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Desmosomal glycoprotein DGI, a component of intercellular desmosome junctions, is related to the cadherin family of cell adhesion molecules

(desmosome/desmoglein/cell–cell junction)

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ABSTRACT **Among the variety of specialized intercellular junctions, those of the adherens type have the most obvious association with cytoskeletal elements. This may be with the actin microfilament system as in the zonula adherens or with intermediate filaments as in the macula adherens, or desmosome. In the former case, it is clear that transmembrane glycoproteins of the cadherin family are important adhesive components of the molecular assembly. We now show for desmosomes that a major glycoprotein component (desmosomal glycoprotein DGI) has extensive homology with the cadherins, defining an extended family, but also has unique features in its cytoplasmic domain that are likely to be relevant to the association with intermediate rather than actin filaments. A novel 282-residue extension contains repeats of ≈29 amino acid residues predicted to have an antiparallel β-sheet structure, followed by a glycine-rich sequence. As in the cadherins, the extracellular domain contains possible Ca^{2+}-binding sequences and a potential protease processing site. The cell adhesion recognition region (His-Ala-Val) of the cadherins is modified to Arg-Ala-Leu.**

The molecules that are involved in cell–cell adhesion are now becoming known. Thus the Ca^{2+}-dependent cadherin cell adhesion molecules (CAMs) have been distinguished from the immunoglobulin-related proteins such as neural (N-) CAM (1). Little is known, however, of the molecules involved in adhesion mediated by the desmosome, which is one of the major adhesive junctions of epithelial tissues with a characteristic and well-defined morphology (2). Two classes of desmosomal proteins are candidates for a role in cell adhesion: (*i*) the glycosylated proteins DGII (M_r 120,000) and DGIII (M_r 110,000), which have been shown to be related to one another by peptide mapping and immunological criteria; and (*ii*) another glycosylated protein, DGI (desmoglein; M_r 150,000) (2). These proteins are, like the cadherins, Ca^{2+}-binding transmembrane proteins, unlike other desmosomal proteins such as plakoglobin and the desmoplakins, which are present in an electron-dense plaque zone underneath the cell membrane. These latter proteins may provide a link to the intermediate filaments with which desmosomes interact.

Here we provide detailed information on the structure of the desmosomal glycoprotein DGI, through the isolation and sequence analysis of cDNA clones.§ Our results show that DGI is indeed related to the cadherin family of cell adhesion proteins, albeit more distantly than other members of the family, and is thus implicated as having a role in adhesion. DGI differs from the cadherins in having a unique large cytoplasmic domain, which may be involved in interactions with the plaque or intermediate filaments. In contrast, the cadherin family of proteins has been shown to codistribute with actin bundles in the adherens type of cell junction (3).

METHODS

Generation and Screening of Antibodies. Desmosomes were isolated from bovine muzzle epidermis (4). Rabbit anti-DGI serum was generated against DGI purified by SDS/PAGE and electroelution; its specificity was similar to the guinea pig sera previously described (4). The serum specifically recognized DGI on immunoblots of cow's nose desmosomes, precipitated a 150-kDa protein from metabolically labeled MDCK canine kidney epithelial cells, and stained MDCK cells in a punctate cell junctional pattern by immunofluorescence microscopy (data not shown; ref. 4). Polyclonal antibodies to DGI were affinity-purified on DGI immobilized on Sepharose CL-4B (CNBr-Sepharose; Pharmacia).

A DGI-specific IgG mouse hybridoma (3D1) was generated against bovine DGI. The antibody produced by this hybridoma recognized DGI on immunoblots of desmosomes, but did not immunoprecipitate well, and gave distinct punctate cell border staining on epithelial cell types, especially if the cells were unfixed (data not shown). Cell permeabilization was required for staining, suggesting that the antibody recognized intracellular epitopes (data not shown).

For the preparation of antibodies to TrpE fusion proteins, a *Sty* I–*Xho* I fragment [base pairs (bp) 2218–3422] from clone G4, encoding the intracellular part of the DGI molecule, including the DGI-specific repeats, was subcloned in frame into the *Xba* I–*Sal* I restriction sites of the pATH2 expression vector (5). The TrpE fusion protein was expressed, separated in a polyacrylamide gel, excised, and electroeluted. Rabbits were immunized and the resulting sera were preabsorbed with Sepharose-bound proteins from *Escherichia coli* expressing the pATH2 vector alone. Immunoblot analysis of fusion proteins expressed by putative DGI recombinants was carried out according to Price *et al.* (6).

Peptide Sequencing. Bovine DGI purified by SDS/PAGE and electroelution was dialyzed against 50 mM Mops buffer (pH 7) containing 0.05% SDS and was concentrated using an Amicon CX30 immersible concentrator. The protein was digested with trypsin or *Staphylococcus aureus* V8 protease (Boehringer Mannheim) (1–5% enzyme/substrate weight ratio) overnight at 37°C. Fragments were resolved by SDS/PAGE and transferred to Immobilon membrane (0.45 μm; Millipore). After staining with Coomassie brilliant blue, the

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Abbreviation: CAM, cell adhesion molecule.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. X56654).

fragments were cut out and sequenced using an Applied Biosystems 477A sequenator with on-line phenylthiohydantoin (PTH) analyzer model 120A.

Library Construction and Screening. A λgt11 cDNA library (Clontech) prepared using mRNA from cultured human keratinocytes was screened with affinity-purified anti-DGI antibodies (see above), essentially as detailed (7). The initial G1 clone was plaque-purified and shown to have an insert size of 1.1 kilobases (kb). To obtain clones covering more of the coding sequence, we constructed a cDNA library in λgt11, using random hexanucleotide primers with 1 μg of mRNA isolated from human keratinocytes (Amersham cDNA synthesis and cloning kit). This library and the poly(A)$^+$ library were probed with a ^{32}P-labeled G1 cDNA and yielded several overlapping clones.

Northern and Southern Analysis. For Northern blot analysis, poly(A)$^+$ RNA was isolated from cultured human epidermal keratinocytes essentially by the guanidinium isothiocyanate method of Chirgwin *et al.* (8). Total RNA was isolated from a myeloma cell line (JKAg8) that does not express DGI. The RNA was electrophoresed in a 1% agarose gel containing 2.2 M formaldehyde and transferred to Hybond-N by standard procedures (9). Hybridizations were carried out for 16 hr at 48°C with ^{32}P-labeled probes in 1.5× SSC (0.225 M NaCl/0.0225 M trisodium citrate, pH 7.0) containing 50% (vol/vol) formamide, 1% (wt/vol) dried milk (Marvel, U.K.), 50 mM Tris Cl (pH 7.6), 3% (wt/vol) SDS, 10% (wt/vol) dextran sulfate, and boiled salmon sperm DNA at 25 μg/ml. Filters were washed once with 1× SSC/0.2% SDS for 30 min and three times with 0.1× SSC/0.1% SDS for 30 min at 65°C. They were then dried and exposed to Kodak XAR-5 film.

For Southern blotting, human genomic DNA was prepared from EDTA-treated blood of healthy male donors according to standard procedures (9). DNA (5 μg) was digested with various restriction enzymes, electrophoresed in 1% agarose gels, and transferred to Hybond-N membranes. Filters were hybridized with various fragments of the DGI cDNA, ^{32}P-labeled by the random primer method (9). PCR-mediated amplification of cDNA fragments was carried out in a volume of 50 μl according to the supplier's recommendation (Cetus), using cDNA clone pG4 as a source.

DNA Sequencing. Phage λ DNA was purified from plate lysates (9), digested with *Eco*RI, separated in agarose gels, purified using Geneclean (Bio 101, La Jolla, CA), and subcloned into M13 mp10 and pBluescript KS(+). The DNA sequence was determined by the dideoxynucleotide chain-termination method (10) using Sequenase (United States Biochemical). The initial G1 clone was sequenced by subcloning restriction fragments into M13. Subsequent clones were sequenced using appropriate oligodeoxynucleotides (synthesized with an Applied Biosystems synthesizer) as primers. The whole of clone G4 was sequenced in both directions.

RESULTS

Isolation and Characterization of DGI cDNA. A human keratinocyte λgt11 library was screened using affinity-purified rabbit anti-DGI antibodies (see *Methods*). Screening of ≈150,000 recombinants identified 3 positive clones. The fusion proteins encoded by these recombinants were analyzed by Western immunoblotting; the results were the same for all three clones. The fusion protein of λ clone G1 showed a molecular mass of ≈150 kDa (Fig. 1*A*, lane 1) when probed with an anti-β-galactosidase antibody; the lower bands were probably due to degradation. The fusion protein reacted

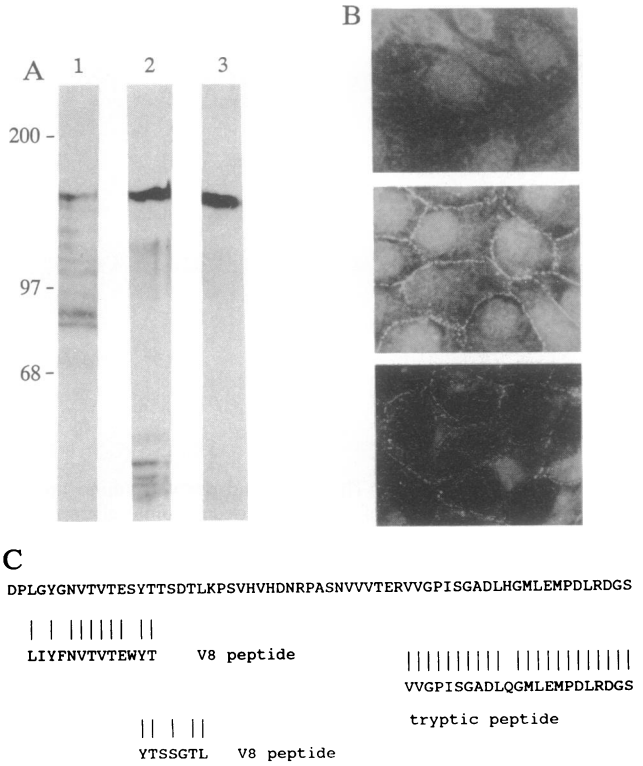

FIG. 1. (*A*) Phage λ clone G1 was expressed in *E. coli* strain C600 (6) and immunoblotted with polyclonal anti-β-galactosidase (lane 1), monoclonal anti-DGI antibody 3D1 (lane 2), and monoclonal anti-DGI antibody DG3.10 [Progen (Heidelberg)] (lane 3). (*B*) Immunofluorescence of SVK14 human epithelial cells after reaction with normal rabbit serum (*Top*), polyclonal guinea pig anti-DG1 antiserum (*Middle*), or rabbit anti-TrpE fusion protein serum (*Bottom*). Primary antibodies were detected with fluorescein-conjugated secondary antibodies. (*C*) Comparison of human DGI amino acid sequence with that derived from protein sequencing.

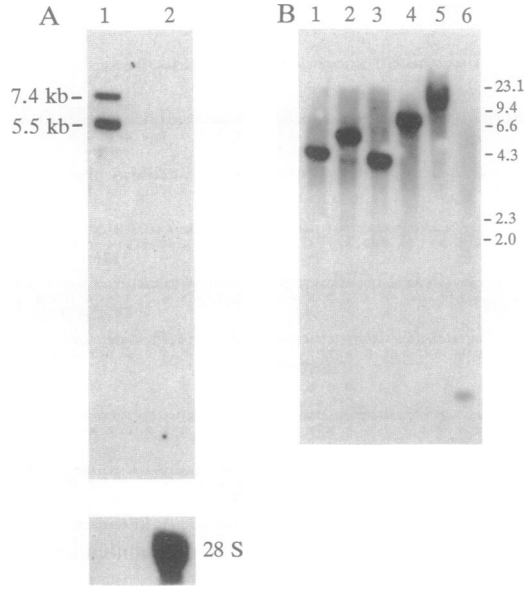

FIG. 2. (*A*) (*Upper*) Northern blot analysis of poly(A)$^+$ RNA (≈3 μg) extracted from human keratinocytes (lane 1) and total myeloma JKAg8 RNA (30 μg; lane 2) probed with G1 cDNA. (*Lower*) The blot was stripped and then hybridized with radiolabeled *Xenopus* rDNA. (*B*) Southern blot analysis of human male DNA digested with *Bgl* II (lane 1), *Eco*RI (lane 2), *Hin*dIII (lane 3), *Pst* I (lane 4), *Bam*HI (lane 5), or *Taq* I (lane 6). The hybridization probe was a purified PCR fragment (cDNA positions 2385–3067) that overlaps with pG1 by 488 bp. The probe was prepared using the primers JA70 (5′-GGTGTACTGCATCCTAAG-3′) and JA71 (5′-GCACGGTACTATACTTTGT-3′). Markers at right are in kilobases.

strongly with two anti-DGI monoclonal antibodies (Fig. 1*A*, lanes 2 and 3) and also with affinity-purified guinea pig anti-DGI antibody (data not shown). The insert from all three clones was a single *Eco*RI fragment of 1.1 kb. The insert of λ clone G1 was subcloned into M13 mp10 in both orientations and into pBluescript KS(+) vectors. DNA probes derived from the G1 insert were used to screen the original poly(A)$^+$ library and a random-primed λgt11 human keratinocyte library, yielding a number of additional cDNA clones of which λ clone G4 was the largest (3.6 kb).

The cDNA of G1 hybridized to two mRNA species of approximately 5.5 and 7.4 kb (Fig. 2*A*). These transcripts

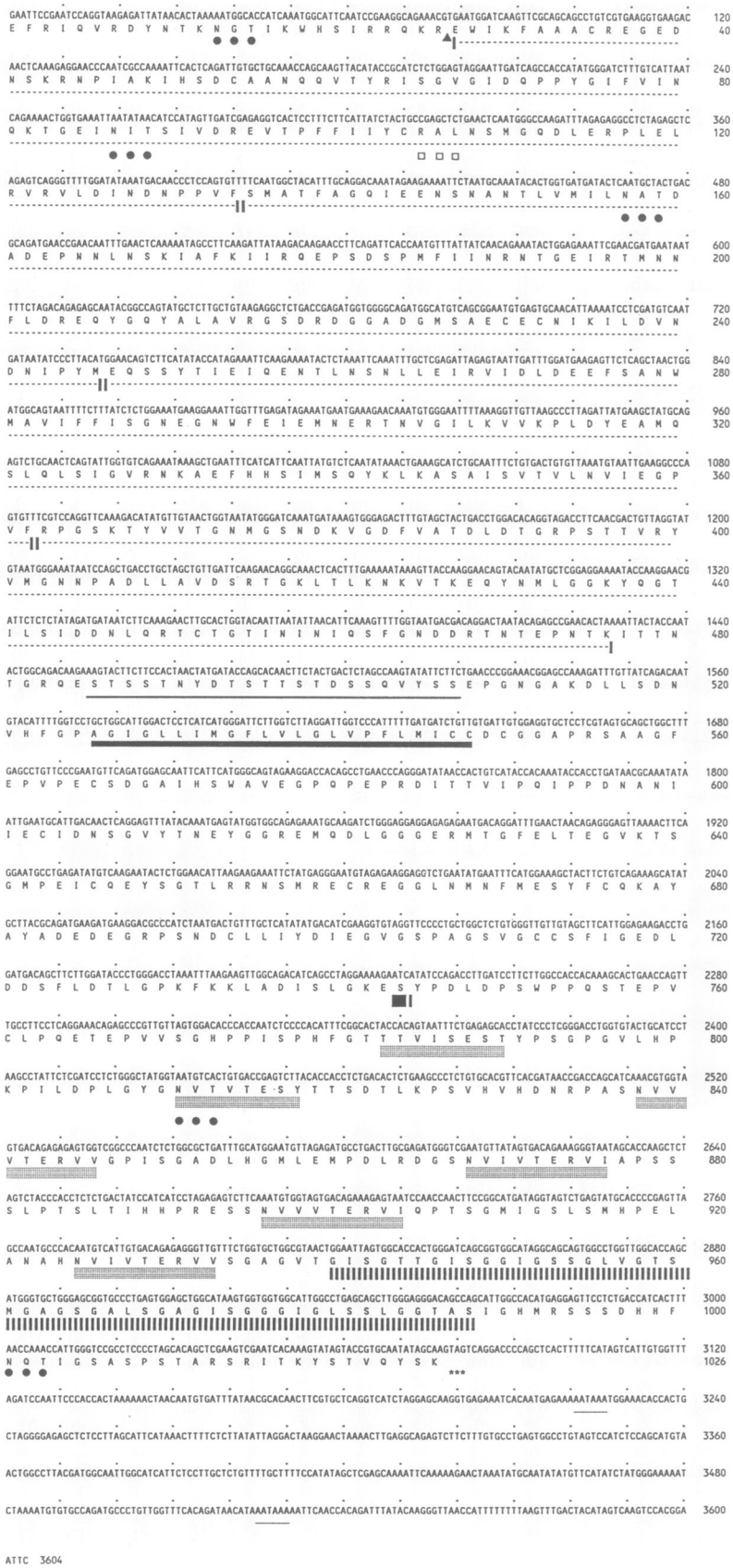

FIG. 3. The DNA and derived amino acid sequence of the human desmosomal glycoprotein DGI clone G4. This is our longest clone and codes for a mature protein of 107,578 Da but does not extend to the amino terminus and lacks a signal sequence. The black bar underlines the putative transmembrane region, the stippled bar underlines the core sequences of the six cytoplasmic domains with internal homology, the striped bar underlines the glycine/serine-rich region, and the dashed line is below the four external putative Ca^{2+}-binding domains that have homology with the cadherins. The end of homology with the cadherins (■ ■), the mature protein cleavage site (▲), and potential N-linked glycosylation sites (●●●) are indicated, and the potential O-linked glycosylation site is underlined. The Arg-Ala-Leu sequence in the analogous position to the His-Ala-Val cell adhesion recognition sequence in cadherin is marked (□ □ □). Two potential polyadenylylation signals in the 3′ untranslated region are underlined. The termination codon is marked with asterisks.

were present in human keratinocytes, which express DGI, but not in RNA from the myeloma cell line JKAg8. On genomic Southern blots a 682-bp fragment amplified from clone G4 by PCR and overlapping G1 by 488 bases hybridized to only one band, suggesting that there is one DGI gene and that the two species of mRNA are derived from it (Fig. 2*B*).

The nucleotide sequence of clone G4 contains a single long open reading frame that ends with a stop codon at nucleotide 3079 (Fig. 3). A putative start site has not yet been reached. Translation of the open reading frame of G4 predicts a mature protein of 1000 amino acids (Fig. 3) with a mass of 107,578 Da. Peptide sequence derived from trypsin and V8 protease fragments of SDS/PAGE-purified bovine DGI matches closely to the predicted amino acid sequence from the coding region of human clone G4 (Fig. 1*C*). The sequence of G4 shows a single hydrophobic region (residues 526–548) long enough to span the plasma membrane and contains three potential N-glycosylation sites in its putative extracellular domain.

Antisera were prepared against a TrpE fusion protein encoding cytoplasmic sequences of DGI. They were reactive against fusion protein and DGI by immunoblotting (data not shown) and stained desmosomes by indirect immunofluorescence in a punctate pattern similar to a polyclonal serum against DGI (Fig. 1*B*; ref. 4).

DISCUSSION

We report here the primary structure of desmosomal glycoprotein DGI, a glycosylated transmembrane protein found solely in desmosome junctions. The conclusion that the sequence is that of DGI is supported by a number of observations. (*i*) Amino acid sequence obtained from trypsin and *S. aureus* V8 digests of purified bovine DGI correlates well with the predicted human amino acid sequence. (*ii*) Fusion proteins from clone G1 react in Western blots with two independent anti-DGI monoclonal antibodies. (*iii*) Messages are large enough to code for a protein of the correct size (4) and they are specifically found in cultured human epidermal keratinocytes and not in myeloma cells. We have not found evidence for alternative exon splicing in any of the regions sequenced, although there is evidence for the use of two alternative polyadenylylation sites (G.N.W., unpublished observations). (*iv*) Sera made by immunizing rabbits with TrpE fusion proteins containing the cytoplasmic portion of DGI react with authentic DGI and give a punctate immunofluorescent staining typical of desmosomes.

Analysis of the deduced amino acid sequence of DGI reveals 29% identity between residues 27–744 of DGI and the whole of mature human N-cadherin. Fig. 4 shows comparisons of DGI with the amino-terminal and cytoplasmic portions of human E-, P-, and N-cadherin as well as with the cytoplasmic region of DGII, which we have cloned and also find to be a member of the cadherin superfamily (11). No other significant homologies were found. The vertebrate cadherin superfamily is involved in cell–cell adhesion and includes E-cadherin, N-cadherin, and P-cadherin (1). The amino acid identity between different cadherins of the same species is ≈45%. E-cadherin and N-cadherin can also be concentrated at cell–cell junctions—namely, at actomyosin-associated adherens junctions in the junctional complexes of epithelial tissues (16) and at the intercalated discs of cardiac muscle (17), respectively.

Additionally, DGI has a large domain (≈282 amino acids) at its cytoplasmic, carboxyl end that shows no significant homology with any known protein (Fig. 3) (searched against the University of Leeds protein data base, April 1990). There are six repeats of an ≈29-residue motif, predicted to consist of an anti-parallel β-sheet, followed by a glycine- and serine-rich domain that has a different repeat structure from that of the glycine- and serine-rich regions of the keratins. This domain may interact with some component of the desmosomal plaque (plakoglobin, the desmoplakins, or other plaque proteins) or with intermediate filaments. DGI has been shown by electron microscopy to extend into the plaque (18, 19). Cadherins have been shown to interact with three proteins called the catenins and indirectly with the microfilament network (20), an interaction critically dependent on their highly conserved cytoplasmic domain. The equivalent region of DGI is less homologous to the cadherins, as is that of DGII

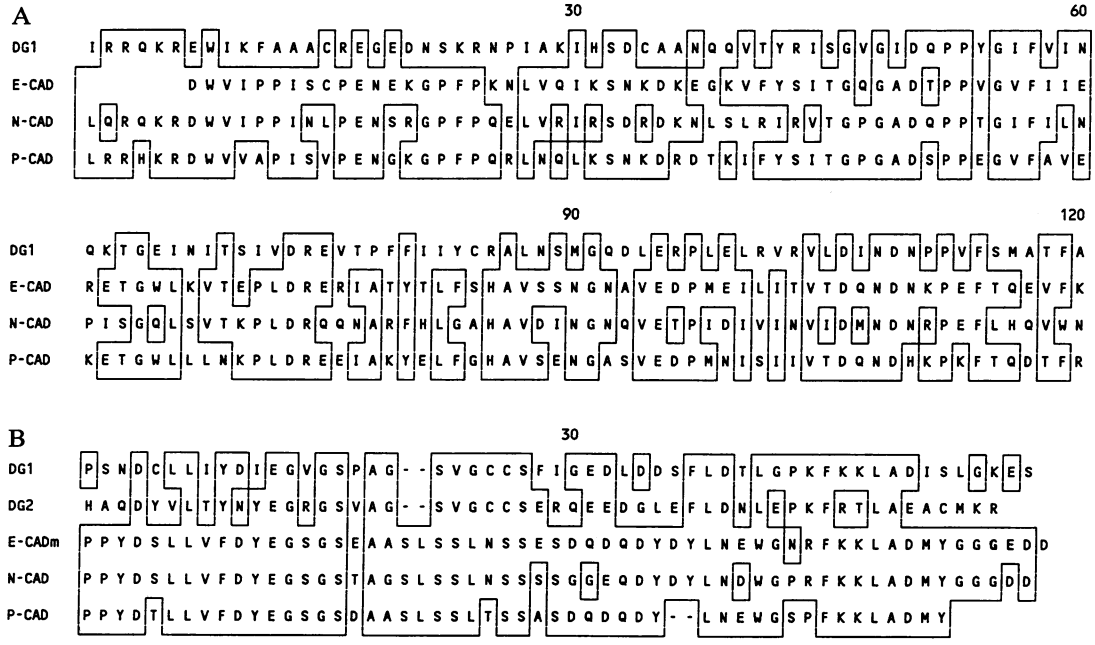

FIG. 4. Comparison of DGI sequence with amino and carboxyl termini of various cadherins and the carboxyl terminus of DGII. The human DGI sequence is compared with the amino termini of the mature form of human E-cadherin (E-CAD, ref. 12), human N-cadherin (N-CAD, ref. 13), and human P-cadherin (P-CAD, ref. 14) (*A*) and with the carboxyl termini of human DGII, mouse E-cadherin (E-CADm, ref. 15), and human N- and P-cadherin (*B*).

(Fig. 4), and this may contribute to the selectivity of the desmosomal glycoproteins for intermediate filaments. The cytoplasmic domain of DGI is also cysteine-rich, unlike the cadherins, which are devoid of cysteine in this region.

This paper provides evidence that cadherin-like molecules can interact with components of the cytoskeleton other than microfilaments. Recently, a novel integrin subunit β4 with a very long cytoplasmic domain (1000 amino acids) has been cloned (21) and has been localized to hemidesmosomes of epithelial cells (22), which interact with intermediate filaments. The cytoplasmic domains of DGI and β4 were found to have no significant homology. It has been suggested that desmoplakins might interact directly with intermediate filaments via a repeated domain near the carboxyl terminus (23), although such an interaction has not been detected (24). No similar domain is found in the sequence of DGI.

The similarity in structure of DGI with cadherins suggests that DGI is likely to have cell-adhesive properties. DGI, unlike the cadherins, does not have the His-Ala-Val sequence, which has been proposed as a cell adhesion recognition sequence (25). This may suggest that DGI does not have an adhesive role or, more likely, that a different sequence is used by DGI. Indeed, in the corresponding region of DGI, the sequence Arg-Ala-Leu is found. DGI and DGII/III bind Ca^{2+} on blots (19, 26) and desmosomal assembly is Ca^{2+}-dependent (27). The cadherins require calcium for their stability and function and they possess four repeated putative Ca^{2+}-binding domains (15). Related domains exist in the extracellular domain of DGI (Fig. 3), which may account for its Ca^{2+}-binding properties and suggest a direct role for DGI in Ca^{2+}-mediated desmosome assembly.

Cadherins undergo posttranslational proteolytic processing during transport to the cell surface (28). We did not find evidence for such processing of DGI in cultured cells, although DGII and DGIII do undergo a similar modification (4). However, recent data have revealed the presence of only a short prosequence for DGI (G.N.W., unpublished observations), the loss of which would probably have gone unnoticed in our previous analysis. The protease cleavage site in human E-cadherin has been identified by amino-terminal sequencing of the mature protein (29), and a comparison to DGI in this region reveals close similarity (Fig. 4*A*). The predicted sequence of the mature DGI codes for a protein of 107,578 Da, which is considerably smaller than its size (140 kDa) estimated by SDS/PAGE. However, all the cadherins have been found to be smaller than their predicted size by SDS/PAGE. DGI has been found to contain two to four N-glycans as well as O-glycans (4). The putative extracellular domain of the DGI cDNA has three potential N-glycosylation sites (Fig. 3). In addition, there is a serine- and threonine-rich region near the transmembrane domain (residues 486–507) that could serve as the site of O-glycosylation. A similar region is found in the muscle-specific domain of N-CAM (30) and in the low density lipoprotein-receptor (31) but not in the cadherin sequences determined previously.

The sequence of bovine DGI has been reported (32) and a comparison with the human sequence reveals 81% identity. The only significant difference is in the serine/threonine putative O-linked glycosylation site just external to the transmembrane domain. The bovine sequence apparently lacks 30–40 C-terminal residues present in the human sequence. However, this discrepancy has recently been corrected after further sequencing of bovine clones (W. Franke and P. Koch, personal communication).

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