Corneodesmosin gene ablation induces lethal skin-barrier disruption and hair-follicle degeneration related to desmosome dysfunction

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Summary
Corneodesmosin (CDSN) is specific to desmosomes of epithelia undergoing cornification, mainly the epidermis and the inner root sheath of the hair follicles. CDSN nonsense mutations are associated with hypotrichosis simplex of the scalp, a rare disease that leads to complete baldness in young adults. CDSN displays adhesive properties, mostly attributable to its N-terminal glycine-rich domain, and is sequentially proteolyzed as corneocytes migrate towards the skin surface. K14-promoter driven Cre-mediated deletion of Cdsn in mice resulted in neonatal death as a result of epidermal tearing upon minor mechanical stress. Ultrastructural analyses revealed a desmosomal break at the interface between the living and cornified layers. After grafting onto nude mice, knockout skin showed a chronic defect in the epidermal permeability barrier. The epidermis was first hyperproliferative with a thick cornified layer, then, both the epidermis and the hair follicles degenerated. In adults, Cdsn deletion resulted in similar histological abnormalities and in a lethal barrier defect. We demonstrate that Cdsn is not essential for skin-barrier formation in utero, but is vital throughout life to preserve this barrier by maintaining desmosome integrity. The strong adhesive function that the protein confers on corneodesmosomes also seems necessary for maintaining the architecture of the hair follicle.

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Introduction
Lessons from human diseases and engineered mouse models have established the importance of desmosomes in tissues subjected to high mechanical stress. Indeed, these intercellular junctions provide strong cohesion between adjacent cells. Mutations in genes encoding desmosomal components are invariably responsible for skin diseases, often associated with heart defects (for reviews, see Kottek et al., 2006; Garrod and Chidgey, 2008). In the epidermis, desmosomes undergo profound morphological and biochemical changes throughout keratinocyte differentiation. In particular, during cornification, a specific cell death process that transforms keratinocytes into anucleated, flattened corneocytes, considerable modifications of the desmosomes occur, accounting for their designation as corneodesmosomes (Serre et al., 1991).

Corneodesmosin (CDSN) is a protein specific to desmosomes that will undergo transformation into corneodesmosomes, which, in humans, comprises desmosomes of the epidermis, the three layers of the inner root sheath (IRS) of the hair follicles and the hard palate epithelium. Indeed, the protein is not expressed in noncornified squamous epithelia (Serre et al., 1991). After its secretion by granular keratinocytes via the lamellar granules, CDSN is incorporated into the desmoglea of the desmosomes shortly before their transformation into corneodesmosomes, most probably reinforcing the cell-cell adhesion mediated by the desmosomal cadherins already present in the structure. In the course of stratum corneum maturation, CDSN is progressively degraded, its degradation being a prerequisite for desquamation (Simon et al., 2001).

Cloning of its cDNA has revealed that CDSN is located on chromosome 6, in the major psoriasis susceptibility locus PSORS1 (Guerrin et al., 1998). Some CDSN single nucleotide polymorphisms (SNPs) have been associated with psoriasis in many genetic studies (reviewed by Capon et al., 2002), and a recent study revealed that only two genes from PSORS1, HLAC and CDSN, yield protein alleles that are unique to risk haplotypes (Nair et al., 2006). However, according to the latter study and others, the exact identity of the PSORS1 gene remains controversial (Veal et al., 2002; Capon et al., 2003; Helms et al., 2005; Orrú et al., 2005; Nair et al., 2006). Intriguingly, the only monogenic disease identified so far to be associated with mutations in CDSN revealed a hair phenotype and did not affect the epidermis (Levy-Nissenbaum et al., 2003); hypotrichosis simplex of the scalp (HSS; OMIM 146520) is a rare autosomal-dominant disease characterized by progressive loss of scalp hair resulting in almost complete baldness by the third decade.
To date, three different nonsense mutations have been reported in four families from Israel, Denmark and Mexico (Lai-Cheong et al., 2007). These mutations lead to the production of a truncated form of CDSN that has been detected as deposits beneath the epidermis basement membrane and deeper in the dermis, at the periphery of the hair follicles (Levy-Nissenbaum et al., 2003). Although the pathophysiological mechanism of this disorder is not understood, CDSN obviously has an important role in hair physiology.

A striking feature of CDSN is its very high serine and glycine content (27.5% and 16%, respectively), particularly at the N-terminus of the protein (residues 60–171). It has been suggested that similar serine- and glycine-rich domains described at both termini of keratins form structural motifs, the so-called ‘glycine loops’, that mediate intermolecular adhesion by acting like Velcro (Steinert et al., 1991). Furthermore, it has been demonstrated, in vivo and in vitro, that CDSN displays homophilic adhesive properties (Jonca et al., 2002; Caubet et al., 2004). Moreover, recombinant CDSN forms highly stable homo-oligomers that dissociate only partially in 8 M urea, possibly reflecting cis and/or trans interactions in vivo. The N-terminal glycine loop domain was shown to be necessary and sufficient, and thus responsible, for both the oligomerization of CDSN and its homophilic adhesive properties in vitro (Caubet et al., 2004).

In vivo, CDSN could thus mediate cell-cell adhesion by reinforcing the resistance of the desmosomes. It has also been proposed that CDSN contributes to the structural modifications that characterize the transformation of desmosomes into corneodesmosomes (Guerrin et al., 1998) or prevents premature desquamation by protecting desmosomal proteins from proteolysis (Lundström et al., 1994). Inactivation of mouse Cdsn by mutational insertion was very recently reported to induce neonatal death (Matsumoto et al., 2008), and defective corneodesmosome formation was proposed to be responsible for stratum corneum detachment.

We developed two epidermal-selective Cdsn-deficient mouse models. Somatic Cdsn inactivation in mouse epidermis was achieved using human keratin 14 (K14)-promoter driven Cre-mediated deletion (Li et al., 2001). Temporally controlled Cdsn deletion in epidermal keratinocytes was achieved using a tamoxifen-inducible Cre recombinase under the control of the same promoter (Li et al., 2004). Moreover, recombinant CDSN forms highly stable homo-oligomers that dissociate only partially in 8 M urea, possibly reflecting cis and/or trans interactions in vivo. The N-terminal glycine loop domain was shown to be necessary and sufficient, and thus responsible, for both the oligomerization of CDSN and its homophilic adhesive properties in vitro (Caubet et al., 2004).

Results

Generation of Cdsn-deficient mice

To investigate the role of CDSN in the barrier function and physiology of the hair follicle, we developed Cdsn-deficient mice by using K14-promoter driven Cre-mediated loxP recombination (Fig. 1A). The human KRT14 promoter is active during mouse epidermal fetal development as early as embryonic day (E)9.5, and is strongly activated by E14.5 onwards (Vassar et al., 1989). Similarly to many other markers of epidermis differentiation, Cdsn mRNA was first detected at E16.5 and becomes prominent at E18.5 (H.G., unpublished results). Therefore, the chosen strategy would induce the deletion of the second exon of Cdsn before the gene is expressed in the developing epidermis and in hair follicles.

We first generated mice bearing floxed Cdsn alleles (Cdsnfl/fl), which were bred to homozygosity. These animals appeared normal, indicating that the genetic manipulation did not affect the function of the floxed gene. Generation of Cdsn-deficient mice was obtained by crossing Cdsnfl/fl mice with K14-Cre transgenic mice (Li et al., 2001). The resulting animals bearing the K14-Cre and heterozygous for the floxed Cdsn allele were selected and mated again with Cdsnfl/fl mice. Four different genotypes were thus obtained (Fig. 1B): K14-Crefl/fl.Cdsnwt/wt and K14-Crefl/fl.Cdsnfl/fl, both denoted hereafter as wild-type (WT), K14-Crefl/fl.Cdsnfl/fl, denoted hereafter...
Inactivation of CDSN in mouse skin results in early postnatal death
KO pups suffered from severe skin detachment (Fig. 2A) and died within 1 hour of birth or were immediately eliminated by their mother. To delineate the causes of neonatal lethality, we systematically performed caesarian deliveries at E18.5. Under these conditions, KO embryos were indistinguishable from their littermates when handled carefully (Fig. 2B). However, during grooming by surrogate mothers we observed the rapid appearance of skin detachment that was similar to that observed after natural birth. Skin rupture started in the ventral area, paws and snout and extended to the flanks (Fig. 2C). The peeled skin was red, shiny, and became somewhat sticky to the touch. On the dorsal part of the pups, the skin retained a normal macroscopic aspect until death, which occurred within 2 hours. A standard procedure for analysis of the phenotype was thus established: after caesarian delivery, all neonates were given to a surrogate mother and euthanized after 1 hour.

Inactivation of CDSN in mouse skin leads to a drastic barrier defect
To measure the effects of the mutation on skin permeability, we performed a Toluidine blue penetration assay on E18.5 embryos (Fig. 3A). When the assessment was carried out immediately after caesarian delivery, before any skin phenotype onset, complete dye exclusion was observed in KO pups, as in their control littermates, reflecting an intact barrier at that stage (Fig. 3A, left panels). However, when mice were given to a surrogate mother for 1 hour, large areas showed toluidine blue penetration, particularly in the ventral part of the embryos (Fig. 3A, right panels). Thus, as soon as a skin phenotype became macroscopically visible in KO animals, the dye extensively penetrated the skin. This drastic perturbation of the barrier could be perfectly superimposed with the zones of detachment of the stratum corneum in KO animals (see Fig. 2C). Similarly, transepidermal water loss (TEWL) measured on whole pups was similar at first in WT, HET and KO pups immediately after caesarian delivery but dramatically increased by a factor greater than ten in KO mice after phenotype onset (Fig. 3B). To maintain conditions of minimal mechanical stress, KO neonates were isolated at 37°C for 4 hours, immediately after caesarian section. Under these conditions, only one out of nine KO pups died. The remaining pups did not develop any noteworthy skin detachment, and five of them maintained a TEWL value that was roughly similar to that of the WT and HET neonates, between 0 and 4 g m⁻² per hour. These experiments demonstrate that the skin phenotype is closely linked to mechanical stress from the environment. To assess the mechanical resistance of the skin, the amount of protein detached from the skin by tape stripping was measured. Three times more protein was extracted from KO skin than from WT or HET skin (Fig. 3C). Hence, even macroscopically intact KO skin is extremely fragile, and tears under mechanical stress.

Histological analysis of KO mouse skin reveals a detachment of the stratum corneum from the living layers of epidermis
In zones of skin rupture, such as the ventral region, a complete absence of the stratum corneum was observed (Fig. 4A). In some areas, the skin abrasion was more pronounced and also concerned part of the living layers (data not shown). On the dorsal skin, the

as heterozygous mice (HET), and K14-Cre<sup>αβ</sup>; Cdsn<sup>ßγ</sup>, denoted as knockout (KO) mice. The genotyping of 232 newborns from 12 intercrosses revealed that WT, HET and KO pups were produced in the expected mendelian ratios, indicating no embryonic lethality for the KO mice. Excision of the floxed Cdsn allele in the skin of newborn pups was controlled by PCR analysis (Fig. 1C). As expected, in skin of KO neonates, Cdsn mRNA level measured by quantitative real-time PCR was very low, confirming efficient ablation of Cdsn (Fig. 1D). In HET pup skin, Cdsn expression was roughly 50% of the WT level, demonstrating the absence of dose compensation. Western blot analysis (Fig. 1E) using the F28-27 anti-CDSN monoclonal antibody (mAb) confirmed the absence of CDSN in the skin of KO animals, whereas the full-length 54 kDa protein was detected in skin extracts from WT and HET animals. The smaller amount of protein in HET mice is consistent with results of RT-PCR analysis, which indicate inactivation of one Cdsn allele. A lower molecular mass peptide of ~45 kDa was also detected in both WT and HET pup extracts, which corresponds to a first step of the CDSN proteolytic cleavage in the course of stratum corneum maturation (Simon et al., 1997).
Morphological changes accompanying cornification occurred both in WT and KO animals, the same characteristic symmetrical triangular structure of regular desmosomes was observed (Fig. 5C,F). The ultrastructure of desmosomes and corneodesmosomes was analyzed at higher magnification. In granular keratinocytes from stratum granulosum and stratum corneum (Fig. 5J; and data not shown).

The thickness of stratum corneum from KO mice was also unchanged, with 10 to 13 layers (Fig. 5A,B). Some blisters, already detected by optical microscopy, were present between the stratum granulosum and stratum corneum (Fig. 5J; and data not shown). The ultrastructure of desmosomes and corneodesmosomes was analyzed at higher magnification. In granular keratinocytes from both WT and KO animals, the same characteristic symmetrical triangular structure of regular desmosomes was observed (Fig. 5C,F). Morphological changes accompanying cornification occurred similarly in both WT and KO mice. Typical transitional junctions were found, with classical dense fibrils of keratin filaments attached to the electron-dense intracellular desmosomal plaque in the granular keratinocyte, densification of the intercellular portion, and an electron-dense line at the other side of the junction corresponding to the cornified envelope of the upper transitional keratinocyte (Fig. 5D,G). The number of these transitional desmosomes at the interface between the stratum granulosum and stratum corneum was not significantly different in WT and KO samples. We made counts on 30 fields at magnification ×20,000, corresponding to the length of 3-4 corneocytes, and found 59 transitional desmosomes in the KO skin section versus 61 in the WT. Hence, in newborn KO mice, Cdsn deficiency seems to have no consequences on the transformation of desmosomes into corneodesmosomes. In the cornified layers, the morphology of the corneodesmosomes from KO mouse also appeared classical (Fig. 5E,H). By contrast, numerous split desmosomes could be observed at the transition from stratum granulosum to stratum corneum, either isolated (Fig. 5I) or adjacent in zones of detachment (Fig. 5J). The main structure of the split transitional desmosomes remained attached to the granular keratinocyte, and no cell lysis was observed. Desmosome breaking seemed to occur specifically at the stratum granulosum to stratum corneum transition, because no split corneodesmosomes were observed higher in the stratum corneum. Thus, abnormalities due to the absence of CDSN seem to occur only when cornification takes place, and lie in adhesive defects.

Absence of CDSN does not affect expression of differentiation markers and desmosomal proteins in neonatal epidermis

Histological analysis revealed no thickening of the living layers in the epidermis from KO neonate dorsal skin. To further investigate
the effect of the null mutation on epidermal differentiation, we examined the expression of differentiation markers and desmosomal proteins by western blot, quantitative real-time PCR and immunohistochemistry (Fig. 6). No significant differences in the amount of early (keratin K10, involucrin, desmoglein 1) and late (loricrin, filaggrin) differentiation markers were observed in skin from WT, HET or KO neonates by western blot analysis (Fig. 6A). Expression level analysis by qRT-PCR of Dsc1, Dsg1a and Dsg1b, coding for desmosomal proteins present in the suprabasal layers of epidermis, did not reveal any differences between the dorsal skin of WT and KO neonates (Fig. 6B). Immunohistochemical analyses (Fig. 6C) were consistent with the western blot and qRT-PCR data. Again, no differences in the distribution or the expression level of differentiation markers were observed between WT and KO neonate dorsal skin. As expected, we observed a dramatic reduction in the expression level of all the differentiation markers in the ventral area of KO animals (data not shown), which is consistent with the sudden loss of the most suprabasal keratinocytes once the stratum corneum had been shed (see Fig. 4A). In conclusion, the absence of CDSN does not affect the expression of differentiation markers and desmosomal proteins as long as the integrity of the epidermis is preserved.

Long-term phenotype of mouse skin lacking CDSN after grafting onto nude mice

The drastic barrier defect that occurs in KO mice does not allow the long-term consequences of Cdsn excision to be analyzed in the epidermis and hair follicle. To address this issue, full-thickness dorsal skin from WT and KO neonates was grafted onto the back of nude mice (Fig. 7). Absence of CDSN in the epidermis and IRS of hair follicles from KO grafts was controlled by immunohistochemistry (data not shown). Macroscopically, newborn KO skin developed some sparse hair that progressively disappeared and became totally absent 9 weeks after grafting. In parallel, the graft became hard, devoid of softness, and formed a scab that was continually renewed (Fig. 7A). In contrast to the WT, KO epidermis first showed a prominent hyperplasia with papillomatosis (Fig. 7B). Hyperkeratosis and parakeratosis were obvious and infiltration of inflammatory cells was observed in the dermis, together with dermal cysts, which characteristically result from degenerative hair follicles.
performed. Values measured from 4 to 9 weeks post grafting ranged from 1.5 to 5 g m\(^{-2}\) per hour for the WT grafts versus 11.5 to 22.5 g m\(^{-2}\) per hour for the KO grafts, suggesting a strong permeability defect. Histological and immunohistological data suggested that the KO graft first behaved like a healing epidermis. However, whereas wound healing leads to barrier restoration and a return of keratinocytes to a normal differentiation program, the KO graft was unable to produce an efficient barrier. However, the epidermis of nude mice at the border of the scab showed acanthosis and perfectly mimicked a healing epidermis.

CDSN is also required in adult mice for epidermis integrity and barrier function

To investigate the consequences of CDSN loss in adult mice, we developed an additional model using a K14-promoter driven CreER\(^{T2}\) recombinase, the activity of which is efficiently induced by 4-hydroxy-tamoxifen (OHT), but not natural estrogen receptor ligands (Li et al., 2000). Mice homozygous for the floxed Cdsn allele and transgenic for K14CreER\(^{T2}\)-0 were generated following the same breeding protocol described previously. Resulting animals were wild-type mice (K14CreER\(^{T2}\)-0:Cdsn\(^{wt/+}\)) or K14CreER\(^{T2}\)-0:Cdsn\(^{fl/fl}\), heterozygous pre-mutant mice (K14CreER\(^{T2}\)-0:Cdsn\(^{wt/+}\) and homozygous pre-mutant mice (K14CreER\(^{T2}\)-0:Cdsn\(^{fl/fl}\)) denoted hereafter HOMO. In the absence of treatment, HOMO mice did not develop any skin or hair phenotype for at least 6 months, denoting no leakiness of uninduced CreER\(^{T2}\). Generalized or localized temporally controlled Cdsn excision was achieved in the skin of adult HOMO mice by intraperitoneal injection of tamoxifen (Tam) or topical application of OHT, respectively. In both cases, immunodetection of CDSN revealed a loss of Cdsn expression in epidermal keratinocytes and in the IRS of hair follicles (Fig. 8C; Fig. 9C). HOMO mice injected with Tam or locally treated with OHT developed a skin phenotype with variable kinetics according to the individuals but, once the phenotype had initiated, it progressed within 5 days to a critical situation. Wild-type mice treated in the same conditions never developed any phenotype, denoting the absence of toxicity of Tam or OHT in our experimental conditions. We attributed the variation in the kinetic of the HOMO mice phenotype onset to the genetic background heterogeneity of the mice. For this reason, we used TEWL values to evaluate the onset and progress of the phenotype.

Typically, TEWL values reached 4-5 g m\(^{-2}\) per hour when the first scales became visible, and rose to 33 g m\(^{-2}\) per hour as the scales became more numerous and were shed. After generalized induction of Cdsn deletion, the scales were first visible on the ventral side and snout, and then extended to the back and the whole body, consistent with the level of mechanical stress applied to these different body areas. Within 5 days of the abnormal skin phenotype onset, mice became prostrate, lost weight and were euthanized. A representative aspect of a ventral skin region with a TEWL value of 13 g m\(^{-2}\) per hour is presented in Fig. 8. At the histological level, acanthosis, hyperkeratosis and parakeratosis of the epidermis accompanied scaling (Fig. 8A,B,D, left panel). Topical applications of OHT were made, with the aim of analyzing the phenotype for a longer period of time (Fig. 9). Skin phenotype progression is presented at early (Fig. 9, middle panels) or late (Fig. 9, right panels) stages in comparison with the control (Fig. 9, left panels). The early stage showed a moderate barrier defect (TEWL of 11 g m\(^{-2}\) per hour) associated with the development of acanthosis, hyperkeratosis and parakeratosis, together with a strong expression of the hyperproliferative keratin K6 (Fig. 9, middle panel). At the late
Corneodesmosomal defects in Cdsn–/– mice

stage, corresponding to a TEWL value of 33 g m⁻² per hour, ulcerations appeared and were associated with the shedding of large scales; histological analysis revealed almost complete disappearance of the epidermis, replaced by a scab (Fig. 9, right panel). At the early stages, moderate inflammation was observed, which we further characterized by immunohistochemistry for CD3 and F4/80 (supplementary material Fig. S1). It mainly consisted of CD3⁺ T cells infiltrating the epidermis (supplementary material Fig. S1A) and macrophages and Langerhans cells in the dermis, at proximity of the epidermis (supplementary material Fig. S1B). The increasing barrier defect led to a typical inflammatory response, with ulceration, dilation of capillaries and subcorneal abscesses with neutrophils (see Fig. 9B, right panel).

Localized temporally controlled Cdsn excision in adult mouse skin also induced a notable morphological modification of the hair follicles. At early stages, they appeared to be distended and associated with hypertrophic sebaceous glands (Fig. 9B, middle panel). This was also the case when generalized Cdsn excision was induced (Fig. 8D). At later stages, hair follicles disappeared, as evidenced by the absence of K6 labeling (Fig. 9B,D, right panel). These observations are reminiscent of the neonatal-skin-grafted model, which is characterized by cyst formation and hair-follicle degeneration.

**Discussion**

In this study, we performed conditional ablation of Cdsn in mouse skin using K14-promoter driven Cre-mediated loxP recombination. Our results demonstrated that CDSN is necessary for epidermis integrity and barrier function in adult and neonatal mouse skin. Cdsn is also excised in the IRS of the hair follicles. At birth, hair follicles from KO mice appeared normal. However, they degenerated in KO
Fig. 8. Phenotype of generalized temporally controlled Cdsn KO in adult mouse skin. Shaved adult HOMO mice were injected with vehicle only (control) or with Tam (TAM) for 5 consecutive days, and checked daily for phenotype onset by clinical observation and TEWL measurement. (A) Macroscopic appearance of the mice. The ventral skin of the control mouse has no phenotype, whereas scales are visible on the ventral area of the TAM mouse. (B) Representative skin sections from ventral areas of control and TAM mice were stained with hematoxylin and eosin (H&E), showing acanthosis, hyperkeratosis and parakeratosis of the TAM mouse epidermis. In the dermis of TAM mouse, the hair follicles display an altered morphology; their upper part from the infudibulum to the skin surface is distended (arrows), and they are associated with hypertrophic sebaceous glands (arrowheads). D, dermis; E, epidermis. (C) Immunohistochemical staining with F28-27 mAb (CDSN) of ventral skin sections from control and TAM mice shows an effective loss of CDSN expression in the stratum granulosum of the epidermis and the IRS of the hair follicles in TAM mouse. (D) Higher magnification corresponding to the areas boxed in B shows in detail hyperproliferation, hyperkeratosis and abnormally distended hair follicles (left panel), hypertrophic sebaceous glands (right panel). Scale bars: 100 μm (B,C), 20 μm (D).

grafted skin as well as in the adult KO models, revealing that CDSN is also required for hair-follicle integrity.

Cdsn inactivation is useful to examine the function of CDSN within the corneodesmosome. As previously shown (Matsumoto et al., 2008), the major consequence observed here was an epidermal tear at sites of trauma and friction. Our detailed analysis of the neonatal phenotype showed that, although the greatly reduced mechanical resistance of the stratum corneum is an intrinsic feature caused by Cdsn deficiency, epidermal tear, leading to lethal barrier defect, occurred only under mechanical stress encountered after birth. At the histological level, blisters were found as soon as cornification occurred, that is, at the stratum granulosum to stratum corneum transition. Ultrastructural analysis revealed numerous split junctions with the main structure remaining attached to the granular keratinocytes, suggesting that the cohesive defect lies in the upper side of the junction. Thus, although CDSN is already present in the extracellular core of desmosomes from the stratum granulosum, it seems to have a fundamental role only when cornification is complete. Intriguingly, we did not observe thinning of single or bundled corneocytes throughout the stratum corneum, but detachment of the whole stratum corneum from the subjacent stratum granulosum. The stratum granulosum to stratum corneum interface might actually be the most fragile zone because it links two epidermal layers with different junctional organizations and mechanical characteristics: desmosomes and keratin intermediate filaments that are organized in taut cables in the stratum granulosum and rigid cornified envelopes linked by corneodesmosomes in the stratum corneum.

The underlying molecular mechanism by which CDSN assumes its adhesive function within the corneodesmosome has not been fully elucidated. In vivo, CDSN is known to be covalently linked to the cornified envelope (Serre et al., 1991). On the one hand, the previously demonstrated homophilic adhesive properties of CDSN and the strong resistance of aggregates formed by bacterially recombinant CDSN to highly denaturing conditions (Jonca et al., 2002; Caubet et al., 2004) suggest that, in vivo, CDSN reinforces cohesion by its own adhesive properties. On the other hand, adhesion provided by glycine-loop domains has been suggested to mediate reversible and constantly adjustable intermolecular links similar to Velcro (Steinert et al., 1991). Consistent with this, CDSN could give the junction the elasticity essential to prevent breaking as soon as the cell envelope rigidifies. Unlike the recent Cdsn inactivation study (Matsumoto et al., 2008), our ultrastructural analysis did not show any significant differences in the number of transitional desmosomes between WT and KO neonates. Moreover, the electron density of the corneodesmosomes appeared unchanged (Fig. 5). Thus, CDSN does not seem indispensable for the morphogenesis of corneodesmosomes, but appears essential to their function as adhesive structures.

Our Cdsn KO mice developed a phenotype that was very close to that displayed by Spink5–/– mice deficient for the serine protease inhibitor LekTI. These mice exhibited fragile stratum corneum and perinatal death due to dehydration, with detachment of the stratum corneum from the subjacent epidermis layers and split desmosomes (Yang et al., 2004; Descargues et al., 2005). It was proposed that, in the absence of LekTI, the premature proteolysis of CDSN or Dsg1 contributed significantly to desmosomal fragility, epidermal detachment and skin-barrier defects. It is interesting to note that, in both models, the breaking points mainly lie in the junction between living and cornified layers. This emphasizes the importance of corneodesmosomes as cornification takes place. However, the timing of the barrier defect differs between the two models: E18.5 for Spink5–/– mice but only after birth for our somatic Cdsn–/– mouse model. It is tempting to suggest that in the Spink5–/– model, the biochemical defect (absence of protease inhibitor) induces a dysfunction of the corneodesmosomes in utero, as soon as cornification occurs. By contrast, the absence of CDSN leads to a mechanical deficiency and thus the barrier impairment only takes place in the environment experienced after birth.

Grafting experiments demonstrated that the barrier defect extended postnataally. Skin from Cdsn-deficient grafts first developed acanthosis and hyperkeratosis. Increased expression of various
Acanthosis, hyperkeratosis and parakeratosis are histological features more generally encountered in inflammatory epidermis, such as during wound healing or in psoriatic lesions. Although wound healing is transitory and rapidly leads to orthokeratotic epidermis and barrier repair, psoriatic epidermis maintains acanthosis, hyperkeratosis and parakeratosis with barrier defect. The situation has been described as a chronically persistent epidermal-healing state (Nickoloff et al., 2006). Our model of induction of Cdsn KO in adult mouse showed a persistent barrier defect similar to that observed in psoriasis. However, although we observed acanthosis, hyperkeratosis and parakeratosis at early stages, there was no formation of prominent and elongated rete ridges, only a weak inflammatory cell infiltrate, and the phenotype evolved towards ulceration and disappearance of the epidermis. In psoriasis, the barrier defect is thought to be associated with intrinsic immunological defects. The lack of any distinctive feature at the immunological level in our inducible KO model might contribute to the differences in the intensity of the inflammatory response. Finally, unlike our Cdsn-deficient mouse models, psoriasis is characterized by a significant increase in CDSN expression which is observed in multiple living layers and stratum corneum from lesional psoriatic epidermis (Haftek et al., 1997; Allen et al., 2001). However, the psoriasis-associated SNPs of CDSN could affect the function of the corresponding proteins, thus participating in the development of hyperproliferation as a compensatory mechanism to the barrier impairment. Assessment of the functional consequence of the amino acid substitution in alleles unique to risk haplotypes is needed to further investigate the putative involvement of CDSN in psoriasis pathophysiology.

Spatially controlled somatic Cdsn inactivation in mouse epidermis, using K14-promoter driven Cre-mediated deletion, also allowed inactivation of Cdsn in the developing hair follicle. Mice heterozygous for the excised Cdsn allele were indistinguishable from their wild-type littermates. In particular, they did not develop any hair phenotype up to 8 months. This suggests that the rare autosomal dominant human disease HSS is not caused by Cdsn haploinsufficiency. Moreover, recent work demonstrated that truncated CDSN, which accumulates abnormally as high-order differentiation markers (involucrin, K10), and induced expression of K6, confirmed the altered differentiation and the hyperproliferative state of the grafted epidermis. Hyperproliferation and acanthosis are thought to be compensatory responses to impaired epidermal barrier (Proksch et al., 1991). In the case of our Cdsn-deficient graft, these compensatory mechanisms appeared to be ineffective, and were followed by a complete disappearance of the epidermis. This demonstrates that CDSN is necessary for maintaining the integrity and barrier function of the postnatal epidermis. In our inducible models, the epidermal phenotype was very similar to that of our Cdsn KO skin graft model, although it progressed faster (within days). This might be due to the different location of the zone of interest, the interscapular area in the case of the grafts, and the flank in the inducible model, which is subjected to higher mechanical stress. Finally, in our inducible Cdsn KO models, barrier restoration remained unsuccessful, compromising the vital prognosis of the mice when the whole skin area was affected. Thus, CDSN seems to have a vital role in adult epidermis too.

Acanthosis, hyperkeratosis and parakeratosis are histological defects frequently observed in psoriatic lesions. The presence of these defects is thought to be a consequence of an impaired epidermal barrier function. In our inducible KO model, the epidermal phenotype was very similar to that observed in psoriasis. However, although we observed acanthosis, hyperkeratosis and parakeratosis at early stages, there was no formation of prominent and elongated rete ridges, only a weak inflammatory cell infiltrate, and the phenotype evolved towards ulceration and disappearance of the epidermis. In psoriasis, the barrier defect is thought to be associated with intrinsic immunological defects. The lack of any distinctive feature at the immunological level in our inducible KO model might contribute to the differences in the intensity of the inflammatory response. Finally, unlike our Cdsn-deficient mouse models, psoriasis is characterized by a significant increase in CDSN expression which is observed in multiple living layers and stratum corneum from lesional psoriatic epidermis (Haftek et al., 1997; Allen et al., 2001). However, the psoriasis-associated SNPs of CDSN could affect the function of the corresponding proteins, thus participating in the development of hyperproliferation as a compensatory mechanism to the barrier impairment. Assessment of the functional consequence of the amino acid substitution in alleles unique to risk haplotypes is needed to further investigate the putative involvement of CDSN in psoriasis pathophysiology.
assemblies in the dermis of patients, organizes into proto-fibrillar structures in vitro and is toxic to keratinocytes (Cécile Caubet, Luc Bousset and G.S. et al., unpublished). Common features between CDSN mutant aggregates and amyloid deposits support HSS as a new potential amyloid disease and allow a better understanding of the HSS pathophysiology. Analysis of skin sections from newborn Cdsn KO mice showed a similar morphology and number of hair follicles to those in skin from WT littermates. The KO neonates also displayed normal whiskers. The only difference was that, in KO pup skin, no hair follicles expressed CDSN in the IRS. Therefore, CDSN seems to be dispensable for hair-follicle morphogenesis, as was the case for barrier establishment in the absence of external stress. The progressive degeneration of hair follicles and the formation of cysts observed from 4 weeks after grafting, together with the altered morphology and disappearance of hair follicles in adult mouse skin induced for Cdsn excision, suggest that CDSN is necessary for normal hair-follicle integrity. Mutation of another gene encoding a structural protein of the IRS, DSG4, is responsible for localized autosomal recessive hypotrichosis (LAH; OMIM 607903) (Kljuc et al., 2003). LAH is allelic with the lanceolate hair (lah) mouse, which fails to grow any normal hair and completely lacks vibrissae. Finally, the inactivation of genes encoding other desmosomal cadherins also leads to hair-follicle abnormalities. Mice deficient for Dsg3 or Dsc3, normally detected in the outer root sheath of the hair follicle, suffer from acantholysis between the two cell layers surrounding the telogen hair club, leading to telogen hair loss (Koch et al., 1998; Chen et al., 2008). Absence of Dsc1 in the IRS of hair follicles from Dsc1–/– mice induces localized hair loss associated with formation of utriculi and dermal cysts denoting hair-follicle degeneration (Chidgey et al., 2001). Altogether, these data emphasize the importance of desmosomes and corneodesmosomes in hair-follicle integrity. In our different models, the hyperproliferative and inflammatory environment could aggravate the hair-follicle phenotype. A mouse model with specific inactivation of Cdsn in the IRS would be useful for an in-depth study of the consequences of Cdsn deletion on hair-follicle integrity.

Materials and Methods

Mice, tamoxifen treatment and skin grafting

Animals were bred according to the institutional guidelines and policies. The Cdsn mutant mouse line was established at the MCI/ICS (Mouse Clinical Institute/Institut Clinique de la Souris, Illkirch, France; http://www.mci-strasbourg.fr). The targeting vector was constructed as follows. A 3 kb fragment encompassing most of the coding region (exon 2) and corresponding to the floxed region was amplified by PCR (from 129S2/SvPas genomic DNA) and subcloned in an MCI proprietary vector, resulting in step 1 plasmid. The 5’ (4.7 kb) and the 3’ (3.5 kb) homologous arms were subcloned successively into the step 1 plasmid to generate the final targeting vector. All the coding regions were sequenced. The MCI vector has a flipped neomycin-resistance selection marker that was flanked by Frt sites. For K14-Cre and K14-CreERT2 were described (Indra et al., 1999, Li et al., 2001). All experiments were performed on isolated mice. Tamoxifen (Tam) and 4-hydroxy-tamoxifen (OHT) (Sigma) were prepared as previously described (Indra et al., 1999). For K14-CreERT2 transgene activation, 8- to 10-week-old transgenic mice were either intraperitoneally injected with Tam (0.1 mg in 100 μl sunflower oil), or subjected to topical application of 40 mM Tris-HCl, 10 mM EDTA, 8 M urea, 0.25 mM PMSF, pH 7.4) with 0.1% v/v protease inhibitor cocktail (Sigma) by the FastPrep system (MP Biomedicals). Equal quantities of protein were separated by 12.5% SDS-PAGE and transferred to nitrocellulose membrane. The blots were probed with primary antibodies and HRP-conjugated secondary antibodies (Invitrogen). Detection was performed with ECL reagent (Amer sham Pharmacia Biotech). Skin-barrier function assays

TEWL was measured using an EP1 Evaporimeter (Servo Med, AB Stockholm, Sweden). The dye exclusion assay was performed as previously described (Hardman et al., 1998; Marshall et al., 2000). The mice were then photographed using a Sony DSC-W50 camera. For quantification of stratum corneum removal by tape stripping, an area of about a D-square disk (19.6 mm2) was placed on the flank of neonates and stripped off, and the process was repeated once on the same area. Tape disc fragments were incubated with shaking in 250 μl extraction buffer (40 mM Tris-HCl, 10 mM EDTA, 8 M urea, 50 mM dithiothreitol, pH 7.4) for 1 h at 70°C, then centrifuged for 5 minutes at 12,000 r.p.m. and the protein content was measured in the supernatant using a colorimetric protein assay (Bio-Rad).

Transmission electron microscopy

Specimens were fixed for at least 4 hours in 2% glutaraldehyde in Sorenson’s buffer (pH 7.4), washed with Sorenson’s buffer, cut into pieces of ~1 mm3, postfixed for 1 hour in 2% osmium and embedded in araldite resin. Ultrathin 90 nm sections cut on a Reichert Ultracut ultramicrotome were stained with uranyl acetate and lead citrate and examined with an electron microscope (Hitachi HU12A).

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simplex of the scalp is associated with nonsense mutations in CDSN encoding corneodesmosin. 


