Journal of Cell Science 109, 3069-3078 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JCS1311

Cadherin binding sites of plakoglobin: localization, specificity and role in targeting to adhering junctions

Regina B. Troyanovsky, Nikolai A. Chitaev and Sergey M. Troyanovsky*

Department of Dermatology, Washington University Medical School, Campus Box 8123, 660 S. Euclid Ave, St Louis, MO 63110, USA

*Author for correspondence

SUMMARY

Plakoglobin directly interacts with cadherins and plays an essential role in the assembly of adherens junctions and desmosomes. Recently we have reported that multiple cadherin binding sites are localized along the *arm* repeat region of plakoglobin. To demonstrate functionality and specificity of these sites in vivo we constructed a set of chimeric proteins containing a plakoglobin sequence fused with the transmembrane vesicular protein synaptophysin. Plakoglobin fused upstream or downstream from synaptophysin (PgSy and SyPg, chimeras, respectively) is exposed on the cytoplasmic surface of synaptic-like vesicles and is able to associate with E-cadherin, and with two desmosomal cadherins, desmoglein and desmocollin. Moreover, plakoglobin targets these vesicles to cell-cell junctions. Insertion of synaptophysin within plakoglobin (PSyG chimeras) can interfere with cadherin binding of the resulting chimeric proteins, dependent on the position of the insertion. Insertion of synaptophysin in the first three *arm* repeats selectively inactivates plakoglobin binding to desmoglein and desmocollin. An insertion of synaptophysin within the next two repeats inactivates E-cadherin and desmocollin binding but not desmoglein binding. This localization of the desmoglein and E-cadherin binding sites was further confirmed by replacement of plakoglobin *arm* repeats with the corresponding sequence derived from the plakoglobin homologue, β-catenin, and by deletion mutagenesis. Insertion of synaptophysin in most sites within *arm* repeats 6-13 does not change plakoglobin binding to cadherins. It does, however, strongly inhibit association of the resulting vesicles either with desmosomes and adherens junctions or with desmosomes only. Using in vitro binding assays we demonstrate that *arm* repeats 6-13 contain two cryptic cadherin binding sites that are masked in the intact protein. These observations suggest that the *arm* repeat region of plakoglobin is comprises two functionally distinct regions: the 1/5 region containing desmoglein and E-cadherin specific binding sites and the 6/13 region implicated in targeting of plakoglobin/cadherin complexes into junctional structures.

Key words: Cadherin, Cell-cell adhesion, Desmosome, Plakoglobin

INTRODUCTION

Epithelial cells exhibit two major types of adhering junctions, adherens junctions anchoring actin microfilaments and desmosomes anchoring intermediate filaments (IF) to the plasma membrane. In adherens junctions intercellular adhesion is caused by classical cadherins (e.g. E-cadherin), while in desmosomes it is maintained by desmosomal cadherins, desmogleins (Dsg1-3) and desmocollins (Dsc1-3) (Green and Jones, 1990; Schwarz et al., 1990; Geiger and Ayalon, 1992; Garrod, 1993; Koch and Franke, 1994).

Intracellular plaques of both types of adhering junctions contain one common component plakoglobin (Cowin et al., 1986). It consists of three structurally distinct regions, the unique amino- and carboxyl-terminal segments, and a 560-amino-acid-long central region comprising 13 *arm* repeats (Franke et al., 1989; McCrea et al., 1991; Butz et al., 1992; Peifer et al., 1994). This latter region is 85% homologous to a corresponding segment of β-catenin, a protein found only in adherens junctions. Recent experiments showed that plakoglobin is involved in a complex pattern of interactions with other proteins. A soluble cytoplasmic form of plakoglobin (and β-catenin) associates with α-catenin and/or the tumor suppressor protein APC via the central repeat domain (Hinck et al., 1994; Hulsken et al., 1994; Rubinfeld et al., 1995). In the adherens junction, plakoglobin is associated with E-cadherin and α-catenin, which provide anchorage for F-actin and α-actinin (Ozawa et al., 1989, 1990; Knudsen and Wheelock, 1992; Nagafuchi et al., 1994; Knudsen et al., 1995; Rimm et al., 1995). Recently a tyrosine kinase substrate, p120, was also found in the E-cadherin/plakoglobin/α-catenin complex (Reynolds et al., 1994; Shibamoto et al., 1995; Staddon et al., 1995). In desmosomes plakoglobin specifically interacts with Dsg and Dsc and apparently does not bind to α-catenin or p120 (Korman et al., 1989; Troyanovsky et al., 1993, 1994a,b; Kowalczyk et al., 1994; Mathur et al., 1994). Notably, β-catenin is unable to interact with desmosomal cadherins.

Experiments with dominant negative cadherin mutants showed that the association of plakoglobin with cadherins is a critical step in the assembly of adherens junctions and desmo-

somes. Over-expression of the intracellular segment of classical cadherins in epithelial cells inhibits cadherin-dependent adhesion, but not desmosome assembly (Kinter, 1992; Fujimori and Takeichi, 1993; Amagai et al., 1995). Similarly, expression of the chimeric protein CoDsg, comprising the transmembrane domain of connexin32 and the carboxyl-terminal intracellular segment of the Dsg1, inhibited formation of endogenous desmosomes, but not adherens junctions (Troyanovsky et al., 1993, 1994a). Catenin-binding domains of classical cadherins and a homologous C-domain of Dsg1, both of which directly interact with plakoglobin, are responsible for these effects (Troyanovsky et al., 1994a; Mathur et al., 1994; Aberle et al., 1994; Stappert and Kemler, 1994; Jou et al., 1995). Disruption of adherens junctions and desmosomes by cadherins suggested the existence of distinct Dsg-specific and classical cadherin-specific binding sites in plakoglobin. The first direct evidence to support this hypothesis was obtained recently. It was shown that a carboxyl-terminal truncated mutant of plakoglobin, containing only three complete amino-terminal *arm* repeats, is able to associate with Dsg (Chitaev et al., 1996; Witcher et al., 1996), while at least six *arm* repeats are required for binding to classical cadherins (Sacco et al., 1995; Wahl et al., 1996). In the present work we show that distinct E-cadherin and Dsg binding sites are localized in the first five *arm* repeats of the plakoglobin molecule. The rest of the plakoglobin *arm* repeat region, containing at least two cryptic cadherin binding sites, is important for its targeting.

MATERIALS AND METHODS

Plasmid construction

The plasmid BlSyPg, encoding a chimeric protein consisting of the synaptophysin and plakoglobin, has been described (Chitaev et al., 1996). For synaptophysin insert mutagenesis a plasmid BlSySM, containing the unique *Sal*I and *Mlu*I sites at the positions of the start and stop codons of synaptophysin, respectively, was constructed. Then this plasmid was cut by *Mlu*I/*Xba*I and the insert was inserted into corresponding positions of the wild-type plakoglobin cDNA (Franke et al., 1989) using PCR-mediated site-directed mutagenesis.

For β-catenin/plakoglobin replacement mutagenesis a complete cDNA encoding mouse β-catenin (Butz et al., 1992) was amplified from mouse liver cDNA. The following steps in this mutagenesis, as well as deletion mutagenesis of the Dsg-binding region, were done using PCR-mediated site-directed mutagenesis.

The construction of the expression plasmids coding for the intact plakoglobin (Pg) and its fragments (Pg1/580, Pg1/147, Pg114/292, Pg305/505, Pg505/672, Pg580/744) or cytoplasmic segments of Dsg, Dsc1a and E-cadherin (HCDg, HCDc, HCUv) were as described (Chitaev et al., 1996). Some additional clones were constructed using conveniently located restriction endonuclease sites. The plasmid pQPg505/744 was constructed by ligation of the *Bgl*II/*Hin*dIII fragment of the pQPg580/744 with the appropriately digested pQPg505/672 vector. The 1,100 bp *Bcl*I/*Sac*I fragment was excised from pQPg and inserted into the appropriate sites of the vector pQE-32 (Qiagen, Chatsworth, CA). The resulting plasmid was cut by *Sac*I/*Hin*dIII and ligated with the corresponding fragment of the pQ580/744, to obtain a plasmid pQ305/744. For construction of the pQPg505/683, the 500 bp *Bgl*II/blunt-ended *Hin*dIII fragment of the pQPg505/744 was replaced with the *Bgl*II/blunt-ended *Bgl*I fragment taken from the same plasmid. The recombinant histidine-tagged proteins were isolated and analyzed by SDS-PAGE, essentially as described (Chitaev et al., 1996). Correct construction of all recombinant plasmids was verified by restriction endonuclease mapping and/or nucleotide sequencing.

Cell culture, DNA transfections and immunological methods

Transfection of the A-431 cells, as well as the selection, growth, immunofluorescence microscopy and immunoprecipitation of stably transfected cell clones, have been described (Troyanovsky et al., 1993; Chitaev et al., 1996). The following primary antibodies were used: (i) polyclonal rabbit and murine monoclonal Sy38 antibodies against synaptophysin (DAKO, Hamburg, FRG); (ii) rabbit and monoclonal murine U114 antibodies against recombinant human Dsc1a (kindly provided by M. Demler and A. Schmidt, Heidelberg, FRG); (iii) rabbit pan-cadherin antibody (Sigma, St Louis, MO); (iv) rabbit antibodies recognizing α-catenin (kindly provided by Dr Wysolmerski, St Louis, MO); (v) murine mAb Dg.3.10 against bovine and human Dsg; murine mAb Dsc 210.2.9 against bovine Dsc; murine mAb PG5.1 against human plakoglobin; mixture of murine mAbs 2.15, 2.17 and 2.19 against desmoplakin (for references of these monoclonal antibodies, see Troyanovsky et al., 1994); (vi) murine mAbs against E-cadherin, α-catenin and p120 (Transduction Laboratories, Lexington, KY); monoclonal antibodies H1 against cytokeratin 8 (Troyanovsky et al., 1989).

Solid-phase and overlay binding assays

In vitro binding assays were described previously (Chitaev et al., 1996). In brief, plakoglobin or its fragments were immobilized on a 96-well dish and incubated with increasing or fixed amounts of the different recombinant cadherin tails or fragment Pg580/744. Binding was detected by an ELISA assay with either DC210.2.9 or PG5.1 monoclonal antibodies. In competition experiments, immobilized plakoglobin or its fragments were incubated with the fixed amount of HDsg in presence of increasing amounts of the Pg580/744. For the overlay assay, plakoglobin fragments (0.8 μg per lane) were separated by SDS-PAGE and electroblotted onto nitrocellulose. Membranes after treatment with 3% bovine serum albumin were incubated with 20 μg/ml Pg580/744 for 60 minutes at room temperature. The binding was detected using the alkaline phosphatase system (Promega, Madison, WI).

RESULTS

Identification of Dsg-, E-cadherin- and Dsc-specific cadherin binding sites in human plakoglobin in vivo

We have described a chimeric protein, SyPg, where synaptophysin is fused with the entire plakoglobin molecule. Synaptophysin is an integral transmembrane protein of the presynaptic vesicles containing four membrane-spanning regions (transmembrane domains) and cytoplasm-exposed amino and carboxyl termini (Fig. 1A,D). The presence of transmembrane domains is sufficient for accurate targeting of synaptophysin into small synaptic-like vesicles (Leube et al., 1994; Leube, 1995). Fusion of either of the synaptophysin termini with different proteins allows their exposure on the surface of these vesicles (Troyanovsky et al., 1993; Leube, 1995). We have previously found that the SyPg chimera efficiently integrates into the vesicles of transfected epithelial cells and binds to E-cadherin, Dsg, Dsc, p120 and α-catenin, similar to the wild-type plakoglobin. As a result, SyPg-containing vesicles associate with desmosomal and adherens junction plaques (Chitaev et al., 1996), while vesicles containing synaptophysin alone are randomly distributed through the cytoplasm of the transfected cells. Identical results were obtained when plako-

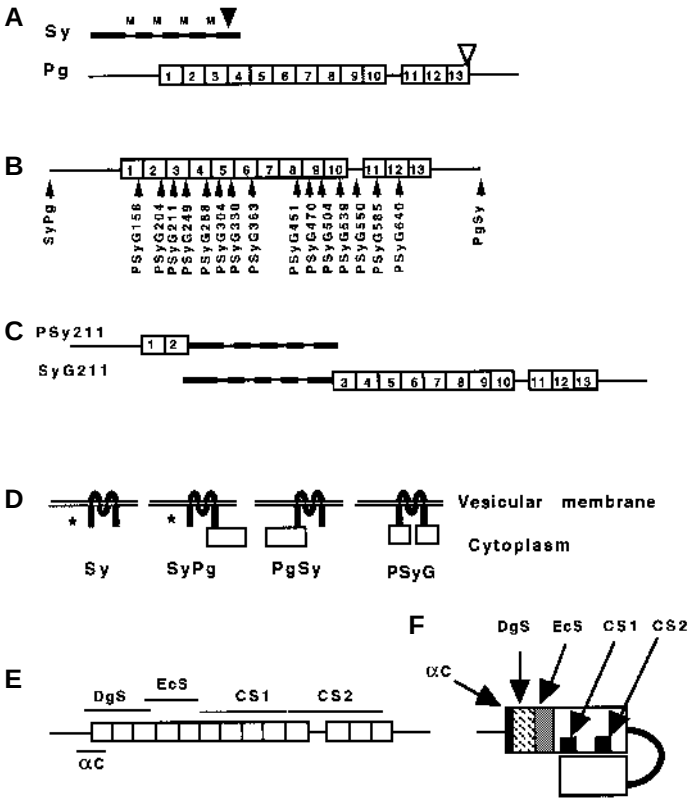

Fig. 1. Synaptophysin insertion mutagenesis (A-C) and proposed plakoglobin model (E,F): (A-C) Structure of plakoglobin-synaptophysin chimeric proteins, and (D) their organization in vesicular membranes. (A) Full-size rat synaptophysin (Sy) and 744 amino acids long human plakoglobin (Pg). Four transmembrane regions of synaptophysin are indicated by thin lines with M above. Thirteen individual *arm* repeats of plakoglobin are represented by open boxes. Nonrepeat sequences, including a short 22-amino-acid insertion, are marked by solid line. The positions of the epitopes used for immunological detection of each of these proteins are marked by large inverted arrowheads. (B) Schematic representation of the chimeric proteins. Positions of synaptophysin insertion in plakoglobin are indicated by solid arrows. The numbers in the construct names of PSyG chimeras correspond to the position of the amino acid residue preceding the synaptophysin insert. In two constructs, SyPg and PgSy, synaptophysin was placed either upstream or downstream of plakoglobin, respectively. (C) The structure of the chimeric proteins, PSy211 and SyG211, with deletions of the plakoglobin segments. (D) Organization of synaptophysin (solid line, Leube et al., 1994) and plakoglobin (open box)-synaptophysin chimeric proteins inserted into a vesicular membrane. (E) Localization of α-catenin (αC), desmoglein (DgS), E-cadherin (EcS) and two cryptic cadherin (CS1 and CS2) binding sites relative to arm repeats of plakoglobin. (F) Proposed model whereby CS1 and CS2 sites are rendered inactive due to intramolecular interactions. See text for details.

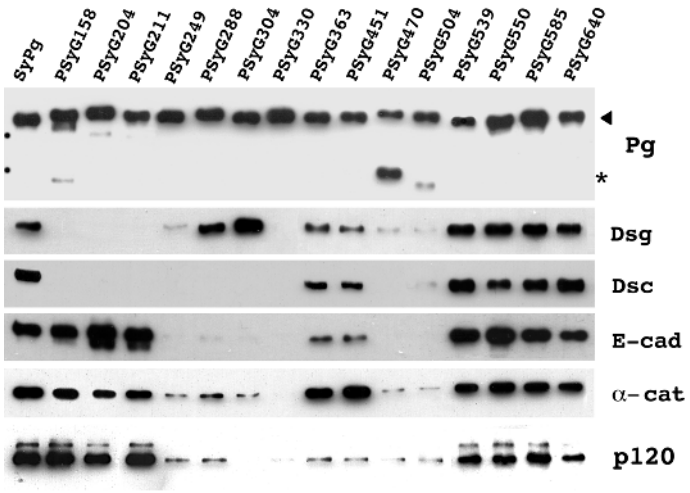

Fig. 2. Western blot analysis of immunoprecipitates obtained from A-431 cells stably expressing plakoglobin-synaptophysin chimeric proteins. Immunoprecipitates were obtained from corresponding stably transfected cells, as described in Materials and Methods, using polyclonal anti-synaptophysin antibodies and were separated by 8% SDS-PAGE. Nitrocellulose blots were developed with monoclonal antibodies against plakoglobin (Pg), desmoglein (Dsg), desmocollin (Dsc), E-cadherin (E-cad), α-catenin (α-cat) or p120 (p120). Chimeric proteins visualized by anti-plakoglobin antibody are indicated by the arrowhead. The position of the endogenous plakoglobin is indicated by an asterisk and it is not present in these immunoprecipitates. Dots in Pg indicate the positions of molecular mass markers, 116 and 97.4 kDa.

globin was translocated to the amino terminus upstream from synaptophysin in the PgSy construct.

Recent experiments with plakoglobin mutants (Hulsken et al., 1994; see also our results below) demonstrated strong intramolecular interactions within this molecule. The data indicate that conformational abnormalities can be induced by large deletions within plakoglobin. Our experiments with synaptophysin-plakoglobin chimeras (Chitaev et al., 1996) made it possible to develop a novel approach to study plakoglobin structure, a synaptophysin insertion mutagenesis. The insertion of synaptophysin into various positions of plakoglobin results in the formation of synaptic-like vesicles coated by plakoglobin with a functionally affected domain, leaving the unaffected domains of plakoglobin exposed on the cytoplasmic surface and involved in appropriate interactions. Here we introduce this approach, in conjunction with more conventional strategies, to characterize cadherin-binding sites of plakoglobin.

15 chimeric plakoglobins (PSyG chimeras) containing a synaptophysin insertion in different positions of the arm repeat region were constructed (Fig. 1B). Expression of these chimeras in A-431 cells resulted in a single polypeptide with a predicted molecular mass of 130 kDa (Fig. 2). A significant degradation product was observed only in the chimera PSyG470. Association of these chimeras with Dsg, Dsc, E-cadherin, p120, α-catenin and endogenous plakoglobin was examined by co-immunoprecipitation using anti-synaptophysin antibodies (see Table 1 for a summary). Western-blot analysis (Fig. 2) shows that PSyG-type proteins expressed in stably transfected cells do not associate with endogenous plakoglobin. Three PSyG-type chimeras, PSyG158, PSyG204 and PSyG211, containing a synaptophysin insertion in the first, second or beginning of the third *arm* repeats, respectively, interact with E-cadherin, α-catenin and p120 but not with Dsg or Dsc. Deletion of the sequences either upstream or downstream from the synaptophysin insertion in the PSyG211 chimera (chimeras PSy211 and SyG212, Fig. 1) completely abolished its binding with E-cadherin (not shown). This result

Table 1. Phenotypic characteristics of the PSyG-type chimeras

Constructs	Des	AJ	Dsg	Dsc	ECad	α	p120
SyPg	++	++	++	++	++	++	++
PSyG158	–	–	–	–	++	++	++
PSyG204	–	++	–	–	++	++	++
PSyG211	–	++	–	–	++	++	++
PSyG249	–	–	–	–	–	+	+
PSyG288	–	+	++	–	–	+	+
PSyG304	–	+	++	–	–	+	–
PSyG330	–	–	–	–	–	–	–
PSyG363	–	–	+	+	+	++	+
PSyG451	–	–	+	+	+	++	+
PSyG470	–	–	–	–	–	+	+
PSyG504	–	–	–	–	–	+	+
PSyG539	–	++	++	++	++	++	++
PSyG550	–	++	++	++	++	++	++
PSyG585	–	+	++	++	++	++	++
PSyG640	+	+	++	++	++	++	++
PgSy	++	++	++	++	++	++	++

A-431 cell clones synthesizing corresponding chimeric proteins were analyzed by immunofluorescence microscopy for the co-localization of chimeras together with desmosomes (Des) or with adherens junctions (AJ), and by immunoprecipitation with synaptophysin antibodies for the capacity of the chimeras to coprecipitate endogenous Dsg (Dsg), Dsc (Dsc) E-cadherin (ECad), α-catenin (α) or p120 (p120).

demonstrates that cooperation between two fragments of plakoglobin exposed on the vesicular surface of PSyG211-expressing cells is required for the formation of the E-cadherin/plakoglobin complex.

Insertion of synaptophysin into the end of *arm* repeat 4 or the beginning of *arm* repeat 5 did not change binding of plakoglobin with Dsg, but completely abolished its interaction with Dsc and E-cadherin, and strongly reduced its binding with α-catenin and p120. Four chimeras (PSyG249, PSyG330, PSyG470 and PSyG504) containing a synaptophysin insert either at the end of *arm* repeats 3 and 5, or in two positions in *arm* repeat 9, respectively, show very weak or no binding to any of the above ligands. All other chimeric proteins (PSyG363, PSyG451, PSyG539, PSyG550, PSyG585 and PSyG640) show an interaction pattern similar to that of wild-type plakoglobin (Fig. 2). These data clearly indicate that different plakoglobin sequences are involved in the binding of E-cadherin, Dsg and Dsc. Insertion of synaptophysin into either of the first three *arm* repeats affects the interaction of plakoglobin with Dsg and Dsc, but not with E-cadherin. Insertion of synaptophysin into repeats 4 or 5 affects binding to E-cadherin and Dsc but not Dsg.

Localization of the Dsg-binding and E-cadherin-binding sequences within *arm* repeats 1-3 and 4-5, respectively

A very high level of sequence identity between β-catenin and plakoglobin and the fact that β-catenin associates only with classical but not with desmosomal cadherins provides an approach to investigate the structure of desmosomal cadherin binding sites in plakoglobin. To map a Dsg-binding site, we substituted a segment of β-catenin for a homologous segment of plakoglobin (Met^{1}-Arg^{233}) to obtain the SyPgBC1/233 chimera (Fig. 3). The replaced plakoglobin segment includes the first two and a half *arm* repeats that have the highest affinity for Dsg in vitro (Chitaev et al., 1996), and its disruption by

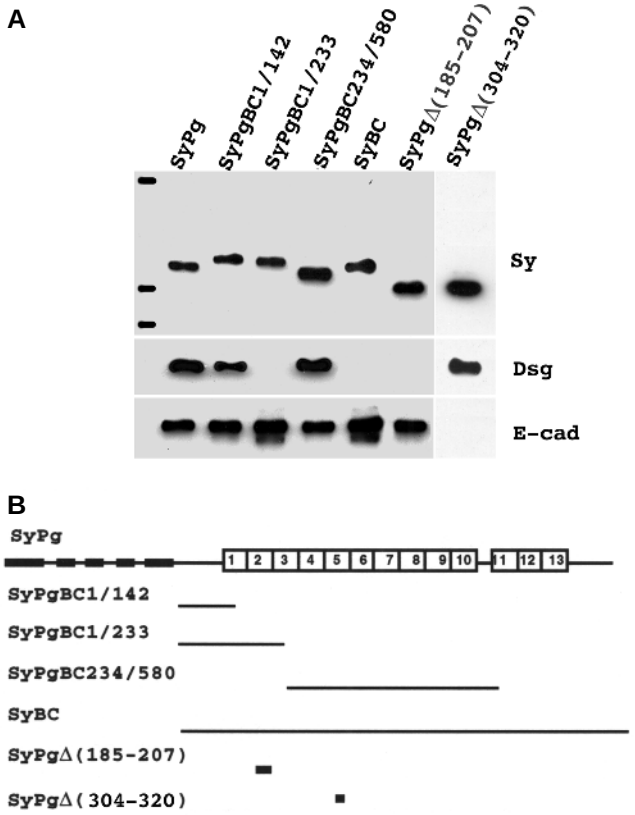

Fig. 3. Western blot analysis of immunoprecipitates obtained from A-431 cells stably expressing plakoglobin-synaptophysin-β-catenin chimeric proteins (the names of the proteins are indicated above the corresponding lanes). (A) Immunoprecipitates were obtained and analyzed as described in Fig. 2. Nitrocellulose blots were developed with monoclonal antibodies against synaptophysin (Sy), Dsg (Dsg), or E-cadherin (E-cad). The bars at the left indicate positions of molecular mass markers, 205, 116 and 97.4 kDa. Note disappearance of Dsg in immunoprecipitates obtained from SyPgBC1/233-, SyBC- and SyPgΔ(185-207)-expressing cells. (B) Structure of plakoglobin-synaptophysin-β-catenin chimeric proteins. The symbols in the SyPg chimera were described in Fig. 1. In other chimeras only mutagenised regions are shown. The thin lines represent β-catenin regions substituted for corresponding regions of plakoglobin in SyPg chimera. A filled box indicates a position of the internal deletion in SyPgΔ(185-207) chimera.

synaptophysin insertion completely abolished plakoglobin-Dsg binding (see above). Co-immunoprecipitation analysis showed that the SyPgBC1/233 chimera binds to E-cadherin, but not to Dsg (Fig. 3), similar to the chimera SyBC containing the entire sequence of β-catenin. The chimeric protein SyPgBC1/142, where only 142 amino acid residues (Met^{1}-Arg^{142}) of plakoglobin were replaced with the corresponding sequence of β-catenin, was able to co-precipitate Dsg, but to a lesser extent than SyPg (Fig. 3). In addition, substitution of β-catenin *arm* repeats 4-10 for the corresponding segment of plakoglobin in the chimera SyPgBC234/580 did not affect its ability to bind to Dsg and E-cadherin. These data suggest that the plakoglobin sequence Arg^{142}-Arg^{233} is essential for binding to Dsg. In agreement with this conclusion, deletion of the 21 most divergent amino acid residues (Leu^{185}-Ser^{207}) within the second *arm* repeat of the SyPg chimera completely

abolished its interaction with Dsg (see Fig. 3), but did not affect its affinity to bind E-cadherin or α-catenin. These data are in agreement with the synaptophysin insertion mutagenesis experiments and support the conclusion that a Dsg-specific binding site is located within the *arm* repeats 1-3 of plakoglobin. To further confirm the presence of E-cadherin-binding site in *arm* repeats 4 and 5, we deleted a 17-amino-acid-long sequence (Leu304-Arg320) within this region from the SyPg chimera. The resulting chimera SyPgΔ(304-320) is completely unable to bind E-cadherin, but interacts normally with Dsg (Fig. 3), in agreement with results obtained using the synaptophysin insertion mutagenesis approach.

Expression of PSyG chimeras with an Inactivated E-cadherin binding site abolishes incorporation of Dsg into endogenous desmosomes

To understand the functional effect of the synaptophysin insertion on plakoglobin, the cells stably expressing PSyG-type proteins were analyzed by double immunofluorescent microscopy. As expected, chimeras unable to bind cadherins (PSyG249, PSyG330, PSyG470 and PSyG504) were localized in small vesicle-like structures randomly distributed in the cytoplasm but not in the cell-cell contacts, similar to wild-type synaptophysin (not shown). Of three chimeras unable to bind Dsg, PSyG158 was randomly distributed in the cytoplasm while the other two (PSyG204 and PSyG211) efficiently integrated into cell-cell contacts and were co-distributed with E-cadherin (Fig. 4), α-catenin and p120 (data not shown). The normal morphology of these cells indicate that the presence of the PSyG204 or PSyG211 chimeras does not detectably affect cell-cell adhesion.

Two chimeras, PSyG288 and PSyG304, able to bind Dsg but not E-cadherin and Dsc, were evenly distributed between the cytoplasm and cell-cell contacts. A significant portion of Dsg in these cells was co-distributed with the chimeric proteins along cell-cell contacts (Fig. 5a,a′). Double immunostaining of these cells with desmoplakin/Dsg antibodies revealed multiple desmosome-like structures containing desmoplakin, which in some cases have very fine Dsg staining (Fig. 5b,b′). These structures were smaller then most desmosomes of the parental A-431 cells and anchor only fine keratin bundles compared to normal desmosomes (Fig. 5c-e). In this respect, desmosomes in the PSyG288- or PSyG304-expressing cells are similar to a population of small-size desmosomes found in A-431 cells that interact only with thin bundles of IF (Fig. 7d,d′ and f,f′). This observation suggests the interesting possibility that PSyG288 or PSyG304 chimeras inhibit growth of desmosomes. The role of the *arm* repeats 4 and 5 of plakoglobin in desmosome assembly needs more detailed investigation.

Plakoglobin *arm* repeats 6-13 are required for the targeting of plakoglobin/cadherin complexes into cell-cell contacts and contain cryptic cadherin binding sites

Although most of the chimeric proteins containing a synaptophysin insert in *arm* repeats 6-13 (PSyG363, PSyG451, PSyG539, PSyG550, PSyG585, PSyG640) were able to associate with all tested plakoglobin binding proteins, they failed to integrate efficiently into desmosomes. Only two chimeras of this group (PSyG539 and PSyG550), containing the synaptophysin insert either at the very end of *arm* repeat 10 or in the unique sequence separating repeats 10 from 11, faithfully accumulated in cell-cell contacts and co-localized with E-cadherin (Fig. 6b,b′), α-catenin and p120 (not shown).

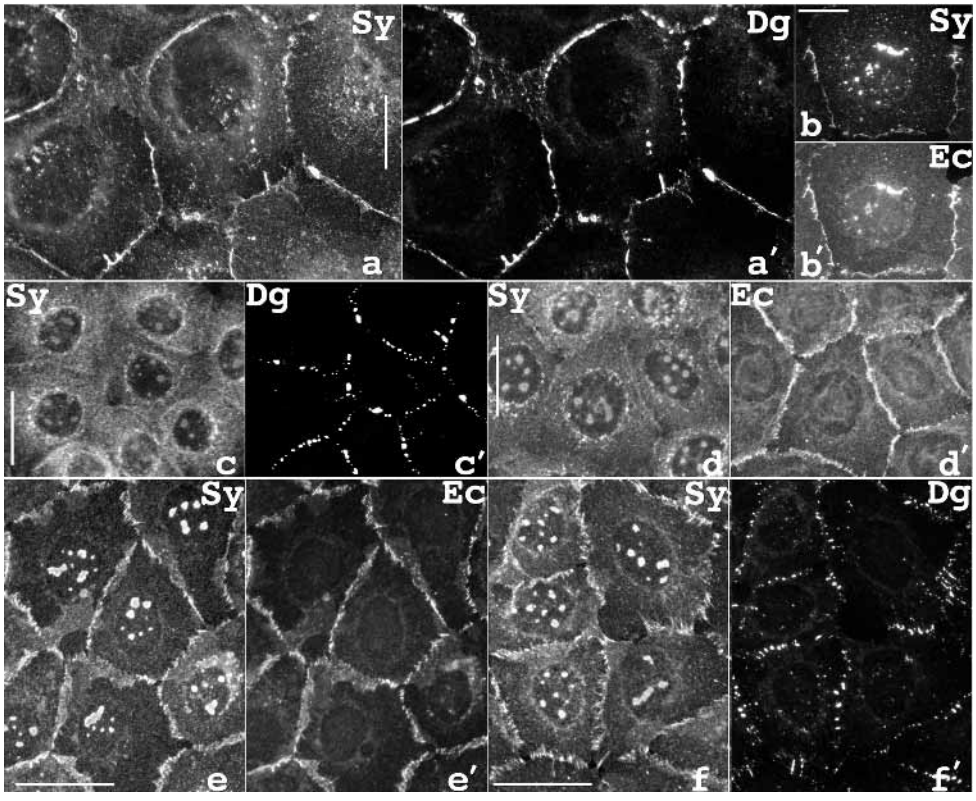

Fig. 4. Double-label immunofluorescence microscopy of A-431 cells stably expressing plakoglobin-synaptophysin chimeric proteins using rabbit anti-synaptophysin (Sy) and mouse monoclonal anti-Dsg (Dg) or anti-E-cadherin (Ec) antibodies. (a,a′ and b,b′) Co-localization of the SyPg chimera (a and b) with Dsg (a′) or with E-cadherin (b′). (c,c′ and d,d′) Co-localization of the PSyG158 chimera (c and d) with Dsg (c′) or with E-cadherin (d′). (e,e′ and f,f′) Co-localization of the PSyG211 chimera (e and f) with E-cadherin (e′) and Dsg (f′). Note that while SyPg vesicles associate with both adherens junctions and desmosomes, vesicles containing PSyG158 or PSyG211 are present either in the cytoplasm or in adherens junctions, respectively. Punctate staining of nucleoli in c,d,e and f is not specific and appears from time to time, depending on the lot of anti-synaptophysin antibody available. Bars: 20 μm (a,b); 40 μm (c-f).

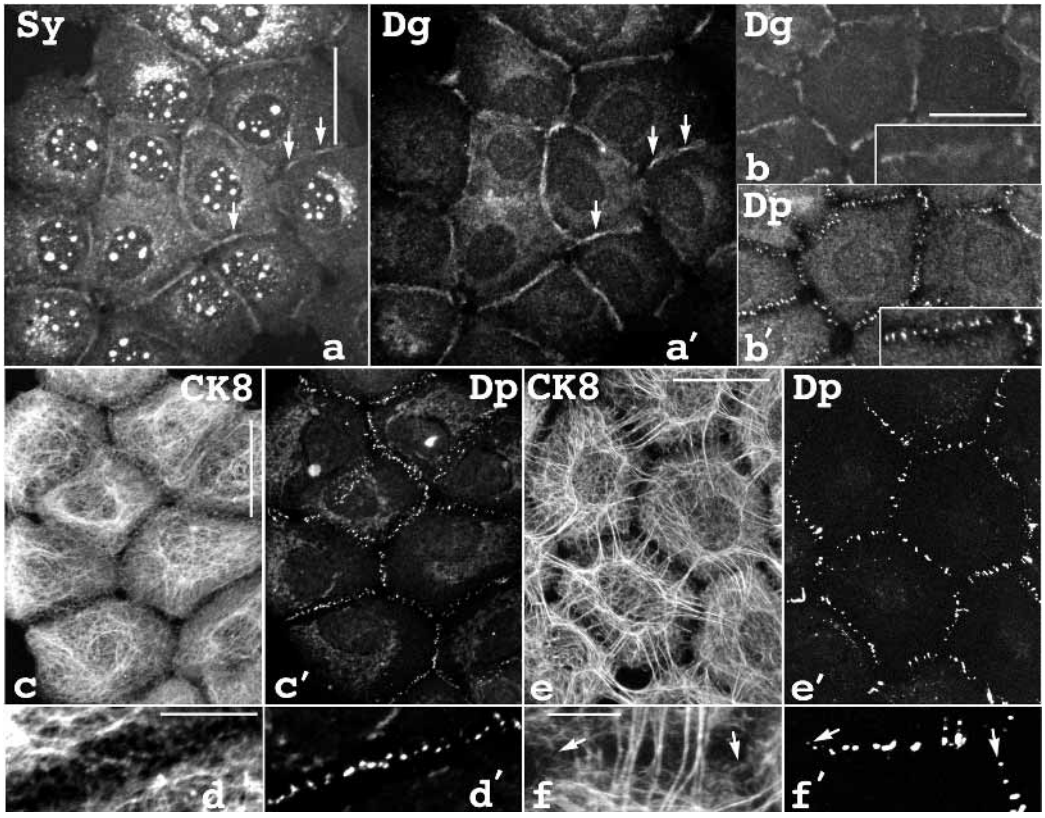

Fig. 5. Double-label immunofluorescence microscopy showing the distribution of the desmosomal and IF proteins in A-431 subclones stably producing the chimeric protein PSyG288 (a-d′) and in parental A-431 cells (e-f′). (a,a′) Detection of PSyG288 chimera with rabbit antibodies against synaptophysin (a) and of Dsg with monoclonal antibody Dg3.10 (a′). (b,b′) Double labelling of the same cells by monoclonal murine Dg3.10 (b) and polyclonal guinea pig antibodies against desmoplakin (b′). The high magnification of the selected regions are given in lower right corners. Note different distribution of the two desmosomal proteins in PSyG288-expressing cells. While Dsg is co-distributed with the PSyG288 chimera at the cell-cell contacts, desmoplakin is detected in the desmosome-like structures. The same PSyG288-expressing cells (c,c′ and d,d′) or normal A-431 cells (e,e′ and f,f′) are stained with antibody H1 against human cytokeratin 8 (CK8) to reveal IF (c,d,e, and f) and polyclonal anti-desmoplakin antibody (c′,d′,e′, and f′). (d,d′ and f,f′) Higher magnifications of the selected regions of c,c′ and e,e′, respectively. Note that desmosomes in PSyG288-expressing cells and the smallest desmosomes in normal A-431 cells (arrows) are connected only with fine IF bundles. Bars: 40 μm (a-c,e); 10 μm (d and f).

In contrast to SyPg (or PgSy), both these chimeras induced detectable defects in desmosomes. Desmosomes in cells expressing these proteins were less abundant and Dsg was no longer associated with desmosomes exclusively, but a detectable pool of this protein was distributed randomly throughout cel-cell contact regions (Fig. 6a,a′).

Among six chimeric proteins that bind to all the tested cadherins, only PSyG363 and PSyG451 were localized exclusively to the cytoplasm, similar to wild-type synaptophysin (not shown). The majority of chimeric proteins PSyG585 and PSyG640, containing the synaptophysin insertion in *arm* repeats 11 and 12, were also found in the cytosol and only occasionally seen in desmosomes or adherens junctions (Fig. 6c,c′). No abnormalities in the localization of the desmoglein or desmoplakin in these cells were detected. These observations indicate that binding to cadherins is not sufficient to target plakoglobin to the adherens junctions or desmosomes.

Recently we have showed (Chitaev et al., 1996; Fig. 1) that intact recombinant plakoglobin may contain at least three independent cadherin binding sites. One of these sites corresponds to the Dsg-specific site described above. Two other sites, exhibiting a similar affinity to the C-domains of Dsg1, Dsc1a and E-cadherin, were mapped to a region spanning *arm* repeats 6-12. However, as reported above, disruption of this region by synaptophysin insertion did not change plakoglobin binding to cadherins in vivo. To solve this discrepancy we studied the binding of cadherins to the C-terminal or N-terminal truncated plakoglobin mutants using a solid-phase binding assay described previously (Chitaev et al., 1996). The C-terminal truncation of plakoglobin (mutant Pg1/580 lacking repeats 11-13 and a unique carboxyl-end region) did not affect binding to Dsc and Dsg, but slightly increased binding to E-cadherin compared to the entire plakoglobin molecule (Fig. 7). Cadherin binding was abolished by amino-terminal deletion of *arm* repeats 1-4 (Fig. 7, mutant Pg305/744, Met^{1}-Leu^{304} deletion). The latter result is in agreement with our in vivo data that mapped Dsg and E-cadherin binding sites within *arm* repeats 1 to 5, but contradict our previous results showing that mutants Pg305/505 and Pg505/672 have strong cadherin binding properties (Chitaev et al., 1996, see also Fig. 7). This apparent discrepancy can be explained if the plakoglobin sequence Lys^{673}-Ala^{744}, which is absent in these two mutants, inhibits cadherin binding activity of mutant Pg305/744. To test this possibility, we inserted the Lys^{673}-Ala^{744} fragment into the Pg505/672 mutant and demonstrated that the insertion eliminated the cadherin-binding activity of Pg505/672 protein (mutant Pg505/744, Fig. 7). Further deletion-mapping experiments showed that a similar inhibitory effect on cadherin binding can be achieved by insertion of ten amino acid residues K^{673}-Q^{683} (mutant Pg505/683, Fig. 7). Based on these data we hypothesized that the inhibitory effect of the ten amino acid residues K^{673}-Q^{683} from *arm* repeat 13 on cadherin binding is due to its interaction with the upstream cadherin binding sites and masking their activity. The results presented in Fig. 8 support

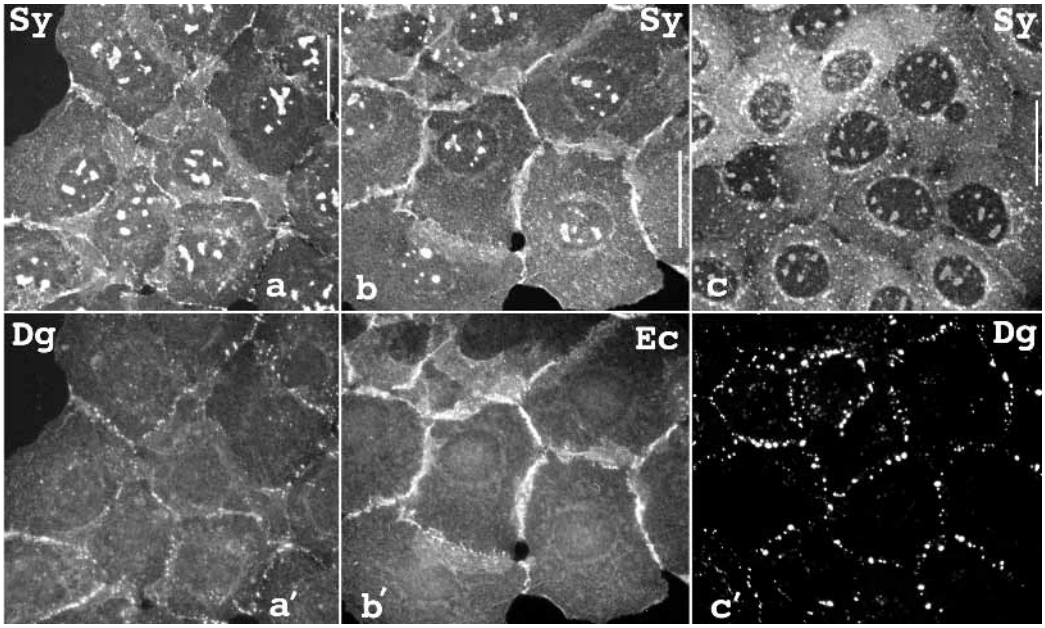

Fig. 6. Double-label immunofluorescence microscopy of A-431 cells stably expressing the PSyG550 (a-b′) and PSyG640 (c,c′) chimeras using rabbit anti-synaptophysin (a-c) and mouse monoclonal anti-Dsg (a′,c′) or anti-E-cadherin (b′) antibodies. Note that the PSyG550 chimera associates with adherens junctions and has an obvious negative effect on desmosomes. The major portion of the PSyG640 chimera is present in the cytoplasm and has no effect on desmosomes. Bars, 40 μm.

this conclusion and demonstrate that the plakoglobin fragment Pg580/744 interacts strongly with the upstream fragments Pg305/505 and Pg505/672. Its binding to the fragment Pg114/292, containing 1-4 *arm* repeats, was significantly weaker. No binding was detected with the Pg1/147 fragment containing the unique amino-terminal region and the first *arm* repeat. Similar results were obtained using both solid-phase and overlay assays (Fig. 8A,B).

To investigate further the inhibitory effect of the C-terminal *arm* repeat on the activity of upstream cadherin binding sites we examined the ability of the Pg580/744 fragment, which does not bind to cadherins (Fig. 7), to compete with the binding of plakoglobin fragments for the C-domain of Dsg. Experiments presented in Fig. 8C show that the Pg580/744 fragment competed with binding of Dsg to the plakoglobin fragments Pg305/505 or Pg505/672. The Pg580/744 fragment had little or no effect on Dsg binding to Pg114/292 or to the entire plakoglobin molecule. These experiments demonstrate that the 11/13 *arm* repeats segment, containing the inhibitory fragment K^{673}-Q^{683}, directly interacts with upstream *arm* repeats 5-10 to negatively affect the activity of cadherin binding sites located in this region.

DISCUSSION

Topology of the cadherin binding sites in plakoglobin

Using deletion mapping analysis of SyPg-type chimeras, we have shown (Chitaev et al., 1996) that the first three *arm* repeats of plakoglobin (Arg^{141}-Met^{234}) contain a Dsg binding site. Synaptophysin insertion mutagenesis and homologous

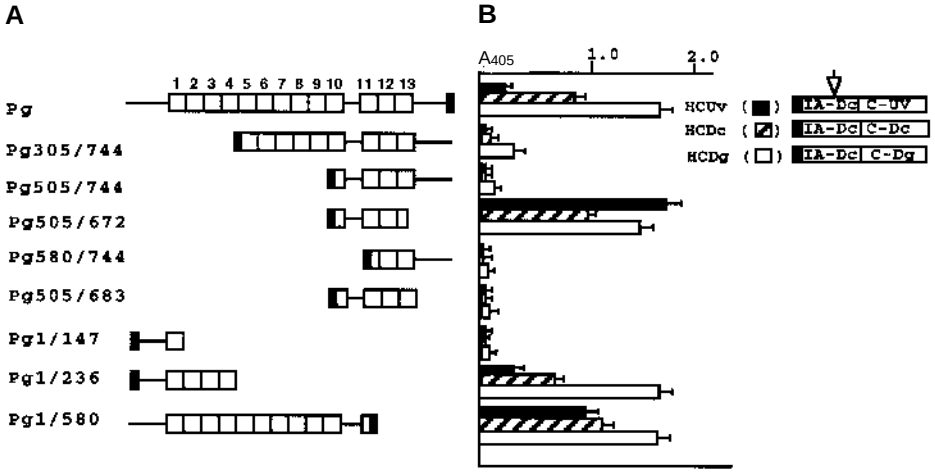

Fig. 7. Deletion mutagenesis of cadherin binding sites in plakoglobin. Structure of the constructs carrying various plakoglobin deletions is shown in A. The effect of each deletion on the interaction of plakoglobin with recombinant cadherin tail fragments HCUv (filled bar), HCDc (striped bar) or HCDg (open bar) in solid-phase assay is shown in B. The numbers in construct names correspond to the first and last amino acid residues of plakoglobin present. The poly(hystidine) tag localized either at the amino or carboxyl terminus is shown as a filled box. Other elements of the structure are marked as in Fig. 1. Each construct of cadherin tails (their structures are given at right upper corner of B) contain an anchor domain of bovine Dsc1 (IA-Dc) followed by a C-domain from either bovine Dsc1a (C-Dc in HCDc) or bovine Dsg1 (C-Dg in HCDg) or murine E-cadherin (C-Uv in HCUv). The position of the epitope for mAb D210 used for immunological detection of recombinant proteins in binding assays is indicated by the open arrow. The experiments were carried out in triplicate and one of seven independent experiments is shown. Bars indicate standard deviation. A_{405}, absorbance at 405 nm, arbitrary units.

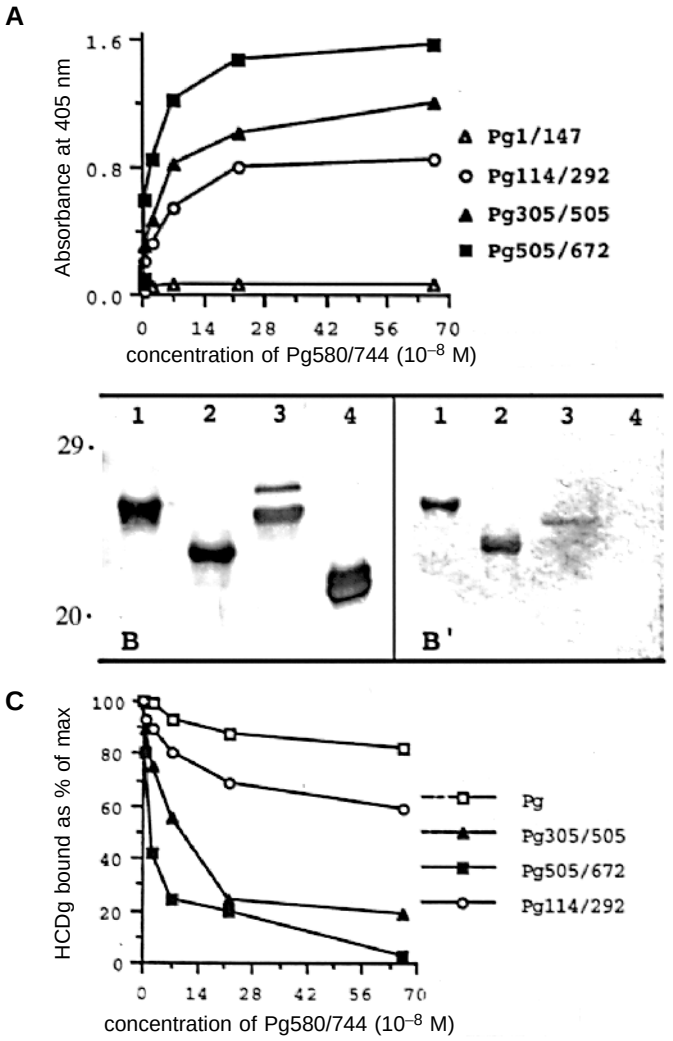

Fig. 8. Interaction of the carboxyl-terminal plakoglobin fragment Pg580/744 with upstream plakoglobin fragments, Pg1/147, Pg114/292, Pg305/505 and Pg505/672 (for structure see Fig. 7 and Chitaev et al., 1996). (A) For the solid-phase binding assay, 100 μl of a 4.5×10^{-7} M solution of purified plakoglobin fragments were applied to each well. Plates were incubated with increasing amounts of Pg580/744 as indicated. The binding was monitored using PG5.1 monoclonal antibody recognizing the C-terminal epitope within the Pg580/744 fragment (see Fig. 1). Experiments were repeated in quadruplicate. Each point represents the average of 4 determinations. (B,B′) For the overlay-binding assay, 0.8 μg of plakoglobin fragments (Pg305/505, lane 1; Pg505/672, lane 2; Pg114/292, lane 3; and Pg1/147, lane 4) were separated by 15% SDS-PAGE and either stained with Coomassie Blue (B) or electroblotted to a nitrocellulose membrane (B′). Blots were incubated with Pg580/744 fragment (20 μg/ml) and binding was detected with PG5.1 antibodies. Positions of molecular mass standards are indicated by dots on the left. (C) Competition of the HCDg binding to intact or deleted plakoglobins with increasing amounts of Pg580/744. Multiwell dishes were coated with either plakoglobin (open squares), Pg114/292 (open circles), Pg305/505 (filled triangles) or Pg505/672 (filled squares) and incubated with HCDg (0.5×10^{-7} M) in the presence of increasing amounts of Pg580/744, as described in Materials and Methods. The binding of HCDg was determined using DC210.2.9 antibody. One of seven independent experiments is shown. All assays were performed in duplicate and expressed as a percentage of HCDg bound in the absence of Pg580/744 competitor. The results shown are the mean of duplicate determinations.

plakoglobin/β-catenin replacement experiments presented here confirm this observation. In addition, we show that this region is not involved in plakoglobin binding with E-cadherin and α-catenin, since synaptophysin insertions in positions 158, 204 and 211 specifically abolished only Dsg and Dsc binding. Elimination of plakoglobin binding to Dsg upon deletion of 21 residues (Leu^{185}-Ser^{207}) demonstrate that the end of the second *arm* repeat is an essential element of the Dsg binding site. These conclusions are in agreement with our data demonstrating that deletion of this sequence in myc-tagged plakoglobin also abolished its binding to desmosomal cadherins (S.M. Troyanovsky, unpublished results). Reduction in the Dsg binding by the chimeric protein SyPgBC1/142 compared to SyPg indicates that the plakoglobin sequence upstream from Thr^{142} can also participate in binding to Dsg. This result raises the possibility that the Dsg binding site partially overlaps with the α-catenin binding sequence in the Gln^{109}-Ala^{137} region (Aberle et al., 1996). An overlap between α-catenin and Dsg-binding sites was also suggested recently by Witcher et al. (1996). These data taken together suggest that a relatively long sequence (about 100 amino acids), including most of the first three *arm* repeats, is involved in Dsg-binding (Fig. 1E,F).

Selective inactivation of E-cadherin binding activity in the chimeras PSyG288, PSyG304 and SyPgΔ(304-320) suggests that *arm* repeats 4 and 5 (the sequence Leu^{250}-Val^{334}) are involved in E-cadherin binding. Interestingly, the PSyG-type chimeras containing the insert in Dsg- or E-cadherin-binding regions are not able to interact with Dsc. Additional mutagenesis experiments will be needed to determine whether the Dsc-binding site overlaps with both E-cadherin and Dsg binding sites. Alternatively, binding of Dsc to plakoglobin could be dependent on the initial binding of plakoglobin to other cadherins or could be mediated through unidentified protein(s). Moreover, the question remains as to whether Dsg and E-cadherin binding sites are totally distinct. Simultaneous inactivation of the Dsg and E-cadherin binding activities in the chimera PSyG249 suggests that the end of the third *arm* repeat may constitute a structural component of both sites. On the other hand the negative effect of the synaptophysin insertion in this position could be due to steric hinderance. A similar mechanism may be responsible for the simultaneous inactivation of the Dsg and E-cadherin binding sites in the other PSyG-type chimeras (PSyG330, PSyG470 and PSyG504). Synaptophysin inserts in the chimeras PSyG288 and PSyG304 do not affect the known α-catenin-binding sequence (Aberle et al., 1996). At the same time they both co-immunoprecipitate an unexpectedly low amount of α-catenin. Very weak binding to α-catenin was also found for SyPgΔ(304-320) (R.B. Troyanovsky, unpublished). Both observations support the hypothesis that α-catenin- and Dsg-binding to plakoglobin are mutually exclusive. Whether the complexes consisting of α-catenin and plakoglobin lacking E-cadherin binding are unstable or simply do not form remains to be determined.

Localization of the Dsg binding site of plakoglobin to the *arm* repeats 1-3 is in good agreement with our previous experiments showing high affinity binding of the plakoglobin fragment Pg114/292, encompassing the sequence of the first three *arm* repeats, to Dsg in vitro (Chitaev et al., 1996). In addition, in vitro binding experiments show that two non-overlapping plakoglobin fragments, Pg305/505 and Pg505/672, both derived from *arm* repeats 6-13, exhibit strong binding to

Dsc, Dsg and E-cadherin C-domains. Since deletion of *arm* repeats 1-3 completely abolished Dsg binding, we hypothesized that in intact plakoglobin, cadherin binding sites localized in *arm* repeats 6-13 are hidden (Chitaev et al., 1996). The data presented here strongly support this possibility. We found that the plakoglobin fragment Pg580/744 (containing *arm* repeats 11 to 13 and the carboxyl terminus of plakoglobin) is unable to bind to cadherins but binds to fragments Pg305/505 and Pg505/672, and efficiently competes for their interaction with cadherins. These observations suggest that in intact plakoglobin, *arm* repeats 11-13 interact with upstream repeats 6-10, masking cadherin binding sites present in this segment of plakoglobin (Fig. 1F). Although it is not clear whether activation of these two cryptic sites is required for desmosome assembly, it is possible that their activation during desmosome or adherens junction assembly leads to cross linking of several cadherins into one supramolecular complex.

Plakoglobin/cadherin complexes can associate with the protein kinase substrate p120 (Reynolds et al., 1994; Shibamoto et al., 1995; Staddon et al., 1995). Synaptophysin insertion mutagenesis did not reveal a specific binding site for p120 in plakoglobin. We have found that most of the PSyG chimeras that are able to form complexes with E-cadherin co-precipitate a large amount of p120. This is in agreement with the observation of Daniel and Reynolds (1995), demonstrating a direct interaction between p120 and catenin-binding region of E-cadherin. At the same time, two observations suggest that plakoglobin may also have a binding site for p120. First, chimera PSyG288, unable to bind E-cadherin, co-precipitates p120. Second, chimeras PSyG363 and PSyG451, able to bind E-cadherin, precipitate only a residual amount of p120. This binding site can also contribute to the association of p120 with plakoglobin/E-cadherin complexes.

Plakoglobin is responsible for targeting of cadherins into cell-cell junctions

Experiments with dominant negative mutants of Dsg and classical cadherins (Kinter, 1992; Troyanovsky et al., 1993, 1994a; Fujimori and Takeichi, 1993) demonstrate an important role of β-catenin and plakoglobin in the assembly of adherens junctions and desmosomes. One of the functions of β-catenin and plakoglobin is to couple classical cadherins with α-catenin. That, in turn, mediates the attachment of the adherens junctions to the cortical actin cytoskeleton (Nagafuchi et al., 1994; Knudsen et al., 1995; Rimm et al., 1995). The function of plakoglobin in desmosome assembly is not well understood.

Synaptophysin insertion mutagenesis divides the *arm* repeat region of plakoglobin into two functionally distinct regions, encompassing *arm* repeats 1 to 5 and 6 to 13 (regions 1/5 and 6/13, respectively). Five of seven mutants containing the synaptophysin insert within the 1/5 region were unable to associate either with E-cadherin or with Dsg, while six out of eight mutants containing the insert in 6/13 region bind equally to all cadherins studied. As discussed above, the 1/5 region of plakoglobin includes distinct binding sites that appear to overlap or be closely juxtaposed. This region mediates direct linkage of plakoglobin with Dsg or with E-cadherin and α-catenin. The role of the 6/13 region is probably more complex. Despite their strong binding with cadherins, chimeras PSyG363, PSyG451, PSyG585 and PSyG640 containing synaptophysin in the 6/13 region, were unable to associate with desmosomes and adherens junctions. Vesicles formed by these chimeras were localized exclusively in the cytoplasm and had no influence on desmosome or adherens junction assembly. Deletion of the *arm* repeats 11-13 was also found to affect correct destination of the SyPg chimera into cell-cell junctions (Chitaev et al., 1996). Therefore, the 6/13 region may function in the targeting of plakoglobin/cadherin complexes to cell-cell junctions. Whether cryptic cadherin binding sites, which are hidden or disrupted in plakoglobins containing synaptophysin in the 6/13 region, are involved in the targeting mechanism remains to be determined.

In addition, our experiments demonstrate clear differences in the assembly of desmosomes and adherens junctions. First, PSyG-type chimeras, regardless of the site of synaptophysin insertion or cadherin binding, are unable to integrate efficiently into desmosomes, whereas several of them can be integrated into adherens junctions. Second, typical epithelial morphology and normal distribution of the adherens junction proteins in the A-431 clones stably expressing any of the PSyG-type chimeras, suggest that these chimeras are not able to affect this type of junction. In contrast, immunofluorescence microscopy shows interesting changes in desmosomes of cells transfected with PSyG288, PSyG304, PSyG539 and PSyG550. In the first two chimeras, PSyG288 and PSyG304, synaptophysin was inserted into the E-cadherin binding site of plakoglobin. Upon expression in A-431 cells, these two chimeric proteins bind to Dsg but not Dsc and produce desmosomes that are smaller in size. These desmosomes are unable to bind normal IF bundles. Chimeras PSyG539 and PSyG550 carry a synaptophysin insertion in the unique 22-amino-acid-long region, separating *arm* repeats 10 and 11. These proteins bind both the desmosomal cadherins, Dsg and Dsc. Their expression in A-431 cells results in a dramatic reduction in the number of desmosomes. All four chimeric proteins were efficiently sorted into cell-cell contact regions but did not incorporate into desmosomes. The mechanism of the dominant negative effect of these two groups of mutants is not clear since other similar chimeric proteins that bind desmosomal cadherins do not negatively affect desmosome formation. To explain these findings an attractive hypothesis is that plakoglobin contains two functionally distinct domains, a binding 1/5 region and a targeting 6/13 region. Occupation of the Dsg or E-cadherin specific sites in the binding domain induces two different 6/13 domain-dependent mechanisms responsible for correct and efficient targeting of cadherins to either desmosomes or adherens junctions.

We would like to thank Dr G. Goldberg, Dr A. Eisen (St Louis, MO) and Dr W.W. Franke (Heidelberg, FRG) for valuable discussion. The work has been supported in part by a Pfizer Pharmaceutical Career Development Award from Dermatology Foundation.

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.

Aberle, H., Schwartz, H., Hoschuetzky, H. and Kemler, R. (1996). Single amino acid substitutions in proteins of the armadillo gene family abolish their binding to α-catenin. *J. Biol. Chem.* **271**, 1520-1526.

Amagai, M., Fujimori, T., Masunaga, T., Shimizu, H., Nishikawa, T., Shimuzu, N., Takeichi, M. and Hashimoto, T. (1995). Delayed assembly

of desmosomes in keratinocytes with disrupted classic-cadherin-mediated cell adhesion by a dominant negative mutant. *J. Invest. Dermatol*. **104**, 27-32.

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

Chitaev, N. A., Leube, R. E., Troyanovsky, R. B., Eshkind, L. G., Franke, W. W. and Troyanovsky, S. M. (1996). The binding of plakoglobin to desmosomal cadherins: Patterns of binding sites and topogenic potential. *J. Cell Biol.* **133**, 359-369

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

Daniel, J. M. and Reynolds, A. B. (1995). The tyrosine kinase substrate p120^{cas} binds directly to E-cadherin but not to the adenomatous polyposis coli protein or α-catenin. *Mol. Cell Biol*. **15**, 4819-4824.

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.

Fujimori, T. and Takeichi, M. (1993). Disruption of epithelial cell-cell adhesion by exogenous expression of a mutated nonfunctional N-cadherin. *Mol. Biol. Cell* **4**, 37-47.

Garrod, D. R. (1993). Desmosomes and hemidesmosomes. *Curr. Opin. Cell Biol*. **5**, 30-40.

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

Green, K. J. and Jones, J. C. R. (1990). Interaction of intermediate filaments with the cell surface. In *Cellular and Molecular Biology of Intermediate Filaments* (ed. R. D. Goldman and P. M. Steinert), pp. 147-174. Plenum Publishing Corp., New York.

Hinck, L., Nathke, 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.

Hulsken, 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.

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.

Kintner, C. (1992). Regulation of embryonic cell adhesion by the cadherin cytoplasmic domain. *Cell* **69**, 225-236.

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 α-actinin with the cadherin/catenin cell-cell adhesion complex via α-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 Sranley, J. R. (1989). Demonstration of an adhering-junction molecule (plakoglobin) in the autoantigens of pemphigus foliaceous 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.* **49**, 31214-31223.

Leube, R. E., Leimer, U., Grund, C., Franke, W. W., Harth, N. and Wiedenmann, B. (1994). Sorting of synaptophysin into special vesicles in non-neuroendocrine epithelial cells. *J. Cell Biol*. **127**, 1589-1601.

Leube, R. E. (1995). The topogenic fate of the polytopic transmembrane proteins, synaptophysin and connexin, is determined by their membrane-spanning domains. *J. Cell Sci*. **108**, 883-894

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.

Nagafuchi, A., Ishihara, S. and Tsukita, S. (1994). The roles of catenins in the cadherin-mediated cell adhesion: functional analysis of E-cadherin-α catenin fusion molecules. *J. Cell Biol.* **127**, 235-245.

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., Ringwald, M. and Kemler, R. (1990). Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. *Proc. Nat. Acad. Sci. USA* **87**, 4246-4250.

Peifer, M., Berg, S. and Reynolds., A. B. (1994). A repeating amino acid motif shared by proteins with diverse cellular roles. *Cell* **76**, 789-791.

Reynolds, A., Daniel, J., McCrea, P. D., Wheelock, M. J., Wu, J. and Zhang, Z. (1994). Identification of a new catenin: the tyrosine kinase substrate p120^{cas} associates with E-cadherin complexes. *Mol. Cell. Biol.* **14**, 8333-8342.

Rimm, D. L., Koslov, E. R., Kebriaei, P., Cianci, C. D. and Morrow, J. S. (1995). Alpha 1(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 with α-catenin. *J. Biol. Chem.* **270**, 20201-20206.

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.

Shibamoto, S., Hayakawa, M., Takeuchi, K., Hori, T., Miyazawa, K., Kitamura, N., Johnson, K. R., Wheelock, M. J., Matsuyoshi, N., Takeichi, M. and Ito, F. (1995). Association of p120, a tyrosine kinase substrate, with E-cadherin/catenin complexes. *J. Cell Biol.* **128**, 949-957

Smith, D. B. and Johnson, K. S. (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusion with glutathione S-transferase. *Gene* **67**, 31-40.

Staddon, J. M., Smales, C., Schlze, C., Esch, F. and Rubin, L. L. (1995). p120, a p120-related protein (p100), and the cadherin/catenin complex. *J. Cell Biol.* **130**, 369-381.

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.

Troyanovsky, S. M., Guelstein, V. I., Tchipisheva, T. A., Krutovskikh, V. A. and Bannikov, G. A. (1989). Patterns of expression of keratin 17 in human epithelia: dependency on cell position. *J. Cell Sci*. **93**, 419-426.

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

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

Wahl, J. K., Sacco, P. A., McGranahan-Sadler, T. M., Sauppe, L. M., Wheelock, M. J. and Johnson, K. R. (1996). Plakoglobin domains that define its association with the desmosomal cadherins and the classical cadherins: identification of unique and shared domains. *J. Cell Sci*. **109**, 1143-1154.

Witcher, L., L., Collins, R., Puttagunta, S., Mechanic, S., E., Munson, M., Gumbiner, B. and Cowin, P. (1996). Desmosomal cadherin binding domains of plakoglobin. *J. Biol. Chem*. **271**, 10904-10909.

(Received 8 August 1996 - Accepted 16 September 1996)