Plakoglobin domains that define its association with the desmosomal cadherins and the classical cadherins: identification of unique and shared domains

**James K. Wahl, Paula A. Sacco, Tammy M. McGranahan-Sadler, Laura M. Sauppé, Margaret J. Wheelock*,†
and Keith R. Johnson†**

Department of Biology, University of Toledo, 2801 W. Bancroft St, Toledo, OH 43606, USA

*Author for correspondence (e-mail: mwheelo@uoft02.utoledo.edu)
†K. R. Johnson and M. J. Wheelock contributed equally to this research

SUMMARY

Two cell-cell junctions, the adherens junction and the desmosome, are prominent in epithelial cells. These junctions are composed of transmembrane cadherins which interact with cytoplasmic proteins that serve to link the cadherin to the cytoskeleton. One component of both adherens junctions and desmosomes is plakoglobin. In the adherens junction plakoglobin interacts with both the classical cadherin and with α-catenin. Alpha-catenin in turn interacts with microfilaments. The role plakoglobin plays in the desmosome is not well understood. Plakoglobin interacts with the desmosomal cadherins, but how and if this mediates interactions with the intermediate filament cytoskeleton is not known. Here we compare the domains of plakoglobin that allow it to associate with the desmosomal cadherins with those involved in interactions with the

classical cadherins. We show that three sites on plakoglobin are involved in associations with the desmosomal cadherins. A domain near the N terminus is unique to the desmosomal cadherins and overlaps with the site that interacts with α-catenin, suggesting that there may be competition between α-catenin and the desmosomal cadherins for interactions with plakoglobin. In addition, a central domain is shared with regions used by plakoglobin to associate with the classical cadherins. Finally, a domain near the C terminus is shown to strongly modulate the interactions with the desmosomal cadherins. This latter domain also contributes to the association of plakoglobin with the classical cadherins.

Key words: Cadherin, Adherens Junction, Desmosome, Plakoglobin

INTRODUCTION

Cell-cell adhesion is a complex process with desmosomes and adherens junctions as the primary structural entities (Schwartz et al., 1990; Garrod et al., 1990; Kaiser et al., 1993). Each of these structures is composed of transmembrane members of the cadherin family that serve to bind two cells together extracellularly and cytoplasmic proteins that link the cadherin to the cytoskeleton.

The transmembrane component of the adherens junction is a classical cadherin; these proteins generally mediate homotypic cell-cell adhesion (Geiger and Ayalon, 1992; Grunwald, 1993; Takeichi, 1988, 1991). Desmosomes have two transmembrane components, desmoglein and desmocollin, which are members of the cadherin superfamily. In the cytoplasm, classical cadherins associate with three proteins known as α-, β-, and γ-catenin (Magee and Buxton, 1991; Ozawa et al., 1989; Ozawa and Kemler, 1992); γ-catenin is identical to plakoglobin (Knudsen and Wheelock, 1992; Peifer et al., 1992). The desmosomal cadherins have been shown to directly interact with plakoglobin (Korman et al., 1989; Mathur et al., 1994; Kowalczyk et al., 1994; Troyanovsky et al., 1993,

1994a,b); plakoglobin is the only protein identified to date that is a component of the cytoplasmic plaque of both desmosomes and adherens junctions (Cowin et al., 1986). The cytoplasmic components of the adherens junction and the desmosome are important in mediating interactions with the appropriate cytoskeletal elements of the junction; i.e. actin and intermediate filaments, respectively (Geiger et al., 1985; Hitt and Luna, 1994; Staehelin, 1974).

In the past few years, efforts have been made to understand the molecular interactions that allow these complex adhesion structures to assemble. Studies have identified a 30 amino acid stretch in the cytoplasmic domain of the classical cadherins that is necessary for the interactions with β-catenin or plakoglobin (Stappert and Kemler, 1994). In addition, the domains on β-catenin and plakoglobin that are primarily responsible for binding to the classical cadherins have been mapped to a region within the repetitive central domain of these two proteins (Aberle et al., 1994; Hülsken et al., 1994; Sacco et al., 1995; Jou et al., 1995). Both β-catenin and plakoglobin associate with α-catenin through a domain located near the N terminus (Aberle et al., 1994; Sacco et al., 1995). Alpha-catenin, in turn, associates with the actin cytoskeleton both directly by inter-

acting with filamentous actin (Rimm et al., 1995) and indirectly through an interaction with α-actinin (Knudsen et al., 1995). In previous studies we have shown that a central domain of plakoglobin is required for its interactions with N-cadherin (Sacco et al., 1995). In this report we extend our studies to show that a domain near the N terminus is also necessary for plakoglobin to associate with classical cadherins. In addition, we show that a domain near the C terminus plays a role in the association of plakoglobin with the classical cadherins such that the interaction can withstand a harsh wash during immunoprecipitation reactions.

The linkage between the classical cadherins and the actin cytoskeleton via the catenins is better understood than the analogous connection of the desmosomal cadherins to the intermediate filament cytoskeleton. A short stretch of 37 amino acids in the C terminus of desmocollin-1 was recently shown by Troyanovsky et al. (1994a) to be necessary for it to associate with plakoglobin and to recruit intermediate filaments to the plasma membrane. Using similar experiments, they also identified a 10 amino acid sequence in desmocollin-1 near the plasma membrane that is necessary for desmoplakin to be recruited into the desmosomal plaque structure (Troyanovsky et al., 1994a). In addition, Mathur et al. (1994) mapped a 19 amino acid region in approximately the center of the cytoplasmic domain of desmoglein-1 that is essential for binding plakoglobin in vitro. The domain identified by Mathur et al. (1994) was also reported by Troyanovsky et al. (1994b). To date, the reciprocal domains on plakoglobin that are necessary for its interaction with the desmosomal cadherins have not been identified.

In this study, we identify the regions of plakoglobin that allow it to remain associated with desmoglein and desmocollin when cells are extracted with non-ionic detergents. At least three regions on plakoglobin are involved in these interactions. They include: (1) a domain near the N terminus that overlaps with the domain used to associate with α-catenin in the adherens junction, suggesting that competition between α-catenin and desmosomal cadherins may partially contribute to the exclusion of α-catenin from the desmosome; (2) a sequence near the C terminus that may coincide with a domain involved in the association of plakoglobin with the classical cadherins; and (3) the previously identified classical cadherin association site located within the central part of plakoglobin. The first domain is unique to the desmosomal cadherins, the second domain modulates in different ways the association of plakoglobin with the classical and desmosomal cadherins and the third domain overlaps with regions used by plakoglobin to associate with the classical cadherins.

MATERIALS AND METHODS

Cells

A-431 human epidermoid carcinoma cells and HT1080 human fibrosarcoma cells were obtained from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium (Sigma Chemical Co., St Louis, MO) supplemented with 5% fetal calf serum (Hyclone, Logan, UT). Transfectants were maintained in the same medium supplemented with 10^{-7} M dexamethasone (Sigma) and 1 mg/ml G-418 (Life Technologies, Gaithersburg, MD). Cells were metabolically labeled as described (Wheelock et al., 1987).

Molecular constructions and transfections

The full-length human plakoglobin clone in pBluescript (HPG Ca2.1; Franke et al., 1989) was a gift from Dr Werner W. Franke, German Cancer Research Center, Heidelberg. The expression vector pLKneo (Hirt et al., 1992) was used for these studies. For each clone described in the text, the extent of the deletion was determined by nucleotide sequencing. Where PCR products were used, each was subcloned and sequenced. The sequence of individual PCR subclones was shown to be consistent with the published plakoglobin sequence (GenBank accession number M23410) prior to their use. Primers were from DNA International, Inc. (Lake Oswego, OR).

The methods used for the preparation of the C-terminal truncations have been described (Sacco et al., 1995). The C-terminal deletions described in the text (see Table 1) were generated as follows: 707, 632, 472, 375 and 303, Exonuclease III; 687, 572 and 233, restriction sites; and 414 and 334, PCR.

For N-terminal deletions, the plakoglobin cDNA was first subcloned into pSPUTK (Falcone and Andrews, 1991) which provided a 5′ untranslated region from *Xenopus* β-globin, a start codon and a multiple cloning site. The clone labeled 234 (see Table 1) was formed by deleting an *Sph*I restriction fragment from the pSPUTK subclone. The clone labeled 123 was generated by PCR. The other N-terminal deletions listed in Table 1 or discussed in the text were made with timed Exonuclease III digestion. Clones where the remaining plakoglobin sequence was in-frame with the start codon were identified by sequencing. The N-terminal deletions contained several amino acids derived from pSPUTK prior to the beginning of the plakoglobin sequence.

Chimeric plakoglobin and chicken β-catenin (Johnson et al., 1993) cDNAs were constructed by cutting plakoglobin at the unique *Sma*I

Table 1. Association of plakoglobin deletion mutants with cadherins

| Deletion mutant | Associates with: | |
	classical cadherins	desmosomal cadherins
C-terminal deletions (number of amino acids remaining)		
707AA	+	+
687AA	+	+
632AA	+	±
572AA	+	−
472AA	+	−
414AA	+	n/d
375AA	±	−
334AA	−	n/d
303AA	−	n/d
233AA	−	−
N-terminal deletions (number of the first amino acid)		
58AA	+	+
113AA	+	+
116AA	+	+
123AA	+	+
130AA	+	±
134AA	+	−
151AA	+	−
191AA	+	−
234AA	−	−

Data from many of the deletion mutants used in these studies are summarized. C-terminal deletions all begin at amino acid number 1 and are listed as the number of amino acids remaining. N-terminal deletions show the number of the first plakoglobin amino acid retained in the construct and all extend to the C-terminal amino acid 745. Immunoprecipitations were done with antibodies against the cadherins. Plus means the deletion mutant retained its ability to associate with the cadherin; minus means the deletion mutant did not associate with the cadherin; plus/minus means the deletion mutant slightly associated with the caherin; n/d, not done.

site and β-catenin at the unique *Eco*72I site. Reciprocal fragments were then ligated together to produce two chimeras; one contained the N terminus of plakoglobin joined to the C terminus of β-catenin and the other contained the N terminus of β-catenin joined to the C terminus of plakoglobin (see Fig. 9). Cell cultures were transfected using calcium phosphate precipitation (Chen and Okayama, 1987) with materials from Stratagene (La Jolla, CA) and G418-resistant clones were isolated. All data in this manuscript were obtained with stable cell lines.

Antibodies and other reagents

The rat monoclonal antibody against human E-cadherin has been described (Wheelock et al., 1987). Mouse monoclonal antibodies 4F11 against the N terminus of plakoglobin and 6A9 against the extracellular domain of P-cadherin have been described (Sacco et al., 1995; Johnson et al., 1993). The Pg5.1 antibody against plakoglobin was a kind gift from Dr Werner W. Franke. The monoclonal antibody (6D8) against desmoglein was generated as described for P-cadherin (Lewis et al., 1994). The monoclonal antibody (7G6) against the extracellular domain of desmocollin-2 (Parker et al., 1991) was generated against a maltose-binding protein fusion protein as described for other antibodies (Johnson et al., 1993). This antibody may also recognize other isoforms of desmocollin. The desmocollin-2 cDNA clone was kindly provided by Dr Kathleen J. Green, Northwestern University. The anti-desmoplakin antibody was purchased from PROGEN (Heidelberg, Germany).

Antibody epitope mapping

The complete open reading frame of plakoglobin cDNA was inserted into pMAL-c2 (New England BioLabs, Beverly, MA). Timed Exonuclease III digestion was used to delete sequences encoding the C terminus. Bacteria containing selected clones were induced to express the fusion protein and extracts were immunoblotted with monoclonal antibody Pg5.1. The plasmids were sequenced in order to determine the extent of the deletion. Among the clones that were tested, the shortest clone positive with Pg5.1 contained amino acids 1 through 681; the longest negative clone contained amino acids 1 through 668. The 4F11 epitope was initially mapped between amino acids 48 and 114 using bacterially-expressed C-terminal deletions. It was more finely mapped using extracts of A-431 cells that had been transfected with N-terminal deletions. Using this assay, plakoglobin missing 57 N-terminal amino acids was positive with 4F11 while a form missing 74 amino acids was negative.

Detergent extraction of cells

Monolayers of cells were washed with phosphate buffered saline (PBS) at room temperature. The cells from a T225 flask were then scraped in 5 ml 10 mM Tris acetate, pH 8.0, 1 mM EDTA (TE) containing 2 mM phenylmethylsulfonyl fluoride (PMSF) and Dounce homogenized for 20 strokes using a Dounce homogenizer and the tight pestle. The insoluble material was pelleted at 15,000 *g* for 15 minutes at 4°C, washed 1× in TE containing PMSF and extracted on ice with 5 ml 10 mM Tris acetate, pH 8.0, containing 0.5% Nonidet

P40 (NP40; BDH Chemicals Ltd, Poole, England), 1 mM EDTA, and PMSF. Insoluble material was removed by centrifugation at 15,000 *g* for 15 minutes at 4°C.

Electrophoresis and immunoblotting

Polyacrylamide slab gel electrophoresis in the presence of SDS (SDS-PAGE) was done according to the procedure of Laemmli (1970). Materials were from Bio-Rad (Richmond, CA). Molecular mass markers were from Sigma. SDS-PAGE-resolved proteins were transferred to nitrocellulose and immunoblotted as described (Knudsen and Wheelock, 1992).

Immunoprecipitation

A 300 μl aliquot of NP-40 extract was mixed with 300 μl monoclonal antibody supernatant at 4°C. After 30 minutes 50 μl packed antimouse IgG-Sepharose (Organon Teknika-Cappel, Durham, NC) was added and mixing was continued for 30 minutes. The Sepharose-bound immune complexes were washed 5 times with either non-stringent buffer composed of 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Tween-20 or stringent buffer (RIPA buffer) composed of 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% deoxycholate, 1% Triton X-100, 0.1% SDS. The pellets were boiled in 2× Laemmli sample buffer and resolved by SDS-PAGE.

Immunofluorescence

Immunofluorescence was done as previously described (Knudsen et al., 1995).

RESULTS

Mapping regions of plakoglobin that allow it to associate with P-cadherin, desmoglein and desmocollin

A-431 cells express two classical cadherins, E-cadherin and P-cadherin (Johnson et al., 1993), and three desmosomal cadherins, desmoglein-2 and desmocollins-1 and -2 (Nuber et al., 1995; Schäfer et al., 1994). Plakoglobin is expressed by these cells and associates equally well with E-cadherin and P-cadherin in order to form adherens junctions. In addition, it associates with both desmoglein and the desmocollins to form desmosomes. Although the components of desmosomes are only partially soluble in non-ionic detergents, we were able to extract sufficient quantities of each component to observe the interactions of plakoglobin with E-cadherin, P-cadherin, desmoglein and desmocollin (see Figs 2 and 3). Our goal in this study was to define the domains on plakoglobin that are necessary for it to associate with the cadherins. To accomplish this goal it was necessary to work with the non-ionic detergent soluble fraction of the cadherin/plakoglobin complexes in order to preserve protein-protein interactions. However, we
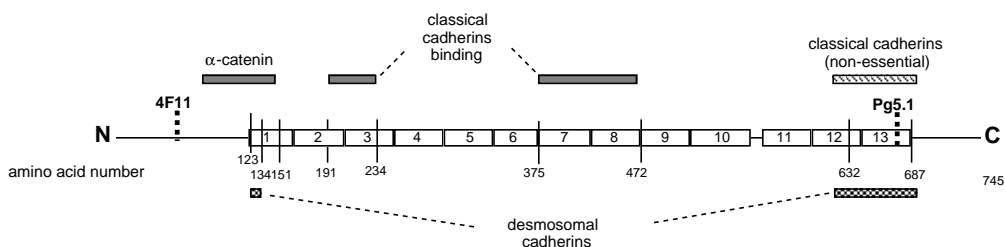
Fig. 1. Associations of plakoglobin with classical and desmosomal cadherins. The 745 amino acid sequence of human plakoglobin is represented as a line. The 13 imperfect repeats are shown as numbered boxes. The domains on plakoglobin necessary for association with various partners are indicated. The antibody binding sites for monoclonal antibodies 4F11 and Pg5.1 are indicated as bold broken lines.
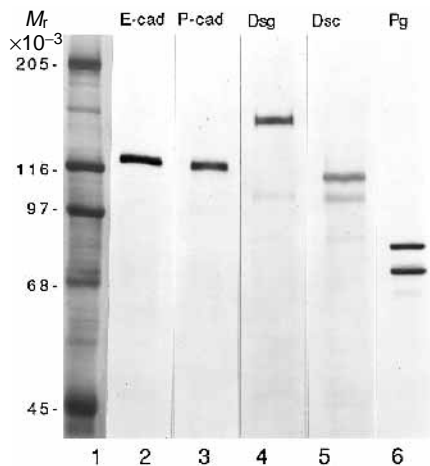
Fig. 2. Non-ionic detergent extraction of cadherins and plakoglobin from A-431 cells. A-431 cells transfected with a C-terminal deletion of plakoglobin retaining 707 amino acids were Dounce homogenized and the insoluble fraction was extracted with NP-40. Proteins in the NP-40 extract were resolved by 7% SDS-PAGE, transblotted to nitrocellulose, and probed with various monoclonal antibodies. Lane 1, molecular mass standards; lane 2, anti-E-cadherin; lane 3, anti-P-cadherin; lane 4, anti-desmoglein; lane 5, anti-desmocollin; lane 6, anti-plakoglobin 4F11. In lane 6, both the endogenous plakoglobin at 82 kDa and the truncated plakoglobin at 70 kDa were detected.

have shown that each deletion that retained its ability to associate with the desmosomal cadherins was capable of being incorporated into the detergent insoluble fraction (see Fig. 7 and related discussion).

In order to minimize the possibility that protein-protein interactions were obscured by inappropriate protein folding rather than removal of specific plakoglobin domains, we made use of a large number of deletion mutants. In most cases the difference in the number of amino acids between the last mutation that bound to the cadherin and the one that did not bind was less than 50 amino acids (see Table 1). Although this strategy does not completely rule out the possibility that we are looking at artifacts due to protein misfolding, it minimizes the possibility.

Deletions of plakoglobin were generated by cutting the cDNA at restriction sites, by polymerase chain reaction, or by exonuclease III digestion resulting in C-terminal or N-terminal truncations of plakoglobin as described in Materials and Methods. Each deleted construct was transfected into A-431 cells and clones were propagated. Extracts of cells stably transfected with each mutant were examined by co-immunoprecipitation reactions. The transfectants expressed both full-length, endogenous plakoglobin as well as the truncated version. To save space, only selected experiments have been shown in the following figures. Fig. 1 presents a map of plakoglobin with each functional domain indicated. Table 1 summarizes selected deletion mutations that were generated and indicates their ability to associate with classical and desmosomal cadherins.

To determine if we were efficiently extracting E-cadherin, P-cadherin, the desmosomal cadherins, and both the endogenous and truncated plakoglobins, NP-40 extracts of the transfected cells were resolved by SDS-PAGE, transferred to nitrocellulose and immunoblotted with the antibodies of interest.

Fig. 2 presents an immunoblot of A-431 cells transfected with a C-terminally truncated construct of plakoglobin retaining 707 amino acids. The molecular mass markers are in lane 1. Lane 2 was immunoblotted with anti-E-cadherin, lane 3 with anti-P-cadherin, lane 4 with anti-desmoglein, lane 5 with anti-desmocollin (both the longer and the shorter splice variants were visible), and lane 6 with 4F11 anti-plakoglobin. Both the endogenous plakoglobin at 82 kDa and the truncated version at about 70 kDa were identified by the 4F11 antibody. Each of the truncated plakoglobin molecules used in this study, whether they were produced as C-terminal deletions or N-terminal deletions, was stably expressed along with the full-length endogenous plakoglobin in A-431 cells. The expression levels of the deletions were always sufficient to be immunoprecipitated with the cadherins and identified by immunoblot analysis. The relative amounts of full-length plakoglobin vs truncated plakoglobin varied, making it necessary to compare ratios of full-length plakoglobin to truncated plakoglobin when analyzing the ability of truncated proteins to remain associated with the cadherins. Each deletion of plakoglobin was also transfected into HT1080 cells to determine its ability to remain associated with N-cadherin when cells were extracted with non-ionic detergent (data not shown but see Sacco et al., 1995, for C-terminal deletions). We detected no differences between the association of plakoglobin deletion mutants with either N-cadherin, E-cadherin or P-cadherin.

Direct interactions between plakoglobin and the classical cadherins (Sacco et al., 1995; Aberle et al., 1994) and between plakoglobin and the desmosomal cadherins (Troyanovsky et al., 1994a; Troyanovsky et al., 1994b; Mathur et al., 1994; Kowalczyk et al., 1994) have been reported. We have previ-
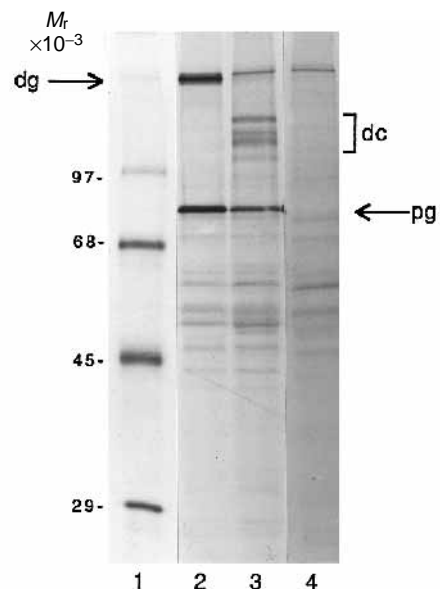

Fig. 3. Association of plakoglobin with desmosomal cadherins. A-431 cells were grown in the presence of [35S]cysteine and [35S]methionine, extracted with NP-40 and immunoprecipitated with anti-desmoglein (lane 2), anti-desmocollin (lane 3) or no antibody (control, lane 4). Proteins were resolved by 7% SDS-PAGE, fixed and en3Hanced and exposed to X-ray film at −70°C. Bands corresponding to desmoglein (dg), desmocollin (dc) and plakoglobin (pg) are indicated.

ously shown that in A-431 cells our anti-P-cadherin (6A9) and anti-E-cadherin (E9) antibodies co-immunoprecipitate plakoglobin, β-catenin and α-catenin but no other major proteins. In order to show that immunoprecipitations done with our anti-desmoglein (6D8) and anti-desmocollin (7G6) monoclonal antibodies included the desmosomal cadherin and plakoglobin but no other major bands, we immunoprecipitated extracts of [^{35}S]cysteine/methionine-labeled cells. Fig. 3 shows an anti-desmoglein immunoprecipitation in lane 2 and an anti-desmocollin immunoprecipitation in lane 3. A control reaction without a primary antibody is shown in lane 4. Desmoglein, desmocollin and plakoglobin were the only bands specifically immunoprecipitated in these experiments (compare lanes 2 and 3 with lane 4).

The expression of truncated plakoglobin molecules did not interfere with the ability of A-431 cells to interact with one another as indicated by morphological analysis and by the staining patterns of E-cadherin and desmoplakin in immunofluorescence light microscopy. Fig. 4 compares the parental A-431 cells with those transfected with C-terminally truncated plakoglobin retaining 707 amino acids. Phase micrographs are shown in Fig. 4A and B; the localization of E-cadherin is compared in Fig. 4C and D and the localization of desmoplakin is compared in Fig. 4E and F. The transfected cells display the close cell-cell associations typical of squamous epithelial cells. In addition, the localizations of both E-cadherin and desmo-

plakin in the transfectant are similar to those seen in untransfected cells as well as in cells transfected with vector alone (not shown).

To determine if the truncated plakoglobin molecules retained their ability to associate with classical cadherins and desmosomal cadherins when cells were extracted with non-ionic detergent, we immunoprecipitated P-cadherin, desmoglein and desmocollin from cell extracts, resolved the immunoprecipitation reactions by SDS-PAGE and immunoblotted the immunoprecipitation reactions with 4F11 anti-plakoglobin. Fig. 5 shows a western blot of immunoprecipitation reactions of A-431 cells transfected with two different C-terminal truncation mutants of plakoglobin, one retaining 707 amino acids (lanes 1-4) and one retaining 632 amino acids (lanes 5-8). Lanes 1 and 5 show the relative amounts of the full-length plakoglobin compared to the truncated version in the NP-40 extracts used for immunoprecipitation reactions. As expected, the full-length, endogenous plakoglobin co-immunoprecipitated with P-cadherin, desmoglein and desmocollin. The 707 amino acid construction also associated with P-cadherin (lane 2), desmoglein (lane 3) and desmocollin (lane 4). Full-length plakoglobin and the truncated versions are indicated by arrows. We have also done similar experiments with a truncation retaining 687 amino acids of plakoglobin (see Table 1). This truncation behaved the same as the 707 amino acid truncation (not shown).
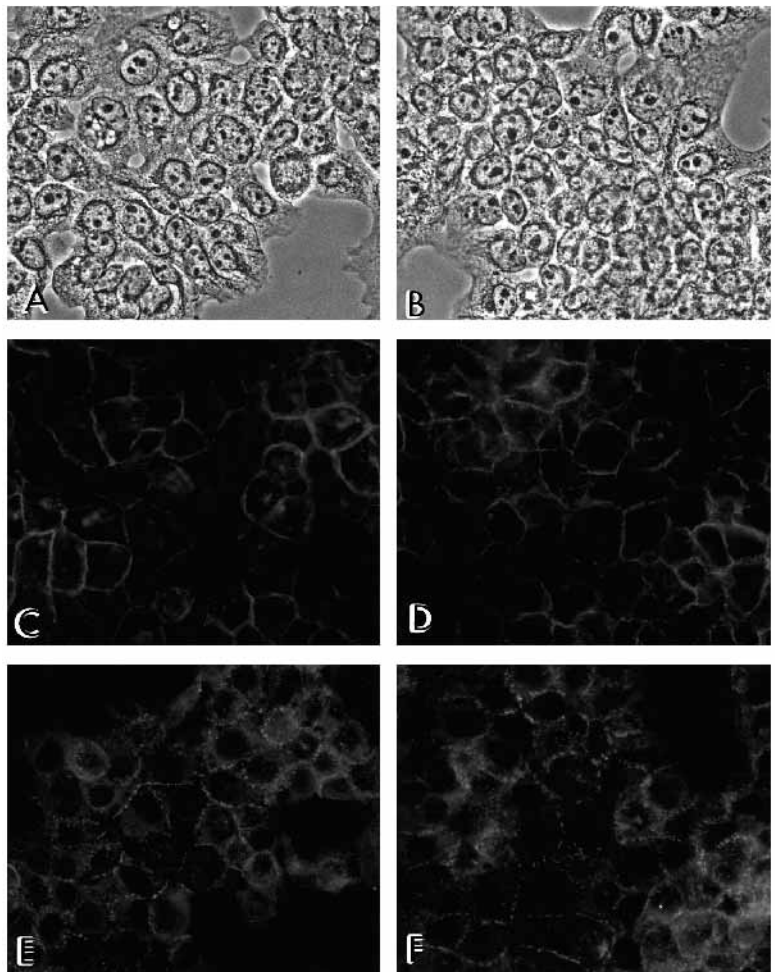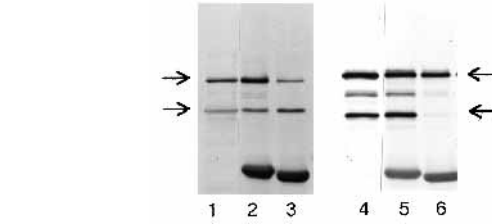

Fig. 4. Microscopic analysis of wild-type A-431 cells and A-431 cells transfected with truncated plakoglobin. Wild type A-431 cells (A,C,E) and A-431 cells transfected with a C-terminal deletion of plakoglobin retaining 707 amino acids (B,D,F) were grown on glass coverslips and processed for immunofluorescence. Phase microscopy (A and B) indicates that transfection with truncated plakoglobin did not alter the morphology of the cells. Immunofluorescence microscopy indicates that the localization of E-cadherin (C and D) and desmoplakin (E and F) is identical in A-431 cells and transfected A-431 cells.
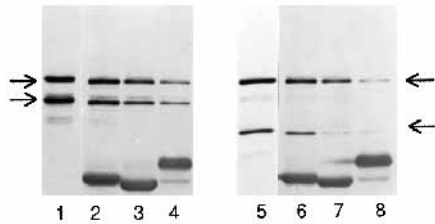
Fig. 5. C-terminal deletions retaining 632 amino acids show decreased association with desmosomal but not classical cadherins. A-431 cells transfected with C-terminal deletions of plakoglobin retaining 707 amino acids (lanes 1-4) or 632 amino acids (lanes 5-8) were Dounce homogenized and the insoluble fraction was extracted with NP-40. The extract was immunoprecipitated with antibodies against P-cadherin (lanes 2 and 6), desmoglein (lanes 3 and 7) or desmocollin (lanes 4 and 8). Proteins were resolved by 7% SDS-PAGE, transblotted to nitrocellulose, and probed with monoclonal antibody 4F11 which is directed against the N terminus of plakoglobin. Lanes 1 and 5 contained the NP-40 extract and show the relative amounts of the endogenous plakoglobin and the faster-migrating truncated versions. The truncated plakoglobin retaining 707 amino acids remained associated with P-cadherin (lane 2), desmoglein (lane 3) and desmocollin (lane 4) during the immunoprecipitation reaction. The truncated plakoglobin retaining 632 amino acids remained associated with P-cadherin (lane 6), but showed a markedly decreased association with desmoglein (lane 7) or desmocollin (lane 8). Full-length plakoglobin and the truncated versions are pointed out by arrows. Also prominent at the bottom of the immunoblot were the antibody heavy chains from the immunoprecipitation reactions which were detected by the alkaline phosphatase-labeled secondary antibodies.

When similar experiments were done with the 632 amino acid C-terminally truncated plakoglobin molecule, it associated with P-cadherin as efficiently as did the full-length plakoglobin (Fig. 5, lane 6). However, this truncated plakoglobin only slightly associated with either desmoglein (lane 7) or desmocollin (lane 8). Truncations smaller than 632 amino acids were associated even less with the desmosomal cadherins but continued to associate with classical cadherins and α-catenin. Truncations smaller that 375 amino acids failed to associate with any cadherin although they remained associated with α-catenin down to 151 amino acids (Sacco et al., 1995). Table 1 presents these data.

N-terminal deletion mutants were generated and examined for their ability to associate with P-cadherin, desmoglein and desmocollin. We found that distinct amino-terminal regions were required for desmosomal cadherin association (amino acids 123-133) and classical cadherin association (amino acids 191-233, see Table 1). Fig. 6 presents a typical experiment to test N-terminal deletion mutants. Extracts from transfected A-431 cells were immunoprecipitated with antibodies against the cadherins. The immunoprecipitation reactions were resolved by SDS-PAGE and immunoblotted with Pg5.1, an anti-plakoglobin monoclonal antibody that recognizes the C terminus of plakoglobin. Fig. 6, lanes 1 and 4, shows the relative amounts of full-length plakoglobin and the truncated versions in the NP-40 extract used for immunoprecipitation reactions. These proteins are pointed out by arrows. Lanes 4 and 5 also contained a band migrating between full-length plakoglobin and the deleted version that is presumably a plakoglobin breakdown product. This experiment demonstrated that plako-

Fig. 6. Plakoglobin deletion lacking 133 amino acids from the N terminus does not co-immunoprecipitate with desmosomal cadherins. A-431 cells transfected with N-terminal deletions of plakoglobin removing 112 amino acids (lanes 1-3) or 133 amino acids (lanes 4-6) were Dounce homogenized and the insoluble fraction was extracted with NP-40. The extract was immunoprecipitated with antibodies against P-cadherin (lanes 2 and 5) or desmoglein (lanes 3 and 6). Proteins were resolved by 7% SDS-PAGE, transblotted to nitrocellulose, and probed with monoclonal antibody Pg5.1 which is directed against the C terminus of plakoglobin. Lanes 1 and 4 contain the NP-40 extract and show the relative amounts of the endogenous plakoglobin and the faster migrating truncated versions. The truncated plakoglobin with 112 amino acids removed from the N terminus remained associated with P-cadherin (lane 2) and desmoglein (lane 3) during the immunoprecipitation reaction. The truncated plakoglobin with 133 amino acids removed remained associated with P-cadherin (lane 5), but showed a markedly decreased ability to co-immunoprecipitate with desmoglein (lane 6). The band seen in lanes 4 and 5 that migrates between the full-length plakoglobin (arrow) and the truncated version (arrow) is likely a plakoglobin break-down product.
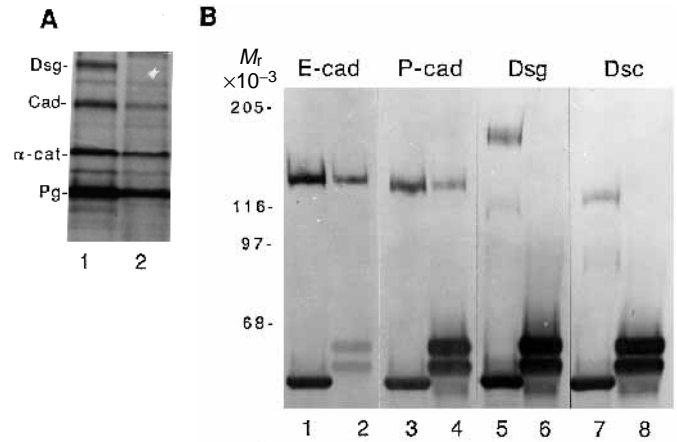
globin missing 112 amino acids associated with P-cadherin (lane 2), desmoglein (lane 3) and desmocollin (not shown). Plakoglobin missing 57 amino acids behaved identically to plakoglobin missing 112 amino acids (not shown; see Table 1). Plakoglobin missing 133 amino acids associated with P-cadherin (Fig. 6, lane 5) but not with desmoglein (lane 6) or desmocollin (not shown). Additional experiments performed with plakoglobin missing 115 or 122 amino acids showed that these truncations retained their ability to associate with both classical cadherins and desmosomal cadherins while a truncation missing 129 amino acids retained its ability to associate with classical cadherins but only slightly associated with desmosomal cadherins (data not shown; see Table 1). Similar experiments were performed with clones missing 150, 190 or 233 amino acids. In these cases, the clones missing 150 or 190 amino acids co-immunoprecipitated with P-cadherin while the clone missing 233 amino acids did not (not shown, see Table 1).

In A-431 cells the desmosomal cadherins and plakoglobin are only partially extractable in non-ionic detergents. The insoluble cadherins and plakoglobin are presumed to be stably incorporated into junctions. To determine if the truncated plakoglobin molecules were able to be incorporated into mature junctions, we examined the detergent extractibility of the proteins. Cells were extracted with non-ionic detergent and the insoluble material was re-extracted with SDS. Fig. 7 shows the detergent soluble (S) and insoluble fractions (I) of three representative clones resolved on SDS-PAGE and immunoblotted with anti-plakoglobin antibodies. Fig. 7A contains samples from the N-terminal deletion that removes 233 AA. This truncated plakoglobin does not associate with
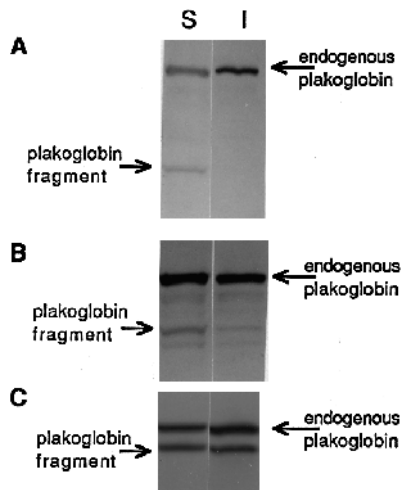
Fig. 7. Plakoglobin deletion mutants that retain the ability to associate with cadherins become insoluble in non-ionic detergent. A-431 cells were transfected with plakoglobin deletions known to differentially associate with the cadherins (see Table 1). Clones of A-431 transfectants were extracted with NP-40 and separated into NP-40 soluble (S) and insoluble (I) fractions. The proteins in each fraction were resolved on 7% SDS-PAGE, transblotted to nitrocellulose and probed with monoclonal antibodies against plakoglobin. (A) A-431 cells were transfected with the N-terminal truncation beginning at amino acid 234 which did not co-immunoprecipitate with either class of cadherin (Table 1). In contrast to the endogenous plakoglobin, the deletion is found only in the NP-40 soluble fraction. (B) A-431 cells were transfected with the C-terminal deletion retaining 572 amino acids which co-immunoprecipitated with the classical but not the desmosomal cadherins (Table 1). When compared with the endogenous plakoglobin, the truncation was found at low levels in the NP-40 insoluble fraction. (C) A-431 cells were transfected with the N-terminal deletion beginning at amino acid 58 which co-immunoprecipitated with both classes of cadherin (Table 1). This deletion was found distributed between the soluble and insoluble fractions equivalent to endogenous plakoglobin.

Fig. 8. The monoclonal antibody Pg5.1 does not co-immunoprecipitate the desmosomal cadherins. (A) A-431 cells were grown in the presence of [^{35}S]cysteine and [^{35}S]methionine, extracted with NP-40 and immunoprecipitated with 4F11 (lane 1) or Pg5.1 (lane 2). Proteins were resolved by 7% SDS-PAGE, fixed, en^{3}Hanced and exposed to X-ray film at −70°C. Bands corresponding to desmoglein (Dsg), classical cadherins (Cad), α-catenin (α-cat) and plakoglobin (Pg) are pointed out.
(B) Immunoblot analysis of 4F11 immunoprecipitations (lanes 1, 3, 5, and 7) and Pg5.1 immunoprecipitations (lanes 2, 4, 6 and 8). Lanes 1 and 2 were immunoblotted with anti-E-cadherin; lanes 3 and 4 were immunoblotted with anti-P-cadherin; lanes 5 and 6 were immunoblotted with anti-desmoglein; and lanes 7 and 8 were immunoblotted with anti-desmocollin.

classical or desmosomal cadherins (see Table 1). All of the fragment (arrow) is found in the soluble fraction; as expected, the endogenous plakoglobin is found both in the NP-40 soluble fraction and in the insoluble fraction. Fig. 7B contains samples from the C-terminal deletion that retains 572AA. This truncated plakoglobin associates with classical cadherins but not with desmosomal cadherins (see Table 1). This fragment (arrow) is found both in the soluble and insoluble fractions. Fig. 7C contains samples from the N-terminal deletion that removes 57 AA. This truncated molecule (arrow) associates both with classical cadherins and with desmosomal cadherins and is found in both the soluble and insoluble fractions. A comparison of Fig. 7B and C shows that truncated plakoglobin that retained its ability to associate with desmosomal cadherins was less soluble in NP-40 than truncated plakoglobin that could only associate with classical cadherins; i.e. there is more fragment in the insoluble fraction in Fig. 7C than there is in Fig. 7B. Thus, the plakoglobin fragments that are capable of associating with the cadherins become incorporated into the detergent insoluble fraction of the cell extract, presumably because they are incorporated into junctions (Fig. 7B and C)

while those that do not associate with cadherins remain soluble (Fig. 7A).

Fig. 1 presents a diagram of the most informative deletion mutants and the ability of these shortened proteins to associate with classical and desmosomal cadherins. Since E-cadherin and P-cadherin are highly homologous to one another, especially in the cytoplasmic domain where plakoglobin associates, we examined only P-cadherin as our classical cadherin. We have also examined both the C- and N-terminal truncations of plakoglobin for their association with N-cadherin in HT1080 cells (Sacco et al., 1995; and data not shown) and include these data in Fig. 1 and Table 1. In each case the deletion mutants that retained their ability to associate with N-cadherin also associated with P-cadherin. We examined the association of each truncated version of plakoglobin with both desmoglein and desmocollin because these two cadherins differ substantially in their cytoplasmic domains (Koch and Franke, 1994). We found no evidence that plakoglobin interacts differently with the two desmosomal cadherins. Interestingly, the region of plakoglobin that is necessary for association with the desmosomal cadherins maps to the same region that we have previously shown to be necessary for association with α-catenin in the classical cadherin-catenin complex (Sacco et al., 1995).

Characterization of the Pg5.1 epitope

We first suspected that the association of plakoglobin with the desmosomal cadherins differs from its association with the classical cadherins when we used the Pg5.1 antibody to immunoprecipitate plakoglobin from A-431 cells. Only the classical cadherins co-immunoprecipitated with plakoglobin

when Pg5.1 was used. Fig. 8A shows immunoprecipitations of [^{35}S]cysteine/methionine-labeled A-431 cell extract comparing the 4F11 monoclonal antibody (lane 1) with Pg5.1 (lane 2). E- and P-cadherin co-migrated at around 120 kDa and are pointed out (Cad). Desmocollins migrated at approximately 116 kDa and are not distinguishable from the E- and P-cadherin band. Desmoglein migrated at approximately 160 kDa and is pointed out (Dsg). The band at 160 kDa was not present when Pg5.1 was the immunoprecipitating antibody (lane 2) even though the bands corresponding to the classical cadherins were clearly visible. Also evident in the autoradiogram were α-catenin (α-cat) and plakoglobin (pg).

To confirm the identity of the proteins that co-immunoprecipitated with plakoglobin, we resolved immunoprecipitation reactions by SDS-PAGE and immunoblotted individual lanes with antibodies against E-cadherin (Fig. 8B, lanes 1 and 2), P-cadherin (lanes 3 and 4), desmoglein (lanes 5 and 6), or desmocollin (lanes 7 and 8). Antibody 4F11 co-immunoprecipitated both of the classical cadherins and both of the desmosomal cadherins (lanes 1, 3, 5 and 7) while Pg5.1 co-immunoprecipitated the classical cadherins (lanes 2 and 4) but not the desmosomal cadherins (lanes 6 and 8). Pg5.1 did recognize the plakoglobin molecules that co-immunoprecipitated with desmoglein (see Fig. 9, lane 3) demonstrating that the Pg5.1 binding site had not been altered by post-translational modification. Taken together, these data suggest that the epitope recognized by Pg5.1 is inaccessible when plakoglobin is associated with either desmoglein or desmocollin but freely accessible when plakoglobin is associated with one of the classical cadherins. In contrast, the 4F11 epitope is always accessible to its antibody. Note in Fig. 8B, lane 7 that only the larger desmocollin isoform co-immunoprecipitated with plakoglobin. It has been previously reported that the smaller isoform does not recruit plakoglobin to the plasma membrane (Troyanovsky et al., 1994a).

To determine where the Pg5.1 antibody bound to plakoglobin, we prepared exonuclease III deletions of the full-length plakoglobin cDNA inserted in the pMAL-c2 vector and expressed the truncated proteins in bacteria. Bacterial extracts were resolved by SDS-PAGE and immunoblotted with Pg5.1. The epitope was present on protein that included amino acids 1 through 681 of plakoglobin but was missing on protein that included amino acids 1 through 668 (data not shown). In support of this mapping data, the plakoglobin truncation retaining 687 amino acids expressed in A-431 was recognized by Pg5.1 on immunoblots while the 632 amino acid truncation was not (data not shown). Thus, the Pg5.1 epitope maps within the C terminus at the extreme end of repeat 13 (see Fig. 1). Using extracts of A-431 cells transfected with N-terminal deletion mutants of plakoglobin, we mapped the 4F11 monoclonal antibody binding site to amino acids 58 through 74 (data not shown).

Our interpretation of these immunoprecipitation results is that the region of plakoglobin including amino acids 669 to 681 is closely associated with the desmosomal cadherins such that the Pg5.1 antibody does not have access to its binding site. Note that the Pg5.1 binding site was removed when going from the 687 amino acid construct to the 632 amino acid construct concomitant with a significant loss in ability of the deletion to associate with the desmosomal cadherins. The domain surrounding the Pg5.1 site is not essential for association with the

classical cadherins (see Fig. 1 and Table 1), however, this region of plakoglobin is essential for the classical cadherin-plakoglobin complex to be stable in RIPA buffer as discussed below.

Both the N and C termini of plakoglobin are necessary for association with desmosomal cadherins

In order to further characterize the association between cadherins and plakoglobin we constructed chimeric molecules between plakoglobin and β-catenin. A *Sma*I site in plakoglobin cDNA coincided with an *Eco*72I site in chicken β-catenin cDNA. Thus two chimeric molecules were formed by joining the sequences at arginine 572 of plakoglobin which corresponds to arginine 582 of β-catenin (Fig. 9A). The cDNAs encoding these two chimeric molecules were transfected into A-431 cells and immunoprecipitations were performed with
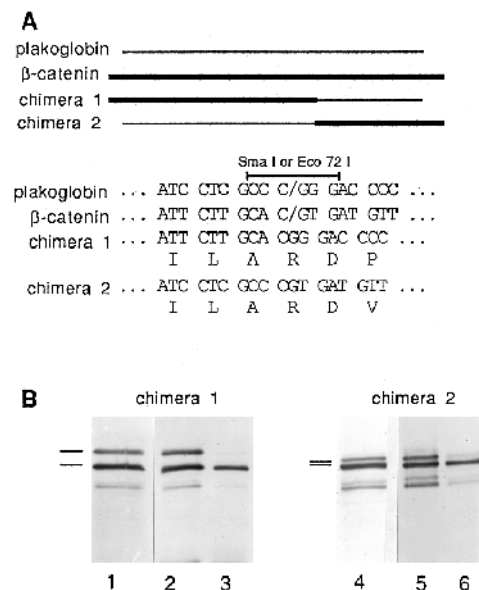
Fig. 9. Both N-terminal and C-terminal sites are essential for plakoglobin to associate with desmosomal cadherins. (A) Chimeric molecules between plakoglobin and β-catenin were constructed taking advantage of the *Sma*I and *Eco*72I sites in corresponding positions in plakoglobin and β-catenin, respectively. Shown are cartoons of the chimeric molecules as well as the sequences of plakoglobin, β-catenin and the two chimeras. (B) A-431 cells transfected with the above chimeric molecules were Dounce homogenized and the insoluble fraction was extracted with NP-40. The extract was immunoprecipitated with antibodies against P-cadherin (lanes 2 and 5) or desmoglein (lanes 3 and 6). Proteins were resolved by 7% SDS-PAGE, transblotted to nitrocellulose, and probed with monoclonal antibody Pg5.1 which is directed against the C terminus of plakoglobin (lanes 1-3) or monoclonal antibody 4F11 which is directed against the N terminus of plakoglobin (lanes 4-6). Lanes 1 and 4 contained NP-40 extract. The chimeric molecules and endogenous plakoglobin are pointed out. Each chimera migrated a little slower than endogenous plakoglobin, and chimera 1 migrated slower than chimera 2. Lanes 1 and 4 are included to indicate the relative amounts of the chimeric proteins and the endogenous plakoglobin. Both chimeras co-immunoprecipitated with P-cadherin (lanes 2 and 5) but neither co-immunoprecipitated with desmoglein (lanes 3 and 6). The bands that migrate faster than full-length plakoglobin are likely plakoglobin break-down products.

anti-P-cadherin or anti-desmoglein monoclonal antibodies. The proteins in the immunoprecipitation reactions were resolved by SDS-PAGE and immunoblotted with antibodies specific for plakoglobin (Fig. 9B). We confirmed the identity of the chimeric molecules by immunoblotting with anti-β-catenin antibodies (not shown). Note that the chimeras migrated more slowly on SDS-PAGE than did the endogenous plakoglobin. It is likely that the bands seen in lanes 1, 2, 4 and 5 that migrate more rapidly than full-length plakoglobin are breakdown products. As expected, the endogenous plakoglobin co-immunoprecipitated with P-cadherin and desmoglein. However, the chimeras differentially associated with the cadherins. Both of the chimeric molecules co-immunoprecipitated with P-cadherin (Fig. 9B, lanes 2 and 5), but neither co-immunoprecipitated with desmoglein (Fig. 9B, lanes 3 and 6).

The constructs presented in Fig. 9 make a smooth transition from plakoglobin to β-catenin or vice versa. Both of these constructs associated fully with E-cadherin, P-cadherin and α-catenin. We presume these molecules assumed a properly folded conformation since they were full-length and associated as efficiently with the classical cadherins as did either endogenous plakoglobin (Fig. 9, compare lanes 1 and 4 with lanes 2 and 5) or β-catenin (not shown). However, neither of these chimeric molecules associated with desmosomal cadherins. Taken together with our deletion studies, these data suggest that both the C-terminal association site and the N-terminal association site of plakoglobin are essential for it to stably associate with desmosomal cadherins and that neither site can be replaced with β-catenin sequence.

Further characterization of the C-terminal cadherin-association sites

The immunoprecipitations presented above as well as those previously reported for the association of plakoglobin with N-cadherin (Sacco et al., 1995) were washed using a non-stringent buffer (see Materials and Methods). We examined the ability of the immunoprecipitated protein complexes to withstand washing with the more stringent RIPA buffer. Fig. 10 shows a typical immunoprecipitation reaction comparing stringent and non-stringent washes. In lanes 1-4, an extract of A-431 cells that had been transfected with plakoglobin C-terminally truncated at amino acid 707 was immunoprecipitated with anti-P-cadherin (lanes 1 and 2) or anti-desmoglein (lanes 3 and 4) and washed non-stringently (lanes 1 and 3) or stringently (lanes 2 and 4). In each case the endogenous full-length plakoglobin and the transfected 707 amino acid plakoglobin are co-immunoprecipitated with the cadherin and remain associated during the washes. In lanes 5 and 6, an extract of A-431 cells transfected with plakoglobin C-terminally truncated following amino acid 632 was immunoprecipitated with anti-P-cadherin antibodies and washed non-stringently (lane 5) or stringently (lane 6). As before, the endogenous full-length plakoglobin was co-immunoprecipitated with the cadherin and remained associated during the washes. In contrast, the transfected 632 amino acid plakoglobin was seen only when the anti-P-cadherin immunoprecipitation reaction was washed non-stringently. Since the 632 amino acid deletion mutant hardly associated with the desmosomal cadherins using even non-stringent conditions (see Fig. 5), we have not presented stringent washes when immunoprecipitating with anti-desmoglein.
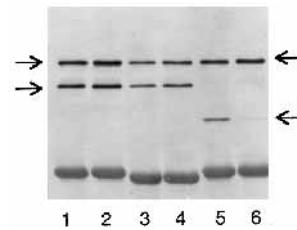
Fig. 10. A domain near the C terminus of plakoglobin modulates its association with both the classical cadherins and the desmosomal cadherins. A-431 cells transfected with C-terminal deletions of plakoglobin retaining 707 amino acids (lanes 1-4) or 632 amino acids (lanes 5-6) were Dounce homogenized and the insoluble fraction was extracted with NP-40. The extract was immunoprecipitated with antibodies against P-cadherin (lanes 1, 2, 5 and 6) or desmoglein (lanes 3 and 4). The immunoprecipitation reactions were washed either non-stringently (lanes 1, 3 and 5) or stringently (lanes 2, 4 and 6). Proteins were resolved by 7% SDS-PAGE, transblotted to nitrocellulose, and probed with monoclonal antibody 4F11 which is directed against the N terminus of plakoglobin. The truncated plakoglobin retaining 707 amino acids remained associated with P-cadherin (lanes 1 and 2) or desmoglein (lane 3 and 4) whether the immunoprecipitation reaction was washed non-stringently (lanes 1 and 3) or stringently (lanes 2 and 4). The truncated plakoglobin retaining 632 amino acids remained associated with P-cadherin when the immunoprecipitation reaction was washed non-stringently (lane 5) but showed a markedly decreased ability to remain associated with P-cadherin when the immunoprecipitation was washed stringently (lane 6). Full-length plakoglobin and the

These data suggest that, in addition to the N-terminal and central domains that are essential for plakoglobin's association with the classical cadherins, an additional domain located near the C terminus is also involved in these interactions. This domain is not essential for plakoglobin to bind to the classical cadherins since truncations containing or lacking this region associate equally well with P-cadherin (Fig. 10). However the domain is required for plakoglobin to remain associated with P-cadherin in RIPA buffer. Earlier we showed that this domain is critical for plakoglobin to associate with the desmosomal cadherins (Fig. 5) and that the Pg5.1 epitope, contained within this C-terminal domain, is inaccessible when plakoglobin is associated with the desmosomal, but not the classical, cadherins (Fig 8). Taken together, these data suggest that the C terminus of plakoglobin is in a different conformation or environment when associated with the desmosomal cadherins than when associated with the classical cadherins.

We also have examined N-terminal deletions for the strength of their association with the cadherins. N-terminal deletion mutants were co-immunoprecipitated with the cadherin and washed either non-stringently or stringently with RIPA buffer. We saw no difference in the ability of any N-terminal deletion to remain associated with the complex using these two washing procedures.

DISCUSSION

Sequence analysis has revealed that plakoglobin and β-catenin are members of the *armadillo* family of proteins (Riggleman et al., 1989; Franke et al., 1989; McCrea et al., 1991; Butz et

A

```
plakoglobin (116-133)      LAEPSQLLKSAIVHLINY
β-catenin                  ••••••M••H•V•N••••
```

B

```
plakoglobin (633-687)      LLHSRNEGTATYAAAVLFRISEDKNPDYRKRVSVELTNSLFKHDPAAWEAAQSMI
β-catenin                  ••••••••V••••••••••M••••PQ••K••L••••••S•••RTE•M••NETADLG
```

Fig. 11. Comparison of the N-terminal and C-terminal desmosomal cadherin association sites on plakoglobin with corresponding sites on β-catenin. (A) The sequence of the N-terminal domain of human plakoglobin that we have identified as important in associating with both α-catenin and desmosomal cadherins is compared with the corresponding sequence of β-catenin. This region of β-catenin has been identified as part of the α-catenin-binding domain (Aberle et al., 1994). (B) The sequence of the C-terminal domain of human plakoglobin that we have identified as essential for associating with the desmosomal cadherins is compared to the corresponding domain in β-catenin. The amino acids identified as part of the Pg5.1 binding site are underlined.

al., 1992). *Armadillo* was originally identified as one of a group of genes that influences segment polarity in *Drosophila* (Nüsslein-Volhard and Wieschaus, 1980). Recent studies identifying *Drosophila* cadherins and catenins have made it clear that Armadillo is not only a signaling molecule but is also a structural component of the *Drosophila* junctional complex (reviewed by Peifer, 1995). Additional studies of *Xenopus* development have shown that plakoglobin and β-catenin play important roles in axis formation suggesting they, like Armadillo, play signaling roles (Karnovsky and Klymkowsky, 1995; Heasman et al., 1994; Funayama et al., 1995).

Plakoglobin and β-catenin are 65% identical (Fouquet et al., 1992) and share a similar structure where a central repetitive core, composed of about 13 repeats, is flanked by non-repetitive N- and C-terminal domains. These proteins are thought to substitute for one another in the classical cadherin/catenin complex (Sacco et al., 1995; Hinck et al., 1994; Butz and Kemler, 1994). The structural details of the classical cadherin/catenin complexes are not known, but a model has been constructed based upon the work of Birchmeier, Behrens and co-workers (Hülsken et al., 1994). They used co-immuno-precipitation experiments to investigate the interactions of β-catenin with E-cadherin. Two deletion mutants of β-catenin, neither of which could interact with E-cadherin when tested alone, interacted with E-cadherin in combination. In addition, these two β-catenin deletion mutants, one lacking the N terminus plus repeats 1 through 7 and the other lacking the C terminus plus repeats 11 through 13, interacted with one another independently of their interaction with E-cadherin. This and other analyses led them to propose a model in which β-catenin is wrapped around the cadherin cytoplasmic domain, with several internal Armadillo repeats interacting with the cadherin and the more C-terminal Armadillo repeats associating with each other.

Plakoglobin is of particular interest to us because, as a component of both the adherens junction and the desmosome (Cowin et al., 1986), it is capable of interacting with desmosomal cadherins as well as classical cadherins. Plakoglobin functions in the adherens junction to link the transmembrane cadherin molecule to α-catenin which then links the entire complex to the microfilaments (Knudsen et al., 1995; Rimm et al., 1995; Wheelock et al., 1996). Although the transmembrane components of the desmosome are members of the broadly-defined cadherin family of proteins, the cytoplasmic domains of these cadherins differ significantly from the cytoplasmic domains of the classical cadherins (Magee and Buxton, 1991).

The molecular makeup of the desmosomal plaque is quite different from that of the adherens junction; the only component known to be present in the plaques of both is plakoglobin.

In this paper we show that the interactions between plakoglobin and cadherins are complex. We also show that some regions of plakoglobin required for association with the desmosomal cadherins differ from those required for association with the classical cadherins. Most strikingly, the plakoglobin domain that associates with α-catenin in the classical cadherin/catenin complex is required for association with the desmosomal cadherins. Sites near the N terminus and the middle of plakoglobin are necessary for the plakoglobin/classical cadherin interaction (Fig. 1). In addition, a domain spanning the Pg5.1 binding site (see Fig. 1) is required for the classical cadherin complex to withstand RIPA buffer. Plakoglobin interacts in a similar fashion, although using different domains, with the desmosomal cadherins. Truncated plakoglobin molecules, missing either an N-terminal domain (construction 134 in Fig. 1) or a C-terminal domain (construction 375 in Fig. 1), do not associate with the desmosomal cadherins. The same two Armadillo repeats (repeats 7 and 8) involved in interaction with classical cadherins play a role in the association with desmosomal cadherins. However, in contrast to the classical cadherins, the sequence that surrounds the Pg5.1 binding site plays a more significant role in the association with desmosomal cadherins. The fact that Pg5.1 does not co-immunoprecipitate the desmosomal cadherins but does co-immunoprecipitate E-, P- and N-cadherin underscores the importance of this domain for association with desmoglein and desmocollin.

Our data suggest a reason α-catenin is not found in desmosomal plaques: plakoglobin's α-catenin binding site, near the N terminus (Sacco et al., 1995; Aberle et al., 1994), overlaps with the site required for association with the desmosomal cadherins. This likely prevents α-catenin from binding to a plakoglobin/desmosomal cadherin complex. Beta-catenin, like plakoglobin, interacts with α-catenin. But unlike plakoglobin, β-catenin does not interact with desmosomal cadherins. A comparison of a portion of the α-catenin-binding site on β-catenin (identified by Aberle et al., 1994) with the α-catenin/desmosomal cadherin-association site on plakoglobin is shown in Fig. 11A. Beta-catenin and plakoglobin differ in 4 residues in this region. Site-directed changes, making plakoglobin more β-catenin-like, may alter its binding capacity such that it would not be capable of interacting with the desmosomal cadherins.

Analysis of the desmosomal cadherin-association site near the C terminus of plakoglobin (Fig. 11B) reveals that it differs substantially from the corresponding region of β-catenin. As a result, this domain may be more difficult to analyze than the N-terminal domain. Rather than changing a few amino acids, an alternative strategy for analyzing the C-terminal domain is to exchange regions in plakoglobin for the corresponding regions in β-catenin. We have constructed two chimeric molecules between β-catenin and plakoglobin (see Fig. 9). Immunoprecipitation studies showed that each chimeric protein fully associated with the classical cadherins but neither associated with the desmosomal cadherins. Thus, domains near both the N and C termini of plakoglobin are essential for it to associate with the desmosomal cadherins. We are constructing additional chimeric molecules to better address the question of what promotes or allows plakoglobin to associate with desmosomal cadherins.

Beta-catenin and plakoglobin each bind to a number of the same partners; both bind α-catenin and classical cadherins but also bind APC (Hülsken et al., 1994; Rubinfeld et al., 1995) and c-erbB2 (Kanai et al., 1995). Beta-catenin has been reported to associate with the epidermal growth factor receptor via its central core domain and it has been suggested that plakoglobin may also interact with the epidermal growth factor receptor (Hoschuetzky et al., 1994). In contrast, only plakoglobin associates with the desmosomal cadherins. Beta-catenin neither co-localizes with desmosomal markers in immunofluorescence nor does it co-immunoprecipitate with desmoglein or desmocollin. It is interesting to speculate on what restricts β-catenin from interacting with desmosomal cadherins. Beta-catenin may lack the information necessary for association with the desmosomal cadherins and/or may contain negative information that prevents it from associating with desmosomal cadherins. Plakoglobin and β-catenin are similar in their repetitive domains but differ significantly in their N- and C-terminal tails. We are using additional chimeric molecules to address the question of why β-catenin does not associate with desmosomal cadherins.

As mentioned above, the associations of the various molecules that make up the adherens junction have been studied extensively. The picture with the desmosome is not as clear. For example, it is not known whether the extracellular interactions of the desmosomal cadherins are homotypic or heterotypic. It is thought that both desmoglein and desmocollin are essential for a cell to form a desmosome, suggesting that the interactions between these cadherins may have a heterotypic component. We show in this report that the interactions of plakoglobin with desmoglein and desmocollin is dependent on domains near both the N terminus and the C terminus of plakoglobin. How (or if) plakoglobin then interacts with the intermediate filament cytoskeleton in order to allow the organization of a desmosome is not known. Troyanovsky et al. (1994a) have shown the cytoplasmic domain of desmocollin-1a that was present on a chimeric plasma membrane protein recruits both plakoglobin and desmoplakin to plaque-like structures. Desmoplakin has been shown to interact with both intermediate filaments and the plasma membrane; it is conceivable that desmoplakin alone is sufficient to link the desmosome to the intermediate filaments (Stappenbeck and Green, 1992; Stappenbeck et al., 1994). If this is the case, the structural role of plakoglobin is uncertain. Its signaling role(s),

if any, remains to be explored. Further studies on the role plakoglobin plays in the desmosome are needed in order to sort out its structural and/or signaling functions in this structure.

REFERENCES

Aberle, H., Butz, S., Stappert, J., Weissig, H., Kemler, R. and Hoschuetzky, H. (1994). Assembly of the cadherin-catenin complex in vitro with recombinant proteins. *J. Cell Sci.* **107**, 3655-3663.

Butz, S., Stappert, J., Weissig, H. and Kemler, R. (1992). Plakoglobin and β-catenin: distinct but closely related. *Science* **257**, 1142-1144.

Butz, S. and Kemler, R. (1994). Distinct cadherin-catenin complexes in Ca^{2+}-dependent cell-cell adhesion. *FEBS Lett.* **355**, 195-200.

Chen, C. and Okayama, H. (1987). High-efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell. Biol.* **7**, 2745-2752.

Cowin, P., Kapprell, H.-P., Franke, W. W., Tamkun, J. and Hynes, R. O. (1986). Plakoglobin: a protein common to different kinds of intercellular adhering junctions. *Cell* **46**, 1063-1073.

Falcone, D. and Andrews, D. W. (1991). Both the 5′ untranslated region and the sequences surrounding the start site contribute to efficient initiation of translation in vitro. *Mol. Cell. Biol.* **11**, 2656-2664.

Fouquet, B., Zimbelmann, R. and Franke, W. W. (1992). Identification of plakoglobin in oocytes and early embryos of *Xenopus laevis:* maternal expression of a gene encoding a junctional plaque protein. *Differentiation* **51**, 187-194.

Franke, W. W., Goldschmidt, M. D., Zimbelmann, R., Mueller, H. M., Schiller, D. L. and Cowin, P. (1989). Molecular cloning and amino acid sequence of human plakoglobin, the common junctional plaque protein. *Proc. Nat. Acad. Sci. USA* **86**, 4027-4031.

Funayama, N., Fagotto, F., McCrea, P. and Gumbiner, B. M. (1995). Embryonic axis induction by the armadillo repeat domain of beta-catenin: evidence for intracellular signaling. *J. Cell Biol.* **128**, 959-968.

Garrod, D. R., Parrish, E. P., Mattey, D. L., Marston, J. E., Measures, H. R. and Vilela, M. J. (1990). Desmosomes. In *Morphoregulatory Molecules* (ed. G. M. Edelman, B. A. Cunningham and J. P. Thiery), pp. 315-339. John Wiley and Sons, New York.

Geiger, B., Avnur, Z., Volberg, T. and Volk, T. (1985). Molecular domains of adherens junctions. In *The Cell in Contact. Adhesions and Junctions as Morphogenetic Determinants* (ed. G. M. Edelman and J. P. Thiery), pp. 461-489. John Wiley and Sons, New York.

Geiger, B. and Ayalon, O. (1992). Cadherins. *Annu. Rev. Cell Biol.* **8**, 307-332.

Grunwald, G. B. (1993). The structural and functional analysis of cadherin calcium-dependent cell adhesion molecules. *Curr. Opin. Cell Biol.* **5**, 797-805.

Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, P., Gumbiner, B., McCrea, P., Kintner, C., Noro, C. Y. and Wylie, C. (1994). Overexpression of cadherins and underexpression of beta-catenin inhibit dorsal mesoderm induction in early Xenopus embryos. *Cell* **79**, 791-803.

Hinck, L., Näthke, I. S., Papkoff, J. and Nelson, W. J. (1994). Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. *J. Cell Biol.* **125**, 1327-1340.

Hirt, R. P., Poulain-Godefroy, O., Billotte, J., Kraehenbuhl, J.-P. and Fasel, N. (1992). Highly inducible synthesis of heterologous proteins in epithelial cells carrying a glucocorticoid-responsive vector. *Gene* **111**, 199-206.

Hitt, A. L. and Luna, E. J. (1994). Membrane interactions with the actin cytoskeleton. *Curr. Opin. Cell Biol.* **6**, 120-130.

Hoschuetzky, H., Aberle, H. and Kemler, R. (1994). β-catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. *J. Cell Biol.* **127**, 1375-1380.

Hülsken, J., Birchmeier, W. and Behrens, J. (1994). E-cadherin and APC compete for the interaction with β-catenin and the cytoskeleton. *J. Cell Biol.* **127**, 2061-2069.

Johnson, K. R., Lewis, J. E., Li, D., Wahl, J., Soler, A. P., Knudsen, K. A.

and Wheelock, M. J. (1993). P- and E-cadherin are in separate complexes in cells expressing both cadherins. *Exp. Cell Res.* **207**, 252-260.

Jou, T.-S., Stewart, D. B., Stappert, J., Nelson, W. J. and Marrs, J. A. (1995). Genetic and biochemical dissection of protein linkages in the cadherin-catenin complex. *Proc. Nat. Acad. Sci. USA* **92**, 5067-5071.

Kaiser, H. W., Ness, W., Jungblut, I., Briggaman, R. A., Kreysel, H. W. and O'Keefe, E. J. (1993). Adherens junctions: demonstration in human epidermis. *J. Invest. Dermatol.* **100**, 180-185.

Kanai, Y., Ochiai, A., Shibata, T., Oyama, T., Ushijima, S., Akimoto, S. and Hirohashi, S. (1995). c-erbB-2 gene product directly associates with β-catenin and plakoglobin. *Biochem. Biophys. Res. Commun.* **208**, 1067-1072.

Karnovsky, A. and Klymkowsky, M. W. (1995). Anterior axis duplication in *Xenopus* induced by the over-expression of the cadherin-binding protein plakoglobin. *Proc. Nat. Acad. Sci. USA* **92**, 4522-4526.

Knudsen, K. A. and Wheelock, M. J. (1992). Plakoglobin, or an 83-kD homologue distinct from β-catenin, interacts with E-cadherin and N-cadherin. *J. Cell Biol.* **118**, 671-679.

Knudsen, K. A., Soler, A. P., Johnson, K. R. and Wheelock, M. J. (1995). Interaction of alpha-actinin with the N-cadherin/catenin cell-cell adhesion complex via alpha-catenin. *J. Cell Biol.* **130**, 67-77.

Koch, P. J. and Franke, W. W. (1994). Desmosomal cadherins; another growing multigene family of adhesion molecules. *Curr. Opin. Cell Biol.* **6**, 682-687.

Korman, N. J., Eyre, R. W., Klaus-Kovtun, V. and Stanley, J. R. (1989). Demonstration of an adhering-junction molecule (plakoglobin) in the autoantigens of pemphigus foliaceus and pemphigus vulgaris. *New Eng. J. Med.* **321**, 631-635.

Kowalczyk, A. P., Palka, H. L., Luu, H. H., Nilles, L. A., Anderson, J. E., Wheelock, M. J. and Green, K. J. (1994). Posttranslational regulation of plakoglobin expression; influence of the desmosomal cadherins on plakoglobin metabolic stability *J. Biol. Chem.* **269**, 31214-31223.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Lewis, J. E., Jensen, P. J. and Wheelock, M. J. (1994). Cadherin function is required for human keratinocytes to assemble desmosomes and stratify in response to calcium. *J. Invest. Dermatol.* **102**, 870-877.

Magee, A. I. and Buxton, R. S. (1991). Transmembrane molecular assemblies regulated by the greater cadherin family. *Curr. Opin. Cell Biol.* **3**, 854-861.

Mathur, M., Goodwin, L. and Cowin, P. (1994). Interactions of the cytoplasmic domain of the desmosomal cadherin Dsg1 with plakoglobin. *J. Biol. Chem.* **269**, 14075-14080.

McCrea, P. D., Turck, C. W. and Gumbiner, B. (1991). A homolog of the *armadillo* protein in *Drosophila* (plakoglobin) associated with E-cadherin. *Science* **254**, 1359-1361.

Nuber, U. A., Schäfer, S., Schmidt, A., Koch, P. J. and Franke, W. W. (1995). The widespread human desmocollin Dsc2 and tissue-specific patterns of synthesis of various desmocollin subtypes. *Eur. J. Cell Biol.* **66**, 69-74.

Nüsslein-Volhard, C. and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila. Nature* **287**, 795-801.

Ozawa, M., Baribault, H. and Kemler, R. (1989). The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J.* **8**, 1711-1717.

Ozawa, M. and Kemler, R. (1992). Molecular organization of the uvomorulin-catenin complex. *J. Cell Biol.* **116**, 989-996.

Parker, A. E., Wheeler, G. N., Arnemann, J., Pidsley, S. C., Ataliotis, P., Thomas, C. L., Rees, D. A., Magee, A. I. and Buxton, R. S. (1991). Desmosomal glycoproteins II and III. Cadherin-like junctional molecules generated by alternative splicing. *J. Biol. Chem.* **266**, 10438-10445.

Peifer, M., McCrea, P. D., Green, K. J., Wieschaus, E. and Gumbiner, B. M. (1992). The vertebrate adhesive junction proteins β-catenin and plakoglobin and the *Drosophila* segment polarity gene *armadillo* form a multigene family with similar properties. *J. Cell Biol.* **118**, 681-691.

Peifer, M. (1995). Cell adhesion and signal transduction: the Armadillo connection. *Trends Cell Biol.* **5**, 224-229.

Riggleman, B., Wieschaus, E. and Schedl, P. (1989). Molecular analysis of the *armadillo* locus: uniformly distributed transcripts and a protein with novel internal repeats are associated with a *Drosophila* segment polarity gene. *Genes Dev.* **3**, 96-113.

Rimm, D. L., Koslov, E. R., Kebriaei, P., Cianci, C. D. and Morrow, J. S. (1995). α₁(E)-catenin is an actin-binding and bundling protein mediating the attachment of F-actin to the membrane adhesion complex. *Proc. Nat. Acad. Sci. USA* **92**, 8813-8817.

Rubinfeld, B., Souza, B., Albert, I., Munemitsu, S. and Polakis, P. (1995). The APC protein and E-cadherin form similar but independent complexes with α-catenin, β-catenin and plakoglobin. *J. Biol. Chem.* **270**, 5549-5555.

Sacco, P. A., McGranahan, T. M., Wheelock, M. J., and Johnson, K. R. (1995). Identification of plakoglobin domains required for association with N-cadherin and α-catenin. *J. Biol. Chem.* **270**, 20201-20206.

Schäfer, S., Koch, P. J. and Franke, W. W. (1994). Identification of the ubiquitous human desmoglein, Dsg2, and the expression catalogue of the desmoglein subfamily of desmosomal cadherins. *Exp. Cell Res.* **211**, 391-399.

Schwarz, M. A., Owaribe, K., Kartenbeck, J. and Franke, W. W. (1990). Desmosomes and hemidesmosomes: constitutive molecular components. *Annu. Rev. Cell Biol.* **6**, 461-491.

Staehelin, L. A. (1974). Structure and function of intercellular junctions. *Int. Rev. Cytol.* **39**, 191-283.

Stappenbeck, T. S. and Green, K. J. (1992). The desmoplakin carboxyl terminus coaligns with and specifically disrupts intermediate filament networks when expressed in cultured cells. *J. Cell Biol.* **116**, 1197-1209.

Stappenbeck, T. S., Lamb, J. A., Corcoran, C. M. and Green, K. J. (1994). Phosphorylation of the desmoplakin COOH terminus negatively regulates its interaction with keratin intermediate filament networks. *J. Biol. Chem.* **269**, 29351-29354.

Stappert, J. and Kemler, R. (1994). A short core region of E-cadherin is essential for catenin binding and is highly phosphorylated. *Cell Adhes. Commun.* **2**, 319-327.

Takeichi, M. (1988). The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. *Development* **102**, 639-655.

Takeichi, M. (1991). Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* **251**, 1451-1455.

Troyanovsky, S. M., Eshkind, L. G., Troyanovsky, R. B., Leube, R. E. and Franke, W. W. (1993). Contributions of cytoplasmic domains of desmosomal cadherins to desmosome assembly and intermediate filament anchorage. *Cell* **72**, 561-574.

Troyanovsky, S. M., Troyanovsky, R. B., Eshkind, L. G., Leube, R. E. and Franke, W. W. (1994a). Identification of amino acid sequence motifs in desmocollin, a desmosomal glycoprotein, that are required for plakoglobin binding and plaque formation. *Proc. Nat. Acad. Sci. USA* **91**, 10790-10794.

Troyanovsky, S. M., Troyanovsky, R. B., Eshkind, L. G., Krutovskikh, V. A., Leube, R. E. and Franke, W. W. (1994b). Identification of the plakoglobin-binding domain in desmoglein and its role in plaque assembly and intermediate filament anchorage. *J. Cell Biol.* **127**, 151-160.

Wheelock, M. J., Knudsen, K. A. and Johnson, K. R. (1996). Membrane-cytoskeleton interactions with cadherin cell adhesion proteins; roles of catenins as linker proteins. *Curr. Topics Membr.* (in press).

Wheelock, M. J., Buck, C. A., Bechtol, K. B. and Damsky, C. H. (1987). Soluble 80-kd fragment of cell-CAM 120/80 disrupts cell-cell adhesion. *J. Cell. Biochem.* **34**, 187-202.