Identification of the Plakoglobin-binding Domain in Desmoglein and Its Role in Plaque Assembly and Intermediate Filament Anchorage

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Abstract. The carboxyterminal cytoplasmic portions (tails) of desmosomal cadherins of both the desmoglein (Dsg) and desmocollin type are integral components of the desmosomal plaque and are involved in desmosome assembly and the anchorage of intermediate-sized filaments. When additional Dsg tails were introduced by cDNA transfection into cultured human epithelial cells, in the form of chimeras with the aminoterminal membrane insertion domain of rat connexin32 (Co32), the resulting stably transfected cells showed a dominant-negative defect specific for desmosomal junctions: despite the continual presence of all desmosomal proteins, the endogenous desmosomes disappeared and the formation of Co32-Dsg chimeric gap junctions was inhibited. Using cell transfection in combination with immunoprecipitation techniques, we have examined a series of deletion mutants of the Dsg1 tail in Co32–Dsg chimeras. We show that upon removal of the last 262 amino acids the truncated Dsg tail still effects the binding of plakoglobin but not of detectable amounts of any catenin and induces the dominant-negative phenotype. However, further truncation or excision of the next 41 amino acids, which correspond to the highly conserved carboxyterminus of the C-domain in other cadherins, abolishes plakoglobin binding and allows desmosomes to reform. Therefore, we conclude that this short segment provides a plakoglobin-binding site and is important for plaque assembly and the specific anchorage of either actin filaments in adherens junctions or IFs in desmosomes.

INTERCELLULAR adhering junctions (9) are important structures contributing to stable and positionally ordered cell–cell attachment as well as to the anchorage and specific intracellular arrangement of cytoskeletal filaments and contain calcium-dependent transmembrane cell adhesion molecules, which are members of the cadherin family of proteins. While intercellular contact formation and adhesion is effected by the aminoterminal and N-glycosylated part of the cadherin molecule, the association of cytoskeletal filaments involves the carboxyterminal intracellular portion and is mediated by certain submembranous dense plaques of 10–35 nm thickness. Despite their structural similarities and the fact that all junctions of this group contain a common major plaque protein, plakoglobin, their biochemical composition is markedly different. In epithelial cells, for example, two major types of adhering junctions can be generally distinguished in relation to composition and the specific type of filament attached (4, 6, 8, 16, 20, 47, 52): (a) Actin microfilaments anchor at adherens junctions which occur in diverse sizes, shapes, and positions (e.g., zonula adherens, fascia adherens, and punctum adherens), and contain E- or N-cadherin whose cytoplasmic portion is associated with certain cytoplasmic proteins such as α- and β-catenin, vinculin, α-actinin, and radixin, all of which contribute to plaque formation.

(b) Intermediate-sized filaments (IFs)1 are anchored at desmosomes (maculae adhaerentes) of variable sizes (mostly isodiametric and with diameters in the 0.1–5.0 μm range), the plaque of which contains, in addition to the intracellular portions of the desmosome-specific cadherins, i.e., desmocollin(s) (Dsc) and desmoglein(s) (Dsg), a special set of cytoplasmic proteins such as plakoglobin, desmoplakin, and some other cell type-specific proteins (for examples see 16, 19, 37, 44, 47). Moreover, the desmosomal cadherins represent multigene families whose members can be expressed differently in relation to epithelial differentiation pathways (24, 26), and at least three different genes of each of the Dsg
(Dsgl–3) and the Dsc (Dsc3–3) proteins have been distin-
guished in human tissues (for review and nomenclature see 5, 14, 45).

While in the last few years considerable progress has been made in the identification of the major molecular compo-
nents of the adhering junctions we are still far from under-
standing the molecular principles and regulatory mecha-
nisms involved in junction formation and in the anchorage of the specific kind of cytoskeletal filaments to the plaque. Immunoprecipitation experiments and transfection studies using cDNAs with deletions have shown that the cytoplasmic portion (tail) of E-cadherin is necessary for the formation and function of the zona
da anhaerens (34, 35) and is intimately and stably associated with β-catenin and/or, somewhat less stably, with its close relative plakoglobin (for amino acid [aa] sequence relationship see 3, 10, 12, 30–32, 41, 42) as well as with the vinculin-related protein α-catenin (18, 22, 33–36, 38–40, 52, 54).

Even less is known about the interaction of molecules of desmosomal cadherins with desmosomal plaque proteins and IFs. In cell transfection experiments using cDNA constructs encoding chimeric proteins, we have recently shown that the cytoplasmic carboxyterminal segment of desmocollin Dscla is sufficient for the assembly of a specific plaque and IFs. In cell transfection experiments using cDNA constructs encoding chimeras combining the connexin32 (Cx32) transmembrane portion with a Dsg tail we noticed a stable dominant-negative effect, resulting in the inhibition of formation of chimeric junctions as well as of all endogenous desmosomes (51).

To find the mechanism(s) responsible for the unexpected, inhibitory and dispersive dominant-negative effect of Dsg1 tails on desmosome assembly and thus on intracellular IF ar-
rangement and to identify the Dsg tail domain(s) involved and potential molecules binding to it, we have extended these experiments by transfections of constructs in which the tail was partially deleted. Here we report the identification of a short Dsg domain segment involved in plakoglobin binding which is important for desmosome formation.

Materials and Methods

Plasmid Construction

The construction of a plasmid clone encoding a chimeric polypeptide con-
sisting of the four transmembrane domains of rat liver Co32 and the cyto-
plasmic segment of bovine Dsg1 has been described (51). Carboxytermin-
al deletion mutants were produced from the construct in the Bluescript vector (plasmid clone BICoDg) with the help of conveniently located unique re-
striction endonuclease sites and subsequent integration of the new gene fragments (flanked by HindIII and 3’ by the blunt-ended restriction endonu-
cl ease site used for truncation) between the HindIII and blunt-ended NcoI sites of plasmid derivative B1 containing the synthetic oligonucleotide (5’-AACCGGATCCGATTCGATTCGATTCGCCCGA-3’). The above constructs were inserted into the Bluescript vector. The following restriction endonuclease sites were used for truncation: BstI for construction of BICoDg(209), NdeI for BICoDg(134). Some mutants were constructed with the help of polymerase chain reaction (PCR). For construct BICoDg(168) primers Dg-O-1 (5’-GGAGC~GCC-
TTGCCCTCCCTACGTCCACAGG-3’) and Dg-O-2 (5’TTTGGATCTCAAGACCCCA-
CAGGCC-3’) were used. BICoDg and the resulting PCR product were subjected to SDS-PAGE followed by autoradio-
graphic detection of labeled polypeptides or immunoblot reaction using the alkaline phosphatase system as described (51).

Immunoprecipitation and Immunoblotting

Cells were metabolically labeled with [35S]methionine mostly overnight (150 μCi/10 cm Petri dish in medium with reduced methionine), washed in PBS and briefly incubated in hypotonic buffer (10 mM Tris–HCl, pH 7.4, 2 mM DTT, and 20 μM 4-aminophenoxy-methylsulfonfluoride [APMSF] with or without 2 mM EDTA and 2 mM EGTA). The suspension was homogenized, centrifuged at 1000 g for 5 min, and the supernatant treated with twice concentrated immunoprecipitation lysis buffer (100 mM Tris–HCl, pH 7.5, 2% NP40, 300 mM NaCl, and 20 μM APMSF and 4 mM EDTA), with or without 1% deoxycholate. After centrifugation (20000 g, 10 min) the supernatant were incubated with the appropriate antib-
odies (overnight at 4°C) and then for 1 h at room temperature with 15 μg Protein A-Sepharose (Pharmacia P-L Biotech, Sweden, Uppsala, WI) equilibrated in immunoprecipitation buffer. Beads were then washed five times with PBS, one time with PBS/1% Triton X-100 and the immune complexes were subjected to SDS-PAGE followed either by autoradiography detection of labeled polypeptides or immunoblot reaction using the alkaline phosphatase system as described (51).
Results

Our observation that the introduction of additional desmoglein (Dsg1) tails in the form of Co–Dsg chimeras resulted, in different cultured epithelial cells, in the disappearance of all desmosomes (51) has led to the working hypothesis that this effect may be due to the depletion of a critical endogenous cytoplasmic molecule that binds to Dsg tails (for N-cadherin see also 21). Therefore, we designed experiments in which the chimera-integrated Dsg1 tails were systematically deleted and mutated (Fig. 1). By studying the synthesis and distribution of desmosomal proteins in A-431 cells transfected with cDNA constructs carrying such deletions and by immunoprecipitation of Dsg complexes we wanted to identify possible partner molecules binding in vivo to certain Dsg segments. In addition, we assayed for cell–cell transfer through the connexons of gap junctions by microinjection of Lucifer Yellow dye and found that Co–Dsg transfected cells were as poorly coupled as normal, i.e., non-transfected A-431 cells whereas cells transfected with desmocollin tail-containing constructs such as Co-Dsc (cf. 51) show markedly increased dye transfer (Fig. 2). All results described in the following have been obtained from stably transfected cell clones.
Figure 3. Immunofluorescence microscopy showing the distribution of desmosomal proteins in untransfected human A-431 cells (a and b) in comparison to A-431 cells transfected with a DNA clone producing the chimeric protein Co–Dsg(209) (c–d'). Untransfected cells were reacted with murine mAbs to desmoplakin (Dp, a) or Dsg (Dg, b) showing the typical punctate distribution of desmosomes. Transfected A-431 cells shown here were double-stained with rabbit antibodies to Co32 (Co, c and d) and monoclonal antibodies to either desmoglein (Dg, c') or desmoplakin (Dp, d'). Note the absence of dotted arrays of desmosomal staining in most transfected cells, with the exception of a small area with some very small desmosome-like dots in a cluster of cells showing drastically reduced immunofluorescence reaction of the transgene product (arrows in c' and lower right part of d and d'). Note also the codistribution of chimera Co–Dsg(209) with the endogenous Dsg2 of A-431 cells, most of which appears to be at the cell surface. The desmoplakin reaction in d' is diffusely spread over the cytoplasm in most cells but the fluorescence intensity shown here has been reduced to optimize the appearance of the brilliantly fluorescent recovered desmosomes in the lower right. Bars, 50 μm.
In Co–Dsg- and Co–Dsg(209)-expressing Cells Desmosomal Proteins Are Continually Synthesized but Not Assembled into Desmosomes

Recently, we reported a cDNA clone encoding a chimeric protein containing the four transmembrane domains of rat liver Co32 and the entire cytoplasmic tail region of bovine Dsg1. Expression of this clone in the notoriously desmosome-rich human epithelial cells of line A-431 led to two spectacular effects: (a) the inability of the synthesized chimeric proteins to assemble into chimeric junctions as, for example, observed for similar constructs with Dscla (51), and (b) the disappearance of all the numerous endogenous desmosomes (51).

To study the molecular basis of these effects and the contribution of individual Dsg subdomains we transfected cells with Co–Dsg deletion mutants lacking the codons for the last 262 aa, including the epitope of mAb Dg3.10 (23). Expression of this cloned construct Co–Dsg(209), which encodes a new and much shorter chimeric protein (Fig. 1), also resulted in the disappearance of punctate desmoplakin immunostaining at cell–cell borders and of all desmosomal structures, leaving only a weak and homogenous cytoplasmic fluorescence (data not shown; compare also Fig. 3, a and b with c and c'). Similar to Co–Dsg, Co–Dsg(209) codistributed with plakoglobin at the cell surface (Fig. 4). The endogenous Dsg, which in this cell line is mostly, if not exclusively Dsg2 (26, 45), was now selectively detected with mAb Dg3.10 and again appeared rather evenly dispersed over the cell surface membrane, particularly along cell–cell contacts (Fig. 3 c'). Double label immunofluorescence microscopy showed that this distribution was similar to that of the chimera encoded by Co–Dsg(209), as detected by antibodies specific for rat Co32 (Fig. 3, e and c'). In addition, some weakly immunostained, dot-like, probably vesicular cytoplasmic structures were also noted at variable frequency. These results indicate that the last 262 aa, with all the three Dsg-specific carboxy-terminal domains, including the repeating units (13, 23), did not contain any information necessary for the negative-dominant effects of desmosome disruption and inhibition of desmosome assembly.

While cells with intense synthesis of the Co–Dsg chimera were totally negative for punctate desmosomal protein immunostaining, small groups of cells that were only weakly positive for Co–Dsg(209) had recovered and showed finely punctate arrays of Dsg and desmoplakin immunostaining along cell–cell borders (compare Fig. 3, c and c', d and d'). Furthermore, Dsg and desmoplakin colocalized in the same small desmosomal dots (not shown) which, however, here appeared to be smaller than those detected in untransfected A-431 cells (compare Fig. 3, a and b with c' and d'). This pattern of heterogeneity suggests that low expression of certain Co–Dsg constructs in individual cell colonies allows the recovery of desmosomal structures.

To examine whether the observed changes of desmosome assembly and of desmoplakin staining were due to alterations in the expression and/or the stability of the proteins involved, we performed comparative immunoblot analyses of total cellular proteins (Fig. 5). These results showed that in steady state culture conditions the total amounts and the sizes of the desmosomal proteins were not considerably affected and that neither down-regulation of expression nor degradation of protein had taken place (Fig. 5, a and b, show examples for Dsg and desmoplakin). We have previously
shown that in these cells the amounts of the chimeric Co-Dsg protein and the endogenous Dsg were similar (51).

**Co-Dsg Chimeras Disrupt Desmosomes Selectively**

We then examined whether the expression of the Co-Dsg chimeras and the disappearance of all desmosomes had any effect on the assembly of other cell contact structures, particularly the actin-filament anchoring adhering junctions containing E-cadherin and vinculin. Immunofluorescence microscopy using antibodies to actin, E-cadherin and vinculin showed these proteins in their characteristic localizations, indistinguishable from those seen in nontransfected A-431 cells (Fig. 6). Many of the actin filament cables were intimately associated with plasma membrane attachment structures in regions of cell-cell contact (Fig. 6 a) which in turn were strongly positive for E-cadherin (Fig. 6, b and c) and vinculin (not shown). Double-label immunolocalization revealed the specific effect of transfections with Co-Dsg and Co-Dsg(209) with particular clarity (Fig. 6, c and c'): Adherens junctions, as identified by the presence of E-cadherin, are present (Fig. 6 c), whereas desmosomes are totally absent (Fig. 6 c').

The level of connexin(s) in untransfected A-431 cells and in A-431 cells expressing Co-Dsg (clone B5) was below the level of detection with antibodies against human Co32 and Co43. We therefore transfected B5 cells with a Co43-encoding gene construct. Immunofluorescence microscopy of selected double-transfected cell clones showed the efficient incorporation of both transgene products: as in B5 cells, Co-Dsg was rather evenly distributed over the plasma membrane but Co43 now formed large gap junctions, many of which were negative for Co-Dsg (results not shown), resulting in effective cell-to-cell coupling (Fig. 2). In parallel cotransfection experiments, Co43 colocalized well with the connexin-desmocollin chimera Co-Dsc (see 51).

Although the assembly of connexons is still poorly under-

**Figure 6.** Fluorescence microscopy of A-431 cells (clone B5) transfected to produce chimeric protein Co-Dsg, comparing the distribution of actin-containing filaments (a), visualized with FITC-labeled phalloidin, and of E-cadherin (b and c) with that of desmoplakin as a marker of desmosomes (c' shows the same field as c) visualized by immunofluorescence using rabbit antibodies (b and c) or, in double-label immunofluorescence, with a monoclonal murine antibody (c'). Two large and representative fields are shown in b and c, presenting extended membrane staining mostly in small distinct junctions of the *punctum adhaerens* type, in the absence of desmosomes (compare c and c'). Note also the typical cable arrays of actin filament bundles in a, with apparent anchorage sites at cell contact points identifiable by E-cadherin antibodies (as in b and c). Bars, 50 μm.
stood, our data suggest that the Dsg1 tail in Co–Dsg, but not that of Dsc in Co–Dsc chimeras (51), interferes with the ability of the transmembrane part of this connexin to cluster into paracrystalline gap junctions and also prevents the integration of these chimeras into preexisting gap junctions.

The C Domain of the Dsg Tail Is Responsible for Desmosome Disruption

To understand which part of the Dsg tail is important for desmosome disruption we prepared several deletion mutants (Fig. 1). As shown, mutant Co–Dsg(209) lacking the Dsg-specific end domains of 262 aa is still able to disrupt desmosomes. In contrast, neither chimera Co–Dsg(168) which lacks further 41 aa representing the end of the C-domain, nor mutant Co–Dsg(134) lacking the entire C-domain affected desmosome formation, as determined by immunolocalization with desmplakin and Dsg antibodies in transfected cell lines (Fig. 7). While in some places the Co–Dsg chimeric proteins showed colocalization with plakoglobin we also encountered many plasma membrane regions that were strongly positive for plakoglobin (Fig. 7 a') but negative for Co32 (Fig. 7 a).

To test whether this short, 41-aa-long segment of the C-domain is sufficient for desmosome destruction we constructed mutant Co–Dsgd(168–210), with an internal deletion of these aa (Fig. 1). The expression of this chimera did not disrupt desmosomes (Fig. 8). Surprisingly, however, protein Co–Dsgd(168–210) was not only transported to the cell surface but formed gap junction-like clusters (Fig. 8 a–c) which, however, were not stained by desmplakin (Fig. 8 a') and plakoglobin (Fig. 8 b') and did not appear to anchor cytokeratin fibrils (Fig. 8 c'). The specificity of this effect was also demonstrated by the introduction of another internal deletion mutant (construct CoDsgd(32–75); cf. Fig. 1) into A-431 cells which induced the same desmosome-negative phenotype as chimera Co–Dsg (not shown).

Plakoglobin Can Bind to the C Domain of Dsg

To determine which polypeptide(s) directly interact with the cytoplasmic Dsg1-tail, in particular with the 41 aa of the C-domain, we performed a series of immunoprecipitation experiments. Using Co32 antibodies, we were, for example, able to precipitate protein Co–Dsg from metabolically labeled A-431 cells of clone B5, as seen by autoradiography (Fig. 9 a) and by immunoblot analyses using Dsg and Co32 antibodies (not shown). In these experiments we consistently and specifically observed coimmunoprecipitation of only one additional polypeptide of molecular mass ~83 kD together with Co–Dsg, independent from the specific calcium and desoxycholate addition (Fig. 9 b). Immunoblot analysis with mAb PGS.1 showed that this polypeptide was plakoglobin (Fig. 9 c). An identical reaction was obtained with mAb 11E4 which recognizes a different plakoglobin epitope (not shown; see also Materials and Methods). Under the same conditions E-cadherin antibodies co-precipitated two major proteins of 90 and 100 kD, i.e., α- and β-catenin, and a miniscule amount of plakoglobin (Fig. 9 b), confirming and extending the data of previous authors (e.g., 22, 34, 39; see also Introduction).

Immunoprecipitation experiments in which the different Co32–Dsg1 mutant chimeras were compared (Fig. 9 c)
Figure 9. Autoradiograms (a and b) and immunoblot reaction (c) of immunoprecipitates from transfected A-431 cells. Immunoprecipitates were separated by SDS-PAGE (relative positions of marker proteins are mostly as in Fig. 3 and indicated on the left margin: β-galactosidase, phosphorylase b, BSA, ovalbumin, and carbonic anhydrase). (a) Immunoprecipitates from Co-Dsg-producing cells (clone B5) obtained with rabbit antibodies against Co43 (lane 1) or Co32 (lane 2). Note that a specific Co-Dsg band is only seen in lane 2 and that this also contains plakoglobin (these two polypeptides are indicated by dots: Co-Dsg, top, plakoglobin, bottom). (b) Immunoprecipitates from A-431 cells (clone B5) transfected to express chimera Co-Dsg, using rabbit antibodies to rat Co32 (lane 1) or to E-cadherin (lane 2). Note coprecipitation (arrowheads) of Co-Dsg (top) with plakoglobin (bottom), in contrast to the coprecipitation of E-cadherin (top) with catenin(s) (bottom) and a very low amount of plakoglobin. (c) Immunoblot reaction of murine monoclonal plakoglobin antibody with proteins in immunoprecipitates obtained with rabbit antibodies against Co32 from A-431 cell lines stably expressing Co-Dsg (lane 1), Co-Dsgd(209) (lane 2), Co-Dsgd(168) (lane 3) and Co-Dsgd(168-210) (lane 4). Immunoblot reaction, visualized by the alkaline phosphatase detection system, is only seen in lanes 1 and 2.

Figure 8. Double label immunofluorescence microscopy of A-431 cells producing the chimeric deletion protein Co-Dsgd(168–210). The left panel (a–c) shows the distribution of the mutant deletion protein, reacted with rabbit Co32 antibodies, in comparison with structures immunostained with mAbs to desmoplakin (a', Dp), plakoglobin (b', Pg) or cytokeratins (c', CK). Although Co-Dsgd(168–210) can form gap junction-like plaque structures these are not codistributed with desmosomal proteins and do not appear as specific sites of intermediate filament anchoring. Bars, 50 μm.

demonstrated that Co32 antibodies also precipitated chimera Co-Dsg(209) in association with plakoglobin, whereas plakoglobin was not detected in association with immunoprecipitated chimeric proteins Co-Dsg(168) and Co-Dsgd(168–210). This was in agreement with our double label immunofluorescence experiments in which plakoglobin colocalized with Co-Dsg and Co-Dsg(209) but not with Co-Dsg(168) or Co-Dsgd(168–210), indicating that the last 41 aa of the C-domain contribute significantly to plakoglobin binding.

Discussion

Desmoglein Dsg1 binds plakoglobin (27, 42, this study). Our detailed mutational analysis now has localized the Dsg1 tail region that is responsible, in transfected cells, for the dominant-negative effect on desmosome formation and stability to a relatively short segment of 41 aa at the end of the intracellular cadherin-specific domain (ICS domain; cf. 23, 45; C-domain in Fig. 1) which is 285 aa away from the carboxyterminus of this protein. This region is homologous to the carboxyterminal domain characteristic of the much shorter desmocollins Dscl–3 type a and the classical cadherins, is most conserved in aa sequence between the diverse cadherins (Fig. 10) (cf. 15, 17, 23–26, 45, 49) and has been shown in E-cadherin to be contained in the region responsible for the binding of plakoglobin and/or the catenins and thus, directly or indirectly, for the specific anchoring of actin microfilaments (3, 18, 20, 22, 30–36, 38–41, 52, 54). The binding of catenins and plakoglobin to other cadherins such as N-cadherin also takes place at the C-domain (cf. 18, 22, 38, see also 21). In a different study, we have further shown that it is this C-domain which in Dsc is needed for the binding of plakoglobin, notably in the central part containing the repeating units (cf. 10, 12, 41), and the assembly of a plaque competent in the specific anchorage of IFs (51, Troyanovsky, S. M., R. B. Troyanovsky, L. G. Eshkind, R. E. Leube, and W. W. Franke, manuscript in preparation). We conclude that the terminal section of the C-domain contains both information common to different cadherins such as that for the binding of plakoglobin and distinguishing information such as that for the exclusion of catenins from desmosomal cadherins and for the specific interaction with other components of the desmosomal plaque.

Considering the high sequence homology in the C-domain of the diverse cadherins, including members of the Dsc and Dsg subfamilies (Fig. 10), it is remarkable to note that this region displays discrete differences in its interaction with
various members of the plakoglobin/β-catenin/armadillo gene product family of proteins: in E-cadherin and some other classical cadherins it can bind β-catenin and/or—although with seemingly lesser stability—plakoglobin (22, 30–32, 42), whereas in desmogleins (this study, 42) and desmocollins (Troyanovsky, S. M., R. B. Troyanovsky, L. G. Eshkind, R. E. Leube, and W. W. Franke, manuscript in preparation) it seems to bind preferentially plakoglobin. A third and even different binding pattern is observed in the APC tumor suppressor gene product of colorectal carcinomas which apparently binds effectively β-catenin (43, 48).

In addition to the C-domain, other regions of the Dsg tail have also a marked influence on the topogenic behavior of the Co–Dsg chimeras. For example, although both C-domain-deficient chimeras Co–Dsg(168) and Co–Dsgd(168–210) cannot bind plakoglobin, the latter protein forms gap junction-like structures in the transfected cells whereas the former does not. A possible explanation might be that the large Dsg-specific domain of 261 aa contributes to the intramembranous lateral assembly of complex, i.e., gap junction-like structures whereas the extensively truncated forms are unable to cluster in this specific way.

The mechanism by which the introduction of Dsg tail domains interferes so dramatically with desmosome assembly and the arrangement of the IF system is still unknown. We can exclude, however, several simple explanations. Clearly, the synthesis of all major desmosomal proteins continues in the cells suffering from the dominant-negative effect and the resulting proteins are sufficiently stable and correctly inserted into the plasma membrane where they tend to concentrate at cell–cell boundaries but do not aggregate in the form of junctions. Both the normal endogenous desmosomal proteins as well as the chimeric proteins are also not enriched in endocytotically derived vesicles as it has been shown for epithelial cells uncoupled in the presence of low calcium concentrations (for references see 6, 16, 47).

In our previous paper (51) we have mentioned the depletion by competition hypothesis that also appeared attractive to Kintner (21) as an explanation for his results with various deletion forms of N-cadherin in Xenopus laevis embryos. Our results showing that the inhibition phenotype correlates with plakoglobin binding are obviously compatible with the hypothesis that the plakoglobin concentration in the cytoplasm and/or at the plasma membrane is critical so that the entrapment of some of it by the extra Dsg tail segments introduced would generally disturb the equilibrium with plakoglobin bound to E-cadherin and the desmosomal cadherins. However, there are still other arguments questioning this explanation and alternative explanations have been discussed elsewhere (51). We are currently analyzing in greater detail the complexes of endogenous Dsg and its mutants that form in the transfected cell and also try to determine the stereochemistry and relative binding affinities of these molecules in vitro.

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Note Added in Proof. After acceptance of this manuscript we learned of an article reporting plakoglobin binding in vitro to the same region of desmoglein that we have identified in living cells in the present paper: Mathur, M., L. Goodwin, and P. Cowin. 1994. Interactions of the cytoplasmic domain of the desmosomal cadherin Dsg1 with plakoglobin. J. Biol. Chem. 269:14075–14080.

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**Figure 10.** Comparison of the amino acid sequences (one letter code) of the carboxyterminal portion of the tail domains of human desmocollin (Dscl; from reference 50), desmoglein 1 (Dsg1; from reference 53) and E-cadherin (E-cad; from reference 2) in an optimized alignment to show amino acids identical (asterisks) or conservatively exchanged (dots) between Dsg1 and Dscl (top) or Dsg1 and E-cadherin (bottom).
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