

Current insights into the formation and breakdown of hemidesmosomes

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Hemidesmosomes are multiprotein adhesion complexes that promote epithelial stromal attachment in stratified and complex epithelia. Modulation of their function is of crucial importance in a variety of biological processes, such as differentiation and migration of keratinocytes during wound healing and carcinoma invasion, in which cells become detached from the substrate and acquire a motile phenotype. Although much is known about the signaling potential of the $\alpha 6 \beta 4$ integrin in carcinoma cells, the events that coordinate the disassembly of hemidesmosomes during differentiation and wound healing remain unclear. The binding of $\alpha 6 \beta 4$ to plectin has a central role in hemidesmosome assembly and it is becoming clear that disrupting this interaction is a crucial event in hemidesmosome disassembly. In addition, further insight into the functional interplay between $\alpha 3 \beta 1$ and $\alpha 6 \beta 4$ has contributed to our understanding of hemidesmosome disassembly and cell migration.

Introduction

The keratinocytes of the basal layer of the skin are firmly attached to the underlying basement membrane through protein complexes called hemidesmosomes (Box 1 provides further details of skin structure). Hemidesmosomes were first defined at the ultrastructural level as small electron-dense domains in the plasma membrane, connecting the extracellular basement membrane to the intracellular intermediate filament system [1,2]. The hemidesmosomal protein complex provides stable adhesion of the epidermis to the underlying dermis and ensures resistance to mechanical stress when applied to the skin. In the skin, hemidesmosomes contain the integrin $\alpha 6 \beta 4$, the type XVII collagen BP180, the tetraspanin CD151 and the two plakin family members plectin and BP230 [1–3]. It is the $\alpha 6 \beta 4$ integrin that connects the cells to a major component of the basement membrane, laminin-5 (now also referred to as laminin-332 [4]), whereas the cytoplasmic proteins plectin and BP230 connect to the keratin filaments, thereby creating a stable anchoring complex. Although the binding of BP180 to laminin-332 has been shown *in vitro*, this interaction by itself is not sufficiently strong to mediate adhesion of cells to laminin-332 in the absence

of $\alpha 6 \beta 4$ [5]. Stable attachment of basal keratinocytes to the basement membrane through hemidesmosomes is of fundamental importance for maintaining skin integrity; inherited or acquired diseases in which either of the hemidesmosome components are affected lead to a variety of skin blistering disorders [6–8].

The $\alpha 6 \beta 4$ integrin has been associated not only with stable adhesion, but also with cell migration [3,9,10]. In all studies investigating keratinocyte migration or carcinoma cell invasion, hemidesmosomes are disassembled, their components are redistributed and $\alpha 6 \beta 4$ is no longer exclusively concentrated at the basal cell surface [9,10]. Here, we focus on recent findings on the structural properties of hemidesmosomes and their constituents, and the regulation of their assembly and disassembly.

Hemidesmosome structure and assembly

The absence of $\alpha 6 \beta 4$ in genetically modified mice results in the loss of hemidesmosomes, and hence of stable epidermal adhesion [11–13]. This is also observed in patients carrying mutations in either the $\alpha 6$ or the $\beta 4$ subunit of the integrin, which can result in devastating diseases characterized by skin fragility and blistering [7]. In affected patients, hemidesmosomes are less robust or completely absent. Notably, two mutations identified in the $\beta 4$ gene (*ITGB4*) of patients with a nonlethal form of junctional epidermolysis bullosa disrupt the binding to plectin, indicating that the interaction of $\beta 4$ with plectin is crucial for the formation of stable hemidesmosomes [14–16]. These two proteins interact at two levels; the primary interaction occurs between the plectin actin-binding domain (ABD) and a region on $\beta 4$ comprising the first pair of fibronectin type III (FnIII) domains and a small region of the adjacent connecting segment, and the secondary between the plakin domain of plectin and a more C-terminal part of the connecting segment of $\beta 4$, and the ultimate C-tail (i.e. the region following the fourth FnIII domain) [14,17–20] (Figure 1). Consistent with the idea that the $\alpha 6 \beta 4$ –plectin connection is crucial for the mechanical stability of hemidesmosomes, plectin-deficient mice show extensive epithelial detachment and die 2–3 days after birth [21]. Patients with mutations in the *PLEC* gene also suffer from skin fragility, although the symptoms are less severe than those observed in plectin-deficient mice. Most of these mutations are located within the rod domain (Figure 1), which is not present in

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Box 1. Structure of the skin

The skin, the largest organ of our body, is crucial for protecting the underlying tissues and organs from the external environment. It is composed of three layers [57]: the epidermis (keratinocytes in various stages of differentiation and melanocytes), the dermis (blood vessels, nerve endings, sebaceous glands and hair follicles, surrounded by connective tissue and collagen fibers) and the subcutaneous layer (connective tissue and fat). The basal layer of cells in the epidermis contains the least differentiated keratinocytes, and among these are the stem cells, which ensure the constant renewal of the skin by their unlimited dividing capacity. Stem cells

produce transit-amplifying cells that are destined to withdraw from the cell cycle and terminally differentiate after a few rounds of division [58,59]. During terminal differentiation, the keratinocytes detach from the basement membrane, migrate upward in the epidermis, gradually flatten, start producing more keratins, lose their nucleus and ultimately die and flake off the skin [60]. The basal keratinocytes are attached to the basement membrane, a meshwork of extracellular macromolecules separating the dermis from the epidermis, through specialized adhesion structures called hemidesmosomes (Figure 1).

a smaller splice variant that is expressed at low levels in cells [17,22]. It is conceivable that this variant can partially compensate for the loss of the canonical plectin molecule in humans. Interestingly, neither BP180 nor BP230 are efficiently recruited into hemidesmosomes when $\beta 4$ cannot bind to plectin [15,17,23]. In addition, simple epithelia (e.g. that of the intestine) contain adhesion complexes consisting of only $\alpha 6\beta 4$ and plectin, described as type II hemidesmosomes [24,25]. This suggests that the $\alpha 6\beta 4$ –plectin interaction is one of the first steps in the formation of hemidesmosomes and occurs before the recruitment of BP180 and BP230 [17].

CD151 is also an early constituent of the hemidesmosomal adhesion structures. It is strongly associated with another laminin-binding integrin on keratinocytes, $\alpha 3\beta 1$, with which it forms ‘pre-hemidesmosomal’ clusters at the

basal cell surface of human keratinocytes [26]. These clusters might serve as a ‘nucleation site’ for the assembly of hemidesmosomes by the integrin $\alpha 6\beta 4$. In contrast to CD151, which becomes a component of mature hemidesmosomes (via binding to $\alpha 6$), $\alpha 3\beta 1$ is recruited into focal contacts or redistributed to cell–cell contacts after hemidesmosomes have been assembled and cell–cell contacts established. Although $\alpha 3\beta 1$ does not appear directly to participate in hemidesmosome assembly, it might, together with other $\beta 1$ -containing integrins, contribute to the efficacy of their formation, possibly by affecting the localization of $\alpha 6\beta 4$ at the basal membrane. Indeed, in $\beta 1$ -deficient mice, the number of hemidesmosomes is reduced, which can partially explain the skin defects that are observed in these mice [27,28]. However, if only $\alpha 3\beta 1$ is absent, as in $\alpha 3$ -null mice, the number and

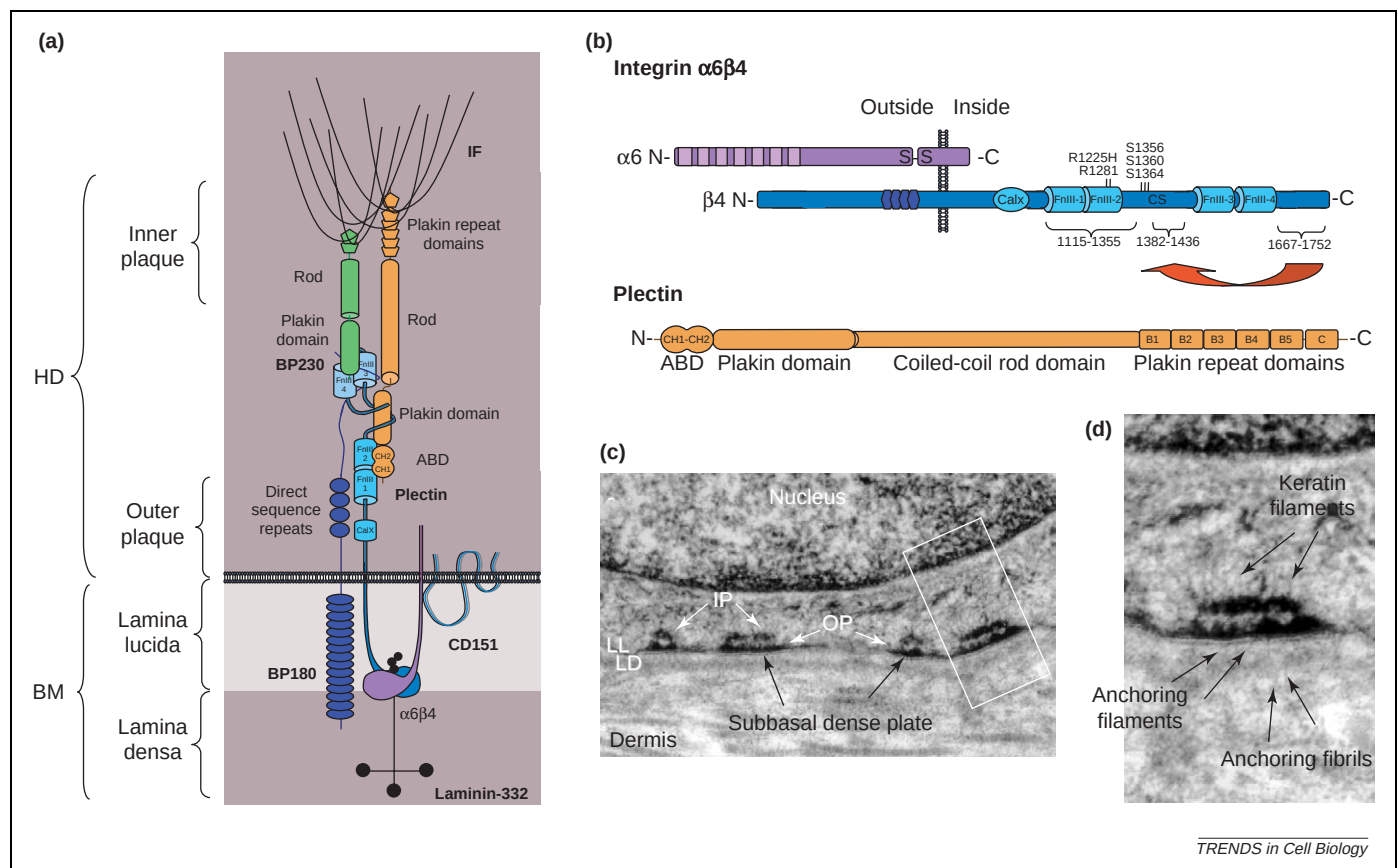


Figure 1. Components and structure of hemidesmosomes. (a) A schematic drawing of the hemidesmosome, showing its six components ($\alpha 6\beta 4$ heterodimer, CD151, BP180, BP230 and plectin) together with its ligand laminin-332 and intracellular intermediate filament partners keratin-5 and -14 (IF). (b) The structures of $\alpha 6\beta 4$ and plectin. The various domains in the proteins are indicated, as well as the residues in the $\beta 4$ cytoplasmic domain that are important for plectin binding and regulation of this interaction. (c) An electron micrograph of several hemidesmosomes. (d) An enlargement of the indicated area in the middle panel. Abbreviations: BM, basement membrane; CH, calponin homology domain; HD, hemidesmosome; IP, inner plaque; LD, lamina densa; LL, lamina lucida; OP, outer plaque.

size of hemidesmosomes appears to be normal [29]. Similarly, no deficiencies in hemidesmosomes were observed in CD151 null mice, and these mice also have no signs of skin fragility [30]. Moreover, in cells expressing $\beta 4$ -chimeras that are unable to associate with $\alpha 6$ and thus CD151, hemidesmosome-like structures, containing plectin, BP180 and BP230, are still formed [31,32]. Thus, in mice and in human cultured keratinocytes, CD151 is not required for the formation of hemidesmosomes, and its precise function with $\alpha 3\beta 1$ in the 'pre-hemidesmosomal' clusters remains unknown.

Interestingly, wound healing was defective in CD151-deficient mice; this was found to be the result of an impaired deposition of laminin-332 into the wound bed and a failure to upregulate $\alpha 6\beta 4$ [33]. Impaired organization of the basement membrane has also been described in $\alpha 3$ -null mice, which hints at an important function of the complexes of $\alpha 3\beta 1$ and CD151 in this process [29]. Intriguingly, although the absence of CD151 in mice has no effect on epidermal integrity, a nonsense mutation in the *CD151* gene in a patient, resulting in a truncated protein, and loss of the integrin binding site, leads to skin blistering, albeit less severe and more localized than in patients with mutations in either $\alpha 6$ or $\beta 4$ [34]. Moreover, it has been shown that the tetraspanin TSP-15, which is similar to mammalian CD151, is required for epidermal integrity in *Caenorhabditis elegans* [35]. It is possible that the truncated CD151 molecule acts in a dominant-negative fashion, with its presence being more deleterious for tissue integrity than is the total lack of CD151. Alternatively, the absence of a functional CD151 molecule in mice, but not in humans, might be compensated by another tetraspanin [30]. Together, these findings indicate that the binding of $\beta 4$ to plectin represents the first step in the formation of hemidesmosomes, and that complexes of $\alpha 3\beta 1$ and CD151 might facilitate this step by providing a platform at the basal surface of keratinocytes through which $\alpha 6\beta 4$ becomes localized and can interact with plectin.

Role of laminin-332 in the formation of hemidesmosomes

In several reports, data have been presented suggesting that the processing of laminin-332 is important for the formation of hemidesmosomes. In quiescent epidermis, the $\alpha 3$ chain of laminin-332 is cleaved, securing the formation of stable $\alpha 6\beta 4$ -containing hemidesmosomes; however, when keratinocytes need to migrate [e.g. following skin wounding (Box 2)], unprocessed laminin-332 is secreted at the leading edge, which stimulates migration through the $\alpha 3\beta 1$ integrin [36,37]. It is generally assumed that when the $\alpha 3$ chain of laminin-322 has not been processed, keratinocytes switch from $\alpha 6\beta 4$ -mediated adhesion to one mediated by $\alpha 3\beta 1$, because the latter binds more efficiently to unprocessed laminin-332. Conversely, $\alpha 6\beta 4$ might bind less efficiently to this unprocessed laminin-322 than to the cleaved form, which might contribute to a higher turnover of hemidesmosomes. In addition, there is evidence that matrix metalloproteinase 19-dependent processing of the laminin- $\gamma 2$ chain can effectuate the above switch, leading to increased migration of keratinocytes [38]. Therefore, whether laminin-332 is processed or not determines which of the two laminin-binding integrins mentioned above becomes engaged, and consequently the turnover of hemidesmosomes. Interestingly, in a recent report it was shown that unprocessed laminin-332 ($\alpha 3$ chain) does indeed prevent the formation of mature hemidesmosomes but not the nucleation of hemidesmosomal plaques [39]. Furthermore, it showed that keratinocytes migrate equally well on both processed and unprocessed laminin-332, whereas mature hemidesmosomes can only be formed after the $\alpha 3$ chain of laminin-332 is processed. Nevertheless, mainly unprocessed laminin-332 is present underneath the leading edge of keratinocytes within a skin wound. It is possible that in the case of excessive production of laminin-332 following wounding, the efficiency of processing will be reduced and the formation of mature hemidesmosomes inhibited, favoring a migratory phenotype.

Box 2. The role of epidermal integrins in wound healing

Injury to the skin causes the transition of quiescent adherent keratinocytes on laminin-332 into migrating cells on the newly formed provisional matrix containing fibrin, fibronectin and vitronectin. During this process, various growth factors and cytokines are secreted to induce healing of the wound [61]. Transforming growth factor β is one of these and contributes to re-epithelialization of skin wounds by modulating integrin expression, thereby stimulating keratinocyte migration towards the provisional wound matrix. As described in this review, stable adhesion of keratinocytes by $\alpha 6\beta 4$ to the basement membrane is lost and the integrin is redistributed from hemidesmosomes to lamellipodia, which might facilitate their stabilization. In addition, the expression of the integrins $\alpha 2\beta 1$ and $\alpha 3\beta 1$, mostly restricted to the lateral membranes of basal keratinocytes in normal epidermis, becomes extended throughout the entire epidermis and their concentration at the basal membrane of basal keratinocytes increases [61–63]. After binding of keratinocytes by $\alpha 3\beta 1$ to newly deposited laminin-332 at the wound edge, the T lymphoma invasion and metastasis 1 (TIAM1)–Rac1 signaling pathway is activated to promote lamellipodia formation and

subsequent spreading [64,65]. The initial spreading induced by $\alpha 3\beta 1$ is followed by the activation of RhoA, which in *in vitro* experiments was found to be required for the adhesion of leading keratinocytes to collagen by $\alpha 2\beta 1$ [66]. However, more recent studies have questioned the importance of $\alpha 2\beta 1$ in wound healing because wound closure is normal in $\alpha 2$ -deficient mice [67,68]. The fibronectin- and vitronectin-binding integrins $\alpha 5\beta 1$, $\alpha v\beta 5$ and $\alpha v\beta 6$ probably have a more important role in wound healing because these integrins are receptors for the various components of the provisional matrix and their expression is induced or strongly increased. Of these integrins, only $\alpha v\beta 5$ is expressed at low levels on proliferating keratinocytes, whereas $\alpha 5\beta 1$ and $\alpha v\beta 6$ are normally absent [69,70]. (Increased) expression of these three integrins results in a more migratory phenotype of the keratinocytes. Interestingly, upregulation of $\alpha v\beta 6$ causes a fibronectin-dependent upregulation of matrix metalloproteinase 9, which might partly be responsible for the increased migration [71]. Although not well documented, a role of the fibronectin- and tenascin-binding integrin $\alpha 9\beta 1$ in epidermal wound repair has been proposed [72,73].

Although the above studies suggest an important function of laminin-332 in the formation and stabilization of hemidesmosomes, studies from several laboratories have indicated that the formation of hemidesmosomes can be driven entirely from within the cell through interaction of the cytoplasmic domain of $\beta 4$ with plectin [31,40–42]. Keratinocytes expressing a $\beta 4$ subunit that is unable to interact with its ligand form structures that contain all of the components of type I hemidesmosomes. Although these data came as a surprise at the time, they do not imply that laminin-332 does not have a role in nucleating hemidesmosomes. Indeed, hemidesmosomes can also be initiated through an interaction of $\alpha 6\beta 4$ with clustered laminin-332 that has been deposited by the cells themselves. Furthermore, ligation of the integrin clearly affects the organization and density of the formed clusters [32,36,38]. When $\alpha 6\beta 4$ is not bound to laminin-332, plectin clusters the integrin into tight complexes. However, upon ligation to laminin-332 in the matrix, the dynamics of $\alpha 6\beta 4$ are reduced and, therefore, the degree of intracellular clustering by plectin is restricted [32]. Thus, the formation of hemidesmosomes is driven both from within the cells through an interaction of the cytoplasmic domain of $\beta 4$ with plectin and from outside the cell through binding of $\alpha 6\beta 4$ to laminin-332.

Basolateral targeting of hemidesmosomal components in polarized epithelia

Evidence is emerging that hemidesmosomes are also affected by mechanisms that control polarized vesicular transport in cells. In polarized epithelia, where tight junctions prevent the free diffusion of plasma membrane proteins between the apical and basolateral membranes, membrane trafficking pathways are used to transport proteins to their respective domains. These trafficking mechanisms are not only important for maintaining, but also for establishing epithelial cell polarity. One of the proteins that has been implicated in directing protein trafficking to the basolateral membrane is the cytoskeletal protein lethal giant larvae 2 (Lgl2). Indeed, this protein is crucial for correct hemidesmosome formation in zebrafish [43]. Lgl2 functions in polarized exocytosis by regulating the function of target membrane soluble N-ethylmaleimide attachment protein receptors (t-SNAREs) at the basolateral plasma membrane [44,45]. At the subapical region, its activity is inhibited as result of phosphorylation by atypical protein kinase C (PKC), which is a member of the Par protein complex [46,47].

Another protein that might have a role in the basolateral targeting of hemidesmosomal proteins is Erbin, which binds to both $\beta 4$ and BP230 [48]. Erbin was originally identified as an ErbB2-associated protein involved in the targeting of this receptor to the basolateral membrane. It is a member of the LAP [leucine-rich repeats (LRR) and postsynaptic density protein-95, Discs-large and zonula occludens-1 (PDZ)] family of proteins, implicated in establishing epithelial cell polarity [49,50]. In addition to the hemidesmosomal proteins $\beta 4$ and BP230, Erbin interacts with components of adherens junctions and desmosomes, both of which are cell–cell adhesion structures at the lateral membrane. These observations

suggest that Erbin might have a general role in the establishment and maintenance of cell–cell and cell–basement membrane adhesions.

Hemidesmosome regulation

Although nothing is known about the dynamics of hemidesmosomes in the epidermis, in cultured keratinocytes these structures appear continuously to renew themselves [32,51]. This dynamic state is thought to be a prerequisite to enable hemidesmosomes to be quickly disassembled when required – for example, for cell migration or differentiation, when cells detach from the underlying basement membrane. Although the role of $\beta 4$ in the migration of keratinocytes, and particularly of carcinoma cells, has been studied extensively, only a few models for hemidesmosome disassembly have been proposed [52,53]. The regulatory requirements for each assembly and disassembly step are not yet clear. It is known that the interaction between plectin and $\beta 4$ is regulated by serine phosphorylation of residues 1356, 1360 and 1364 in the connecting segment of $\beta 4$. Phosphorylation of these residues by PKC α , and possibly other enzymes, results in partial hemidesmosome disassembly owing to loss of interaction with plectin [53]. Most likely, activation of a phosphatase is required to induce binding of plectin and reassembly of hemidesmosomes. This putative phosphatase might also be activated upon ligation of $\alpha 6\beta 4$ to processed laminin-332.

The cytoplasmic domain of $\beta 4$ can adopt a folded conformation by binding of the C-tail to the connecting segment [54] (Box 3). Interestingly, these same sequences in $\beta 4$ are involved in binding to the plectin plakin domain [17,20,54]. Therefore, the intramolecular folding of the $\beta 4$ cytoplasmic domain might also regulate the interaction with plectin. Posttranslational modifications, such as phosphorylation, might determine the conformation of the $\beta 4$ cytoplasmic domain and thereby affect its affinity for other hemidesmosome components. Based on the limited data available, several mechanisms for phosphorylation-induced hemidesmosome disassembly are conceivable (Figure 2).

First, phosphorylation of the connecting segment might disrupt the interaction between $\beta 4$ and the plakin domain of plectin and/or the intramolecular interaction, thereby destabilizing the interaction with plectin (Figure 2a). Second, phosphorylation of $\beta 4$ might increase its affinity for a third protein, which subsequently competes with plectin for binding to the connecting segment and/or interferes with binding of plectin with the first pair of FnIII domains in the integrin C-tail (Figure 2b,c). Indeed, macrophage-stimulating protein-induced serine phosphorylation of the $\beta 4$ connecting segment by PKC- α , leads to hemidesmosome disassembly and relocation of $\alpha 6\beta 4$ to lamellipodia at the leading edge of migrating keratinocytes via binding of 14-3-3 proteins to the phosphorylated connecting segment [52]. Interestingly, macrophage-stimulating protein is one of the growth factors secreted in the wound fluid and might, by inducing the disassembly of hemidesmosomes, contribute to wound repair [55,56] (Box 2). Third, phosphorylation of the connecting segment might induce a significant

Box 3. Structure of hemidesmosomal components

Knowledge of the structure of the protein components of hemidesmosomes has contributed to our understanding of the structure, assembly and disassembly of these adhesion complexes. The cytoplasmic domain of $\beta 4$ is ~1000 residues long and contains four FnIII domains arranged in two tandem pairs separated by a connecting segment (Figure I). The knowledge of the structure of $\beta 4$ is limited to the crystal structure of the first pair of FnIII domains [74]. This region and the first 35 residues of the adjacent connecting segment (1321–1355) constitute the primary plectin binding site in $\beta 4$, a region essential for targeting plectin to hemidesmosomes [14,17,18]. Residues important for the interaction with plectin map onto a continuous area of $\beta 4$ extending over the first and second FnIII domains and define the binding interface (Figure I). Plectin binds to the first pair of FnIII domains of $\beta 4$ via its N-terminal ABD. The ABD is formed by two CH domains arranged in a closed conformation, as shown in the crystal structure [75,76]. Residues crucial for binding to $\beta 4$ are clustered on the surface of the N-terminal CH domain, and overlap partially with one of the actin-binding sequences essential for binding to F-actin, providing a structural basis for the competitive nature of plectin binding to $\beta 4$ and F-actin [14,77,78]. The structure of the complex of the first pair of FnIII domains of $\beta 4$ and the ABD of plectin is not known.

However, a validated model of this binary complex has been proposed in which the N-terminal CH domain contacts $\beta 4$ at the inter-FnIII domain area [78].

The *PLEC* gene contains many alternative first exons, resulting in 11 plectin splice variants differing in their N-terminal sequence [79]. Although it has been shown that both plectin-1A and plectin-1C can interact with $\beta 4$ *in vitro*, only plectin-1A is expressed in basal keratinocytes and is localized in hemidesmosomes [14,77,79,80]. The N-terminal sequence in plectin-1A might be important for regulating the affinity of the ABD for $\beta 4$ and F-actin [77].

The cytoplasmic domain of $\beta 4$ might adopt a closed conformation owing to the interaction between its connecting segment and its C-terminal tail, which might affect the affinity for plectin [54]. For the folded state of $\beta 4$, a strong bending of the second pair of FnIII domains is probably required and might be favored by the 20-residue linker connecting the third and fourth FnIII domains. The $\beta 4$ regions involved in the intramolecular interaction contain binding sites for the plakin domain of plectin located adjacent to the ABD [17,54]. Similarly to $\beta 4$, plectin contains a binding site for BP180, and together these proteins recruit BP180 into hemidesmosomes [23]. Ultimately, BP230 becomes recruited into hemidesmosomes by binding to both $\beta 4$ and BP180, and thus type I hemidesmosomes are formed.

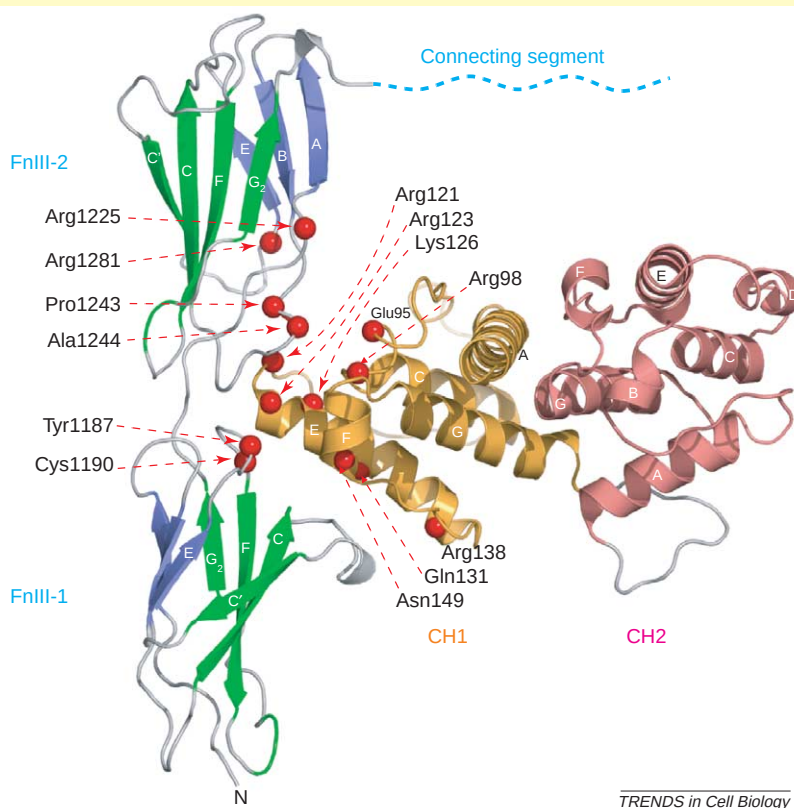


Figure I. Structure of the binding interfaces of integrin $\beta 4$ and plectin. Schematic representation of a model of the complex between the first pair of FnIII domains of $\beta 4$ and the plectin ABD obtained by computational docking methods [78]. The C α atoms of residues whose mutation affects binding are shown as red spheres. The $\beta 4$ residues that are important for binding to plectin are located in the loop EF of the first FnIII domain and in loops BC, EC' and the region preceding strand A of the second FnIII domain. The initial residues of the connecting segment are necessary for plectin binding but are not present in the crystal structure, yet they are likely to be orientated towards the plectin ABD in the model (dashed line). Residues in the plectin ABD relevant for binding to $\beta 4$ are located in a continuous area of the first calponin homology (CH) domain (orange) and include the loop preceding the α -helix C and the α -helices E and F. The second CH domain (pink) does not contain particular residues crucial for binding but its presence is essential for the interaction. Abbreviation: N-term, amino terminus.

conformational change of the $\beta 4$ cytoplasmic domain that would distort the binding sites for the plectin ABD and/or plakin domain (Figure 2d). In the first two models, binding of the plectin plakin domain is lost, whereas binding of the plectin ABD to $\beta 4$ is still possible. In the latter two models,

the binding of the plectin ABD is prevented and the affinity of the plakin domain of plectin for $\beta 4$ is not strong enough to induce binding. In these models, the interaction between plectin and $\beta 4$ has a key role. Obviously, other hemidesmosomal interactions might be subject to

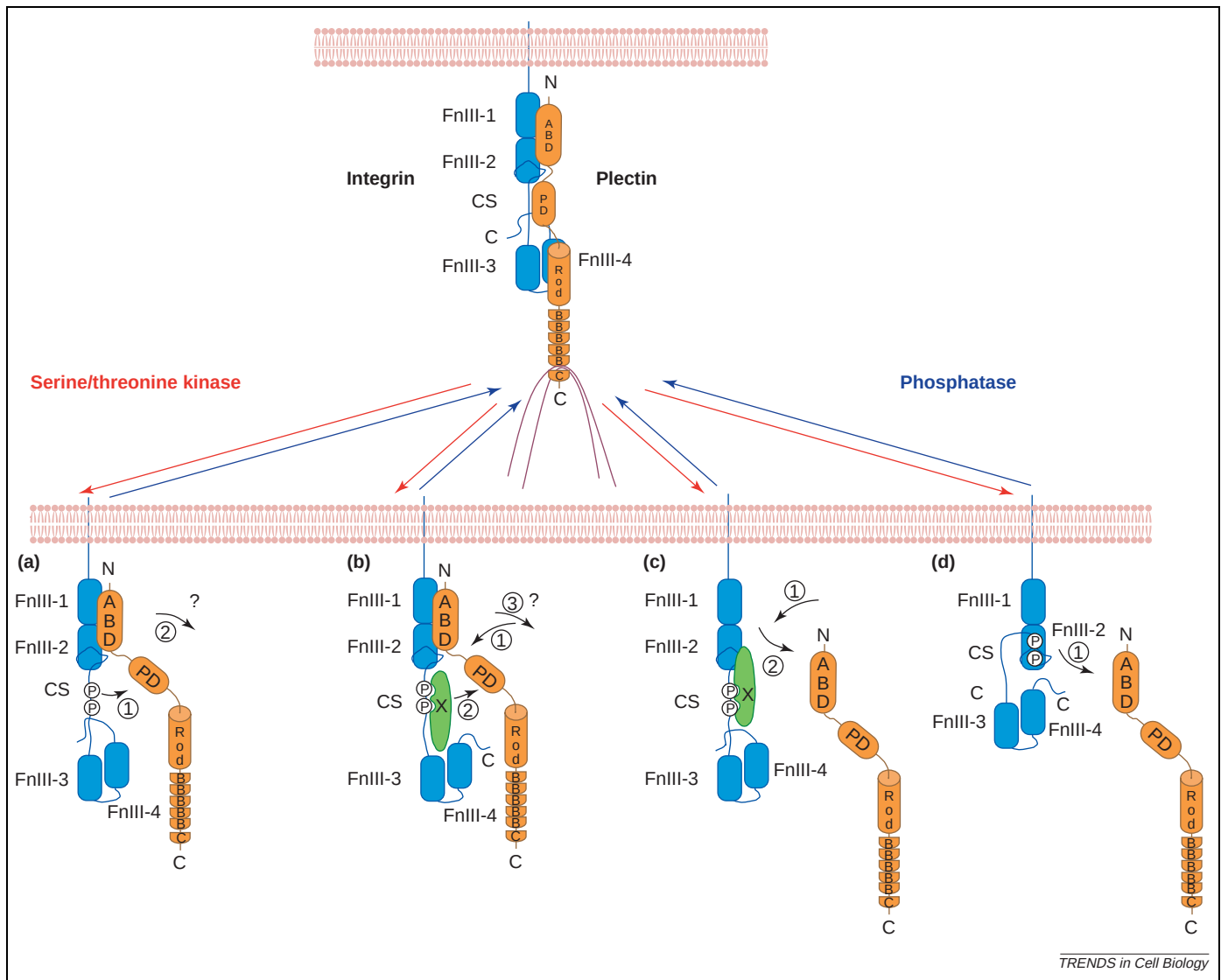


Figure 2. Four putative models for phosphorylation-induced hemidesmosome disassembly. Upon phosphorylation of the $\beta 4$ connecting segment, the interaction with plectin is lost. (a,b) Binding of plectin might disappear by interfering with binding of the plakin domain, thereby destabilizing the interaction between plectin and $\beta 4$. Binding of the plakin domain can be (a) prevented directly by phosphorylation, or (b) prevented indirectly by the binding of a third protein to the phosphorylated connecting segment. (c,d) Alternatively, binding of the plectin ABD might be lost owing to phosphorylation of $\beta 4$. Phosphorylation might (c) indirectly lead to loss of plectin ABD binding through competition with a third protein, or (d) directly lead to loss of binding by inducing a large conformational change. Only the cytoplasmic domain of $\beta 4$ is shown for simplicity. Abbreviations: CS, connecting segment; PD, plakin domain.

regulation, and their posttranslational modifications might contribute to the balance between hemidesmosome formation and disassembly.

Summary

Although research on carcinoma cell lines has informed us about possible pathways for hemidesmosome disassembly, it should be kept in mind that cancer cells have developed mechanisms enabling them to escape pathways that normally regulate proliferation and migration. Therefore, in physiological conditions in the skin, the pathways leading to the disassembly of hemidesmosomes might be entirely different. It is clear that serine phosphorylation of the $\beta 4$ cytoplasmic domain is involved in the disassembly of hemidesmosomes in keratinocytes to initiate migration. However, it is not yet known which signals induce clustering of $\alpha 6 \beta 4$, or subsequent incorporation of other hemidesmosome components, and whether

(de-)phosphorylation of the $\beta 4$ cytoplasmic domain is required for plectin binding. Furthermore, it is not clear whether serine phosphorylation of $\beta 4$ is sufficient to induce hemidesmosome disassembly. The answers to these questions will help to understand the switch in function of the $\alpha 6 \beta 4$ integrin between the formation of stable adhesion structures and induction of migration.

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