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Commentary & View

Advances and perspectives of the architecture of hemidesmosomes

Lessons from structural biology

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Hemidesmosomes (HD) are adhesive protein complexes that mediate stable attachment of basal epithelial cells to the underlying basement membrane. The organization of HDs relies on a complex network of protein-protein interactions, in which integrin α6β4 and plectin play an essential role. Here we summarize the current knowledge of the structure of hemidesmosomal proteins, which includes the structures of the first and second fibronectin type III (FnIII) domains and the calx-β domain of the integrin β4 subunit, the actin binding domain of plectin, and two non-overlapping pairs of spectrin repeats of plectin and BPAG1e. Binding of plectin to the β4 subunit is critical for the formation and the stability of HDs. The recent 3D structure of the primary complex between the integrin β4 subunit and plectin has provided a first insight into the macromolecular recognition mechanisms responsible for HD assembly. Two missense mutations in β4 linked to non lethal forms of epidermolysis bullosa map on the plectin-binding surface. Finally, the formation of the β4-plectin complex induces conformational changes in β4 and plectin, suggesting that their interaction may be subject to allosteric regulation.

HDs are located at the basal side of epithelial cells where they link the extracellular matrix to the intermediate filament network in the cell. Thus, HDs provide stable adhesion of epithelia to the basement membrane and contribute to the resistance to mechanical stress of epithelial tissues. The skin and other complex epithelia assemble type I HDs, which consist of the integrin α6β4, the type XVII collagen BP180, the integrin-associated tetraspanin CD151, plectin and BPAG1e (also known as BP230) (Fig. 1). α6β4, BP180 and CD151 are transmembrane proteins while plectin and BPAG1e are located in the cytoplasm. Intestinal epithelia contain more rudimentary anchoring complexes, termed type II HDs, which contain only α6β4 and plectin.

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α6β4, like other members of the integrin family of receptors, is a non-covalent heterodimer composed of two type I transmembrane subunits.[1] The extracellular moiety of α6β4 binds to laminins and has a preference for laminin-332. The intracellular region of α6β4 consists of the short tail of the α6A isoform and the β4 cyto-domain, which is much larger (~1,000 residues) than that of all other integrin β subunits and shares no similarities with them. The cytoplasmic moiety of β4 contains five globular domains: four FnIII domains and one calx-β domain. The FnIII domains are arranged in two pairs (FnIII-1,2 and FnIII-3,4) separated by a region named the connecting segment (CS); a C-terminal tail extends downstream of FnIII-4. The cytoplasmic domain of β4 mediates most of the intracellular interactions of α6β4, including all the interactions with other hemidesmosomal components described to date. On the other hand, the cytoplasmic tail of the α6A subunit, one of the two splice variants (A and B) of the α6 subunit that is predominantly expressed in the epidermis, has a membrane proximal GFFKR sequence recognized by calreticulin, Rab21, Mss4, BIN1 and other proteins,[2-4] and contains a binding site for the PDZ domain of TIP-2/GIPC at its C-terminus.[5,6]

Plectin and BPAG1e are high molecular weight proteins that belong to the plakin family of cytoskeletal linkers.[7] They have a similar overall tripartite structure consisting of a central rod domain, which mediates self-association, flanked by N- and C-terminal domains that harbor binding sites for other proteins. The N-terminal segment contains a region conserved among plakins named the plakin domain (~1,000 residues), which consists of an array of spectrin repeats (SR) and an SH3 domain inserted in the central spectrin repeat.[8,9] Upstream of the plakin domain, plectin contains an F-actin binding domain (ABD) similar to those present in proteins of the spectrin family. BPAG1e lacks the ABD and the N-terminal SR (SR1). The C-terminal region of plectin and BPAG1e contains six and two copies, respectively, of the plakin repeat domain (PRD).

During the last decade the crystallographic 3D structures of several fragments of hemidesmosomal proteins have been elucidated. These include the structure of the FnIII-1,2 domains[10] and calx-β domain[11] of the β4 cytoplasmic moiety. The FnIII and

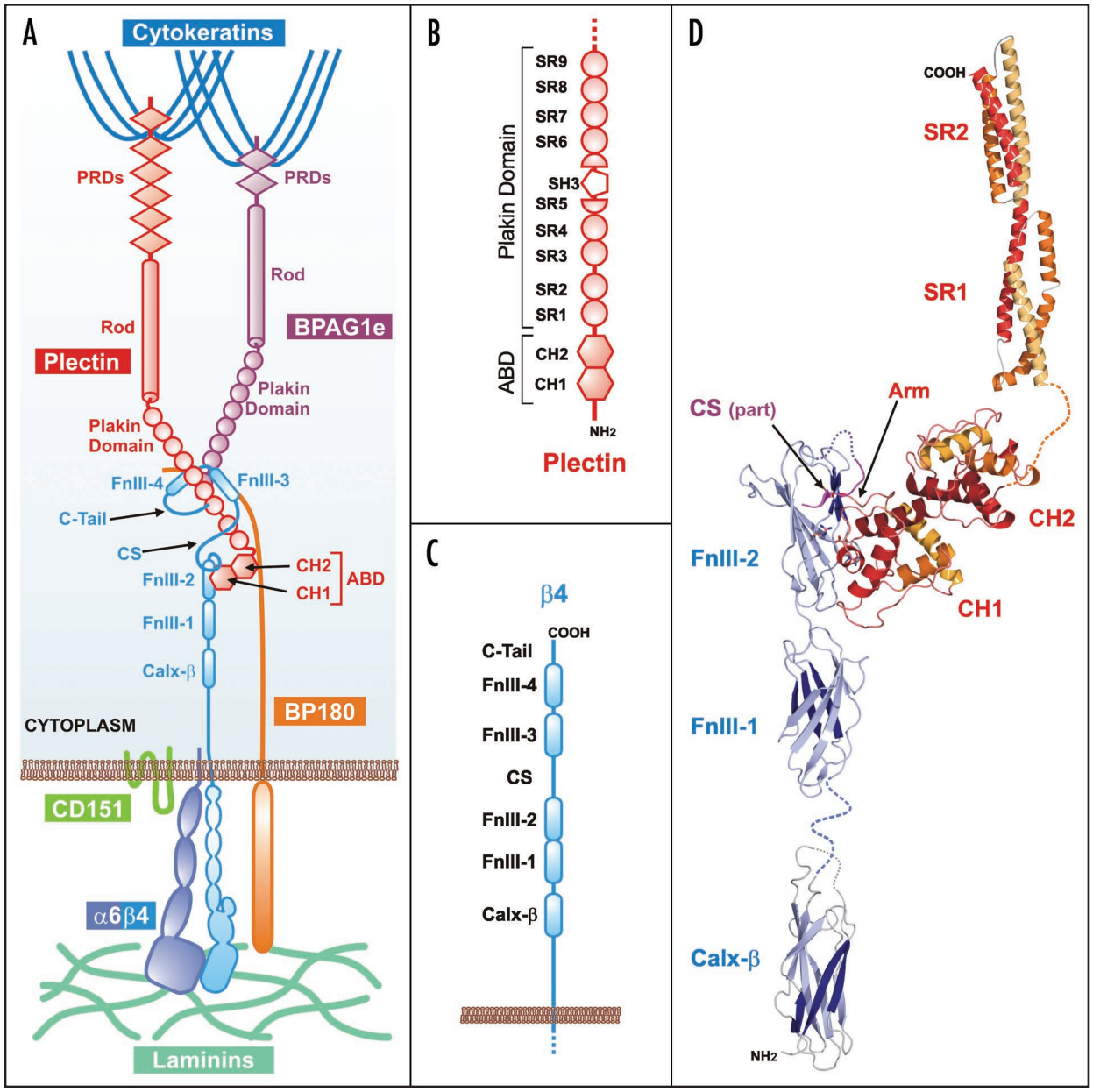

Figure 1. Structural organization of HDs. (A) Schematic representation of the components of the type I HDs and the interactions that link the adhesion receptors to the IF cytoskeleton. Homo-associations have been omitted for clarity. (B) Domain organization of the N-terminal region of plectin and (C) the cytoplasmic region of the integrin β4 subunit. (D) Composite structure of the primary β4-plectin complex (PDB code 3F7P) and adjacent regions, which include the structure of the Calx-β domain of β4 (PDB code 3FQ4) and the first pair of SRs of the plakin domain of plectin (PDB code 2ODU). The relative orientations of the structures of the Calx-β domain of β4 and the SR1-SR2 fragment of plectin with respect to the β4-plectin complex were arbitrarily chosen for representation purposes. The Calx-β is connected to the FnIII-1 domain by a ~20 residues-long linker, while the sequence that connects the CH2 to the SR1 of plectin is ~10 residues-long. In both cases, these linkers (shown as dashed lines) are likely to act as flexible joints.

calx-β domains belong to the immunoglobulin superfamily fold; they are about 100 residues long and their structures consist of a β-sandwich formed by two β-sheets. The calx-β of β4 has homology with the Ca^{2+}-binding domains of the Na^{+}/Ca^{2+} exchangers; but the β4 domain does not bind Ca^{2+}.[11] The structures of the ABD of human and murine plectin have been determined.[12,13] The ABD is formed by two calponin homology domains (CH1 and CH2), each of which is built around a conserved core of four α-helices. The CH1 and CH2 are arranged in a closed conformation establishing extensive contacts between them. The structures of two tandem pairs of SRs of BPAG1e[8] and plectin[9] have revealed the modular organization of the plakin domain and its resemblance to the structure of spectrins. We have identified, by sequence analysis, nine SRs (SR1 to SR9) in the plakin domain of plectin and eight SRs in BPAG1e (SR2-SR9). The crystal structures correspond to the SR1-SR2 of plectin and the SR3-SR4 of BPAG1e. Each SR is a three-helix bundle with up-down-up topology which places the N- and C-termini of each SR at the opposite ends of the

longitudinal axis of the repeat. In the structures of tandem pairs of SRs, such as those of the SR1-SR2 of plectin and the SR3-SR4 of BPAG1, the last α-helix of the N-terminal repeat and the first α-helix of the C-terminal SR are fused to form a single helix that spans both repeats. Thus, tandem pairs and arrays of SRs adopt rod-like structures. The other SRs of the plakin domain of plectin and BPAG1e are predicted to be linked by an inter-repeat helix as observed in the SR1-SR2 and SR3-SR4 junctions; the exceptions are the repeats SR2 and SR3, which are connected by a non-helical linker. Thus, the region SR3-SR9 of the plakin domain forms an array of concatenated SRs that is likely to adopt an elongated structure 30 to 40 nm in length. The plakin domain also contains an SH3 domain, which is inserted between the second and third α-helices of the SR5. Thus, the SH3 domain does not disrupt the tandem array of SRs. Overall, the structure of the plakin domain is highly reminiscent of the structure of proteins of the spectrin family. The elastic properties of the rod-like structure of the plakin domain may contribute to the mechanical stability of HDs. Finally, the structures of two PRDs of desmoplakin, which bind to intermediate filaments and are homologous to the repeats found in the C-terminal region of plectin and BPAG1e, have been described. The PRD is a globular structure build up of 4.5 copies of a 38-amino acid repeat, and the multiple PRDs found in plectin, BPAG1e, and other plakins are likely to be arranged as "beads-on-a-string".[14]

The association of α6β4 with the keratin intermediate filaments at the HDs is not direct, but it is mediated by plectin and BPAG1e. The direct interaction between α6β4 and plectin is required for the stability of HDs and it is likely to also be an initial step in the assembly of HDs. Therefore, it is possible that HD disassembly may be triggered by the inhibition of the α6β4-plectin association. Binding involves multiple sites in the cytoplasmic domain of β4 and plectin. The primary contact occurs between the ABD of plectin and the FnIII-1,2 domains and the N-terminal region of the CS of β4.[15] Without this region of β4, plectin is not targeted to HDs.[16] Two missense mutations in the β4 gene (*ITGB4*) linked to non-lethal forms of epidermolysis bullosa introduce single amino acid substitutions, R1225H and R1281W, in the FnIII-2 domain which compromise the β4-plectin interaction.[17] Additional interactions occur between the plakin domain of plectin and the CS, the FnIII-4, and the C-terminal tail of β4.[18,19] HDs are further stabilized by a network of interactions that include binding of the N-terminal region of BPAG1e to the C-terminal region of the CS and the FnIII-3,4 of β4, and binding of the cytoplasmic domain of BP180 to β4, plectin, and BPAG1e.[20-22]

The ABD of plectin also binds to actin filaments.[12,15,23-25] The binding of the ABD to β4 competes with that to F-actin, which may explain why plectin mediates linkage of HDs to the cytokeratin system and not to the actin filaments.[15] Binding to F-actin induces changes in the arrangement of the CH1 and CH2 domains of the ABD, while plectin binds to β4 in a closed conformation in which the CH1 and CH2 domains are arranged as observed in the structure of the free ABD.[12] Thus, in the selective interaction with F-actin or β4 an allosteric component is included. The ABD of plectin also binds to the first spectrin repeat of nesprin-3α, which is an outer nuclear membrane protein.[26] Binding to nesprin-3α apparently also competes with the interaction between the ABD and F-actin. Thus, when plectin is bound to nesprin-3α, it will link the nucleus to the intermediate filament system.

In spite of the advances in the elucidation of the structures of individual hemidesmosomal proteins and their protein-protein interaction network, little was known about the structural basis of the association between hemidesmosomal components. In order to better understand the molecular recognition mechanisms that govern the assembly and stability of hemidesmosomes we have recently solved the structure of the primary β4-plectin complex.[27]

What has been learned from this crystal structure? First, it has revealed a detailed map of the intramolecular contacts between β4 and plectin. The core interaction occurs between the FnIII-2 of β4 and the CH1 of plectin, while the FnIII-1 and the CS of β4 provide additional contacts with plectin. A short sequence upstream of the CH1 of plectin, termed the N-terminal arm, establishes additional contacts with the FnIII-2 of β4. In contrast, the CH2 of plectin does not contribute directly to the β4-binding surface. Two basic residues located on the surface of the FnIII-2 of β4, R1225 and R1281, which are mutated in patients suffering from epidermolysis bullosa, engage in salt bridges with D151 and E95 in the CH1 domain of plectin (numbering corresponds to the human plectin 1C isoform), respectively. Mutagenesis analysis revealed that these contacts are critical for binding and are hot spots of the binding interface.

Second, the comparison of the free and bound structures has revealed conformational changes in β4 and plectin upon formation of the complex. In the unbound structures of β4, the N-terminal region of the CS forms two β-strands that pack against strands E and C' of the FnIII-2 extending the two β-sheets that form the FnIII fold; while a proline-rich sequence of the CS is highly exposed to the solvent. In the β4-plectin complex, the CS swings over to the opposite side of the FnIII-2 domain and is packed adjacent to the β-strand A where it contacts the CH1 domain of plectin. This conformational change of the CS is necessary for the efficient binding to plectin. Locking β4 in the conformation observed in the free structures reduces the affinity of β4 for plectin to a degree similar to that observed when the CS is not present, suggesting that the β4-plectin interaction may be allosterically regulated by controlling the conformation of β4. For example, binding to plectin could be reduced if the CS were stabilized in the conformation observed in the free structures of β4. Putative mechanisms of β4 allosteric regulation might involve post-translational modification of β4 and/or binding of other proteins to β4. Stimulation of growth factor receptors results in phosphorylation of the β4 subunit mainly at S1356, S1360 and S1364, and in the translocation of α6β4 from HDs, suggesting that phosphorylation of β4 may destabilize its interaction with plectin.[28,29] The β4 fragment (residues 1126–1370) used to obtain the crystal structures of the β4-plectin complex and the free structures of β4, contains S1356, S1360 and S1364. Nonetheless, these residues were disordered in the crystals and were not included in the refined structures. The region 1356–1370 is dispensable for the binding to the ABD. Nevertheless, its proximity to the primary binding site for plectin

suggests that phosphorylation at S1356, S1360 or S1364 might reduce the affinity of the primary β4-plectin interaction.

The conformation of the region upstream of the CH1 domain of plectin is different in the structure of free and β4-bound plectin. This region is coded by several alternative first exons, which produce multiple plectin isoforms by an alternative splicing mechanism. The available structures of the ABD of human plectin contain part of the sequence specific for isoform 1C.[12,27] In the free structure, residues 59–64, which are coded by exon 1C, are part of the N-terminal α-helix of the CH1 domain. On the other hand, in the β4-bound structure, this segment adopts an extended conformation, forming the N-terminal arm that binds in antiparallel fashion to the β-strand E of the FnIII-2 of β4. Despite the contribution of the N-terminal arm to the β4-binding surface it does not increase the affinity of the β4-plectin interaction. On the contrary, the affinity for β4 of an ABD fragment that does not contain the exon 1-coded sequences is slightly higher than that of similar fragments that do contain the ABD and either the 1A or 1C sequences.[25,27] The negative effect of the sequence upstream of the ABD on the interaction with β4 may be related to the energy needed for the helix-to-strand conformational transition that it undergoes upon binding. Despite the fact that the sequences coded by the exons 1A and 1C do not contribute to the binding to the FnIII-1,2 of β4, they might bind to other hemidesmosomal components or regulators. For example, calmodulin has been reported to bind to plectin 1A in a Ca^{2+}-dependent manner and to compete with β4 for binding to plectin.[30]

The 3D structures of individual hemidesmosomal components and that of the α6β4-plectin complex have contributed towards understanding the organization of HDs. Further studies will be required to elucidate the structures of other assemblies of the hemidesmosomal protein network, and to unveil how regulatory mechanisms, such as specific post-translational modifications, induce the disassembly of HDs. In addition, the lessons learned from the structural description of HDs will help to understand the function of hemidesmosomal proteins in other biological events, such as the role of α6β4 in keratinocyte migration and carcinoma invasion.

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