Desmoplakin: an unexpected regulator of microtubule organization in the epidermis

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Despite their importance in cell shape and polarity generation, the organization of microtubules in differentiated cells and tissues remains relatively unexplored in mammals. We generated transgenic mice in which the epidermis expresses a fluorescently labeled microtubule-binding protein and show that in epidermis and in cultured keratinocytes, microtubules stereotypically reorganize as they differentiate. In basal cells, microtubules form a cytoplasmic network emanating from an apical centrosome. In suprabasal cells, microtubules concentrate at cell–cell junctions. The centrosome retains its ability to nucleate microtubules in differentiated cells, but no longer anchors them. During epidermal differentiation, ninein, which is a centrosomal protein required for microtubule anchoring (Dammermann, A., and A. Merdes. 2002. *J. Cell Biol.* 159:255–266; Delgehyr, N., J. Sillibourne, and M. Bornens. 2005. *J. Cell Sci.* 118:1565–1575; Mogensen, M.M., A. Malik, M. Piel, V. Bouckson-Castaing, and M. Bornens. 2000. *J. Cell Sci.* 113:3013–3023), is lost from the centrosome and is recruited to desmosomes by desmoplakin (DP). Loss of DP prevents accumulation of cortical microtubules in vivo and in vitro. Our work uncovers a differentiation-specific rearrangement of the microtubule cytoskeleton in epidermis, and defines an essential role for DP in the process.

Introduction

The microtubule cytoskeleton plays roles in determining cell shape, cell polarity, vesicle trafficking, and cell division. Consequently, microtubule reorganization during differentiation is believed to be essential for morphogenesis. However, in most tissues and differentiated cells, little is known about the organization of microtubules or the mechanisms involved in microtubule reorganization.

The epidermis is a stratified squamous epithelium that acts as a barrier between the internal and external environments. The innermost (basal) layer maintains contact with its underlying basement membrane, separating epidermis from dermis. As basal cells move outward, they enter a program of terminal differentiation that involves the assembly of robust intercellular desmosomal junctions and culminates in the formation of flattened dead cells that are sloughed from the skin surface. During terminal differentiation, both the intermediate filament (IF) and the actin cytoskeleton undergo dynamic reorganization in their associations with desmosomes and adherens junctions, respectively (Perez-Moreno et al., 2003). What happens to the microtubule cytoskeleton in the course of terminal differentiation remains unexplored.

We report the first example of a transgenic mouse with fluorescently labeled microtubules. Using these mice, we have uncovered a differentiation-specific reorganization of the microtubule cytoskeleton that, surprisingly, depends on the desmosomal linker protein desmoplakin (DP). Further implicating DP in microtubule organization is the observation that DP is required for the relocalization of the microtubule-anchoring protein ninein from the centrosome to cell junctions.

Results and discussion

To visualize microtubules in living epidermis and cultured keratinocytes, we engineered transgenic mice expressing the microtubule-binding domain of ensconsin fused to three copies of GFP (EMTB-3GFP). This fusion protein has been previously well characterized and has a fast on-rate for microtubules, but does not significantly alter microtubule dynamics (Faire et al., 1999; Bulinski et al., 2001). To drive its expression, we used the epidermal-specific keratin 14 (K14) promoter (Fig. 1 A; Vasioukhin et al., 1999).

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Abbreviations used in this paper: DP, desmoplakin; e, embryonic day; IF, intermediate filament; mk, mouse keratinocyte; MTOC, microtubule-organizing center.

The online version of this article contains supplemental material.

The Journal of Cell Biology, Vol. 176, No. 2, January 15, 2007 147–154
http://www.jcb.org/cgi/doi/10.1083/jcb.200609109

Mice expressing *K14-EMTB-3GFP* were viable and fertile, with no observable detrimental phenotype. Whole-mount epifluorescence microscopy revealed transgene expression throughout the epidermis and in the dots corresponding to developing hair follicles (Fig. 1 B).

At embryonic day (e) 14.5, the microtubule network of basal cells was significantly concentrated in the apical domain, and it extended downward toward the base of these cells (sagittal views in Fig. 1, C and D, and Video 1, available at http://www.jcb.org/cgi/content/full/jcb.200609109/DC1). Confocal imaging from the surface of living embryos revealed that the microtubule-organizing center (MTOC) of each basal cell localized to its apical domain (Fig. 1 E).

Microtubules were apically organized within the basal epidermal layer whether the epidermis was well stratified (e18.5) or not (e14.5; Fig. 1, D and G [inset]). This organization contrasted markedly from that of stratified cells, where microtubule networks were concentrated at cell–cell borders (Fig. 1, F and G). Because the K14 promoter is most active in basal cells, these distinctions were best visualized at different exposures (Fig. 1 G, inset). Another striking difference between basal and suprabasal microtubule networks was the lack of an obvious MTOC in stratified cells. This is most apparent by confocal imaging through the suprabasal cells (Video 2, available at http://www.jcb.org/cgi/content/full/jcb.200609109/DC1).

Collectively, these data suggested that as epidermal cells differentiate, they undergo a dynamic rearrangement of microtubules, which includes disappearance of the centrosomal array (Fig. 1 H). The organization of microtubules in the epidermis differed significantly from that reported for simple epithelia, where microtubules exhibit a perpendicular (apical–basal) orientation with no obvious MTOC (Musch, 2004).

To determine whether the differentiation-associated changes in microtubule organization can be recapitulated in vitro, primary mouse epidermal keratinocytes (1° mouse keratinocyte [mk]) were cultured from both wild-type (WT) and EMTB-3GFP–expressing mice. In low-calcium media, cells remain proliferative and do not form cell–cell junctions. Under these conditions, microtubules extended radially from the nucleus, which appeared to serve as an MTOC for these cells (Fig. 1 I). This organization was also visualized by immunostaining for α- or β-tubulin in wild-type keratinocytes.

When calcium levels were raised to induce cell–cell adhesion and differentiation, a reorganization of the microtubule cytoskeleton was observed. At early times after induction, microtubules concentrated at areas of developing cell–cell contacts

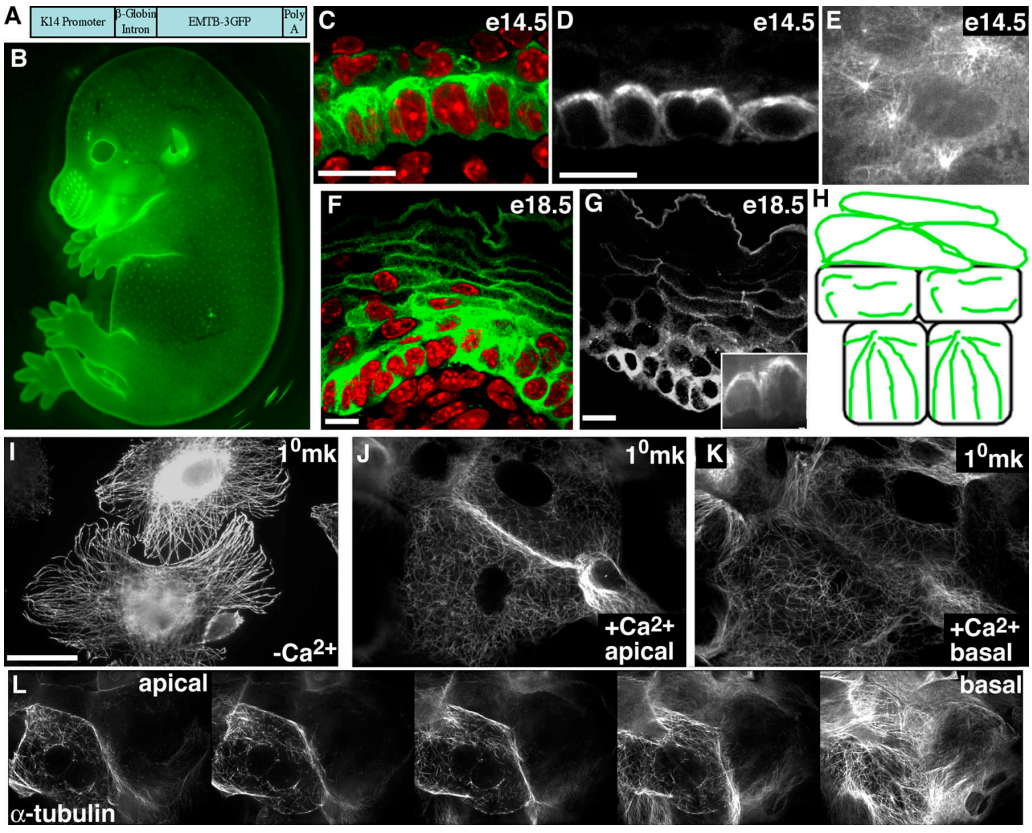

Figure 1. **Microtubule organization in the epidermis.** (A) Diagram of the K14-EMTB-3GFP gene used to generate transgenic mice. (B) Fluorescence image of a transgenic K14-EMTB-3GFP embryo at e16.5. (C and D) K14-EMTB-3GFP fluorescence in fixed sagittal sections of e14.5 epidermis. DNA is labeled with Topro3 in C. (E) K14-EMTB-3GFP fluorescence in living e14.5 epidermis viewed in the plane of the epidermis at the apical region of the basal keratinocytes. (F and G) K14-EMTB-3GFP fluorescence in fixed sagittal sections of e18.5 epidermis. DNA is labeled with Topro3 in G. (H) Diagram of microtubule organization (green) in stratified epidermis. (I–K) K14-EMTB-3GFP in fixed primary cultured keratinocytes (1°mk) grown in low- (I) or high-calcium media for 24 h (J and K). J (apical) and K (basal) are confocal slices of the same cells. (L) Z-stack through an anti–α-tubulin staining of fixed wild-type (WT) 1°mk grown for 72 h in high-calcium media. Bars, 10 μm.

(Fig. 1 J), which were particularly prominent in the apical domains of the cells. By 72 h after induction, cells were stratified, and microtubules were most concentrated at the cell junctions of the suprabasal (apical) cells (Fig. 1 L). Notably, microtubules no longer displayed the radial association with the nucleus that had been prominent in low-calcium conditions (compare Fig. 1, I and K). As judged by confocal microscopy and z-stack imaging, MTOC-like structures were absent in these differentiating cultures (Fig. 1, K and L).

Overall, the microtubule rearrangements seen in cultured keratinocytes bore a strong resemblance to those in intact epidermis, and further substantiated that upon differentiation, microtubules undergo a stereotypical reorganization consisting of loss of centrosomal/nuclear MTOC activity and accumulation of microtubules at intercellular junctions.

The accumulation of microtubules at calcium-stimulated cell junctions suggested a role for cadherin-mediated junctions in this process. To test this hypothesis, we mated mice to obtain embryos that expressed epidermal EMTB-3GFP and had epidermal-specific loss-of-function mutations in *desmoplakin* or *α-catenin* genes. DP is a linker protein known to connect desmosomal cadherin complexes to the IF cytoskeleton (Stappenbeck and Green, 1992; Kowalczyk et al., 1997), and it is essential for the formation of desmosomes (Gallicano et al., 1998; Vasioukhin et al., 2001b). In contrast, α-catenin integrates E-cadherin–β-catenin complexes with actin dynamics, and it is essential for the formation of adherens junctions (Vasioukhin et al., 2001a).

In both wild-type and α-catenin cKO epidermis, the microtubule cytoskeleton was concentrated at the junctions of suprabasal cells (Fig. 2, A and B). In striking contrast, despite the relatively normal organization of microtubules in DP cKO basal cells, the suprabasal cells displayed a paucity of cortical microtubules (Fig. 2 C and Video 3, available at http://www.jcb.org/cgi/content/full/jcb.200609109/DC1). Instead, cytoplasmic aggregates of microtubules were observed (Fig. 2 C, inset). This observation was surprising because desmosomes and DP have not been implicated in microtubule organization. There were small regions of the α-catenin cKO epidermis that displayed reduced cortical staining for microtubules. However, closer inspection revealed that they exhibited a corresponding lack of cortical DP (Fig. S1). Collectively, the cortical microtubule organization observed in suprabasal epidermal layers appeared to be directly attributable to DP.

The dependency of cortical microtubules on DP could also be recapitulated in vitro. Thus, in WT and *α-catenin*–null epidermal cultures grown in a high-calcium medium, microtubules concentrated at sites of cell–cell contact (Fig. 2, D and E). In contrast, DP-deficient keratinocytes in high-calcium media displayed an evenly distributed array of cytoplasmic microtubules with no appreciable border accumulation, even in closely juxtaposed cells, and with no obvious perinuclear organization (Fig. 2 F). In low-calcium media, where cell junctions do not form, a radial microtubule organization extending from the perinuclear area was observed in WT, α-catenin–null, and DP-null keratinocytes (Fig. 2, G–I).

These findings revealed that in high calcium, the loss of a perinuclear MTOC and gain of a cortical microtubule network can be uncoupled when DP is absent. Moreover, the results demonstrate that the microtubule rearrangements that occur in differentiating WT epidermal cells can be dissected into DP-dependent and -independent events.

In many cells, protein complexes containing γ-tubulin induce local microtubule nucleation at the centrosome/MTOC. In cross sections of WT epidermis, basal cells displayed a concentrated focus of anti–γ-tubulin staining at the apical edge of

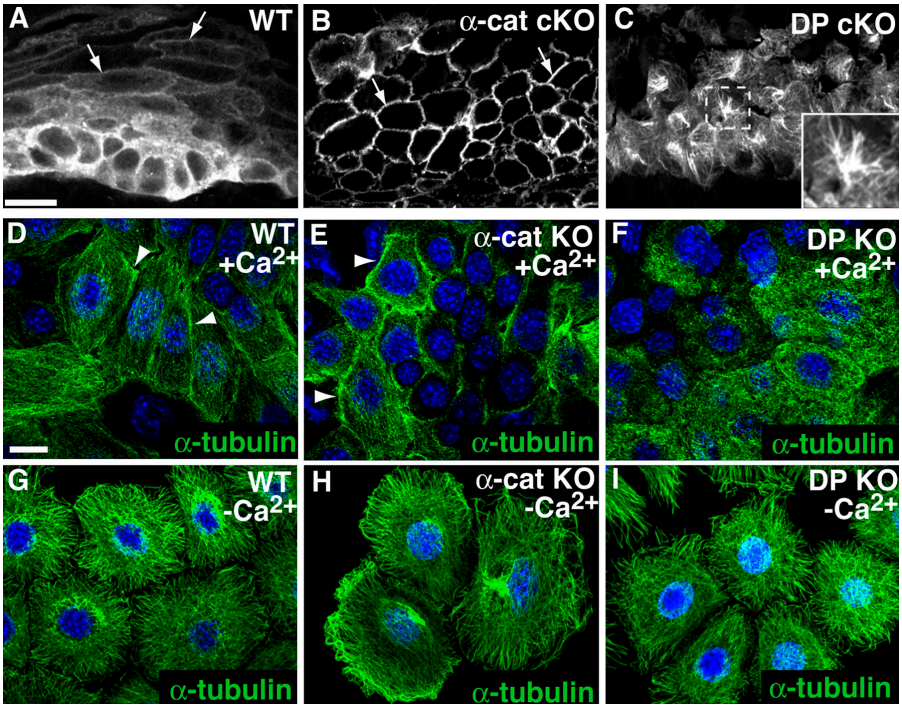

Figure 2. **DP is required for cortical microtubule organization.** (A–C) K14-EMTB-3GFP epifluorescence in sagittal e18.5 skin sections of WT (A), α-catenin conditionally null (α-cat cKO; B), and DP cKO (C) mice. Inset in C is of a microtubule aggregate in the suprabasal DP cKO epidermis. (D–I) Microtubule organization (anti–α-tubulin) in 1°mk grown in high-calcium media for 36 h before fixation (D–F) or in low-calcium media (G–I). Genotypes are as indicated. D–I show all maximum-intensity projections through the cells. Arrows and arrowheads denote border localizations. Bars, 10 μm.

the nucleus (Fig. 3 A). These data provided strong support for our prior localization of the putative MTOC in basal cells (Fig. 1, C–E).

Although no longer apical, γ-tubulin retained its focal perinuclear localization in the differentiating suprabasal cells of the e18.5 epidermis (Fig. 3 A). This was also the case for a centriolar marker, centrin-GFP (Fig. 3 B; Lechler and Fuchs, 2005). For both γ-tubulin and centrin-GFP, the intensity of labeling was reduced as cells moved to the suprabasal layers. However, the persistence of the centrosomes was surprising, given the loss of a discernable MTOC.

In vitro, γ-tubulin and the centriolar proteins centrin and centriolin (Gromley et al., 2003) also remained associated with centrosomes under both low- and high-calcium conditions (Fig. 3, C and D). This was also the case in DP-null keratinocytes (Fig. 3, E and F). Thus, both in vivo and in vitro, the differentiation-induced rearrangements in microtubule organization were not attributable to a loss of the centrosome and/or relocalization of γ-tubulin. Centrosomes remained largely intact, but they did not function as MTOCs when epidermal cells enter their program of terminal differentiation.

Time-lapse imaging of EMTB-3GFP revealed that centrosomes in differentiating epidermal cells are still able to nucleate microtubules (Video 4, available at http://www.jcb.org/cgi/content/full/jcb.200609109/DC1). Additionally, when keratinocytes were released from nocodazole-induced microtubule depolymerization, a burst of microtubule polymerization was detected at centrosomes (Fig. 3, I and J, and Videos 5 and 6). The ability of microtubules to be nucleated from centrosomes was observed in both low- and high-calcium conditions. However, although the centrosome remained associated with some of the microtubules in low calcium, this was not the case in high calcium. These data provide further evidence suggesting that when epidermal cells differentiate, centrosome activities become separated; microtubule nucleation continues to occur, whereas microtubule-anchoring activity is lost.

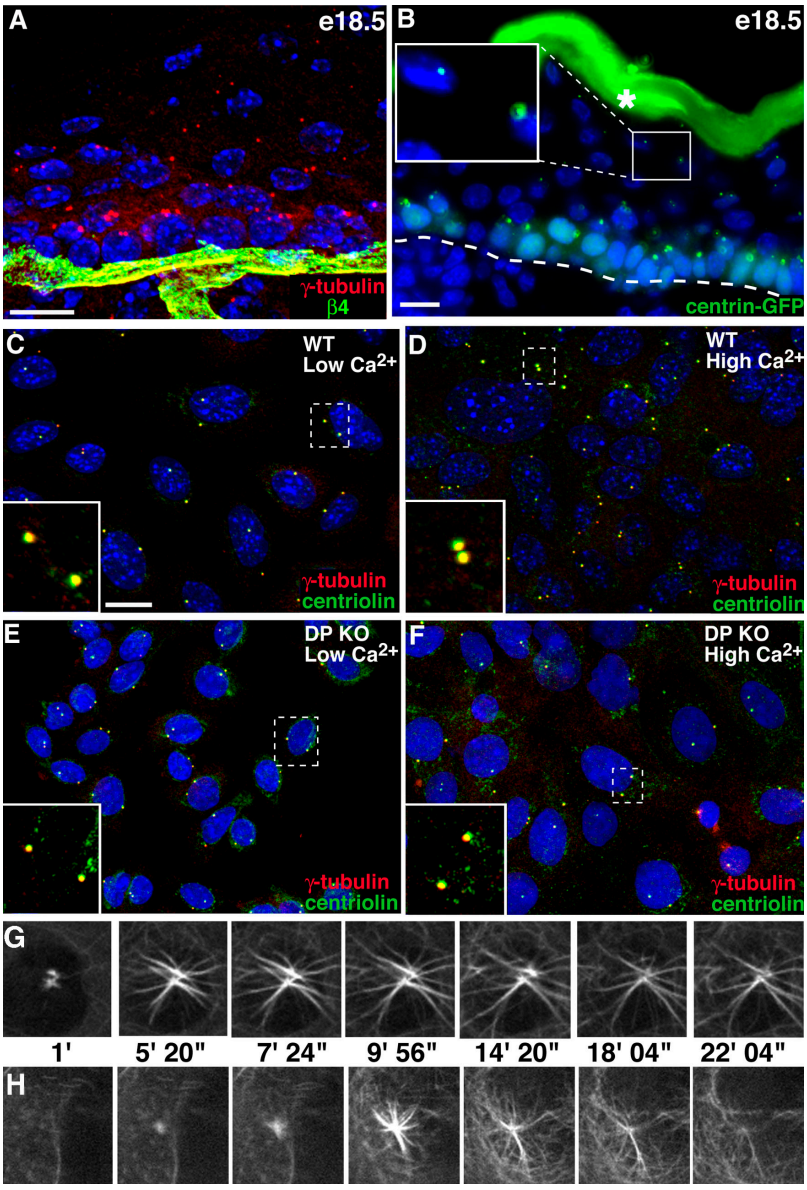

Figure 3. **Differentiating keratinocytes retain a centrosome, but it does not act as a MTOC.** (A) γ-Tubulin localization in sagittal section of e18.5 epidermis. β4-integrin marks the base of the basal layer of the epidermis. (B) Centrin-GFP in e18.5 transgenic epidermis. Inset shows a magnified view to highlight intact centrioles in suprabasal cells. Asterisk denotes autofluorescence of the uppermost layers of the skin surface. (C–F) Anti–γ-tubulin (red) and anti-centriolin (green) immunofluorescence labeling of WT (C and D) and DP KO (E and F) 1°mk grown in low-calcium (C and E) or high-calcium media for 48 h (D and F). Insets are higher magnifications of boxed regions. A–F show all maximum-intensity projections to capture centrosomes in different planes. (G and H) Images of K14-EMTB-3GFP fluorescence taken during the recovery of microtubules after washout of nocodazole in 1°mk grown in low-calcium (G) or in high-calcium media for 48 h (H). Bars, 10 μm.

Several proteins have been implicated in anchoring microtubules at the centrosome (Kim et al., 2004; Delgehyr et al., 2005; Guo et al., 2006; Yan et al., 2006). One of these is the coiled-coil protein ninein, which localizes to the subdistal appendage of the mother centriole (Ou et al., 2002), a preferred site for microtubule attachment. Ninein is a marker for microtubule anchoring not only in proliferative cells but also in differentiated inner ear hair cells, where it colocalizes with microtubule minus ends (Mogensen et al., 2000). Functionally, it has been shown that the levels of ninein at the centrosome determine its microtubule-anchoring activity (Abal et al., 2002; Dammermann and Merdes, 2002). Interestingly, the only reported experimentally induced separation of centrosomal nucleation and anchoring functions (which resembles what happens physiologically in differentiating epidermis) is by expression of mutants of ninein that prevent its targeting to the centrosome (Delgehyr et al., 2005).

To investigate whether ninein might play a role in these microtubule rearrangements, we first examined its localization. In cultured keratinocytes, ninein localized to one of the two centrioles marked by antibodies against γ-tubulin (Fig. 4 A, inset). Such labeling has been observed before in fibroblasts (Piel et al., 2000). However, keratinocytes differed from fibroblasts in their display of anti-ninein labeling around the nuclear envelope, from which the radial array of microtubules emanated in these cells (Fig. 4 A, arrowheads).

Another distinguishing feature was illuminated when epidermal cultures were induced to terminally differentiate. Associated with this process was a depletion of ninein at the centrioles and nuclear envelope, and concomitant ninein relocalization to cell–cell junctions (Fig. 4, B and D). Quantitation of ninein-containing centrioles revealed that both WT and DP-null cells show a characteristic loss of ninein from centrioles upon differentiation (Fig. 4 D). However, at 48 h after calcium addition, DP-null cells have more ninein-positive centrioles than do WT cells. This might be expected given that DP-null cultures did not stratify efficiently and remained more proliferative than their WT counterparts (mitotic index 0.40 ± 0.06 for DP-null vs 0.06 ± 0.03 for WT). In vivo, for both WT and DP cKO epidermis, ~90% of basal cells had detectable ninein at the centrosome, compared with <10% of suprabasal cells ($n > 200$).

Localization of ninein to cell junctions was not an antibody artifact, as an exogenous GFP-ninein fusion protein also localized to cell junctions (Fig. 4 C). This localization did not require either intact microtubules or F-actin (Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200609109/DC1). To further characterize the localization of ninein to cell–cell borders, we costained high-calcium keratinocyte cultures with antibodies against ninein, E-cadherin, and DP. Although ninein displayed a largely nonoverlapping pattern with E-cadherin (Fig. 4 E), it showed extensive colocalization with DP (Fig. 4 F), and with the desmosomal cadherins (not depicted). Based upon these data, ninein's border localization appeared to coincide with desmosomal, rather than adherens junction, markers.

To determine whether DP might act to recruit ninein to desmosomes, we repeated our calcium-induced differentiation experiments in *DP*-null epidermal cultures. As shown in Fig. 4 G, ninein remained largely cytoplasmic and was not recruited to cell–cell borders in the absence of DP. Moreover, this was not a general effect of disrupting intercellular adhesion because

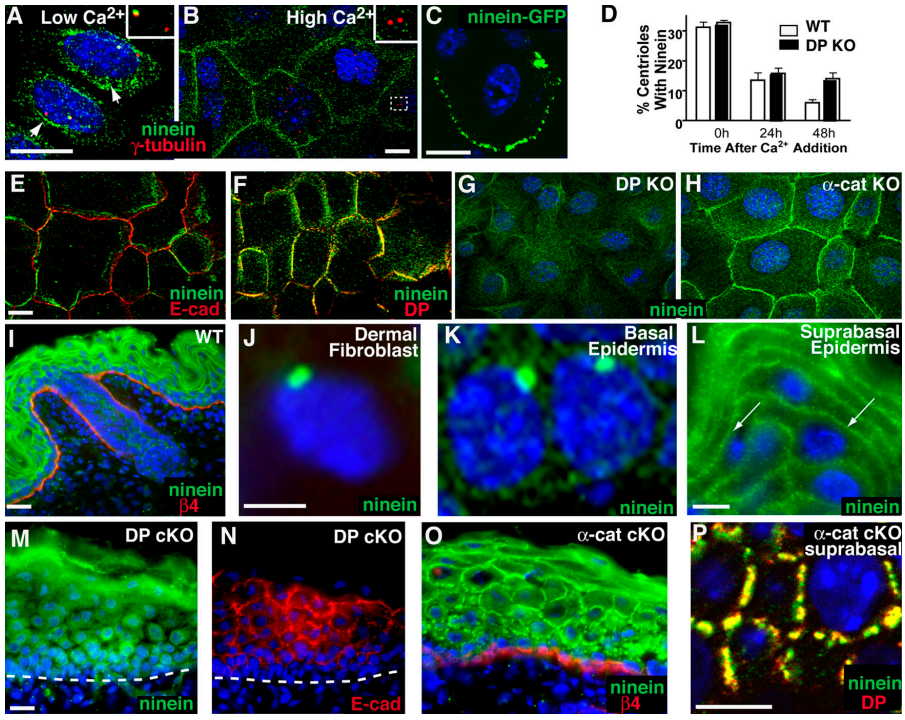

Figure 4. **Ninein localizes to cell–cell borders in a DP-dependent manner.** (A and B) Immunofluorescent localization of antibodies against γ-tubulin (red) and ninein (green) in 1°mk grown in low-calcium media (A) or in high-calcium media for 48 h (B). Arrowheads denote perinuclear labeling. Insets are higher magnification of centrosomes. (C) Localization of ninein-GFP in a transfected keratinocyte grown in high-calcium media. (D) Quantification of centrioles (identified by γ-tubulin staining) that have detectable levels of ninein associated with them. More than 250 centrioles were counted for each experiment. Error bars represent the SD. (E and F) Indirect immunofluorescence analysis of antibodies against ninein (green) and E-cadherin (E-red) or DP (F-red) in 1°mk grown in high-calcium media for 48 h. (G and H) Ninein localization in DP KO (G) and α-catenin KO (H) 1°mk. A–H show maximum-intensity projections through the cells to visualize all of the junctions and centrosomes. (I) Sagittal section of WT e18.5 epidermis labeled with antibodies against ninein (green) and β4 integrin (red), which localizes to the base of basal epidermal cells. (J–L) Higher magnification images of ninein localization to the centrosome in a dermal fibroblast (J), and a basal keratinocyte (K), and to the cell junctions of the suprabasal epidermis (L). Arrows denote cell border labeling. (M and N) Sagittal sections of DP cKO e18.5 skin immunolabeled for ninein (M; green) and E-cadherin (N; red). Dashed lines denote dermal/epidermal boundary. (O and P) Sagittal sections of α-catenin cKO e18.5 skin immunolabeled for ninein (green) and β4 integrin (M) or ninein (green) and DP (N; red). Bars: (A–C, E–I, and K–O) 10 μm; (J and P) 5 μm.

calcium-treated keratinocyte cultures lacking α-catenin still localized ninein at sites of cell–cell contact (Fig. 4 H).

Using deletion mutagenesis and transient transfections, we narrowed down the desmosomal-colocalization domain of ninein to a 368-aa stretch in the central region of ninein (Fig. S2). The insolubility of both desmosomal proteins and ninein, coupled with the ability of these proteins to aggregate, precluded our ability to conduct further biochemical studies on the nature of the interaction with desmosomal components.

In WT skin, ninein localized to the centrosomes in dermal fibroblasts and basal epidermal cells, but to intercellular borders in suprabasal cells (Fig. 4, I–L). The loss of ninein from the centrosomes of suprabasal cells was selective, as γ-tubulin and centrin both remained associated with centrosomes in suprabasal cells and were not detected at cell junctions (Fig. 3, A and B). These data unveiled a striking correlation between the rearrangement of microtubules in differentiating epidermal cells and the selective redistribution of ninein from the centrosomes to cell–cell borders. Moreover, this redistribution was also seen in esophagus (Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200609109/DC1), suggesting that it could be a general phenomenon of epithelial stratification/differentiation.

The distinctive localization of ninein to cell borders was lost in DP-null epidermis, although E-cadherin and cell–cell junctions were still observed (Fig. 4, M and N). In contrast, ninein's colocalization with desmosomal components remained intact in α-catenin–null epidermis (Fig. 4, O and P).

Given that the number of desmosomes increases dramatically as epidermal cells differentiate, one might expect that ninein's localization is determined by a competition between desmosomes and centrosomes. This did not appear to be the case, however, because in DP-null suprabasal cells, ninein did not remain focused at centrosomes (Fig. 4 M). These results suggest that distinct signals govern ninein's centrosomal localization. Based upon these data and previous experiments (Delgehyr et al., 2005), depletion of ninein from the centrosome appears to be physiologically relevant for the subsequent loss of microtubule anchoring at the MTOC. Furthermore, the DP-dependent relocalization of ninein to desmosomal cell–cell contacts may be involved in the reorganization of microtubules to the cell cortex.

DP has been traditionally viewed as an adaptor protein that links to desmosomes through its head domain and links to the IF cytoskeleton through its tail domain (Getsios et al., 2004). To ascertain which of these domains are required for the reorganization of ninein, we transiently transfected DP-null keratinocytes with expression vectors driving either full-length DP or a stable truncated form of DP lacking the C-terminal domain that binds to IFs (Fig. 5 A; Smith and Fuchs, 1998; Vasioukhin et al., 2001b). Both the full-length and tailless forms of DP restored ninein localization to cell junctions, suggesting that tethering of IFs to desmosomes is not required for cortical ninein localization (Fig. 5, B and C). A construct containing only the DP head domain failed to recruit ninein, suggesting that the coiled-coil rod domain is required for this function (Fig. 5 D). These findings suggest a novel function for DP that goes beyond its ability to connect IFs to desmosomes.

In this report, we have described a dramatic rearrangement of microtubules that occurs as epidermal cells differentiate.

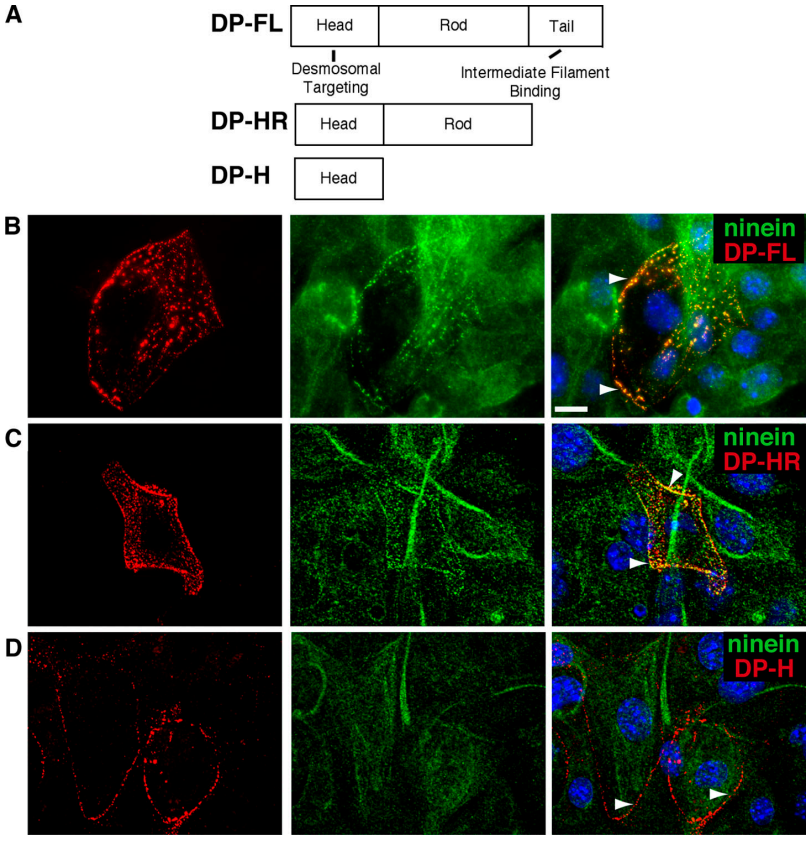

Figure 5. **DP-mediated ninein localization does not require binding IFs.** (A) Domain organization of DP and constructs used in this experiment. DP binds to desmosomes through its head (H) domain and to IFs through its tail domain. (B–D) DP-null 1°mk were transfected with full-length DP (B), DP head + rod (C), or DP head (D). After transfection, cultures were shifted to high-calcium media and fixed in methanol 24 h later. Indirect immunofluorescence reveals colocalization between ninein (green) and DP (red) when the head and rod domains were present. The tail domain was dispensable. B–D are maximum intensity projections. Arrowheads denote cell border localization. Bars, 10 μm.

In suprabasal cells, the microtubule-anchoring protein ninein is released from the centrosome and relocalizes in a DP-dependent manner to developing desmosomes at cell–cell junctions. This leads to a robust cortical network of microtubules in the differentiating layers. That said, ninein need not act alone in eliciting its association with desmosomes and triggering the dramatic reorganization of the microtubule network from the centrosome to the cortex. Several other centrosomal proteins have now been shown to be involved in microtubule-anchoring at centrosomes (Kim et al., 2004; Guo et al., 2006; Yan et al., 2006), and, intriguingly, they include not only minus-end microtubule proteins like ninein but also plus-end microtubule-binding proteins. In this regard, it is noteworthy that an isolated report over a decade ago showed that the plus-end microtubule-binding protein CLIP170 colocalizes with desmosomes (Wacker et al., 1992).

Our work also raises questions about the roles of microtubules in terminally differentiating epidermal cells. Our studies documenting the functional importance of DP in governing microtubule reorganization in differentiating epithelia now pave the way for future studies in dissecting the underlying mechanisms involved, and in evaluating the specific contributions that microtubule remodeling makes in dictating desmosomal functions in the epidermis.

Materials and methods

Generation of mice

A cDNA encoding the ensconsin microtubule-binding domain fused to three copies of GFP was generated previously (Bulinski et al., 2001). This was subcloned into a vector under the control of an epidermal keratin promoter (Vasioukhin et al., 1999). This gene was removed from the vector backbone by digestion with EcoRI and SphI and used for injection via established procedures into fertilized mouse embryos.

Fixation and staining

For visualization of microtubules, tissues were fixed in a solution containing 80 mM PIPES, pH 6.9, 50 mM NaCl, 2 mM $MgCl_2$, 0.4 mM $CaCl_2$, 1% glutaraldehyde, 3% paraformaldehyde, and 0.2% Triton X-100 at 37°C. After fixation, tissues were extensively washed, and then treated with sodium borohydride (0.1%) in PBS for 30 min. Tissues were then washed extensively in PBS before embedding in OCT. For other stainings, tissues were embedded directly in OCT, frozen, sectioned, and fixed in 4% PFA in PBS. Fixation of cultured cells for preservation of microtubules was carried out with the same buffer, except with 0.5% glutaraldehyde and 0.1% Triton X-100. For any stainings involving ninein or γ-tubulin, cells or tissue were fixed in –20°C methanol for 3 min. Antibodies used were rabbit anti-ninein (Rattner Laboratory, University of Calgary, Calgary, Alberta, Canada), mouse anti–γ-tubulin (Sigma-Aldrich), rat anti–α-tubulin (Serotec), mouse anti-DP (ICN Biomedicals), rat anti–β4 integrin (BD Biosciences), rat anti–E-cadherin (Fuchs Laboratory, The Rockefeller University, New York, NY), and rabbit anti-centriolin (Doxsey Laboratory, University of Massachusetts, Worcester, MA). The DP deletion constructs used in this study were previously generated and characterized (Smith and Fuchs, 1998; Vasioukhin et al., 2001b), with the DP-Head including aa 1–1,020 and the head + rod including aa 1–2,000.

Drug treatments

For the microtubule-recovery video, primary cells were cultured in low- or high-calcium media for 72 h, and then treated with 10 μM nocodazole for 1 h at 4°C. Cells were thoroughly washed with four changes of media to remove nocodazole before imaging. For analysis of ninein localization, keratinocyte cultures were treated for 1 h with 2 μg/ml latrunculin-B or 2 μM nocodazole before fixation.

Imaging

Images of fixed cells were taken with an Axioplan with Apotome attachment (both Carl Zeiss MicroImaging, Inc.). A 63×/1.4 NA objective was used, and images were collected with Axiovision software (Carl Zeiss MicroImaging, Inc.). Living cells/tissues were imaged with a spinning disk (Perkin-Elmer), using a 63×/1.4 NA objective at 37°C, and acquired with Perkin-Elmer software. Images were collected on cameras (Orca-ER; Hamamatsu). Videos were rendered using AfterFX software.

Online supplemental material

Fig. S1 shows loss of DP and ninein in small regions of the α-catenin cKO epidermis. Fig. S2 shows that neither F-actin, microtubules, nor the IF-binding domain of DP are required for ninein localization. In Fig. S3, ninein localization to cell junctions in suprabasal esophagus is demonstrated. Video 1 is a z-stack imaging microtubules in e14.5 epidermis. Videos 2 and 3 are z-stacks imaging microtubules in e18.5 epidermis in WT (Video 2) and DP cKO (Video 3) epidermis. Microtubule nucleation at the centrosome is shown in unperturbed differentiated cells in Video 4, and after nocodazole washout in proliferating cells (Video 5) and differentiated cells (Video 6). Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200609109/DC1.

We thank Linda Degenstein for assistance in generating the EMTB-3GFP transgenic mouse, Nicole Stokes and Lisa Polak for their assistance in breeding and handling these mice, which were housed and cared for by the Laboratory animal research center at The Rockefeller University. We also thank Chloe Bulinski, Steven Doxsey, Michel Bornens, and Jerome Rattner for reagents, and Markus Schober for assistance with AfterFX. T. Lechler was a Jane Coffin Childs postdoctoral fellow. E. Fuchs is an investigator of the Howard Hughes Medical Institute.

This work was funded by a grant from the National Institutes of Health (R01-AR27883).

Submitted: 18 September 2006
Accepted: 8 December 2006

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