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Chapter 1

Regulation of hemidesmosome disassembly by growth factor receptors

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Regulation of hemidesmosome disassembly by growth factor receptors

Evelyne Frijns, Coert Margadant, Kevin Wilhelmsen, and Arnoud Sonnenberg

Hemidesmosomes (HDs) promote the stable adhesion of basal epithelial cells to the underlying basement membrane (BM). Critical for the mechanical stability of the HD is the interaction between integrin α6β4 and plectin, which is destabilized when HD disassembly is required, for instance, to allow keratinocyte migration during wound healing. Growth factors such as epidermal growth factor (EGF) can trigger HD disassembly and induce phosphorylation of the β4 intracellular domain. Whereas tyrosine phosphorylation appears to mediate cooperation with growth factor signaling pathways and invasion in carcinoma cells, serine phosphorylation seems the predominant mechanism for regulating HD destabilization. Here, we discuss recent advances that shed light on the residues involved, the identity of the kinases that phosphorylate them, and the interactions that become disrupted by these phosphorylations.

Introduction

Hemidesmosomes (HDs) are specialized multiprotein complexes that provide stable adhesion of basal epithelial cells to the underlying basement membrane (BM) in pseudo-stratified as well as certain complex and simple epithelia [1•]. Two types of HDs can be distinguished on the basis of their components (Figure 1). Type II HDs are found in simple epithelia such as that of the intestine, and consist of integrin α6β4 and plectin (HD1). Type I (classical) HDs are found in (pseudo-) stratified epithelium, for example, in the skin and consist of α6β4, plectin, tetraspanin CD151 and the bullous pemphigoid (BP) antigens 180 (also called BPAG2 or type XVII collagen) and 230 (BPAG1) [1•; 2•]. Integrin α6β4 and BP180 bind with high and low affinity, respectively, to laminin-332 (Ln-332; previously called laminin-5) in the BM, and intracellular HD stabilization occurs via their association with keratin intermediate filaments through the two plakins plectin and BP230, thus creating a stable anchoring complex [1•;2•;3;4]. The importance of HDs in maintaining epithelial integrity is illustrated by two lines of evidence. Firstly, ablation of the genes encoding α6, β4, or plectin in mice results in severe blistering of the skin, causing neonatal death because of an epithelial barrier defect; however, knockout mice lacking BP180 or BP230 display only a mild form of skin blistering [1•;2•]. Secondly, human patients carrying mutations in any of the HD components suffer from a skin blistering disorder known as epidermolysis bullosa. The severity of the disease depends on the type and
location of the mutations, and their consequences at the mRNA and protein levels [5;6]. Despite the role of HDs in mediating stable adhesion, they are highly dynamic structures that can quickly disassemble under conditions in which

Figure 1. Schematic drawing of the structure and components of type I and type II HDs. Type II HDs are present in simple epithelia such as that of the intestine and consist of only the integrin α6β4 and the plakin plectin (HD1). Type I HDs are found in (pseudo-) stratified epithelium such as that of the skin and additionally contain the tetraspanin CD151, the type XVII collagen BP180, and the plakin BP230. BP230 and plectin mediate intracellular stabilization of the HD by binding to keratin intermediate filaments. K, keratin; Ln, laminin.
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(partial) detachment from the BM is required, for example, during cell division, differentiation, or migration [7;8]. Upon disassembly, HD components are no longer concentrated at the basal surface but are diffusely distributed over the plasma membrane or in the cytoplasm, or are translocated to lamellipodia [9-11]. Although the precise mechanisms that lead to HD disassembly remain obscure, it is at least partially triggered by, and dependent on, the phosphorylation of HD components elicited by growth factor stimulation. The phosphorylation of the β4 intracellular domain has been documented in response to hepatocyte growth factor (HGF), macrophage-stimulating protein (MSP), and primarily epidermal growth factor (EGF). However, there are significant controversies in the literature concerning the residues, which are phosphorylated, their role in the regulation of HD destabilization, and the intracellular responses that are triggered by these phosphorylations apart from HD disassembly.

In this review, we will first focus on protein–protein interactions governing HD assembly and then discuss recent insights into how growth factor-induced phosphorylation events have an impact on these interactions, thus to regulate HD disassembly and d6β4-dependent functions in normal keratinocytes and carcinoma cells.

Protein–protein interactions involved in HD assembly

The cytoplasmic tail of β4 is over 1000 amino acids long and consists of a membrane-proximal Na⁺–Ca²⁺ (CalX) exchanger motif and two pairs of fibronectin type III (FNIII) repeats, which are separated by a connecting segment (CS; Figure 2). The cytoskeletal linker protein plectin can associate with either β4 or actin filaments, and these binding events are mutually exclusive [12-15]. The interaction of the actin-binding domain (ABD) of plectin with the first pair of FNIII repeats and the N-terminal 35 amino acids of the CS of β4 (residues 1115–1355) is thought to be the initial step in HD assembly [12;16;17]. This is strengthened by additional interactions of the plectin plakin domain with the CS and the C-tail (Figures 2 and 3). Subsequently, BP180 interacts extracellularly with Ln-332 and intracellularly with plectin and the third FNIII repeat of β4. Lastly, BP230 is recruited through associations with BP180 and a region on β4 comprising the C-terminal 21 amino acids of the CS and the second pair of FNIII repeats [17-20]. In addition to the multiple associations exerted by the cytoplasmic domain of the β4 subunit, the extracellular domain of the α6 subunit interacts with BP180 and CD151 [21]. The crucial event in HD assembly is the interaction between β4 and plectin, as indicated both by the existence of type II HDs which can apparently form in the absence of BP180 and BP230, and the
hypoplastic nature of HDs that are observed in patients with mutations in β4 (R1281W or R1225H) that prevent this interaction [22;23]. Furthermore, in vitro evidence indicates that by preventing the plectin–β4 interaction, the formation of HDs is severely compromised [12;17]. It is therefore probable that HD disassembly in response to growth factor stimulation is primarily achieved by disrupting the plectin–β4 association.

**Growth factor-induced tyrosine phosphorylation of β4**

Several tyrosines located in the β4 cytoplasmic tail have been implicated in processes typically regulated by growth factor receptors (Figure 2). However, this is an area of many conflicting results. For instance, the association of α6β4 with ErbB2 was reported in transformed keratinocytes, carcinoma cells, and ErbB2-transformed fibroblasts, resulting in ErbB2 autophosphorylation, activation of phosphatidylinositol 3-kinase (PI3-K), tumorigenesis and enhanced invasiveness [24-28]. The activation of PI3-K and increased invasion of cells were induced by the ligation of α6β4, and the subsequent phosphorylation of primarily tyrosines 1257 and 1494 [29,30]. Nevertheless, while one study determined Y1494 as the crucial residue, another study reported that the region spanning residues 854–1183 was essential [28;30].

Figure 2. **Structural organization of the integrin α6β4 and plectin.**

Indicated are the various domains and the positions of important tyrosine and serine phosphorylation sites as reported in the literature. The regions involved in the plectin-β4 interaction are indicated by yellow ovals. The arrow indicates intramolecular folding of the β4 cytoplasmic domain. ABD, actin-binding domain; CS, connecting segment; C-tail, carboxy-terminal tail.
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Shp2 to β4, which results in the stimulation of Src and the subsequent phosphorylation of Gab1 on residues that promote Grb2 binding, or via the binding of Shc, which when phosphorylated also recruits Grb2 to the membrane [37;38]. Cooperation between α6β4 and c-Met was independent of the extracellular domain, giving rise to the idea that the β4 cytoplasmic domain functions as a signaling platform for growth factor signaling pathways [34-37]. However, a c-Met–α6β4 association was not detected by other researchers in the same cells. In addition, β4-enhanced invasion was not specific to c-Met, and c-Met could mediate invasion independently of β4 [39]. Furthermore, the role of the β4 intracellular domain as a signaling adaptor is questioned by a recent study showing that β4–Shp2 association was only slightly increased by HGF, and HGF-induced invasion of tumor cells, as well as ERK and PI3-K signaling, were not enhanced by the dimerization of the β4 intracellular domain [40]. It therefore remains unclear how β4 and c-Met exactly cooperate in carcinoma cells.

The role of EGF-induced tyrosine phosphorylation events is also controversial; whereas initial studies suggested that phosphorylation of Y1422 and Y1440 in the CS of β4 mediates HD assembly, a later study by the same group confusingly reported the opposite, namely that these phosphorylations antagonize HD formation [38;41;42]. A subsequent report then again implicated these residues in HD assembly based on the observation that phenylalanine substitutions impaired HD formation in an in vitro organotypic culture model [43]. However, it remains ambiguous whether it is the inability to phosphorylate these residues or whether the mutations themselves caused this effect. Given the available data, the contribution of tyrosine phosphorylation to HD disassembly under physiological conditions (i.e. in normal untransformed keratinocytes) is disputable. This is underscored by the observation that a β4 mutant that was not tyrosine-phosphorylated in response to EGF was not impaired in mediating EGF-stimulated migration and thus HD disassembly in keratinocytes [44]. In addition, in normal keratinocytes as well as in the same transformed cell lines used in the aforementioned studies, tyrosine phosphorylation was absent or only marginally detected by several groups, both in unstimulated conditions and under conditions when HDs are disassembled such as during EGF stimulation. Instead, serine phosphorylation of β4 was evident under steady-state conditions, and increased in the presence of EGF [45;46•;47••;48••].

Thus, the functional relevance of tyrosine phosphorylation of the β4 cytoplasmic domain might be restricted to processes such as carcinoma invasion. Association of α6β4 with a growth factor receptor and tyrosine
phosphorylation of β4 in carcinoma cells may represent aberrant phenomena that are induced by the overexpression of growth factor receptors or the constitutive signaling by hyperactive receptor tyrosine kinases, as commonly observed in transformed cells. Moreover, the HDs in carcinoma cells are often rudimentary and structurally inferior because of decreased expression levels of BP180 and BP230, and α6β4 localization is often no longer confined to the basal surface but is in fact diffusely distributed over the membrane, which may increase its susceptibility to active kinases [2•,49].

Growth factor-induced serine phosphorylations of HD components
Early reports documented a redistribution of HD components from the basal surface to the cytosol upon phorbol ester-induced activation of members of the protein kinase C (PKC) family of serine/threonine kinases, suggesting that PKCs regulate HD disruption [50•]. This was confirmed in later studies demonstrating the breakdown of HDs in carcinoma cells and normal keratinocytes after the activation of PKC-family members or the overexpression of PKC isoforms. In particular PKCα and PKCδ have been

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Table 1. Conservation of the region containing serines 1356, 1360, and 1364 in multiple species.
Residues 1352-1368 of the human β4 sequence were aligned with the same region in the indicated mammalian, bird and fish species using CLUSTAL W.
implicated in this process, with the particular isoform involved seemingly being cell type-dependent [45;46;47;48••].

The β4 cytoplasmic domain is phosphorylated on serines under steady-state conditions, which increases after phorbol myristate acetate-stimulated PKC activation or, physiologically more relevant, EGF stimulation. Serine phosphorylations occur primarily in the CS and the C-tail, and phosphopeptide mapping experiments identified S1356, S1360, and S1364 in the CS as the most prominent sites (Figure 2; [47••;48••]). They are embedded in an amino acid context that is highly conserved, in mammals as well as in evolutionarily more distant species such as fish (Table 1), which suggests a crucial role for this region. Indeed, studies using mutants carrying either a phospho-mimicking aspartic acid or a nonphosphorylatable alanine substitutions revealed that the phosphorylation of two or more of these serines prevents binding of the plectin ABD to β4. In accordance with this, HD formation under steady-state conditions was significantly impaired when in β4 all three serines were substituted by aspartic acid, whereas when substituted by alanine, robust HDs were formed, which were resistant to EGF-induced disruption [48••]. Although PKC is undoubtedly involved, it may not account for the phosphorylation of all three residues. Whereas in one study it was reported that at least two of them were PKC targets, we found that S1360 is the only PKC site on β4, at least in keratinocytes [47••;48••]. In search for additional kinases that may be involved, S1364 was identified as a site for protein kinase A [48••]. However, there is no evidence for protein kinase A activation downstream of the EGF receptor in keratinocytes, whereas the EGF-induced activation of PKC is well established. The exact identity of all kinases triggering β4 serine phosphorylations in response to EGF remains to be determined.

Interestingly, S1356, S1360, and S1364 are not directly involved in the binding of the plectin ABD, and are located in a region that can be deleted without compromising HD formation [16;50•]. There is evidence suggesting that the C-tail of β4 can bind intramolecularly to a 321 amino acid segment including the first pair of FNIII repeats and part of the CS [13;18]. These regions of β4 also bind to a segment of the plectin plakin domain [17], thereby enforcing the interaction between the two proteins. Possibly, this complex is disrupted upon serine phosphorylation of β4, allowing two of the three phosphorylated serines to interact with arginines 1225 and 1281 in the second FNIII repeat (Figure 3). Since the arginines are essential for plectin binding [22;23], the segment of the CS containing the phosphorylated serines thus competes for binding with plectin. Alternatively, β4
Figure 3. **Hypothetical models for HD disassembly induced by serine phosphorylation.**

When not phosphorylated, the β4 intracellular domain interacts with the ABD and the plakin domain of plectin. Upon serine phosphorylation of the β4 CS, binding of the plectin ABD is prevented either by (A) a conformational change leading to intramolecular folding of the β4 cytoplasmic domain, or (B) binding of an alternative protein to the phosphorylated CS of β4. Open circles indicate unphosphorylated serines 1356, 1360, and 1364, and closed circles the phosphorylated ones.

Phosphorylation may increase its affinity for a third protein, that when bound to β4 prevents plectin-binding by steric hindrance (Figure 3; reviewed in [1•]).

Although the stability of HDs mainly depends on the plectin–β4 association, additional associations must be broken for full HD dissolution, including the interactions of β4 with both BP180 and BP230. In this respect, it is noteworthy that BP180 is also phosphorylated by PKC, leading to its translocation from HDs [51]. It is conceivable that other HD components are subject to a similar mode of regulation. In fact, PKC-mediated phosphorylation of α6
has also been reported [45]. Moreover, whereas the emphasis has been on the effects of EGF, it should be noted that EGF alone does not induce complete HD disruption. It is probable that in an *in vivo* situation, for example, during wound healing, additional growth factors known to modulate keratinocyte migration and proliferation induce the activity of other kinases that contribute to HD disassembly. These factors may include MSP and transforming growth factor-α and -β. For the latter factors, there is no evidence to date of their involvement in HD disassembly, but an interesting report has highlighted the role of MSP, a ligand for the receptor tyrosine kinase Ron, in the breakdown of HDs. MSP-Ron signaling regulates multiple processes in keratinocytes including proliferation, survival, and migration. Keratinocyte stimulation with MSP results in the serine phosphorylation of α6β4, causing 14-3-3 protein-dependent mobilization to lamellipodia where it associates with Ron, and the partial breakdown of HDs [52••]. Furthermore, although the role of S1356, S1360, and S1364 is emphasized, they are not the only serines phosphorylated. The phosphorylation of additional serines on β4 may play a role to achieve full HD destabilization. The complete dissolution of HDs is likely to be the result of the concerted efforts of multiple kinases activated by distinct extracellular stimuli.

**Conclusions**

We have discussed recent findings concerning the mechanisms involved in the disassembly of HDs by growth factor receptors, both in normal keratinocytes and carcinoma cells. The mechanisms involved may differ in different cell types: tyrosine phosphorylation seems to mediate activation of growth factor signaling pathways involved in the migration and invasiveness of carcinoma cells, while serine phosphorylation appears to be more relevant under physiological conditions in normal keratinocytes to destabilize HDs. It is possible that serine phosphorylation is also the primary mechanism to disrupt HDs in carcinoma cells, which then releases β4 to be phosphorylated on tyrosines. Serine phosphorylations primarily target the plectin–β4 association and may result in an intramolecular binding of the β4 cytoplasmic domain, which prevents the interaction with plectin. Alternatively, a third protein might bind to β4 when it is phosphorylated, thus preventing plectin binding through competition. Though an important role is established for EGF-induced PKC activation, it does not lead to complete HD disassembly. Additional kinases and extracellular stimuli governing complete HD dissolution remain to be identified.

**Acknowledgements**

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References and recommended reading

Papers of particular interest, published within the period of the review, have been highlighted as:

• of special interest
•• of outstanding interest


Focused review highlighting the molecular interactions involved in HD assembly and disassembly, the regulation of these interactions, and putative models for HD breakdown.


Extensive overview of the many different functions attributed to integrin α6β4, not only in HD formation but also in migration, cell cycle progression, signal transduction, tumorigenesis, survival and invasion of cells.


35. Mainiero F, Pepe A, Wary KK, Spinardi L, Mohammadi M, Schlessinger J, Giancotti FG: Signal transduction by the αβ4 integrin: distinct β4 subunit sites mediate recruitment of Shc/Grb2 and


This study demonstrates that activation of PKCδ in primary mouse keratinocytes mediates redistribution of α6β4 from HDs to the cytosol and reduced attachment to laminin, indicating that serine phosphorylations mediate HD disassembly.

47. Rabinovitz I, Tsomo L, Mercurio AM: *Protein kinase C-α phosphorylation of specific serines in the connecting segment of the beta4 integrin regulates the dynamics of type II hemidesmosomes*. *Mol.Cell Biol*. 2004, 24:4351-4360. The first of two papers using phosphopeptide mapping to identify serines 1356, 1360, and 1364 in the β4 CS as critical residues for HD disassembly. The phosphorylation of these sites was induced by EGF and at least two of the three sites were phosphorylated by PKCq in HaCaT cells. These phosphorylations were correlated with HD destabilization using mutants carrying aspartic acid or alanine substitutions overexpressed in COS-7.


This is an elegant work demonstrating serines 1356, 1360, 1364 as important phosphorylation sites involved in EGF-induced HD disassembly in human keratinocytes. S1360 and S1364 were identified as sites for PKC and PKA, respectively. Using mutants carrying aspartic acid or alanine substitutions, it was shown that the phosphorylation of two or more of the serines prevents binding of the plectin ABD to β4. Whereas triple aspartic acid mutations prevented HD assembly, substitution to the same residues to alanines provided partial protection against EGF-induced HD disassembly.


50. Nikolopoulos SN, Blaikie P, Yoshioka T, Guo W, Puri C, Tacchetti C, Giancotti FG: *Targeted deletion of the integrin β4...

In this study, mice were generated carrying a targeted deletion of the cytoplasmic domain of β4 downstream of residue 1355. Despite epidermal hypoplasia and reduced wound healing, HD formation and stable adhesion to the BM in these mice were not impaired, demonstrating that the part upstream of residue 1355 that interacts with plectin is sufficient for HD assembly, whereas the downstream segment containing the serines and tyrosine described in the literature is not essential.


Very interesting report demonstrating that stimulation of human keratinocytes with the Ron ligand MSP induces serine phosphorylation of both β4 and Ron at specific 14-3-3 binding sites, resulting in the 14-3-3 dependent formation of a Ron-α6β4 complex that result in the translocation of α6β4 from HDs to lamellipodia. This report thus emphasizes the importance of serine phosphorylations on integrin α6β4 in response to a physiological stimulus that induces HD disassembly and wound healing. In addition, it provides potential mechanism for serine phosphorylation-mediated HD disruption.