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(54) Title: FUCOSYL TRANSFERASES INVOLVED IN ADHESION MOLECULE EXPRESSION

(57) Abstract

We disclose DNA sequences designated clone 7.2 and clone 1, which cause cells transformed with them to express 1,3-fucosyl transferases and which are involved in CDX expression. We also disclose protein 7.2 and protein 1 which are encoded by clone 7.2 and clone 1, respectively. We also disclose Pseudo-X and Pseudo-X_2, proteins which cause COS cells and CHO cells to bind to ELAM1 and to be recognized by α-CDX antibodies. We also disclose methods of using these molecules.

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FUCOSYL TRANSFERASES INVOLVED IN ADHESION MOLECULE EXPRESSION

BACKGROUND OF THE INVENTION

This invention relates to the biology of cell adhesion and, in particular, to molecules that are involved in the expression of surface ligands, particularly CDX, a glycoprotein involved in leukocyte binding to the adhesion molecule, ELAM1.

Inflammation characteristically involves, among other things, the adhesion of leukocytes (white blood cells) to the endothelial wall of blood vessels and the infiltration of leukocytes into the surrounding tissues. (Harlan, 1985.) In normal inflammation, the infiltrating leukocytes phagocytize invading organisms or dead cells and play a role in tissue repair. However, in pathologic inflammation, infiltrating leukocytes can cause serious and sometimes deadly damage. (Hough and Sokoloff, 1985; Ross, 1986; Harlan, 1987 and Malech and Gallin, 1987.) Recognizing that leukocyte adhesion is a key step of much inflammation-related pathology, investigators have recently focused attention on the mechanism of leukocyte binding to the endothelial cell surface.

Cell adhesion is mediated by cell-surface molecules on both endothelial cells and leukocytes which act as receptor and ligand. (Harlan et al., 1987; Dana et al., 1986; and Bevilacqua et al., 1987a.)

The molecules on the endothelial cell surface that mediate lymphocyte binding are sometimes called endothelial cell-leukocyte adhesion molecules (ELAMs). (Bevilacqua et al., 1987b.) One ELAM in particular, ELAM1, appears to be a major mediator of PMN adhesion to the inflamed vascular wall in vivo. ELAM1 is a 116 kD cell surface glycoprotein. In HUVECs (human umbilical vein endothelial cells) grown in vitro, it is rapidly synthesized in response to the inflammatory cytokines IL-1 and TNF. (Bevilacqua et al., 1987b; Cotran et al., 1986 and Cotran and Pober, 1988.)

The adhesion of leukocytes to cells expressing ELAM1 suggests the existence on leukocytes of ELAM1 ligands. We reported, in PCT/US 90/02357 (incorporated herein by reference), the isolation of a molecule involved in leukocyte adhesion to endothelial cells (MILA) which is probably the (or an) ELAM1 ligand. The molecule, isolated from HL-60 cells and designated CDX, is a glycoprotein of 150 kD. We isolated CDX using a monoclonal antibody, SGB_3B_4, which we raised against it by immunizing mice with whole HL-60 cells. SGB_3B_4 inhibits the binding of PMNs and HL-60 cells to ELAM1-expressing cells. Furthermore, CDX is present on leukocyte cell types known to adhere to ELAM1 and is absent from leukocyte cell types and other cell types that do not adhere to ELAM1. Thus, CDX is a molecule expressed on certain leukocytes that plays an important role in ELAM1-mediated leukocyte-endothelial cell adhesion.

A growing body of evidence indicates that in addition to their role in inflammation, ELAMs may play important roles in a wide range of pathological states involving cell-cell recognition, including tumor invasion, metastasis and viral infection. (Harlan,

1985; Wallis and Harlan, 1986; Bevilacqua et al., 1987a; and Cotran and Pober, 1988.)

Thus, CDX and ELAM1 play important roles in inflammation and, perhaps, other pathologies. The isolation of molecules that contribute directly or indirectly to their expression will be an important step in the development of therapies aimed at preventing cell adhesion during inflammation or at limiting ELAM1 and CDX involvement in other pathological states.

SUMMARY OF THE INVENTION

This invention provides DNA sequences encoding molecules that cause several cell lines, including COS, CHO and R1.1, both to express surface glycoproteins that are recognized by anti-CDX (α-CDX) antibodies and to bind to ELAM1. This invention provides, in particular, clone 7.2 and clone 1, and protein 7.2 and protein 1, respectively. These proteins appear to be 1,3-fucosyl transferases.

This invention also provides the glycoproteins, Pseudo-X and Pseudo-X_2, which cause COS cells and CHO cells, respectively, to bind ELAM1 and to be recognized by α-CDX antibodies.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the sequence of cDNA coding for protein 7.2 and the deduced amino acid sequence of protein 7.2 derived from pSQ219 and CDX pCDM8 clone 7.2. The nucleotides are numbered 1-2175. In this application we refer to the coding DNA sequence of this figure as the DNA sequence for clone 7.2. We also refer to the polypeptide comprising the amino acid sequence depicted in this figure as protein 7.2.

Figure 2 depicts the sequence of cDNA coding for protein 1 derived from clone 1. The nucleotides are numbered 1-2861. In this application we refer to the coding DNA sequence of this figure as the DNA sequence for clone 1. We also refer to the polypeptide comprising the amino acid sequence depicted in this figure as protein 1.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with this detailed description, the following definitions apply:

Expression control sequence -- A DNA sequence that controls and regulates the transcription and translation of another DNA sequence.

Operatively linked -- A DNA sequence is operatively linked to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

Standard hybridization conditions -- salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. Under standard hybridization conditions the DNA sequences of this invention will hybridize to other DNA sequences having sufficient homology, including homologous sequences from different species. It is

understood that the stringency of hybridization conditions is a factor in the degree of homology required for hybridization.

DNA sequences -- The DNA sequences of this invention refer to DNA sequences prepared or isolated using recombinant DNA techniques. These include cDNA sequences, DNA sequences isolated from their native genome, and synthetic DNA sequences. The term as used in the claims is not intended to include naturally occurring DNA sequences as they exist in Nature.

Expression of recombinant DNA molecules according to this invention may involve post-translational modification of a resultant polypeptide by the host cell. For example, in mammalian cells expression might include, among other things, glycosylation, lipidation or phosphorylation of a polypeptide, or cleavage of a signal sequence to produce a "mature" protein. Accordingly, as used herein, the term "protein" encompasses full-length polypeptides and modifications or derivatives thereof, such as glycosylated versions of such polypeptides, mature proteins, polypeptides retaining a signal peptide, truncated polypeptides having comparable biological activity, and the like.

The molecules of the present invention are involved in the expression of the glycoprotein, CDX, on the surface of certain leukocytes. CDX appears on SDS-PAGE as a single, diffuse band of about 150 kD. A 90 kD protein band was sometimes observed in the bound proteins from HL-60 cells and always in the proteins from neutrophils. We believe this 90 kD band represents a CDX degradation product. We also sometimes observed higher molecular weight bands (i.e., around 170 kD). These may be non-specific bands. When the 150 kD CDX was treated with N-glycanase, the

molecular weight was reduced to approximately 70 kD. When the 150 kD band was treated with N-glycanase and O-glycanase, the molecular weight was not further reduced. Furthermore, when HL-60 cells are treated with sialidase, they lose the ability to bind ELAM1. These results indicate that CDX is a very heavily glycosylated protein and that the glycosylation plays an important role in CDX-ELAM1 interactions.

We have isolated two DNA sequences, clone 7.2 and clone 1, that appear to encode 1,3-fucosyl transferases that glycosylate the CDX polypeptide and impart to it the ability to bind ELAM1. 1,3-fucosyl transferases are highly specific enzymes that function in the Golgi apparatus and endoplasmic reticulum to attach fucosyl moieties to appropriate acceptor carbohydrates through a 1,3 glycosidic linkage. The genetic structure of these sequences is consistent with that of other, known glycosyl transferases. Furthermore, CHO cells transfected with clone 7.2 express fucosyl transferase activity.

Several 1,3-fucosyl transferases are known to the art. (Paulson and Colley, 1989 and Kukowska-Latallo et al., 1990) These proteins of similar activity share little sequence homology between themselves or other glycosyl transferases. (Paulson and Colley, 1989 and Kukowska-Latallo et al., 1990.) Therefore, we would not expect these DNA sequences to share homology with the DNA sequences of this invention. However, other species are likely to contain homologous genes that share significant sequence homology with the DNA sequences disclosed here. One can isolate these homologous genes using the DNA sequences of this invention as probes under standard hybridization conditions. This invention

specifically contemplates and encompasses such sequences.

When COS 7 cells were transfected with either of these two clones, they behaved like cells expressing CDX, that is, they became "visible" to ELAM1 in that they were able to produce a surface glycoprotein to which ELAM1 binds and which are recognized by the α-CDX monoclonal, SGB_3B_4. Using α-CDX monoclonals, we immunoprecipitated a 130 kD glycoprotein from transfected COS cells, which we have designated Pseudo-X. Similarly, CHO cells transfected with clone 7.2 also became visible to ELAM1 and α-CDX. They express a 140 kD glycoprotein which we have designated Pseudo-X_2.

Neither Pseudo-X nor Pseudo-X_2 are CDX. Pseudo-X has a molecular weight of about 130 kD and Pseudo-X_2, of 140 kD. CDX has a molecular weight of 150 kD. When treated with N-glycanase or hydrofluoric acid (which removes all carbohydrate), Pseudo-X shifts to 110 kD. Pseudo-X_2 shifts to approximately 120 kD. CDX shifts to about 70 kD. Neither migrates at 46 kD or 59 kD, the predicted molecular weights of protein 7.2 and protein 1. Pseudo-X and CDX also have different V8 and chymotrypsin digestion patterns.

We isolated clone 7.2 and clone 1 as follows: We created a cDNA library from mRNA of a human cell line, HL-60, that expresses CDX. We enriched this library by using subtraction techniques, as we describe below, with a human cell line that does not express CDX, in this case HeLa cells. This produced a subtracted library containing about 2100 clones. We transfected a monkey kidney cell line, COS 7, with the subtracted library which we assayed in a number of ways.

We incubated the transfected cells with the α-CDX monoclonal antibodies (Moabs) and panned them on plates coated with anti-mouse IgG or IgM (Wysocki and Sato, 1978); cells binding to the plates would be those expressing a molecule recognized by α-CDX Moabs. In this manner, we identified adherent cells transfected with a 2.1 kb DNA insert. We subcloned a portion of this sequence into a sequencing vector and designated it pSQ219. The DNA insert in the pCDM8 clone was designated clone 7.2. We also isolated a 2.9 kb insert by hybridization, which we designated clone 1. These two clones encode protein 7.2 and protein 1, respectively.

Another feature of this invention is the expression of the DNA sequences disclosed herein. As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host.

Such operative linking of a DNA sequence of this invention to an expression control sequence includes the provision of an initiation codon, ATG, in the correct reading frame upstream of the DNA sequence, if it is not already part of the DNA sequence or the expression vector.

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., E.coli plasmids col E1, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAs, e.g.,

the numerous derivatives of phage λ, e.g., NM989, and other phage DNA, e.g., M13 and Filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

Any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC or TRC system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase (e.g., Pho5), the promoters of the yeast α-mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of E.coli, Pseudomonas, Bacillus, Streptomyces, fungi such as yeasts, and animal cells, such as CHO, R1.1, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture.

It will be understood that not all vectors, expression control sequences and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.

In selecting a suitable expression control sequence, a variety of factors will normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular hosts will be selected by consideration of, e.g., their compatibility with the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products.

It will also be recognized that expression of the DNA sequences of the present invention may have different effects in different hosts. For example, whereas clone 7.2 expressed in COS cells leads to the appearance of an ELAM1-binding surface molecule, expression of clone 7.2 in, e.g., prokaryotic host

cells may have no similar effect, since prokaryotes lack internal cell structures (e.g., Golgi apparatus) that may be necessary for the biological functionality of protein 7.2. On the other hand, for isolation and purification of the clone 7.2 expression product intact, host cells in which protein 7.2 does not have a function in the cellular biochemistry (such as the catalytic role of a glycosyl transferase) may be preferred. The practitioner will be able to select the appropriate host cells and expression mechanisms for a particular purpose.

Considering these and other factors, a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the DNA sequences of this invention on fermentation or in large scale animal culture.

Several strategies are available for the isolation and purification of protein 7.2 and protein 1 after expression in a host system. One method involves expressing the proteins in bacterial cells, lysing the cells, and purifying the protein by conventional means. Alternatively, one can engineer the DNA sequences for secretion from cells. For example, Colley et al. (1989) describe purifying a sialyltransferase by engineering the cleavable signal peptide of human gamma-interferon onto the DNA sequence for the transferase. Larsen et al. (1990) fused the DNA sequence for protein A to the amino-terminal end of a fucosyl transferase gene and expressed it as an excreted fusion protein. In these constructions, one can optionally remove the transmembrane region of these proteins that exists near the amino-terminus. After secretion the proteins are purified from the medium. Similar strategies are available for bacteria.

Increasingly scientists are recognizing the value of enzymes as catalysts in organic synthesis. (Wong, 1989.) The 1,3-fucosyl transferases of this invention are useful for enzymatic synthesis of carbohydrates in vitro. Specifically, they are useful for catalyzing the linkage of fucose to appropriate acceptors through a 1,3 glycosidic bond. We describe one set of suitable conditions for this catalysis in Example I, relating to an assay for fucosyl transferase activity. One skilled in the art will recognize other suitable conditions under which the 1,3 fucosyl transferases described herein may be advantageously employed.

It is now clear that the carbohydrate moiety of CDX is important in ELAM1-mediated cell adhesion. A molecule comprising the carbohydrate moiety of CDX, Pseudo-X or Pseudo-X_2, or a fucose-containing portion of that moiety may be sufficient to function as an ELAM1 ligand. Such molecules may be useful in methods, including therapies, directed to inhibiting ELAM1-mediated cell adhesion.

This invention is also directed to small molecules that inhibit the activity of the 1,3-fucosyl transferases described herein, including synthetic organic chemicals, natural fermentation products, peptides, etc. These molecules may be useful in therapies aimed at inhibiting ELAM1-mediated cell adhesion. To identify such molecules, one produces a test mixture by contacting together an inhibitor candidate, a fucose acceptor and a 1,3-fucosyl transferase. The fucose acceptor is, preferably, LacNAc or 2'-fucosyllactose. The 1,3-fucosyl transferase preferably is derived from an extract from a cell transformed with clone 7.2 or clone 1. Then one

assays the test mixture for 1,3-fucosyl transferase activity, such as described in Example I.

In order that one may better understand this invention, we set forth the following examples. These examples are for purposes of illustration and are not to be construed as limiting the scope of the invention in any manner.

EXAMPLE I -- ISOLATION AND CHARACTERIZATION OF CLONE 7.2 AND CLONE 1

We prepared two cDNA libraries in the pCDM8 vector from two types of CDX-expressing cells, HL-60 cells and U937 cells. We isolated the mRNA from these cells and reverse-transcribed it into cDNA using techniques well known to the art. (Gubler and Hoffman, 1983.) Using standard procedures, we ligated double stranded cDNA to a NotI-BstXI linker/adaptor having the following sequence:

5' GCG GCC GCT TTA GAG CAC A 3'
3' CGC CGG CGA AAT CTC 5'

We then size-selected the cDNA on a 4.2 ml 5-20% potassium acetate gradient, 2 mM EDTA, 1 μg/ml ethidium bromide, in a BECKMAN SW60 Rotor for 3 hours at 50,000 rpm at 22°C according to the protocols of Brian Seed. (See also Maniatis, 1982, p. 278.) We pooled the cDNA fragments of greater than 500 base pairs. Then we prepared the vector, pCDM8 (a gift from Brian Seed). We digested this plasmid with BstXI. To remove the 400 base pair stuffer fragment we centrifuged the mixture on a potassium acetate gradient, as above, and isolated the large fragment. We further purified this fragment by agarose gel electrophoresis, and then ligated the cDNA to the vector.

We then prepared an enriched cDNA library by first creating a ^{32}P-labeled cDNA probe from 1 microgram of HL-60 poly A+ mRNA, then subtracting non-CDX related

cDNA sequences from the probe by hybridizing with 30 micrograms of poly A+ mRNA from HeLa cells, which do not express CDX. (See, Davis, 1986.) We used the subtracted probe to screen the pCDM8 cDNA library and thus created an enriched sublibrary from HL-60 cells in E.coli MC1061 P3. We grew about 2100 clones in twenty-two 96-well plates. A U937 enriched sublibrary was prepared in a similar manner, and 1400 clones were obtained.

We divided the colonies from our HL-60 enriched library into 22 pools for transfection of COS 7 cells by spheroplast fusion. (Sandri-Goldin et al. 1981.) We assayed transfected COS 7 cells for ELAM1-binding activity by panning with α-CDX monoclonal antibodies from hybridoma SGC_2E_5 (an antibody similar in function to SGB_3B_4) according to the method of Seed and Aruffo (1987). (See also Aruffo and Seed, 1987 and Wysocki and Sato, 1978). Pool #7 assayed positive, yielding two clones with a 2.1 kb cDNA insert. These were designated clones 7.1 and 7.2.

We obtained the DNA sequence of clone 7.2 by the Maxam and Gilbert technique (Maxim and Gilbert, 1980) from CDX pCDM8 clone 7.2 and from a portion of the 7.2 insert subcloned into the sequencing vector, pNN11. The latter plasmid was designated pSQ219. The DNA sequence obtained is set forth in Figure 1.

We deposited a culture containing the plasmid CDX pCDM8 clone 7.2 under the Budapest Treaty with In Vitro International, Inc., 611 P. Hammonds Ferry Rd., Linthicum, Md. 21090 (USA) on April 26, 1990. The deposit is identified as:

CDX pCDM8 / E. coli MC1061 P3

Accession Number IVI-10242

We also performed a Northern blot on mRNA from HL-60 cells and probed it with clone 7.2. Clone

7.2 hybridized to three mRNA species, two prominent bands at 6.0 kb and 2.4 kb and another band at 3.0 kb. Clone 7.2, a cDNA of 2.1 kb, is not large enough to be a full length cDNA from the 3.0 kb and 6.0 kb species. Therefore, in order to identify DNA sequences for these messages, we probed the enriched cDNA sublibrary from both U937 and HL-60 cells with an oligonucleotide derived from clone 7.2. We isolated several long inserts from the HL-60 library, transfected them into COS 7 cells, and selected clones that bound to ELAM1 and α-CDX. In this way we identified a 2.9 kb insert that could have come from the 3.0 kb message. We called it CDX clone 1.

We determined the DNA sequence of CDX clone 1 by the Maxam and Gilbert technique. The DNA sequence obtained is set forth in Figure 2.

We deposited a culture containing the plasmid CDX clone 1 under the Budapest Treaty with In Vitro International, Inc., 611 P. Hammonds Ferry Rd., Linthicum, Md. 21090 (USA) on October 11, 1990. The deposit is identified as:

CDX clone 1 pCDM8 / E. coli MC1061 P3
Accession Number IVI-10255.

We transfected clone 7.2 and clone 1 into COS 7 and CHO cells. At 48 hours after transfection these cells expressed a glycoprotein on their cell surfaces to which fluorescently labelled α-CDX antibodies bound, as assayed by FACS. These cell surface proteins could be labeled with ^{125}I and immunoprecipitated with α-CDX Moabs. We designated the protein isolated from COS 7 cells, Pseudo-X and from CHO cells, Pseudo-X_2. On SDS polyacrylamide gels, Pseudo-X and Pseudo-X_2 were approximately 130 kD and 140 kD, respectively.

The transfected COS cells also formed rosettes around Sepharose beads coated with recombinant

soluble ELAM1 (rsELAM1); and the rosetting was cation dependent and was inhibited by both BB11 (anti-ELAM1 antibody) and α-CDX. COS cells and CHO cells transfected with pCDM8 alone (without the inserted clone) did not rosette rsELAM1 beads. Also, the COS and CHO cells transfected with clone 7.2 did not rosette to beads coated with bovine serum albumin.

We further characterized clone 7.2 and clone 1 by DNA sequence analysis and enzyme assays. Clone 1 encodes a polypeptide of 530 amino acids (encoded by nucleotides 174-1763 of Figure 2). Clone 7.2 encodes a 405-amino acid polypeptide (encoded by nucleotides 66-1280 in Figure 1). Using UWGCG Sequence Analysis Software Package (version 6.1, Aug. 1989), we searched the NBRF Protein database (release 23, Dec. 1989) using the program FASTA for homology to other proteins. We also searched GenBank (release 63, Mar. 1990) and EMBL (release 19, May 1989) using TFASTA. In these searches we found short regions (e.g., about 23 amino acids) of homology to certain viral envelope proteins including Herpes simplex virus type 1, Dengue virus, yellow fever and other flaviviruses. In general the homology to known proteins was low, and we conclude that the polypeptides are novel.

The portion of the nucleotide sequence of clone 7.2 from nucleotide 9 to nucleotide 2162 (Figure 1) is identical to the portion of the sequence of clone 1 from nucleotide 492 to nucleotide 2645 (Figure 2). The first methionine of protein 7.2 corresponds to the methionine at amino acid 126 of protein 1. One explanation of this homology is that the two inserts represent different transcripts from the same DNA segment.

As we stated earlier, these clones do not code for CDX, Pseudo-X or Pseudo-X_2 -- the polypeptides they encode are not the correct size. Rather, the evidence strongly supports the conclusion that clone 7.2 and clone 1 encode 1,3-fucosyl transferases that glycosylate other proteins, such as CDX, Pseudo-X and Pseudo-X_2, in a way that makes them "visible" (i.e., recognized by or able to bind to) ELAM1 or α-CDX. First, the DNA sequences of clone 1 and clone 7.2 share several structural features with the DNA sequences of known glycosyl transferases. For example, genes encoding known glycosyl transferases commonly have consecutive methionine start sites and are capable of producing more than one mRNA transcript. As mentioned above, we have identified three mRNA transcripts that hybridize to clone 7.2, and clone 1 contains two codons that can serve as transcription start signals. Also, like known glycosyl transferases, the clones have multiple SP1 enhancer sites. The nucleotide sequences for these sites are GGGCGG or CCGCCC; clone 1 has five such sites. Also, like known glycosyl transferases, clones 7.2 and 1 are rich in guanine (G) and cytosine (C). For example, clone 1 is 75% GC rich in the 5' region of the gene and 60% GC rich in the 3' region of the gene. Glycosyl transferases in addition are typically class II membrane proteins, in which the membrane-spanning domain is near the amino terminus and the extracellular portion is near the carboxy terminus. Clone 1 and clone 7.2 encode a polypeptide having a hydrophobic region near the amino terminus. Glycosyl transferases also tend to have molecular weights between 40 kD to 60 kD; clone 1 encodes a polypeptide of about 59 kD and clone 7.2 encodes a polypeptide of about 46 kD. Finally, known glycosyl transferases

usually have one to three N-glycosylation sites; clone 1 and clone 7.2 both encode two such sites.

Second, enzyme assays performed on extracts from CHO cells transfected with clone 7.2 revealed the presence of fucosyl transferases not expressed in untransformed cells. The assays tested the ability of the enzyme to link radioactively labelled fucose to an acceptor molecule. We performed the assays as follows.

We prepared assay samples containing 10 μl enzyme, 8 μl cocktail and 2 μl 10X acceptor. We prepared the enzyme by isolating about 1.5 million CHO cells transfected with clone 7.2 and lysing them by sonication for 15 seconds in 150 μl ice-cold 1% Triton X-100 in water. The cocktail contained 75 μM ^{14}C-GDP fucose, 100 mM ATP, 500 mM L-fucose, 1 M $MnCl_2$ and 1 M cacodylate at pH 6.2. 10X acceptor contained, variously, 200 mM LacNAc, Lac-N-biose, or lactose, 250 mM phenyl-β-D-galactoside, or 50 mM 2'-fucosyllactose. We incubated the assay samples for 1 hour at 37°C. We stopped the reaction by addition of 20 μl ethanol. We diluted the sample with 560 μl water and centrifuged in an EPPENDORF centrifuge for 5 minutes at high speed.

We had prepared a DOWEX 1 X 2-400 column (Sigma Chemical Co.) to separate the unconverted ^{14}C fucose-GDP from the converted. We loaded the matrix into a large column and washed it with 10 volumes of 1N NaOH, followed by 5 volumes of water, followed by 10 volumes of 5% concentrated formic acid. Then we repeated this wash cycle. We used this material to create small columns of 0.4 ml. We prepared the small columns for use by washing them with 10 volumes of water.

We loaded 200 μl of the sample onto the small column, collected the eluate, rinsed with 2 ml water

and collected it into the eluate. We determined the radioactivity of this eluate by scintillation counting.

The results of this assay demonstrated that the induced enzyme is a 1,3-fucosyl transferase. (See Table 1.) The enzyme linked fucose to LacNAc, 2'-fucosyllactose and lactose, acceptors having GlcNAc or glucose moieties with free 3' hydroxyls. It did not link fucose to LacNBiose, whose GlcNAc moiety does not have a free 3' hydroxyl, or phenyl-β-D-galactoside, the negative control acceptor. Control samples from untransfected cells showed only insignificant linking of fucose to these acceptors.

Table 1 -- Efficiency of Fucosylation

Acceptor	picomoles mg Total Protein•hr
LacNAc	1110
Lac-N-Biose	76
2'-Fucosyllactose	151
Lactose	200
PhβDgal	Not detectable

[The enzyme was freshly produced from transfected CHO cells.]

Therefore, both genetic and enzymatic evidence indicate that clone 7.2 and clone 1 encode 1,3-fucosyl transferases.

While we have described herein a number of embodiments of this invention, it is apparent that one of skill in the art could alter our procedures to provide other embodiments that utilize the processes and compositions of this invention. Therefore, one will appreciate that the scope of this invention is to be defined by the claims appended hereto rather than by the specific embodiments that we have presented by way of example.

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CLAIMS:

1. A DNA sequence selected from the group consisting of:

(a) the DNA sequence of Figure 1 from nucleotide 66 to 1280;

(b) the DNA sequence of Figure 1 from nucleotide 69 to 1280;

(c) the DNA sequence of Figure 2 from nucleotide 172 to 1761;

(d) the DNA sequence of Figure 2 from nucleotide 175 to 1761;

(e) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions and have the biological activity of protein 7.2 or protein 1; and

(f) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

2. A recombinant DNA molecule comprising a DNA sequence selected from the group consisting of:

(a) the DNA sequence of Figure 1 from nucleotide 66 to 1280;

(b) the DNA sequence of Figure 1 from nucleotide 69 to 1280;

(c) the DNA sequence of Figure 2 from nucleotide 172 to 1761;

(d) the DNA sequence of Figure 2 from nucleotide 175 to 1761;

(e) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions and have the biological activity of protein 7.2 or protein 1 ; and

(f) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

3. The recombinant DNA molecule according to claim 2 wherein said DNA sequence is operatively linked to an expression control sequence.

4. A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence encoding an amino acid sequence of Figure 1 or Figure 2.

5. A unicellular host of claim 4 selected from the group consisting of E.coli, Pseudomonas, Bacillus, Streptomyces, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, BMT10, insect cells, plant cells, and human cells in tissue culture.

6. A protein produced by the method of expressing in a unicellular host a recombinant DNA molecule according to claim 2.

7. A process for producing a molecule that binds to ELAM1 comprising the step of expressing a DNA sequence encoding the amino acid sequence of Figure 1 or Figure 2 in a eukaryotic host cell.

8. A process for producing a cell that adheres to ELAM1 comprising the step of expressing a DNA sequence encoding the amino acid sequence of Figure 1 or Figure 2 in a eukaryotic host cell.

9. Pseudo-X or a fragment thereof capable of binding to α-CDX.

10. Pseudo-X_2 or a fragment thereof capable of binding to α-CDX.

11. A molecule capable of binding to ELAM1 comprising the carbohydrate moiety of a protein or a fucose-containing portion thereof wherein the protein is selected from the group consisting of CDX, Pseudo-X or Pseudo-X_2.

12. A molecule according to claim 11 wherein the protein is CDX.

13. A method of inhibiting adhesion between leukocytes and endothelial cells in a system containing them comprising the step of introducing in said system an effective amount of a molecule capable of binding to ELAM1, which molecule comprises a carbohydrate moiety of a protein or a fucose-containing portion thereof, wherein the protein is selected from the group consisting of CDX, Pseudo-X and Pseudo-X_2.

14. The method according to claim 13 wherein the protein is CDX.

15. A small molecule that inhibits the activity of the 1,3-fucosyl transferases described herein.

16. A method of identifying small molecules that inhibit the activity of the 1,3-fucosyl transferases described herein comprising the steps of:

(1) contacting together an inhibitor candidate, a fucose acceptor and a 1,3-fucosyl transferase to create a test mixture and

(2) assaying the test mixture for 1,3-fucosyl transferase activity.

17. The method according to claim 16 wherein the fucose acceptor is LacNAc.

18. The method according to claim 16 wherein the fucose acceptor 2'-fucosyllactose.

19. The method according to claim 16 wherein the 1,3-fucosyl transferase is derived from an extract from a cell transformed with clone 7.2 or clone 1.

20. The use of a 1,3-fucosyl transferase in a process for synthesizing an organic compound.

21. A method of producing a 1,3 glycosidic bond between fucose and a fucose acceptor comprising the step of catalysis with a 1,3-fucosyl transferase as described herein.

22. The method according to claim 21 in which the fucose acceptor is a carbohydrate.

23. Protein 7.2, protein 1, non-human homologues of protein 7.2 or protein 1 and biologically active fragments of any of the foregoing proteins.

AMENDED CLAIMS

[received by the International Bureau on 22 August 1991 (22.08.91); new claims 24 and 25 added; other claims unchanged (5 pages)]

1. A DNA sequence selected from the group consisting of:

(a) the DNA sequence of Figure 1 from nucleotide 66 to 1280;

(b) the DNA sequence of Figure 1 from nucleotide 69 to 1280;

(c) the DNA sequence of Figure 2 from nucleotide 172 to 1761;

(d) the DNA sequence of Figure 2 from nucleotide 175 to 1761;

(e) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions and have the biological activity of protein 7.2 or protein 1; and

(f) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

2. A recombinant DNA molecule comprising a DNA sequence selected from the group consisting of:

(a) the DNA sequence of Figure 1 from nucleotide 66 to 1280;

(b) the DNA sequence of Figure 1 from nucleotide 69 to 1280;

(c) the DNA sequence of Figure 2 from nucleotide 172 to 1761;

(d) the DNA sequence of Figure 2 from nucleotide 175 to 1761;

(e) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions and have the biological activity of protein 7.2 or protein 1; and

(f) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

3. The recombinant DNA molecule according to claim 2 wherein said DNA sequence is operatively linked to an expression control sequence.

4. A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence encoding an amino acid sequence of Figure 1 or Figure 2.

5. A unicellular host of claim 4 selected from the group consisting of E.coli, Pseudomonas, Bacillus, Streptomyces, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, BMT10, insect cells, plant cells, and human cells in tissue culture.

6. A protein produced by the method of expressing in a unicellular host a recombinant DNA molecule according to claim 2.

7. A process for producing a molecule that binds to ELAM1 comprising the step of expressing a DNA sequence encoding the amino acid sequence of Figure 1 or Figure 2 in a eukaryotic host cell.

8. A process for producing a cell that adheres to ELAM1 comprising the step of expressing a DNA sequence encoding the amino acid sequence of Figure 1 or Figure 2 in a eukaryotic host cell.

9. Pseudo-X or a fragment thereof capable of binding to α-CDX.

10. Pseudo-X_2 or a fragment thereof capable of binding to α-CDX.

11. A molecule capable of binding to ELAM1 comprising the carbohydrate moiety of a protein or a fucose-containing portion thereof wherein the protein is selected from the group consisting of CDX, Pseudo-X or Pseudo-X_2.

12. A molecule according to claim 11 wherein the protein is CDX.

13. A method of inhibiting adhesion between leukocytes and endothelial cells in a system containing them comprising the step of introducing in said system an effective amount of a molecule capable of binding to ELAM1, which molecule comprises a carbohydrate moiety of a protein or a fucose-containing portion thereof, wherein the protein is selected from the group consisting of CDX, Pseudo-X and Pseudo-X_2.

14. The method according to claim 13 wherein the protein is CDX.

15. A small molecule that inhibits the activity of the 1,3-fucosyl transferases described herein.

16. A method of identifying small molecules that inhibit the activity of the 1,3-fucosyl transferases described herein comprising the steps of:

(a) contacting together an inhibitor candidate, a fucose acceptor and a 1,3-fucosyl transferase to create a test mixture and

(b) assaying the test mixture for 1,3-fucosyl transferase activity.

17. The method according to claim 16 wherein the fucose acceptor is LacNAc.

18. The method according to claim 16 wherein the fucose acceptor 2'-fucosyllactose.

19. The method according to claim 16 wherein the 1,3-fucosyl transferase is derived from an extract from a cell transformed with clone 7.2 or clone 1.

20. The use of a 1,3-fucosyl transferase in a process for synthesizing an organic compound.

21. A method of producing a 1,3 glycosidic bond between fucose and a fucose acceptor comprising the step of catalysis with a 1,3-fucosyl transferase as described herein.

22. The method according to claim 21 in which the fucose acceptor is a carbohydrate.

23. Protein 7.2, protein 1, non-human homologues of protein 7.2 or protein 1 and biologically active fragments of any of the foregoing proteins.

24. A method for identifying molecules that inhibit the binding of leukocytes to endothelial cells comprising the steps of:

(a) contacting a molecule with ELAM1 or with ELAM1-expressing cells to create a first mixture;

(b) contacting said first mixture with cells transfected with clone 7.2 or clone 1 or with an ELAM1-

binding molecule expressed by cells transfected with clone 7.2 or clone 1 to create a second mixture; and

(c) testing said second mixture for the amount of said ELAM1 or ELAM1-expressing cells bound to said transfected cells or ELAM1-binding molecule.

25. A method for identifying molecules that inhibit the binding of leukocytes to endothelial cells comprising the steps of:

(a) contacting a molecule with cells transfected with clone 7.2 or clone 1 or with an ELAM1-binding molecule expressed by cells transfected with clone 7.2 or clone 1 to create a first mixture;

(b) contacting said first mixture with an ELAM1 or with ELAM1-expressing cells to create a second mixture; and

(c) testing said second mixture for the amount of said transfected cells or ELAM1-binding molecule bound to said ELAM1 or ELAM1-expressing cells.

FIG. IA

```
   1  GCGGCCGCCGCTCCTCCACGCCTGCGGACGCGTGGCGAGCGGAGGCAGCG                50
  51  CTGCCTGTTCGCGCCATGGGGGCACCGTGGGGCTCGCCGACGGCGGCGGC               100
                    MetGlyAlaProTrpGlySerProThrAlaAlaAl
 101  GGGCGGGCGGCGCGGGTGGCGCCGAGGCCGGGGGCTGCCATGGACCGTCT               150
      aGlyGlyArgArgGlyTrpArgArgGlyArgGlyLeuProTrpThrValC
 151  GTGTGCTGGCGGCCGCCGGCTTGACGTGTACGGCGCTGATCACCTACGCT               200
      ysValLeuAlaAlaAlaGlyLeuThrCysThrAlaLeuIleThrTyrAla
 201  TGCTGGGGGCAGCTGCCGCCGCTGCCCTGGGCGTCGCCAACCCCGTCGCG               250
      CysTrpGlyGlnLeuProProLeuProTrpAlaSerProThrProSerAr
 251  ACCGGTGGGCGTGCTGCTGTGGTGGGAGCCCTTCGGGGGGCGCGATAGCG               300
      gProValGlyValLeuLeuTrpTrpGluProPheGlyGlyArgAspSerA
 301  CCCCGAGGCCGCCCCCTGACTGCCGGCTGCGCTTCAACATCAGCGGCTGC               350
      laProArgProProProAspCysArgLeuArgPheAsnIleSerGlyCys
 351  CGCCTGCTCACCGACCGCGCGTCCTACGGAGAGGCTCAGGCCGTGCTTTT               400
      ArgLeuLeuThrAspArgAlaSerTyrGlyGluAlaGlnAlaValLeuPh
 401  CCACCACCGCGACCTCGTGAAGGGGCCCCCCGACTGGCCCCCGCCCTGGG               450
      eHisHisArgAspLeuValLysGlyProProAspTrpProProProTrpG
 451  GCATCCAGGCGCACACTGCCGAGGAGGTGGATCTGCGCGTGTTGGACTAC               500
      lyIleGlnAlaHisThrAlaGluGluValAspLeuArgValLeuAspTyr
 501  GAGGAGGCAGCGGCGGCGGCAGAAGCCCTGGCGACCTCCAGCCCCAGGCC               550
      GluGluAlaAlaAlaAlaAlaGluAlaLeuAlaThrSerSerProArgPr
 551  CCCGGGCCAGCGCTGGGTTTGGATGAACTTCGAGTCGCCCTCGCACTCCC               600
      oProGlyGlnArgTrpValTrpMetAsnPheGluSerProSerHisSerP
 601  CGGGGCTGCGAAGCCTGGCAAGTAACCTCTTCAACTGGACGCTCTCCTAC               650
      roGlyLeuArgSerLeuAlaSerAsnLeuPheAsnTrpThrLeuSerTyr
 651  CGGGCGGACTCGGACGTCTTTGTGCCTTATGGCTACCTCTACCCCAGAAG               700
      ArgAlaAspSerAspValPheValProTyrGlyTyrLeuTyrProArgSe
 701  CCACCCCGGCGACCCGCCCTCAGGCCTGGCCCCGCCACTGTCCAGGAAAC               750
      rHisProGlyAspProProSerGlyLeuAlaProProLeuSerArgLysG
 751  AGGGGCTGGTGGCATGGGTGGTGAGCCACTGGGACGAGCGCCAGGCCCGG               800
      lnGlyLeuValAlaTrpValValSerHisTrpAspGluArgGlnAlaArg
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FIG. IB

```
 801  GTCCGCTACTACCACCAACTGAGCCAACATGTGACCGTGGACGTGTTCGG   850
      ValArgTyrTyrHisGlnLeuSerGlnHisValThrValAspValPheGl

 851  CCGGGGCGGGCCGGGGCAGCCGGTGCCCGAAATTGGGCTCCTGCACACAG   900
      yArgGlyGlyProGlyGlnProValProGluIleGlyLeuLeuHisThrV

 901  TGGCCCGCTACAAGTTCTACCTGGCTTTCGAGAACTCGCAGCACCTGGAT   950
      alAlaArgTyrLysPheTyrLeuAlaPheGluAsnSerGlnHisLeuAsp

 951  TATATCACCGAGAAGCTCTGGCGCAACGCGTTGCTCGCTGGGGCGGTGCC  1000
      TyrIleThrGluLysLeuTrpArgAsnAlaLeuLeuAlaGlyAlaValPr

1001  GGTGGTGCTGGGCCCAGACCGTGCCAACTACGAGCGCTTTGTGCCCCGCG  1050
      oValValLeuGlyProAspArgAlaAsnTyrGluArgPheValProArgG

1051  GCGCCTTCATCCACGTGGACGACTTCCCAAGTGCCTCCTCCCTGGCCTCG  1100
      lyAlaPheIleHisValAspAspPheProSerAlaSerSerLeuAlaSer

1101  TACCTGCTTTTCCTCGACCGCAACCCCGCGGTCTATCGCCGCTACTTCCA  1150
      TyrLeuLeuPheLeuAspArgAsnProAlaValTyrArgArgTyrPheHi

1151  CTGGCGCCGGAGCTACGCTGTCCACATCACCTCCTTCTGGGACGAGCCTT  1200
      sTrpArgArgSerTyrAlaValHisIleThrSerPheTrpAspGluProT

1201  GGTGCCGGGTGTGCCAGGCTGTACAGAGGGCTGGGGACCGGCCCAAGAGC  1250
      rpCysArgValCysGlnAlaValGlnArgAlaGlyAspArgProLysSer

1251  ATACGGAACTTGGCCAGCTGGTTCGAGCGGTGAAGCCGCGCTCCCCTGGA  1300
      IleArgAsnLeuAlaSerTrpPheGluArg

1301  AGCGACCCAGGGGAGGCCAAGTTGTCAGCTTTTTGATCCTCTACTGTGCA  1350

1351  TCTCCTTGACTGCCGCATCATGGGAGTAAGTTCTTCAAACACCCATTTTT  1400

1401  GCTCTATGGGAAAAAAACGATTTACCAATTAATATTACTCAGCACAGAGA  1450

1451  TGGGGGCCCGGTTTCCATATTTTTTGCACAGCTAGCAATTGGGCTCCCTT  1500

1501  TGCTGCTGATGGGCATCATTGTTTAGGGGTGAAGGAGGGGGTTCTTCCTC  1550

1551  ACCTTGTAACCAGTGCAGAAATGAAATAGCTTAGCGGCAAGAAGCCGTTG  1600

1601  AGGCGGTTTCCTGAATTTCCCCATCTGCCACAGGCCATATTTGTGGCCCG  1650

1651  TGCAGCTTCCAAATCTCATACACAACTGTTCCCGATTCACGTTTTTCTGG  1700

1701  ACCAAGGTGAAGCAAATTTGTGGTTGTAGAAGGAGCCTTGTTGGTGGAGA  1750
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FIG. IC

```
1751  GTGGAAGGACTGTGGCTGCAGGTGGGACTTTGTTGTTTGGATTCCTCACA  1800
1801  GCCTTGGCTCCTGAGAAAGGTGAGGAGGGCAGTCCAAGAGGGGCCGCTGA  1850
1851  CTTCTTTCACAAGTACTATCTGTTCCCCTGTCCTGTGAATGGAAGCAAAG  1900
1901  TGCTGGATTGTCCTTGGAGGAAACTTAAGATGAATACATGCGTGTACCTC  1950
1951  ACTTTACATAAGAAATGTATTCCTGAAAAGCTGCATTTAAATCAAGTCCC  2000
2001  AAATTCATTGACTTAGGGGAGTTCAGTATTTAATGAAACCCTATGGAGAA  2050
2051  TTTATCCCTTTACAATGTGAATAGTCATCTCCTAATTTGTTTCTTCTGTC  2100
2101  TTTATGTTTTTCTATAACCTGGATTTTTTAAATCATATTAAAATTACAGA  2150
2151  TGTGAAAATAAAAAAAAGCGGCCGC  2175
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FIG. 2A

```
   1  CTGCTCCTGCGCGGCAGCTGCTTTAGAAGGTCTCGAGCCTCCTGTACCTT   50
  51  CCCAGGGATGAACCGGGCCTTCCCTCTGGAAGGCGAGGGTTCGGGCCACA  100
 101  GTGAGCGAGGGCCAGGGCGGTGGGCGCGCGCAGAGGGAAACCGGATCAGT  150
 151  TGAGAGAGAATCAAGAGTAGCGGATGAGGCGCTTGTGGGGCGCGGCCCGG  200
                                   MetArgArgLeuTrpGlyAlaAlaArg
 201  AAGCCCTCGGGCGCGGGCTGGGAGAAGGAGTGGGCGGAGGCGCCGCAGGA  250
      LysProSerGlyAlaGlyTrpGluLysGluTrpAlaGluAlaProGlnGl
 251  GGCTCCCGGGGCCTGGTCGGGCCGGCTGGGCCCCGGGCGCAGTGGAAGAA  300
      uAlaProGlyAlaTrpSerGlyArgLeuGlyProGlyArgSerGlyArgL
 301  AGGGACGGGCGGTGCCCGGTTGGGCGTCCTGGCCAGCTCACCTTGCCCTG  350
      ysGlyArgAlaValProGlyTrpAlaSerTrpProAlaHisLeuAlaLeu
 351  GCGGCTCGCCCCGCCCGGCACTTGGGAGGAGCAGGGCAGGGCCCGCGGCC  400
      AlaAlaArgProAlaArgHisLeuGlyGlyAlaGlyGlnGlyProArgPr
 401  TTTGCATTCTGGGACCGCCCCCTTCCATTCCCGGGCCAGCGGCGAGCGGC  450
      oLeuHisSerGlyThrAlaProPheHisSerArgAlaSerGlyGluArgG
 451  AGCGACGGCTGGAGCCGCAGCTACAGCATGAGAGCCGGTGCCGCTCCTCC  500
      lnArgArgLeuGluProGlnLeuGlnHisGluSerArgCysArgSerSer
 501  ACGCCTGCGGACGCGTGGCGAGCGGAGGCAGCGCTGCCTGTTCGCGCCAT  550
      ThrProAlaAspAlaTrpArgAlaGluAlaAlaLeuProValArgAlaMe
 551  GGGGGCACCGTGGGGCTCGCCGACGGCGGCGGCGGGCGGGCGGCGCGGGT  600
      tGlyAlaProTrpGlySerProThrAlaAlaAlaGlyGlyArgArgGlyT
 601  GGCGCCGAGGCCGGGGGCTGCCATGGACCGTCTGTGTGCTGGCGGCCGCC  650
      rpArgArgGlyArgGlyLeuProTrpThrValCysValLeuAlaAlaAla
 651  GGCTTGACGTGTACGGCGCTGATCACCTACGCTTGCTGGGGGCAGCTGCC  700
      GlyLeuThrCysThrAlaLeuIleThrTyrAlaCysTrpGlyGlnLeuPr
 701  GCCGCTGCCCTGGGCGTCGCCAACCCCGTCGCGACCGGTGGGCGTGCTGC  750
      oProLeuProTrpAlaSerProThrProSerArgProValGlyValLeuL
 751  TGTGGTGGGAGCCCTTCGGGGGGCGCGATAGCGCCCCGAGGCCGCCCCCT  800
      euTrpTrpGluProPheGlyGlyArgAspSerAlaProArgProProPro
```

FIG. 2B

```
 801  GACTGCCGGCTGCGCTTCAACATCAGCGGCTGCCGCCTGCTCACCGACCG   850
      AspCysArgLeuArgPheAsnIleSerGlyCysArgLeuLeuThrAspAr

 851  CGCGTCCTACGGAGAGGCTCAGGCCGTGCTTTTCCACCACCGCGACCTCG   900
      gAlaSerTyrGlyGluAlaGlnAlaValLeuPheHisHisArgAspLeuV

 901  TGAAGGGGCCCCCCGACTGGCCCCCGCCCTGGGGCATCCAGGCGCACACT   950
      alLysGlyProProAspTrpProProProTrpGlyIleGlnAlaHisThr

 951  GCCGAGGAGGTGGATCTGCGCGTGTTGGACTACGAGGAGGCAGCGGCGGC  1000
      AlaGluGluValAspLeuArgValLeuAspTyrGluGluAlaAlaAlaAl

1001  GGCAGAAGCCCTGGCGACCTCCAGCCCCAGGCCCCCGGGCCAGCGCTGGG  1050
      aAlaGluAlaLeuAlaThrSerSerProArgProProGlyGlnArgTrpV

1051  TTTGGATGAACTTCGAGTCGCCCTCGCACTCCCCGGGGCTGCGAAGCCTG  1100
      alTrpMetAsnPheGluSerProSerHisSerProGlyLeuArgSerLeu

1101  GCAAGTAACCTCTTCAACTGGACGCTCTCCTACCGGGCGGACTCGGACGT  1150
      AlaSerAsnLeuPheAsnTrpThrLeuSerTyrArgAlaAspSerAspVa

1151  CTTTGTGCCTTATGGCTACCTCTACCCCAGAAGCCACCCCGGCGACCCGC  1200
      lPheValProTyrGlyTyrLeuTyrProArgSerHisProGlyAspProP

1201  CCTCAGGCCTGGCCCCGCCACTGTCCAGGAAACAGGGGCTGGTGGCATGG  1250
      roSerGlyLeuAlaProProLeuSerArgLysGlnGlyLeuValAlaTrp

1251  GTGGTGAGCCACTGGGACGAGCGCCAGGCCCGGGTCCGCTACTACCACCA  1300
      ValValSerHisTrpAspGluArgGlnAlaArgValArgTyrTyrHisGl

1301  ACTGAGCCAACATGTGACCGTGGACGTGTTCGGCCGGGGCGGGCCGGGGC  1350
      nLeuSerGlnHisValThrValAspValPheGlyArgGlyGlyProGlyG

1351  AGCCGGTGCCCGAAATTGGGCTCCTGCACACAGTGGCCCGCTACAAGTTC  1400
      lnProValProGluIleGlyLeuLeuHisThrValAlaArgTyrLysPhe

1401  TACCTGGCTTTCGAGAACTCGCAGCACCTGGATTATATCACCGAGAAGCT  1450
      TyrLeuAlaPheGluAsnSerGlnHisLeuAspTyrIleThrGluLysLe

1451  CTGGCGCAACGCGTTGCTCGCTGGGGCGGTGCCGGTGGTGCTGGGCCCAG  1500
      uTrpArgAsnAlaLeuLeuAlaGlyAlaValProValValLeuGlyProA

1501  ACCGTGCCAACTACGAGCGCTTTGTGCCCCGCGGCGCCTTCATCCACGTG  1550
      spArgAlaAsnTyrGluArgPheValProArgGlyAlaPheIleHisVal

1551  GACGACTTCCCAAGTGCCTCCTCCCTGGCCTCGTACCTGCTTTTCCTCGA  1600
      AspAspPheProSerAlaSerSerLeuAlaSerTyrLeuLeuPheLeuAs
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FIG. 2C

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1601  CCGCAACCCCGCGGTCTATCGCCGCTACTTCCACTGGCGCCGGAGCTACG  1650
      pArgAsnProAlaValTyrArgArgTyrPheHisTrpArgArgSerTyrA
1651  CTGTCCACATCACCTCCTTCTGGGACGAGCCTTGGTGCCGGGTGTGCCAG  1700
      laValHisIleThrSerPheTrpAspGluProTrpCysArgValCysGln
1701  GCTGTACAGAGGGCTGGGGACCGGCCCAAGAGCATACGGAACTTGGCCAG  1750
      AlaValGlnArgAlaGlyAspArgProLysSerIleArgAsnLeuAlaSe
1751  CTGGTTCGAGCGGTGAAGCCGCGCTCCCCTGGAAGCGACCCAGGGGAGGC  1800
      rTrpPheGluArg
1801  CAAGTTGTCAGCTTTTTGATCCTCTACTGTGCATCTCCTTGACTGCCGCA  1850
1851  TCATGGGAGTAAGTTCTTCAAACACCCATTTTTGCTCTATGGGAAAAAAA  1900
1901  CGATTTACCAATTAATATTACTCAGCACAGAGATGGGGGCCCGGTTTCCA  1950
1951  TATTTTTTGCACAGCTAGCAATTGGGCTCCCTTTGCTGCTGATGGGCATC  2000
2001  ATTGTTTAGGGGTGAAGGAGGGGGTTCTTCCTCACCTTGTAACCAGTGCA  2050
2051  GAAATGAAATAGCTTAGCGGCAAGAAGCCGTTGAGGCGGTTTCCTGAATT  2100
2101  TCCCCATCTGCCACAGGCCATATTTGTGGCCCGTGCAGCTTCCAAATCTC  2150
2151  ATACACAACTGTTCCCGATTCACGTTTTTCTGGACCAAGGTGAAGCAAAT  2200
2201  TTGTGGTTGTAGAAGGAGCCTTGTTGGTGGAGAGTGGAAGGACTGTGGCT  2250
2251  GCAGGTGGGACTTTGTTGTTTGGATTCCTCACAGCCTTGGCTCCTGAGAA  2300
2301  AGGTGAGGAGGGCAGTCCAAGAGGGGCCGCTGACTTCTTTCACAAGTACT  2350
2351  ATCTGTTCCCCTGTCCTGTGAATGGAAGCAAAGTGCTGGATTGTCCTTGG  2400
2401  AGGAAACTTAAGATGAATACATGCGTGTACCTCACTTTACATAAGAAATG  2450
2451  TATTCCTGAAAAGCTGCATTTAAATCAAGTCCCAAATTCATTGACTTAGG  2500
2501  GGAGTTCAGTATTTAATGAAACCCTATGGAGAATTTATCCCTTTACAATG  2550
2551  TGAATAGTCATCTCCTAATTTGTTTCTTCTGTCTTTATGTTTTTCTATAA  2600
2601  CCTGGATTTTTTAAATCATATTAAAATTACAGATGTGAAAATAAAGCAGA  2650
2651  AGCAACCTTTTTCCCTCTTCCCAGAAAACCAGTCTGTGTTTACAGACAGA  2700
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FIG. 2D

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2701  AGAGAAGGAAGCCATAGTGTCACTTCCACACAATTATTTATTTCATGTCT  2750
2751  TTACTGGACCTGAAATTTAAACTGCAATGCCAGTCCTGCAGGAGTGCTGG  2800
2801  CATTACCCTCTGCAGAACAGTGAAAGGTATTGCACTACATTATGGAATCA  2850
2851  TGCAAAAAAAA  2861
```

ANNEX M3

International Application No: PCT/US90 / 06198

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 15, line 22 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2] CDX clone 1 pCDM8 / E. coli MC1061 P3

Further deposits are identified on an additional sheet ☐ [3]

Name of depositary institution [4]

In Vitro International, Inc.

Address of depositary institution (including postal code and country) [4]

611 P. Hammonds Ferry Road
Linthicum, Maryland 21090 (USA)

Date of deposit [5]	**Accession Number** [6]
October 11, 1990	IVI-10255

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of those designations in which a European patent is sought samples of the deposited microorganisms will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

EPO

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

was 27 AUGUST 1991

(Authorized Officer)

(January 1985)

ANNEX M3

International Application No: PCT/ US90 / 06198

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 14, line 32 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2] CDX pCDM8 / E. coli MC1061 P3

Further deposits are identified on an additional sheet ☐ [3]

Name of depositary institution [4]

In Vitro International, Inc.

Address of depositary institution (including postal code and country) [4]

611 P. Hammonds Ferry Road
Linthicum, Maryland 21090 (USA)

Date of deposit [5]	Accession Number [6]
April 26, 1990	IVI-10242

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of those designations in which a European patent is sought samples of the deposited microorganisms will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

EPO

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

was 27 AUGUST 1991

(Authorized Officer)

(January 1985)

INTERNATIONAL SEARCH REPORT

International Applica. No. PCT/US90/06198

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5):A61K 31/70;C07H 17/00;C07K 15/14;C12N 1/21,1/19,5/04,5/06,5/08,5/10,9/10 see attachment.

U.S.CL.:435/15,69.1,97,172.3,193,240.2,240.4;435/243,252.5,252.8,253.3,253.5,255;see attachment.

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/15,69.1,97,172.3, 193, 240.2, 240.4, 435/243,252.5, 252.8, 253.3, 253.5, 255, 320.1; 514/8, 25; 536/27 530/395; 935/14

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

GenBank and UEMBL search of nucleic acid sequences recited in claim 1, parts (a)-(d). CAS Online searches 1) fucosyltransferase # and cDX 2) fucosyltransferase # and(cDNA or clon?) 3) fucosyltransferase # and inhibit

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
E,X	Cell, Vol. 63, No. 3, issued 02 November 1990, J.B. LOWE ET AL., "ELAM-1 dependent cell adhesion to a vascular endothelium determined by a transfected human fucosyl-transferase cDNA", pages 475-484. See Summary.	1-8 & 23
X	The Journal of Biological Chemistry, vol. 264, no. 30, issued 25 October 1989, J.C. PAULSON ET AL., "Glyco syltransferases". See Table I and pages 17618, second full paragraph	20-22

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

15 March 1991

Date of Mailing of this International Search Report

24 APR 1991

International Searching Authority

ISA/US

Signature of Authorized Officer

David A. Saunders ebw

Form PCT/ISA/210 (second sheet) (July 1989)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X Y	The Journal of Biological Chemistry, vol. 265, no. 3, issued 25 January 1990, B. POTVIN ET AL, Transfection of a Human α-(1,3) Fucosyltransferase Gene into Chinese Hamster Ovary Cells, pages 1615-1622. See Abstract; paragraph spanning cols. 1 and 2 of page 1615; and page 1616, col. 2, last full par.	1-8,20-23 15-19
Y	The Journal of Biological Chemistry, vol. 264, no. 29, issued 15 October 1989, M.M. PALCIC ET AL., "A Bisubstrate Analoge Inhibitor for α(1-2)-Fucosyltransferase", pages 17174-17181. See page 17174 cols. 1 and 2; page 17177, col. 2; and page 17178, col. 2.	15-19