env mRNA defines determinants of expression of the mouse mammary tumor virus env gene

AU Majors, John E.; Varmus, Harold E.
CS Dep. Microbiol. Immunol., Univ. California
LO San Francisco, CA 94143, USA
SO J. Virol., 47(3), 495-504
SC 3-2 (Biochemical Genetics)
DT J
CO JOVIAM
IS 0022-538X
PY 1983
LA Eng
AN CA99(21):170484x
AB The nucleotide sequences of large regions of a cloned mouse mammary tumor virus strain C3H provirus that appear to be a DNA copy of env mRNA were detd. In conjunction with the anal. of several addnl. clones of integrated and unintegrated mouse mammary tumor virus DNAs, several conclusions were drawn. The mRNA for env is generated by splicing mechanisms that recognize conventional eukaryotic signals at donor and acceptor sites with a leader of .gtoreq.289 bases. The 1st of 3 possible initiation codons for translation of env follows the splice junction by a single nucleotide and produces a signal peptide of 98 amino acids. The N terminal sequence of the major virion glycoprotein gp52env is confirmed by nucleotide sequencing and is encoded by a sequence beginning 584 nucleotides from the 5' end of env mRNA. The final 17 amino acids at the C terminus of the primary product of env are encoded within the long terminal repeat by the 51 bases at the 5' end of the U3 domain, and bases 2-4 at the 5' end of the long terminal repeat constitute an initiation codon that commences an open reading frame capable of directing the synthesis of a 36-kilodalton protein.

L25 ANSWER 25 OF 49

AN CA99(19):156646v
TI Isolation of cDNA clones for the p33 invariant chain associated with HLA-DR antigens
AU Long, E. O.; Strubin, M.; Wake, C. T.; Gross, N.; Carrel, S.; Goodfellow, P.; Accolla, R. S.; Mach, B.
CS Med. Sch., Univ. Geneva
LO Geneva 1205, Switz.
SO Proc. Natl. Acad. Sci. U. S. A., 80(18), 5714-18
SC 15-7 (Immunochemistry)
DT J
CO PNASA6
IS 0027-8424
PY 1983
LA Eng
AN CA99(19):156646v
AB An invariant glycoprotein of mol. wt. 33,000 (DRp33) is assocd. intracellularly with HLA-DR antigens. A cDNA clone for DRp33, called 33-10, was isolated. Because no amino acid sequence has been detd. for DRp33, the identification of cDNA clone 33-10 was based on selection of mRNA by hybridization, subsequent translation in a rabbit reticulocyte lysate supplemented with microsomes, and translation in microinjected Xenopus oocytes followed by immunopptn. with an anti-DR antiserum. The translation products assembled with DR .alpha. and .beta. chains in oocytes coinjected with all 3 mRNAs. Assembly of DR .alpha. and .beta. chains was also obsd. in the absence of DRp33 mRNA. When compared with DRp33 immunopptd. from a human B-cell line, translation products of the hybrid-selected mRNA showed (i) identical migration in 2-dimensional gel electrophoresis,

(ii) identical apparent mol. wt. in the absence of N-linked glycosylation, and (iii) a very similar 2-dimensional peptide map. Transcription of the DRp33 gene into a mRNA 1,400 nucleotides long was obsd. in B cells but was undetectable in T-cell lines. Thus, DRp33 appears to be coordinately expressed with DR .alpha. and .beta. chains. Hybridization to DNA of mouse-human somatic cell hybrids showed that DRp33 is encoded by a gene that is located outside the major histocompatibility complex.

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L25 ANSWER 26 OF 49

AN CA99(19):153188m
TI Molecular cloning and nucleotide sequence of a cDNA clone coding for the cell attachment domain in human fibronectin
AU Oldberg, Ake; Linney, Elwood; Ruoslahti, Erkki
CS Cancer Res. Cent., La Jolla Cancer Res. Found.
LO La Jolla, CA 92037, USA
SO J. Biol. Chem., 258(17), 10193-6
SC 3-4 (Biochemical Genetics)
SX 13
DT J
CO JBCHA3
IS 0021-9258
PY 1983
LA Eng
AN CA99(19):153188m
AB A cDNA clone coding for the cell-attachment domain in human fibronectin was isolated with synthetic oligonucleotides. Three sets of mixed tetradecamer oligonucleotides were synthesized on the basis of published amino acid sequences in the 108-amino-acid cell-attachment domain. One of these sets was made complementary to amino acids located near the C terminus of the cell-attachment domain and synthesized as a mixt. of 24 sequences. This oligonucleotide mixt. was used to prime cDNA synthesis with mRNA prepd. from a human fibrosarcoma as a template. A cDNA library was constructed with the oligonucleotide-primed sequences in the vector pBR322. Colonies that hybridized with the primer were isolated from the library and further identified by hybridization with oligonucleotides deduced from an amino acid sequence located 45 amino acid residues N-terminal of the primer sequence. One clone, which hybridized to both probes, was characterized in detail. The insert was 380 base pairs, and its nucleotide sequence agreed completely with the corresponding amino acid sequence of human plasma fibronectin, which shows that the sequences for this region are identical in plasma fibronectin and fibronectin from a cell line. This clone should be useful for studies of the expression of fibronectins and may also allow for the prodn. of the biol. active cell-attachment domains of fibronectin in bacteria.

L25 ANSWER 27 OF 49

AN CA99(15):118770y
TI Quantitative in situ hybridization of 3H-labeled complementary deoxyribonucleic acid (cDNA) to the messenger ribonucleic acid of thyroglobulin in human thyroid tissues
AU Berge-Lefranc, Jean Louis; Cartouzou, Guy; Bignon, Christophe; Lissitzky, Serge

CS Inst. Natl. Sante Rech. Med., Fac. Med.
LO Marseille 13385, Fr.
SO J. Clin. Endocrinol. Metab., 57(3), 470-6
SC 9-8 (Biochemical Methods)
SX 14
DT J
CO JCEMAZ
IS 0021-972X
PY 1983
LA Eng
AN CA99(15):118770y
AB Thyroglobulin-specifying mRNA (Tgb mRNA) content was studied in human thyroid tissues by using liq. hybridization and in-situ hybridization. Liq. hybridization revealed no differences in mRNA content, except in the case of colloid adenoma in which a lower amt. of Tgb mRNA was found. Conditions for the quant. in-situ hybridization of [3H]DNA complementary to the mRNA of Tgb are described. In-situ hybridization allowed correlation of the morphol. and functional state of the follicles and their content of Tgb mRNA.

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AN CA99(15):117128h
TI Nucleotide sequence of cloned complementary deoxyribonucleic acid for the .alpha. subunit of bovine pituitary glycoprotein hormones
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SO Biochemistry, 22(20), 4856-60
SC 3-4 (Biochemical Genetics)
SX 2, 13
DT J
CO BICHAW
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PY 1983
LA Eng
AN CA99(15):117128h
AB Recombinant DNA plasmids contg. sequences coding for the .alpha. subunit of the bovine pituitary glycoprotein hormones were isolated. The nucleotide sequences of 3 different cDNA clones were detd. The largest .alpha.-subunit cDNA clone contained 713 bases, which included 77 nucleotides from the 5'-untranslated region, 72 nucleotides coding for a precursor segment, 288 nucleotides coding for the mature .alpha. subunit, and 276 nucleotides from the 3'-untranslated region of the mRNA followed by a poly(A) segment. This cDNA likely represents most of the bovine .alpha.-subunit mRNA sequence. Nucleotide sequences were obtained from the cDNA inserts of 2 other .alpha.-subunit clones, and several differences among the 3 cDNA sequences were detected. These differences in nucleotide sequence might represent either individual variation in genomic sequence or cloning artifacts. The comparison of the bovine .alpha.-subunit cDNA sequence with those of human, rat, and mouse .alpha.-subunit cDNAs reveals that the bovine sequence has)70% homol. with the other cDNAs. The cloned .alpha.-subunit cDNA should provide a useful probe for further studies of the structure and expression of this interesting gene.

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