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Cloning, Expression, and Chromosome Mapping of Human Galectin-7*

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The galectins are a family of β-galactoside-binding proteins implicated in modulating cell-cell and cell-matrix interactions. Here we report the cloning and expression of a novel member of this family (galectin-7) that correspond to IEF (isoelectric focusing) 17 (12,700 Da; pI, 7.6) in the human keratinocyte protein data base, and that is strikingly down-regulated in SV40 transformed keratinocytes (K14). The cDNA was cloned from a λgt11 cDNA expression library using degenerated oligodeoxyribonucleotides back-translated from an IEF 17 peptide sequence. The protein encoded by the galectin-7 clone comigrated with IEF 17 as determined by two-dimensional (two-dimensional gel electrophoresis) analysis of proteins expressed by transiently transfected COS-1 cells, and bound lactose. Alignment of the amino acid sequences with other members of the family showed that the amino acids central to the β-galactoside interaction are conserved. Galectin-7 was partially externalized to the medium by keratinocytes although it has no typical secretion signal peptide. Immunoblotting as well as immunofluorescence analysis of human tissues with a specific galectin-7 antibody revealed a narrow distribution of the protein which was found mainly in stratified squamous epithelium. The antigen localized to basal keratinocytes, although it was also found, albeit at lower levels, in the suprabasal layers where it concentrated to areas of cell to cell contact. Both, its cellular localization as well as its striking down-regulation in K14 keratinocytes imply a role in cell-cell and/or cell-matrix interactions necessary for normal growth control. The galectin-7 gene was mapped to chromosome 19.

The galectins belong to a family of related β-galactoside-binding lectins also referred to as S-type or S-Lac lectins (Barondes *et al.*, 1994a, 1994b). Members of this family have been implicated in a variety of functions that include growth regulation (Wells and Mallucci, 1991), cell adhesion (Cooper *et al.*, 1991; Zhou and Cummings, 1993; Gu *et al.*, 1994), migration (Hughes 1992), neoplastic transformation (Raz *et al.*, 1990), and immune responses (Offner *et al.*, 1990; Cherayil *et al.*, 1990; Liu 1993). To date, four members of the family have been well characterized (Barondes *et al.*, 1994a, 1994b). These include: galectin-1, also known as galaptin, L-14-I, L-14, and BHL, a homodimer with subunit molecular mass of 14,500 which is abundant in smooth and skeletal muscle although it is also found in many other cell types (Couraud *et al.*, 1989); galectin-2, a homodimer with a subunit molecular mass of 14,650 originally described in a hepatoma (Gitt *at al.*, 1992); galectin-3, also known as Mac-2, EPB, CBP-35, CBP-30, and L-29, a monomer with an apparent molecular mass between 26,320 and 30,300 that is abundant in activated macrophages and epithelial cells (Cherayil *et al.*, 1990); and galectin-4, a monomer with a molecular mass of 36,300 containing two carbohydrate-binding domains within a single polypeptide chain (Oda *et al.*, 1993).

In our laboratory we have systematically searched for keratinocyte proteins whose levels are differentially regulated in transformed cells and that may play a role in the maintenance of the normal phenotype. In particular, abundant proteins that are strongly down-regulated in the transformed cells are expected to play a role in the cytoskeleton and cell-cell interactions as it is well known that these cells show altered morphology, are less adherent to neighboring cells, and usually reorganize the extracellular matrix (Bar-Sagi and Feramisco, 1985; Greig *et al.*, 1985; Kamps *et al.*, 1985; Liotta *et al.*, 1991; Birkedal-Hansen *et al.*, 1993; Ponta *et al.*, 1994). Here we report the cloning, expression, and chromosome mapping of an abundant keratinocyte protein whose expression is abrogated in SV40 transformed keratinocytes (Taylor-Papadimitriou *et al.*, 1982) and that corresponds to a new member of the galectin family. The monomeric β-galactoside-binding protein, which has been termed galectin-7, exhibit a very narrow tissue distribution and may play a role in cell-cell and/or cell-matrix interactions necessary for normal growth control.

MATERIALS AND METHODS

Normal Primary Human Keratinocytes—Normal unfractionated keratinocytes prepared as described by Madsen *et al.* (1992) were plated in 35-mm culture dishes (coated with human dermal extract) that contained 3 ml of complete serum-free keratinocyte medium (Life Technologies, Inc.). Keratinocytes were fed with fresh medium every day and loosely attached cells were eliminated by pipetting the medium up and down with the aid of an automatic pipette. Cells were labeled with a mixture of ^{14}C-labeled amino acids on the fifth day. K14[1] cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L07769.

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[1] The abbreviations used are: K14, SV40 transformed keratinocytes; IEF, isoelectric focusing; PBS, phosphate-buffered saline.

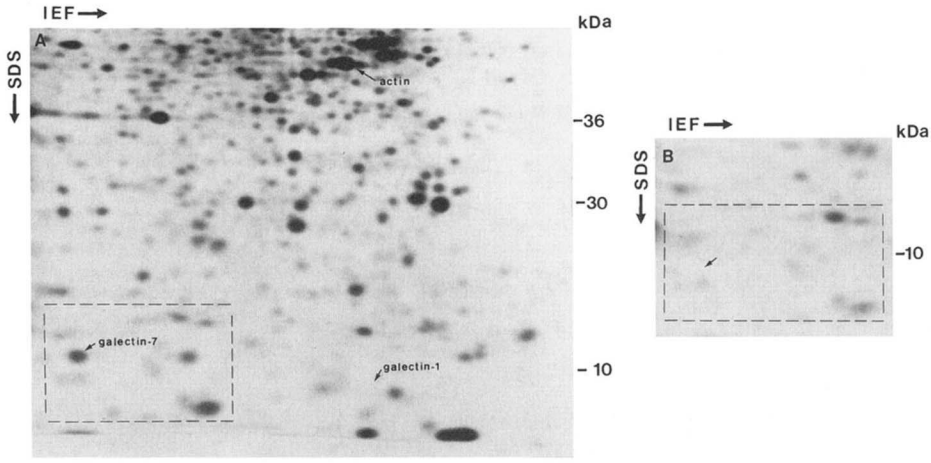

FIG. 1. **Identification of IEF 17 in IEF two-dimensional gels of keratinocyte proteins.** *A*, cellular proteins from primary normal keratinocytes labeled with a mixture of 16 ^{14}C-amino acids. *B*, cellular proteins from K14 keratinocytes labeled with a mixture of 16 ^{14}C-amino acids. Only a fraction of the gels are shown.

FIG. 2. **Nucleotide and corresponding amino acid sequence of the cDNA clone coding for IEF 17.** The cDNA, which contains 483 base pairs, codes for a protein of 136 amino acids starting at nucleotide position 19 and ending with a termination codon at position 427. The protein has a molecular mass of 15,073 daltons and a calculated pI of 7.73. These values are in close agreement with the parameters observed by two-dimensional gel electrophoresis (Celis *et al.*, 1992, 1993). The four partial peptide sequences obtained by microsequencing are *underlined*.

```
1                    M  S  N  V  P  H  K  S  S  L  P  E  G  I          14
1   ACCAACCCGGTCCCAGCCATGTCCAACGTCCCCCACAAGTCCTCGCTGCCCGAGGGCATC       60

15  R  P  G  T  V  L  R  I  R  G  L  V  P  P  N  A  S  R  F  H        34
61  CGCCCTGGCACGGTGCTGAGAATTCGCGGCTTGGTTCCTCCCAATGCCAGCAGGTTCCAT       120

35  V  N  L  L  C  G  E  E  Q  G  S  D  A  A  L  H  F  N  P  R        54
121 GTAAACCTGCTGTGCGGGGAGGAGCAGGGCTCCGATGCCGCCCTGCATTTCAACCCCCGG       180

55  L  D  T  S  E  V  V  F  N  S  K  E  Q  G  S  W  G  R  E  E        74
181 CTGGACACGTCGGAGGTGGTCTTCAACAGCAAGGAGCAAGGCTCCTGGGGCCGCGAGGAG       240

75  R  G  P  G  V  P  F  Q  R  G  Q  P  F  E  V  L  I  I  A  S        94
241 CGCGGGCCGGGCGTTCCTTTCCAGCGCGGGCAGCCCTTCGAGGTGCTCATCATCGCGTCA       300

95  D  D  G  F  K  A  V  V  G  D  A  Q  Y  H  H  F  R  H  R  L       114
301 GACGACGGCTTCAAGGCCGTGGTTGGGGACGCCCAGTACCACCACTTCCGCCACCGCCTG       360

115 P  L  A  R  V  R  L  V  E  V  G  G  D  V  Q  L  D  S  V  R       134
361 CCGCTGGCGCGCGTGCGCCTGGTGGAGGTGGGCGGGGACGTGCAGCTGGACTCCGTGAGG       420

135 I  F  end                                                          136
421 ATCTTCTGAGCAGAAGCCCAGGCGGGCCCGGGGCCTTGGCTGGCAAATAAAGCGTTAGCC       480

481 CGCpoly(A)                                                         483
```

Galectin-7 Transient Expression and Lactose Binding Assay—Galectin-7 cDNA was cloned in the pMT21 expression vector (Kaufman *et al.* 1991), and transiently expressed in COS-1 cells using Lipofectin-AMINE (Life Technologies, Inc.) as transfection agent (Düzgünes and Felgner, 1993). Thirty-six h after transfection the cells were scraped of the 3-cm Petri dishes in 0.3 ml of buffer A (58 mM Na_2HPO_4, 18 mM KH_2PO_4, 75 mM NaCl, 2 mM EDTA, 4 mM β-mercaptoethanol, and 0.2% bovine serum albumin, pH 7.2) and sonicated for 15 s. The extracts were then centrifuged at 15,000 rpm for 15 min at 4 °C and the supernatants were transferred to a new tube and kept at 0 °C. Lactose binding activity of the expressed galectin-7 was assayed by immunodetection of *in situ* binding activity to asialofetuin (Sigma) immobilized on nitrocellulose (Amersham). Thirty μg of asialofetuin dissolved in 3 μl of water were spotted on a 1-cm^2 strip of nitrocellulose. The nitrocellulose pieces were then placed in a 24-well tissue culture plate and incubated overnight in buffer B (58 mM Na_2HPO_4, 18 mM KH_2PO_4, 75 mM NaCl, 2 mM EDTA, and 3% bovine serum albumin, pH 7.2) with constant agitation at 22 °C. Following incubation, the blocking medium was aspirated and the nitrocellulose pieces were washed three times in buffer A. COS-1 cell extracts were prepared containing 1% bovine serum albumin and either with or without 150 mM lactose (105 μl of primary extract, 15 μl of 10% bovine serum albumin in buffer A and either 30 μl of 0.75 M lactose in buffer A or 30 μl of buffer A). The immobilized asialofetuin was incubated with the extracts for 2 h and washed 5 times in buffer A. The nitrocellulose pieces were then fixed in 2% formalin in PBS (58 mM Na_2HPO_4, 18 mM KH_2PO_4, 75 mM NaCl, 2 mM EDTA, pH 7.2) for 1 h to prevent loss of bound galectin-7. Following extensive washing in PBS the pieces were incubated with a rabbit anti-galectin-7 polyclonal serum (R23) diluted 1:100 in PBS for 2 h at 22 °C. The pieces were then washed in PBS and incubated with peroxidase-labeled goat anti-rabbit antibodies (DAKO). Following incubation for 2 h at 22 °C, the pieces were washed in PBS and the substrate was added. Nitrocellulose pieces were incubated until the color developed and the reaction was stopped by washing in distilled water.

Cell Hybrids—Cell hybrids were obtained from Coriell Cell Repositories, Camden, NJ. Hybrids NA09925 through NA09940, NA10567, and NA10324 correspond to human/mouse somatic cell hybrids, whereas hybrid NA10611 is of human/hamster origin. Restriction enzyme digestion of hybrid cell DNA, Southern transfer, and filter hybridization were carried out as described previously (Kruse *et al.*, 1988). The galectin-7 cDNA probe was ^{32}P-labeled by nick translation to a specific activity higher than 10^8 cpm/μg.

Other Procedures—The procedures for two-dimensional gel electrophoresis (O'Farrell, 1975; Celis *et al.*, 1994a), silver staining (Tunon and Johansen, 1984), labeling of cells with [^{35}S]methionine or with a mixture of 16 ^{14}C-amino acids (Bravo and Celis, 1982), two-dimensional immunoblotting (Celis *et al.*, 1994b), production of antibodies in rabbits (Huet, 1994), immunofluorescence (Osborn, 1994), microsequencing (Vandekerckhove and Rasmussen, 1994), RNA purification (Sambrook *et al.*, 1989), screening of cDNA expression libraries (Honoré *et al.*, 1993), and preparation of libraries have been described in detail elsewhere.

```
Galectin 7                                  1 MSNVPHKSSLPEGIRPGTVL 20
Galectin 1                                    1 MACGLVASNLNLKPGECL 18
Galectin 2                                    1 MTGELEVKNMDMKPGSTL 18
Galectin 3                        101 YPATGPYGAPAGPLIVPYNLPLPGGVVPRMLI 133
Galectin 4I                       1 MAYVPAPGYQPTYNP TLPYKRPIPGGLSVGMSI 33
Galectin 4II 161 GTMTIPAYPSAGYNPPQMNSLPVMAGPPIFNPPVPYVGTLQGGLTARRTI 210

              21 RIRGLVPPNASRFHVNLLCGEEQGSDAALHFNPRLDTS E   VVFNSKE 66
              19 RVRGEVAPDAKSFVLNL   GKDSNNLCLHFNPRFNAHGDANTIVCNSKD 64
              19 KITGSIADGTDGFVINL   GQGTDKLNLHFNPRFS    ESTIVCNSLD 61
             134 TILGTVKPNANRIALDF    QRGNDVAFHFNPRFNEN NRRVIVCNTKL 177
              34 YIQGIAKDNMRRFHVNFAVGQDEGADIAFHFNPRFDGW DK  VVFNTMQ 80
             211 IIKGYVLPTAKNLIINFKVG  STGDIAFHMNPRIGD     CVVRNSYM 253
                   *                          * ***         * *

              67 QGSWGREERGPGV PFQRGQPFEVLIIASDDGFKAVVGDAQYHHFRHRL  114
              65 GGAWGTEQREAVF PFQPGSVAEVCITFDQANLTVKLPDGYEFKFPNRL  112
              62 GSNWGQEQREDHL CFSPGSEVKFTVTFESDKFKVKLPDGHELTFPNRL  109
             178 DNNWGREERQSVF PFESGKPFKIQVLVEPDHFKVAVNDAHLLQYNHRVK 226
              81 SGQWGKEEKKKSM PFQKGHHFELVFMVMSEHYKVVVNGTPFYEYGHRL  128
             254 NGSWGSEERKIPYNPFGAGQFFDLSIRCGTDRFKVFANGQHLFDFSHRFQ 303
                   ** * *      *  *                          *

             115 PLARVRLVEVGGDVQLDSVRIF 136
             113 NLEAINYMAADGDFKIKCVAFD 134
             113 GHSHLSYLSVRGGFNMSSFKLKE 135
             227 KLNEISKLGISGDIDLTSASYTMI 250
             129 PLQMVTHLQVDGDLELQSINFLGGQPAASQYP 160
             304 AFQRVDMLEIKGDITLSYVQI 324
```

FIG. 3. **Alignment of galectin-7 with the galectin family.** Sequences compared include galectin-1 (Couraud *et al.*, 1989), galectin-2 (Gitt *et al.*, 1992), galectin-3 (Cherayil *et al.*, 1989), and galectin-4 (Oda *et al.*, 1993). Conserved amino acids that are important for the β-galactoside binding activity are indicated with an *asterisk* (Lobsanov *et al.*, 1993).

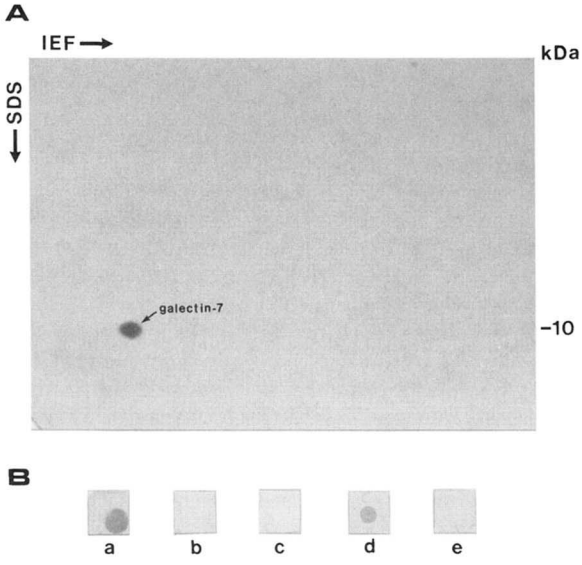

FIG. 4. **Binding of recombinant galectin-7 to asialofetuin.** *A*, specificity of the galectin-7 antiserum determined by IEF two-dimensional gel immunoblotting of total keratinocyte proteins. *B*: *a*, asialofetuin immobilized on nitrocellulose and incubated with extracts containing recombinant galectin-7 expressed in COS-1 cells. The binding was assessed by dot blotting using a galectin-7 antiserum. *b*, extract from COS-1 cell transfected with the pMT21 vector without the insert. *c*, binding of recombinant galectin-7 in the presence of 150 mM lactose. Recombinant expressed galectin-7 spotted on nitrocellulose and reacted with the galectin-7 antiserum. *e*, extract from COS-1 cell transfected with pMT21 lacking the insert and reacted with the galectin-7 antiserum.

RESULTS

As a result of a systematic analysis of keratinocyte proteins using two-dimensional gel electrophoresis we have revealed many proteins that are differentially regulated in SV40 transformed keratinocytes (K14) (Celis and Olsen, 1994) and for which we are actively gathering data in the keratinocyte data base (Celis *et al.*, 1992, 1993). One of these proteins (Fig. 1*A*), corresponding to IEF 17 in the data base, seemed most interesting as (i) its peptide sequences did not match any known protein, (ii) it was partially secreted to the culture medium by normal keratinocytes (see Fig. 5*A*), and (iii) it was highly downregulated in SV40-transformed keratinocytes (K14) that are anchorage independent and unable to differentiate (Fig. 1*B*).

Molecular cloning of IEF 17 was performed by screening a λgt11 cDNA expression library using degenerated oligodeoxyribonucleotides that had been back-translated from one of several peptide sequences derived by microsequencing of the purified protein and stored in the keratinocyte data base (peptide 1, SSLPEGFIRPGTVL; peptide 2, GPGVPFFQR; peptide 3, HRLPLAR; and peptide 4, LVEVGGDVQLDSV). Inosine containing oligodeoxyribonucleotide probes derived from part of the sequence of peptide 4 (EVGGDVQ), were used to screen a λgt11 cDNA library prepared from unfractionated, noncultured psoriatic keratinocytes (Honoré *et al.*, 1993). The nucleotide sequence and deduced amino acid sequence of the fragment from one of the cDNA clones (clone 1949) are shown in Fig. 2. The open reading frame codes for a protein containing 136 amino acids, with a molecular mass of 15,075 and a calculated pI of 7.73. These values are close to those recorded for IEF 17 in the two-dimensional gel data base of keratinocyte proteins (12,700 Da; pI 7.62) (Celis *et al.*, 1992, 1993). The predicted amino acid sequence contains the four tryptic peptides analyzed by microsequencing (*underlined* in Fig. 2). Analysis of the proteins expressed by COS-1 cells transiently transfected with clone 1949 using the eukaryotic expression vector pMT21 (Kaufman *et al.*, 1991) showed that the recombinant protein expressed by clone 1949 comigrated with IEF 17 synthesized by primary keratinocytes (results not shown).

Comparison of the predicted amino acid sequence derived from clone 1949 with protein sequences available in the Swissprot data base, revealed similarity to the galectin family, a group of β-galactoside-binding lectins having related amino acid sequences (Barondes *et al.*, 1994a, 1994b). Alignment of the predicted amino acid sequence encoded by clone 1949 and the galectins (Fig. 3) showed that the amino acid central to the

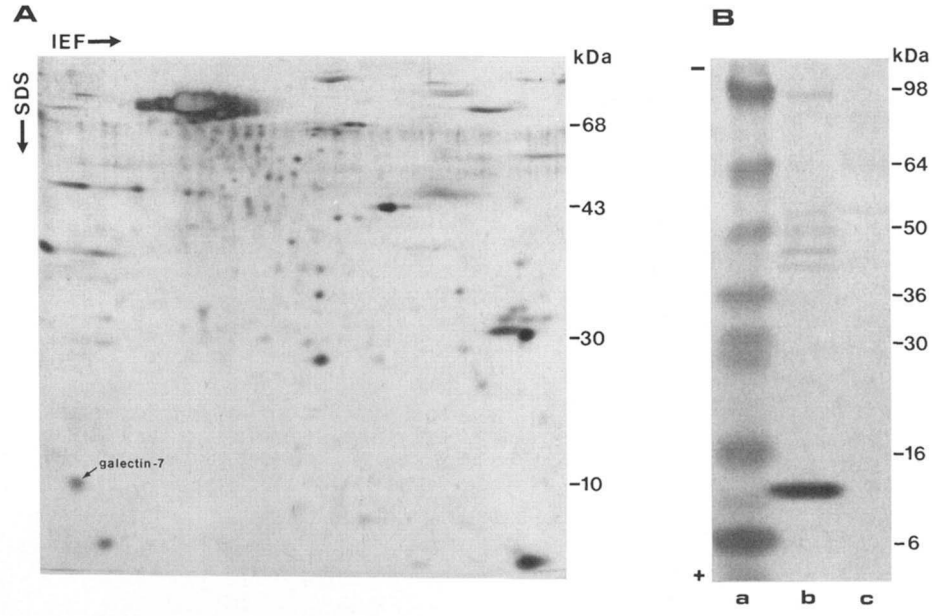

FIG. 5. **IEF two-dimensional gel analysis of proteins externalized to the medium by keratinocytes and COS-1 cells transiently transfected with clone 1949.** *A*, silver-stained gel of proteins found in the medium of keratinocytes kept overnight in K_m basal medium. *B*, one-dimensional immunoblotting of extracts from COS-1 cells transiently transfected with clone 1949. *a*, prestained molecular weight markers stained. *b*, cellular extracts reacted with the galectin-7 antibody. *c*, medium from the transfected cells reacted with the galectin-7 antibody.

β-galactoside interaction with the protein are conserved. These include His-45, Asn-47, Arg-49, Val-56, Asn-58, Trp-65, Glu-68, Arg-70, and Arg-108, using the amino acids numbers from the galectin-2 sequence (Fig. 3) (Lobsanov *et al.*, 1993). IEF 17 does not label with [^{35}S]methionine (results not shown) and therefore, the methionine at the start codon, which is the only methionine in the sequence, must be removed post-translationally as it is the case for other members of the galectin family (Barondes *et al.*, 1994). Upon consultation with researchers in the field (Barondes *et al.*, 1994b), IEF 17 has been termed galectin-7. The clone 1949 cDNA sequence has been submitted to GenBank and has been assigned the accession number L07769.

To confirm that galectin-7 is indeed a bona fide member of the galectin family we performed lactose binding experiments. Asialofetuin immobilized on nitrocellulose was incubated with extracts containing recombinant galectin-7 expressed in COS-1 cell in either the presence or absence of 150 mM lactose, and the binding was assessed by dot blotting using an antiserum prepared by immunizing rabbits with IEF 17 recovered by electroelution from Coomassie Brilliant Blue-stained gels. As shown in Fig. 4*A*, the antibody reacts with galectin-7 in IEF two-dimensional gel blots of total keratinocyte proteins but it does not recognize other members of the family or other keratinocyte proteins, basic polypeptides included (results not shown). As expected, recombinant galectin-7 showed significant binding activity toward asialofetuin (Fig. 4*B, blot a*), while no binding was observed in extracts from COS-1 cell transfected with the pMT21 vector lacking the insert (Fig. 4*B, blot b*). In addition, binding of recombinant galectin-7 to immobilized asialofetuin was completely blocked by the addition of 150 mM lactose (Fig. 4*B, blot c*). Dot blot analysis of extracts from COS-1 cells transfected with galectin-7 cDNA or with pMT21 lacking the insert showed that the galectin-7-specific polyclonal antibody did not react with antigens present in the COS-1 cells (Fig. 4*B, blot d* and *e*), a fact that was also confirmed by indirect immunofluorescence of the transfected cell population.

Two-dimensional gel analysis of the proteins found in the media of keratinocytes kept overnight in serum-free keratinocyte medium indicated that galectin-7 is externalized to the medium (Fig. 5*A*) in spite of the fact that its sequence has no typical secretion signal peptide (Kuchler, 1993). The presence of galectin-7 in the medium is not due to cell death and/or lysis as most cellular proteins, including known extractable proteins such as lactic dehydrogenase, α and β-tubulin, etc., are not present in the media (Fig. 5*A*). Similar results have been observed with quiescent and *in vitro* differentiated keratinocytes (results not shown), suggesting that the protein is externalized by a nonclassical, yet unknown secretory mechanism. COS-1 cells transiently transfected with clone 1949 on the other hand, did not externalize galectin-7 as no immunoreactivity could be detected in the culture medium (Fig. 5*B, lane c*). That transfection worked efficiently was confirmed by the two-dimensional gel analysis of the cellular proteins, which showed abundant expression of the protein (Fig. 5*B, lane b*), and by indirect immunofluorescence of the transfected cells using the galectin-7 antiserum which showed that about 20% of the cells stained with the antibody (results not shown).

One-dimensional gel immunoblotting of several human fetal tissues (brain, large intestine, lung, mesonephric tissue, pancreas, pectoral muscle, skin, small intestine, stomach, thyroid gland, and umbilical cord) showed that only skin extracts reacted with the galectin-7-specific polyclonal antibody yielding a single band of apparent molecular mass of 15 kDa (results not shown). For the major part, these results were also confirmed by IEF two-dimensional gel immunoblotting using the enhanced chemiluminescence detection method (results not shown).

Immunofluorescence analysis of methanol fixed fetal human tissues reacted with the galectin-7 antibody confirmed the restricted occurrence of this protein, which was mainly observed in stratified squamous epithelia. In the skin, the antibody stained all layers of the epidermis, although the basal layer labeled more intensively (Fig. 6*A*). Adult human skin also showed strong staining of the basal layer (Fig. 6*B*), but in some areas of the cryostat sections the staining was also observed in the suprabasal layers and concentrated at sites of cell to cell contact (*upper right* of Fig. 6*B*). The uneven distribution of galectin-7 in some areas of the skin sections most likely reflect particular microenvironmental conditions (cells, matrix, factors, etc.). Staining of suprabasal cells was also observed in the case of psoriatic skin (Fig. 6*C*). Treatment of skin sections for 2 min with 0.1% Triton X-100 prior to methanol or formaldehyde fixation revealed similar results as those depicted in Fig. 6, *A* and *B* (results not shown), suggesting that at least a fraction of galectin-7 is associated with the cytoskeleton. Fetal human

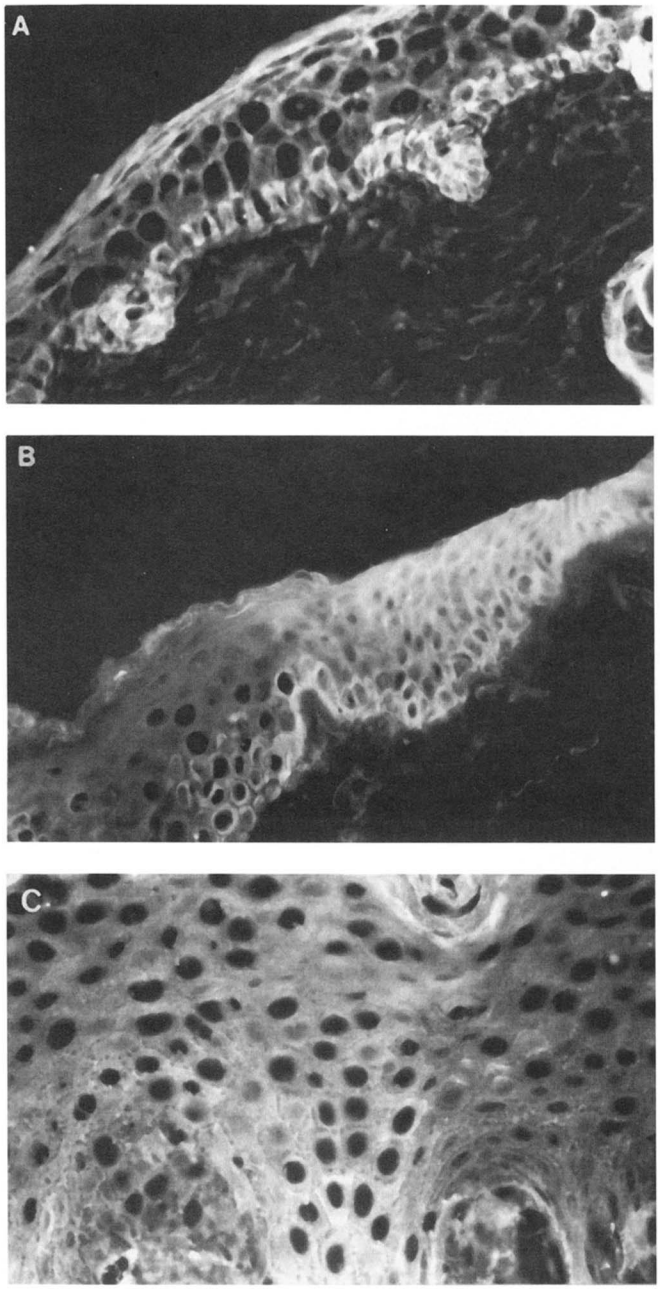

FIG. 6. **Immunofluorescence of cryostat sections of methanol fixed human tissues reacted with the galectin-7 antiserum.** *A*, fetal human skin. *B*, adult human skin. *C*, psoriatic skin.

tissues that did not stain with the antibody included brain, kidney, lung, pancreas, smooth and skeletal muscle, thyroid gland, and umbilical cord (results not shown).

Northern blot analysis of total mRNA extracted from several human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas) and cell lines (K14 and A431 epidermoid carcinoma cells) failed to reveal the galectin-7 transcript in line with the above results. Abundant expression of the galectin-7 transcript was detected in mRNA extracts from unfractionated epidermal cells (results not shown).

DNA samples from 18 different human/rodent cell hybrids were analyzed by Southern blotting using the galectin-7 cDNA probe. Following digestion with *Hin*dIII, two hybridizing bands (10 and 8 kilobases, respectively) were detected in human genomic DNA (Fig. 7, *lane 7*), one (14 kilobases) in mouse DNA (Fig. 7, *lane 1*), and one (7 kilobases) in hamster

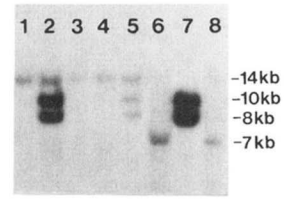

FIG. 7. **Detection of the human galectin-7 gene sequence by Southern blot analysis.** *Lane 1*, NA00347A (mouse); *lane 2*, NA09933 (mouse hybrid); *lane 3*, NA09934 (mouse hybrid); *lane 4*, NA09935A (mouse hybrid); *lane 5*, NA09936 (mouse hybrid); *lane 6*, NA10611 (hamster hybrid); *lane 7*, NAIMR91 (human); *lane 8*, NA10908 (hamster).

(Fig. 7, *lane 8*). *Lanes 2* and *5* show human/mouse hybrids containing the human galectin-7 gene, while *lanes 3, 4,* and *6* show hybrids where only cross-hybridizing bands from either mouse or hamster are observed (Fig. 7). Table I compares the segregation of the human specific bands and the chromosomal content of the hybrid cells. For any given hybrid, only chromosomes totally absent or present in at least 20% of the metaphases analyzed were considered informative. The results showed concordant segregation of chromosome 19 and the galectin-7 gene.

DISCUSSION

Using high resolution two-dimensional gel electrophoresis, microsequencing, and cDNA cloning we have identified a transformation down-regulated human protein that shares identity to the galectins (Barondes *et al.* 1994a, 1994b) and that has been termed galectin-7. Galectin-7 binds lactose and contains all the amino acids that are central to the β-galactoside interaction with the protein. We have shown that the gene encoding for galectin-7 maps to chromosome 19, indicating that genes coding for members of the family are scattered throughout the genome (Raz *et al.*, 1991; Mehrabian *et al.*, 1993).

Galectin-7, like other galectins is externalized to the medium by proliferating quiescent and differentiated keratinocytes in spite of the fact that it does not contain a secretion peptide signal (Kuchler, 1993). Previously, we have identified a few keratinocyte proteins that behave similarly and that include the psoriasin (Madsen *et al.*, 1991), a fatty acid-binding protein highly up-regulated in psoriasis (Madsen *et al.*, 1992) and stratifin, a member of the 14-3-3 family that is expressed only by epithelial cells (Leffers *et al.*, 1993). Also, well studied proteins such as interleukin-1β, basic fibroblast growth factor, and others (Kuchler, 1993) have been shown to be externalized by nonclassical pathway(s), and in the case of interleukin-1β there is evidence indicating that the mature form of the protein is the preferred substrate for secretion, and that some conformational properties of the protein are required for optimal secretion (Siders *et al.*, 1993). Even though the mechanism(s) underlying nonclassical secretion are largely unknown it is thought that there may be multiple mechanisms that operate for different groups of proteins in various cell types or for the same protein in different cell types (Rubartelli *et al.*, 1992; Koronakis and Hughes, 1993; Barondes *et al.*, 1994b). In this context, it is important to emphasize that transiently transfected COS-1 cells did not externalize galectin-7, implying that these cells may not possess the specialized mechanism necessary to secrete this protein.

Presently, all available information indicates that galectin-7 may play a role in cell-cell and cell-matrix interactions as it seems to be the case for other members of the galectin family (Barondes *et al.* (1994), and references therein). The protein localizes to areas of cell to cell contact, particularly in the upper layers of human epidermis, and it is strikingly down-regulated

TABLE I
Chromosome mapping of the galectin-7 gene
Analysis of human chromosomes in 18 human/rodent cell hybrids.

Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Concordant	16	14	11	10	8	12	12	12	11	13	9	6	13	12	13	11	9	12	18	11	8	11	11	13
Discordant	2	4	7	5	9	5	6	5	7	4	9	12	5	6	4	6	9	6	0	6	10	6	7	4

in SV40 transformed K14 keratinocytes that are anchorage independent and unable to differentiate. K14 cells show no strong adhesion to the surrounding cells and attach and detach with ease from other cells as they move. Thus, the lack of expression of galectin-7 by transformed keratinocytes may contribute to their inability to respond to growth control and differentiation signals. Recent experiments from Zhou and Cummings (1993) have shown that L-14 (galectin-1) can promote the adhesion of cultured cells to surfaces coated with laminin, a protein that has been involved in the metastatic invasion process (Liotta *et al.*, 1988; Pauli and Knudson, 1988). The expression of galectin-1 correlates with tumor metastasis potential, and an antibody raised against this protein has been shown to inhibit tumor cell colony formation (Raz *et al.*, 1986). In addition, laminin produced by differentiating mouse muscle cells binds to galectin-1 leading to loss of cell-substratum adhesion (Cooper *et al.*, 1991). At present, we have not determined whether galectin-7 binds to laminin or other glycoproteins but our results showing a strong down-regulation of this protein in transformed keratinocytes suggest a role in modulating cell-cell and cell-matrix interactions necessary for normal growth control.

Studies of Wells and Mallucci (1991, 1992) have shown that galectin-1 may act as an autocrine negative growth factor that regulates cell proliferation. Our unpublished studies,[2] however, indicate that galectin-7 may not possess such a function. For example, proliferating keratinocyte populations synthesize and externalize as much galectin-7 as their quiescent counterparts. Furthermore, primary keratinocyte cultures often contain proliferating melanocytes and dermal fibroblasts, and usually it is necessary to use a special keratinocyte growth medium in order to prevent growth of the latter cells.

To conclude, our studies imply that the expression of galectin-7 may be required for the maintenance of the normal keratinocyte phenotype. Together with other proteins that are involved in cell adhesion, and that are also down-regulated in transformed malignant cells (Ponta *et al.* (1994), Birchmeier and Behrens (1994), and Giancotti and Mainiero (1994), and references therein), the galectin family may represent yet another important component of the network that modulates cell-cell and cell-matrix interactions.

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