The Head Domain of Plakophilin-1 Binds to Desmoplakin and Enhances Its Recruitment to Desmosomes

IMPLICATIONS FOR CUTANEOUS DISEASE*

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The contribution of desmosomes to epidermal integrity is evident in the inherited blistering disorder associated with the absence of a functional gene for plakophilin-1. To define the function of plakophilin-1 in desmosome assembly, interactions among the desmosomal cadherins, desmoplakin, and the armadillo family members plakoglobin and plakophilin-1 were examined. In transient expression assays, plakophilin-1 formed complexes with a desmoplakin amino-terminal domain and enhanced its recruitment to cell-cell borders; this recruitment was not dependent on the equimolar expression of desmosomal cadherins. In contrast to desmoplakin-plakoglobin interactions, the interaction between desmoplakin and plakophilin-1 was not mediated by the armadillo repeat domain of plakoglobin but by the non-armadillo head domain, as assessed by yeast two-hybrid and recruitment assays. We propose a model whereby plakoglobin serves as a linker between the cadherins and desmoplakin, whereas plakophilin-1 enhances lateral interactions between desmoplakin molecules. This model suggests that epidermal lesions in patients lacking plakophilin-1 are a consequence of the loss of integrity resulting from a decrease in binding sites for desmoplakin and intermediate filaments at desmosomes.

Desmosomes are intercellular adhesive junctions that act as anchorage points for intermediate filament networks. The desmosomal cadherins, desmogleins and desmocollins, bind directly to the cytoplasmic protein plakoglobin (1–3), a member of the armadillo gene family that includes the adherens junction protein β-catenin (4). The desmosomal cadherin-plakoglobin complex is coupled to the intermediate filament network by desmoplakin (5, 6), which binds to both plakoglobin and intermediate filaments (7–10).

The disruption of desmosomes by gene ablation in experimental model systems (6, 11–13) or by autoimmune antibodies in human patients (14) severely compromises tissue integrity and function. Recently, the first human genetic disorders involving mutations in desmosomal genes have been reported. Haploinsufficiency of desmoplakin results in the disruption of intermediate filament interactions with desmosomes in palmoplantar epidermis (15). In addition, mutation of both alleles of plakophilin-1 (PKP-1)1 and consequent loss of the protein causes epidermal fragility, perturbed desmoplakin localization, and detachment of intermediate filaments from the plasma membrane of keratinocytes (16). PKP-1 was originally identified as Band 6, a 75-kDa keratin-associated protein component of desmosomes in stratified tissues (17). PKP-1 is also part of the armadillo gene family (18–22). In overlap assays, PKP-1 was recently found to bind to a number of desmosomal components, including desmoplakin and intermediate filament polypeptides (10). However, the mechanism by which PKP-1 contributes to desmosome assembly and attachment to intermediate filaments remains unknown.

We report here that the non-armadillo head domain of PKP-1 binds directly to the amino-terminal domain of desmoplakin and enhances its recruitment to cell junctions. Our data indicate that PKP-1 is a desmoplakin-binding partner in the desmosomal plaque and suggest a model by which PKP-1 recruits desmoplakin to desmosomes by increasing lateral interactions between junction components.

EXPERIMENTAL PROCEDURES

Cell Culture and Immunofluorescence— COS 7 cells (subclone 20) were transiently transfected and processed for immunofluorescence analysis as described (8, 23). To enhance detection of full-length PKP-1 and the PKP-1 head domain, cells were permeabilized with 0.01% saponin before fixation in methanol (24). DP-NTP was detected using the monoclonal antibody M2 directed against the FLAG epitope tag (Eastman Kodak Co., Rochester, NY). Rabbit polyclonal antibodies against recombinant PKP-1 head (Ab 667) and repeat domains (Ab 670) were used to detect the PKP-1 fragments.

cDNA Expression Constructs—A cDNA encoding the first 584 amino acids of desmoplakin (DP-NTP), and an amino-terminally FLAG epitope-tagged version driven by the CMV promotor were described previously (5, 8, 25). DP-NTP with a carboxyl-terminal FLAG tag was generated by PCR, cloned into pBlueScript and sequenced, and then subcloned into the CMV expression vector. Full-length, Myc-tagged plakoglobin and Dg1 were described previously (8), and the full-length “a” form of PKP-1 was assembled and cloned into pCMV-SCRIPT (Stratagene, La Jolla, CA) (19). Plasmids encoding the PKP-1 head and armadillo repeat domains were generated by reverse transcription-PCR using RNA isolated from A431 cells. The resulting cDNAs were sequenced and subcloned into the pCMV5 vector (26) using the BamHI and HindIII restriction sites. The PKP-1 head domain comprises amino acids 1–286 ending with QTYYGL and the repeat domain begins at amino acid 287 (GGICK).

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1 The abbreviations used are: PKP-1, plakophilin-1; Ab, antibody; mAb, monoclonal antibody; CMV, cytomegalovirus; PCR, polymerase chain reaction.

2 M. Hatzfeld, manuscript in preparation.
Co-immunoprecipitation and Immunoblot Analysis—Co-immunoprecipitation was carried out as described previously (8, 23). Monoclonal antibody M2 coupled to agarose beads (Kodak) was used to capture FLAG epitope-tagged DP-NTP. Antibodies to plakoglobin (mouse mAb 11E4) (27) and PKP-1 (rabbit polyclonal Ab 667) were used to precipitate these armadillo proteins and associated polypeptides. Immune complexes were released by incubation in reducing SDS-polyacrylamide gel electrophoresis sample buffer at 95 °C and analyzed by immunoblot using Enhanced Chemiluminescence (Amersham Pharmacia Biotech). Plakoglobin was detected using mAb 11E4, DP-NTP was detected using a rabbit polyclonal antibody NW161 (5), and desmoglein (Dsg1) was monitored using mAb 9E10 directed against the Myc tag (28).

RESULTS

PKP-1 Enhances Desmoplakin Recruitment to Intercellular Junctions—To define the molecular basis of the cutaneous lesions exhibited by patients lacking PKP-1, the role of PKP-1 in desmosome assembly was addressed by transiently expressing junctional proteins in COS cells. We previously demonstrated that a polypeptide comprising the first 584 amino acids of desmoplakin (DP-NTP) is recruited to cell borders when co-expressed with a cadherin and plakoglobin (8, 23). Here, DP-NTP was expressed alone (Fig. 1, A and B) or in combination with full-length PKP-1 (Fig. 1, C and D). When expressed alone, DP-NTP remained in large cytoplasmic aggregates and exhibited minimal cell border localization (Fig. 1A). In the presence of PKP-1, extensive DP-NTP recruitment to intercellular junctions was observed (Fig. 1C), and PKP-1 and DP-NTP co-localized at cell borders (Fig. 1D). Nuclear staining was also observed for PKP-1, consistent with a report that PKP-1 is present in the nucleus in many cell types (24).

To define which domain of PKP-1 was responsible for enhancing DP-NTP recruitment to borders, DP-NTP was co-expressed with either the head domain of PKP-1 (Fig. 1, E and F) or with the carboxyl-terminal armadillo repeat region of PKP-1 (Fig. 1, G and H). Similar to full-length PKP-1, the PKP-1 head domain localized at borders and in the nucleus (Fig. 1F). The head domain also enhanced DP-NTP recruitment to borders, although to a lesser extent than full-length PKP-1. Both full-length PKP-1 and the head domain localized to cell-cell borders even when expressed in the absence of DP-NTP or exogenously expressed cadherins (not shown). The PKP-1 armadillo domain did not enhance DP-NTP recruitment to borders, and both DP-NTP (Fig. 1G) and the PKP-1 armadillo domain exhibited a cytoplasmic distribution. These data indicate that PKP-1 enhances DP-NTP recruitment to cell borders and that this activity is contained within the amino-terminal head domain of PKP-1. Furthermore, this activity does not require co-expression of exogenous desmosomal cadherins.

PKP-1 Co-immunoprecipitates with DP-NTP and the PKP-1 Head Domain Binds to the Amino-terminal Domain of Desmoplakin—To establish the hierarchy of protein-protein interactions in which PKP-1 participates, we addressed first whether PKP-1, like plakoglobin, interacts with desmosomal cadherins and second whether PKP-1 interacts with the amino-terminal domain of desmoplakin. PKP-1 was co-expressed in COS cells with the desmosomal cadherin Dsg1 (Fig. 2) or with DP-NTP (Fig. 3). As reported previously (27, 30), Dsg1 and plakoglobin form complexes as demonstrated by the presence of Dsg1 in the plakoglobin immunoprecipitation (Fig. 2A). In contrast, Dsg1 was not detected when PKP-1 was immunoprecipitated in parallel experiments (Fig. 2B). Furthermore, PKP-1 did not co-immunoprecipitate with Dsg1 when antibodies directed against the Dsg1 extracellular domain were used (not shown). Similar results were obtained when Dsc2a was tested for complex formation with PKP-1 (not shown).

Similar to previous results with plakoglobin, PKP-1 co-immunoprecipitated with DP-NTP (Fig. 3). To determine whether PKP-1 binds directly to DP-NTP or to the desmosomal cadherins, yeast two-hybrid analysis was carried out. The PKP-1 head domain and armadillo domains were tested for interactions with DP-NTP, the Dsg1 cytoplasmic domain, and the Dsc2a cytoplasmic domain. Consistent with the co-immunoprecipitation analysis, the PKP-1 head domain interacted strongly with DP-NTP as determined by a β-galactosidase reporter assay (Fig. 4). The PKP-1 head domain also interacted with the Dsg1 cytoplasmic tail in this assay, but a significant interaction with the Dsc2a cytoplasmic domain was not detected. The PKP-1 armadillo repeat domain did not bind to either DP-NTP or to the desmosomal cadherin cytoplasmic tail domains. This
is in contrast to the armadillo repeat domain of plakoglobin, which does bind to DP-NTP (8, 23). Similar results were obtained using growth in the absence of histidine as the reporter for protein interactions (not shown).

DISCUSSION

The results presented here provide insights into both the molecular basis of a cutaneous disorder in patients lacking PKP-1 (16) and into the role of PKP-1 in desmosome assembly. In addition to binding to keratins (10, 17, 19), PKP-1 may also facilitate intermediate filament attachment to the desmosomal plaque by enhancing desmoplakin recruitment to the desmosome. In patients lacking PKP-1, epidermal fragility and blistering would be predicted because of the loss of intermediate filament-binding proteins in the desmosome. The head domain of PKP-1 but not the armadillo repeat domain binds to and recruits desmoplakin to cell borders (Figs. 1 and 4), highlighting a fundamental difference between PKP-1 and plakoglobin. In the case of plakoglobin, desmoplakin binds directly to the central armadillo motifs, and, although the first three repeats retain some binding ability, the entire collection of 13 repeats is required for a robust interaction (not shown). The fact that the PKP-1 armadillo domain remained diffuse in the cytoplasm was somewhat surprising because the arm repeats of plakoglobin, β-catenin, p120, and p0071 all bind to cadherins (31). PKP-1 does bind to some desmosomal cadherins when tested by in vitro overlay assays (10, 32), and PKP-1 did interact with Dsg1 in the yeast two-hybrid assay. However, the interaction was between Dsg1 and the non-armadillo head domain of PKP-1 (Fig. 4). Furthermore, in contrast to plakoglobin (8, 23), transfection experiments demonstrated that full-length PKP-1 does not require co-expression with a cadherin to localize and recruit DP-NTP to cell-cell borders (Fig. 1). It is possible that PKP-1 preferentially associates with certain isoforms of desmosomal cadherins that are co-expressed with PKP-1 during epidermal differentiation, such as Dsg1 and or Dsc1. However, it appears that the primary binding partner for PKP-1 is desmoplakin and that equimolar amounts of cadherin/PKP-1 are not required for PKP-1 assembly into the plaque.

Previous studies suggest that lateral interactions among desmosomal components are critical to the assembly process (8, 10). Together with the data presented here showing that PKP-1 enhances desmoplakin recruitment to the plasma membrane,
these observations are consistent with the model in Fig. 5. In the absence of PKP-1, the number of available desmosomal cadherin-plakoglobin complexes would limit the total amount of desmoplakin present at the junction. However, the presence of PKP-1 would overcome this limitation by providing additional desmoplakin-binding sites at the membrane. Consistent with this, increased desmosomal localization of endogenous desmoplakin was observed in COS cells transiently expressing PKP-1 (not shown). The model also predicts that PKP-1 could associate laterally with multiple desmoplakin molecules, which might occur if PKP-1 forms dimers or multimers or if each PKP-1 molecule has multiple desmoplakin-binding sites. Because our present data cannot provide quantitative information on the stoichiometry of PKP-1-DP complexes, future work will be needed to test these possibilities. However, if PKP-1 were to associate with at least two desmoplakin molecules, this would provide an explanation for its ability to enhance DP-NTP recruitment to cell borders in the absence of exogenously expressed cadherins and plakoglobin. Secondary interactions between PKP-1 and the desmosomal cadherins might also occur, leading to further strengthening of the adhesive complex (see also Ref. 10).

The data presented in this study also suggest that plakoglobin and PKP-1 exhibit fundamentally different modes of assembly into the desmosomal plaque, with plakoglobin linking desmosomal cadherins to desmoplakin and PKP-1 functioning largely in lateral interactions. Consistent with this hypothesis is the observation that desmosomes in suprabasal layers of the epidermis are larger than simple epithelial desmosomes or desmosomes from basal cells. During epidermal differentiation, desmosomes may increase in size and stability because of insertion of PKP-1/DP-complexes, thereby enhancing desmosome interactions with the cytoskeleton and rendering cells more resistant to mechanical stress. Presumably, this architectural arrangement of both linear and lateral interactions provides for a strong adhesive junction with multiple protein-protein interactions as targets for cellular regulation.

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FIG. 5. A model for desmosome assembly. In the presence of PKP-1, there is an increase in desmoplakin (DP) recruitment and enhanced intermediate filament attachment. In the absence of PKP-1, the number of available cadherin-plakoglobin (PG) complexes would limit desmoplakin accumulation at the membrane. The schematic summarizes current information regarding the hierarchy of interactions among desmosomal components; however, a determination of the absolute stoichiometry of these interactions awaits future study.