UNIVERSITY OF BIRMINGHAM
University of Birmingham Research at Birmingham

Molecular mechanism of intermediate filament recognition by plakin proteins

Mohammed, Fiyaz; Trieber, Catharine; Overduin, Michael; Chidgey, Martyn

DOI:
10.1016/j.bbamcr.2020.118801

License:
Creative Commons: Attribution (CC BY)

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Mohammed, F, Trieber, C, Overduin, M & Chidgey, M 2020, 'Molecular mechanism of intermediate filament recognition by plakin proteins', *Biochimica et Biophysica Acta. Molecular Cell Research*, vol. 1867, no. 11, 118801. https://doi.org/10.1016/j.bbamcr.2020.118801

Link to publication on Research at Birmingham portal

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

•Users may freely distribute the URL that is used to identify this publication.
•Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
•User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
•Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 24. Apr. 2024

BBA - Molecular Cell Research 1867 (2020) 118801

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

journal homepage: www.elsevier.com/locate/bbamcr

Minireview

Molecular mechanism of intermediate filament recognition by plakin proteins

Fiyaz Mohammed[a], Catharine Trieber[b], Michael Overduin[b], Martyn Chidgey[c,*]

[a] Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham B15 2TT, UK
[b] Department of Biochemistry, Faculty of Medicine and Dentistry, 474 Medical Sciences Building, University of Alberta, Edmonton, Alberta T6G 2H7, Canada
[c] Institute of Clinical Sciences, University of Birmingham, Birmingham B15 2TT, UK

ARTICLE INFO

Keywords:
Plakin
Plakin repeat domain
Linker module
Desmoplakin
Arrhythmogenic right ventricular cardiomyopathy
Intermediate filament

ABSTRACT

The plakin family of cytolinkers interacts with intermediate filaments (IFs) through plakin repeat domain (PRD) and linker modules. Recent structure/function studies have established the molecular basis of envoplakin-PRD and periplakin-linker interactions with vimentin. Both plakin modules share a broad basic groove which recognizes acidic rod elements on IFs, a mechanism that is applicable to other plakin family members. This review postulates a universal IF engagement mechanism that illuminates the specific effects of pathogenic mutations associated with diseases including arrhythmogenic right ventricular cardiomyopathy, and reveals how diverse plakin proteins offer tailored IF tethering to ensure stable, dynamic and regulated cellular structures.

1. Introduction

Plakin proteins form cell-cell and cell-matrix junctions and link to organelles such as mitochondria and nuclei by engaging intermediate filaments (IFs), actin microfilaments and microtubules. The seven mammalian members include desmoplakin, envoplakin, periplakin, plectin, bullous pemphigoid antigen 1 (BPAG1), microtubule-actin cross-linking factor 1 (MACF1) and epiplakin. In addition to PRD and linker modules, their architectures comprise plakin, actin-binding, coiled-coil rod, growth-arrest-specific 2-related (GAR) and glycine-serine-arginine-rich (GSR) domains (Fig. 1A), as well as spectrin repeat regions and EF hands. As such, they act as cytoskeletal adapters that facilitate cell adhesion, migration, signalling and the stress response [1–3]. Plakin proteins are widely distributed in tissues including epithelia, cardiac muscle and skeletal muscle and mediate specialized functions. Desmoplakin is essential for cell-cell adhesion in desmosomes, envoplakin and periplakin serve as scaffolds in the cornified envelope, plectin and BPAG1 contribute to cell-matrix adhesion in hemidesmosomes, MACF1 figures in microtubule stabilisation and cell motility and epiplakin supports stress responses [1–3]. Due to their essential role in maintaining tissue integrity and resilience, compromised plakin function can lead to genetic and autoimmune diseases, with the structural mechanisms involved now becoming clearer.

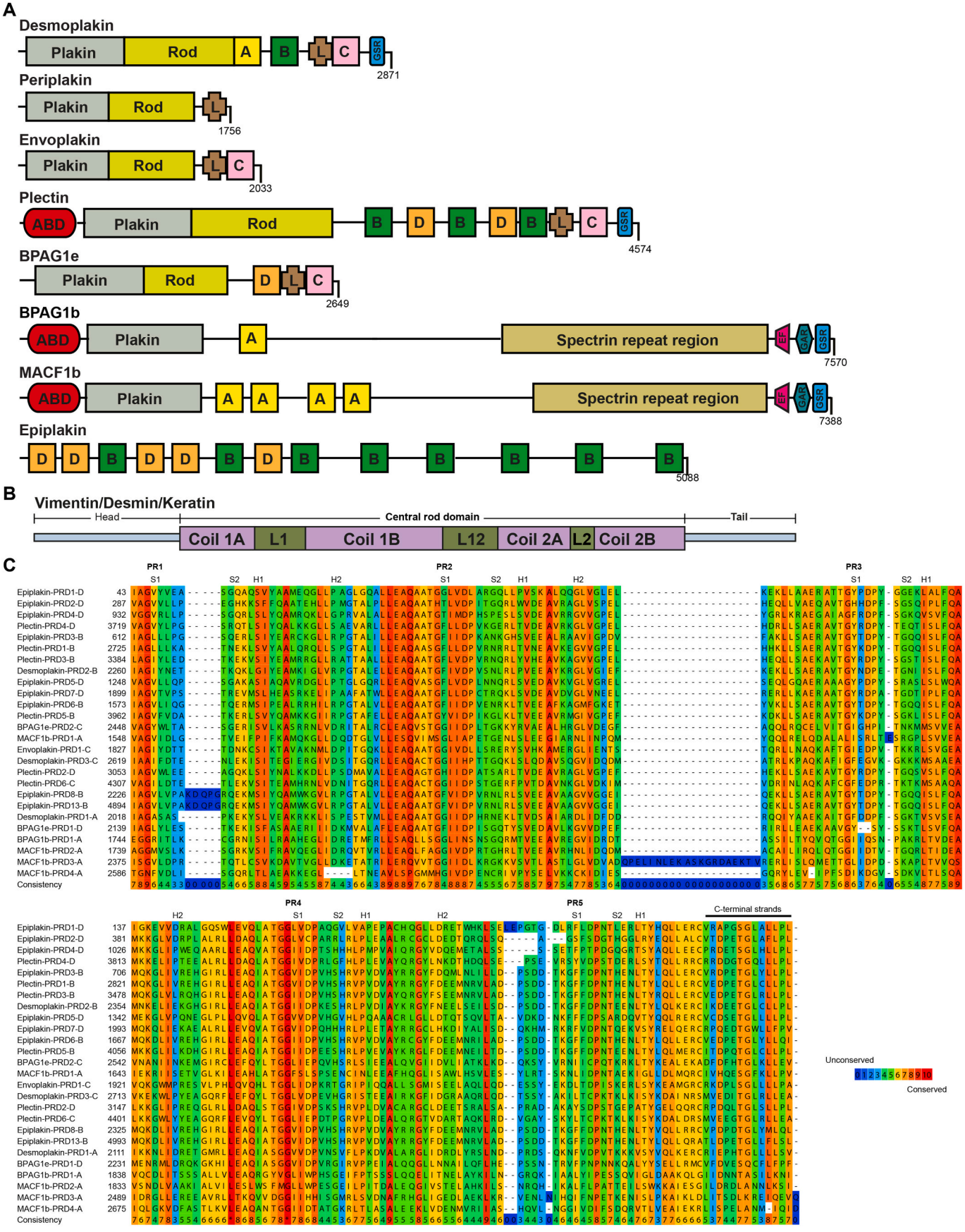
Alternative splicing of plakin proteins endows family members with a myriad of tissue-specific functions. These include not just binding to IFs but also to actin microfilaments *via* N-terminal actin-binding domains, to microtubules by means of C-terminal GSR and GAR motifs, and to transmembrane assemblies through their N-terminal plakin domains [1–3]. Here we focus on recent insights into the mechanisms governing IF interactions, which are mediated by PRD and linker modules, and explain how these interactions are affected by pathogenic disease-causing mutations.

2. Interaction of plakin proteins with IFs

Plakin proteins interact with the cell-type specific IF proteins desmin, keratin and vimentin. These proteins feature a long α-helical rod domain consisting of four heptad repeat containing regions (coils 1A, 1B, 2A and 2B) interspersed by three short intervening segments (L1, L12 and L2) (Fig. 1B). The conserved extremities of the rod domain have critical roles in IF assembly, with even subtle amino acid substitutions leading to dramatic consequences [4]. The central rod domain is flanked by unstructured, poorly conserved N- and C-terminal regions, completing the characteristic tripartite domain structure shared by IF proteins. Their hierarchical assembly involves two elongated protein subunits forming parallel coiled-coil hetero- (keratin) or homo- (desmin and vimentin) dimers which subsequently form antiparallel tetramers. Tetramers then associate laterally and longitudinally

* Corresponding author.
E-mail addresses: F.Mohammed@bham.ac.uk (F. Mohammed), ctrieber@ualberta.ca (C. Trieber), overduin@ualberta.ca (M. Overduin), M.A.Chidgey@bham.ac.uk (M. Chidgey).

https://doi.org/10.1016/j.bbamcr.2020.118801
Received 11 June 2020; Received in revised form 15 July 2020; Accepted 20 July 2020
Available online 23 July 2020
0167-4889/ © 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).



(caption on next page)

Fig. 1. A) Domain architecture of plakin proteins. Mammalian plakins that possess plakin repeat domains (PRDs) and/or linker modules are displayed, with alternatively spliced versions that lack PRDs omitted. The plakin domain consists of a variable number of spectrin repeats and a Src homology 3 (SH3) module, whilst actin-binding domains (ABD) possess two calponin homology modules. Coiled-coil rod domains mediate dimerisation. EF-hand motifs bind calcium, and together with growth-arrest-specific 2-related (GAR) domains and glycine-serine-arginine-rich (GSR) segments are found at the C-termini of some plakin proteins. All plakins except periplakin contain PRDs of which there are four different subtypes (A, B, C and D), and some possess C-terminal linker (L) modules. B) Schematic representation of the tripartite domain structure shared by mammalian vimentin, desmin and keratin IF proteins, which encompasses an α-helical central rod flanked by unstructured 'head' and 'tail' modules. C) Alignment of PRD sequences from human plakins obtained from Uniprot (accession numbers O60437 (periplakin), P15924 (desmoplakin), Q92817 (periplakin), Q15149-2 (plectin), Q03001-3 (BPAG1e), Q9UPN3-1 (MACF1b) and Q03001-7 (BPAG1b)) using the PRALINE toolkit ([57] with colours showing the degree of amino acid conservation. Epiplakin PRDs 9-12 have been omitted as they are identical to PRD8. Secondary structure for the plakin repeat motif is shown (S1, strand1; S2, strand 2, H1, helix 1; H2, helix 2).

to assemble mature 10 nm-wide elongated compacted filaments of varying lengths [5,6].

Plakin proteins possess varying numbers of PRDs from one in envoplakin to thirteen in epiplakin. Periplakin lacks a PRD but does have a linker module, in common with several other plakin proteins (Fig. 1A). Most plakin proteins bind IFs through a cluster of PRD and linker modules at their C-termini. This allows desmoplakin to recognize coil 1 of keratins 5 and 14, keratins 1 and 10, desmin and vimentin [7], and plectin to engage the equivalent region of keratins 5 and 14 [8]. The sole PRD of envoplakin binds to coil 1A of vimentin [9]. Together, this indicates that plakin C-termini generally engage overlapping sequences conserved within the coil 1 region of IF proteins. By contrast, the PRDs of BPAG1b and MACF1b are more centrally located between the plakin domain and the spectrin repeat region and it remains unclear whether they mediate IF interactions [10,11]. Epiplakin is comprised exclusively of PRDs and binds only to IF proteins, particularly keratin [12].

Linker modules are typically found immediately preceding the "PRD-C"-type domain, which is found at the C-terminus of desmoplakin, envoplakin, plectin, and BPAG1e (Fig. 1A). Periplakin is unique in that its linker module is its only means of mediating direct IF binding. The ability of linker domains to bind IFs varies. The periplakin linker associates with IFs in overlay assays, yeast two-hybrid and pull-down assays whereas no such interaction is evident with the envoplakin linker [13,14]. A periplakin construct comprising part of its rod domain and its linker co-localises with IFs in cells whereas an analogous envoplakin construct (including the C-terminal PRD) shows limited, partial co-localisation [13]. When co-transfected, both constructs co-localise with IFs [9,13]. Hence periplakin appears to recruit envoplakin to the IF cytoskeleton, presumably through heterodimerisation of their rod elements.

There are pathological consequences when interactions between plakins and IFs are compromised. Lethal acantholytic epidermolysis bullosa is a skin blistering disease that is caused by recessive heterozygous truncations in the desmoplakin gene that eliminate C-terminal IF binding sub-domains. Keratinocyte cell-cell adhesion is compromised and the subsequent skin blistering causes catastrophic fluid loss and early death [15]. Carvajal syndrome is caused by a recessive homozygous truncation that removes part of the desmoplakin linker module and all of PRD3-C, and clinically manifests as cardiomyopathy, palmoplantar keratoderma and woolly hair [16]. Numerous mutations within desmoplakin's PRD and linker modules result in arrhythmogenic right ventricular cardiomyopathy (ARVC), a common cause of sudden cardiac arrest and death in young adults [17,18], with reduced IF binding being one potential cause of the pathology [19]. Related conditions include epidermolysis bullosa simplex (EBS), a skin blistering disease that is linked to mutations in genes encoding plectin and BPAG1 (although the majority of the mutations that cause EBS occur in keratin 5 and keratin 14). In EBS mutations often lead to IF aggregation but in a subset of cases skin blistering has been attributed to defective IF anchorage at the hemidesmosome [20].

The various IF protein mutations and associated diseases reported in the Intermediate Filament Database (www.interfil.org) include > 60 keratin alterations, > 10 desmin mutations and one vimentin substitution [21]. A further mutation in vimentin has recently been described [22]. The majority of these mutations are thought to be pathogenic due to predicted effects on IF structure and assembly. Keratin mutations primarily affect the skin and hair, and can cause tissue fragility, blistering, hyperkeratosis, hyperproliferation and IF aggregation [23]. Desmin mutations principally affect muscle and the heart and often lead to filament aggregation, progressive muscle damage and cardiomyocyte degeneration [24]. Interestingly desmin mutation E108K, that results in dilated cardiomyopathy, fails to disrupt desmin network architecture in human smooth muscle cells and neonatal rat cardiac myocytes [25]. This residue is conserved in coil 1A of type I-III IF proteins, the region that mediates desmoplakin binding [7], so it is conceivable that defective desmoplakin-desmin binding could explain its pathogenic effect.

3. Structures of plakin repeat domain and linker modules

In addition to the three established classes of PRDs (A/B/C), a fourth subtype (D) is present in plectin, BPAG1e and epiplakin [9] (Fig. 1C). All PRDs contain 4.5 copies of the canonical 38-residue plakin repeat (PR) motif [26], which includes an 11 residue β-hairpin followed by an antiparallel pair of α-helices. The crystal structures of the desmoplakin PRD1-A, PRD2-B, PRD3-C and envoplakin PRD-C modules (Fig. 2A), and the solution structure of plectin PRD6-C (PDB: 2N03), display a large positively charged groove lined with several conserved amino acids that could accommodate IF rods [9,27,28]. PRDs A/B/D also include an additional N-terminal (Nt) PR-like motif characterised by a β-hairpin followed by three antiparallel α-helices. The periplakin linker structure (PDB: 4Q28) features a distinct PR-like motif at each end of the protein. The smaller Nt-PR-like pattern aligns well with the PR2 motif, whereas the larger C-terminal PR-like module resembles the Nt-PR-like elements found in desmoplakin PRDs-A and B. The two PR motifs within the periplakin linker form an elongated bi-lobed domain that frames an electropositive groove (Fig. 2B) that, like that of PRDs, could accommodate an IF rod bearing a multi-acidic surface [28,29].

4. Molecular basis of plakin protein-IF interactions

The mechanism responsible for plakin recognition of IFs involves coiled-coil rods slotting into the basic grooves of PRDs and linkers. This electrostatic complementarity can be disrupted with either charge reversal mutation of basic residues (K1901 and R1914) in the positively charged groove of envoplakin's PRD or of acidic residues (D112 and D119) in the vimentin coil 1A, compromising direct interactions [9]. Accordingly, mutation of the K1901 and R1914 binding determinants delocalise envoplakin constructs in cells [9]. The interface is stabilised by ionic interactions mediated between basic residues emanating from the PRD groove and acidic side-chains projecting from the vimentin coil 1A domain (Fig. 2C). Non-polar residues and other factors such as steric fit may also contribute to the interface as charge reversal substitutions fail to completely abolish PRD-vimentin ligand interactions [9]. Different vimentin fragments can be accommodated by the envoplakin PRD, albeit with various angles of vimentin slotting into the groove. This infers that PRD modules are presented with different opportunities for sliding and locking onto filaments, facilitating the dynamic assembly and disassembly of junctional complexes between epithelial cell

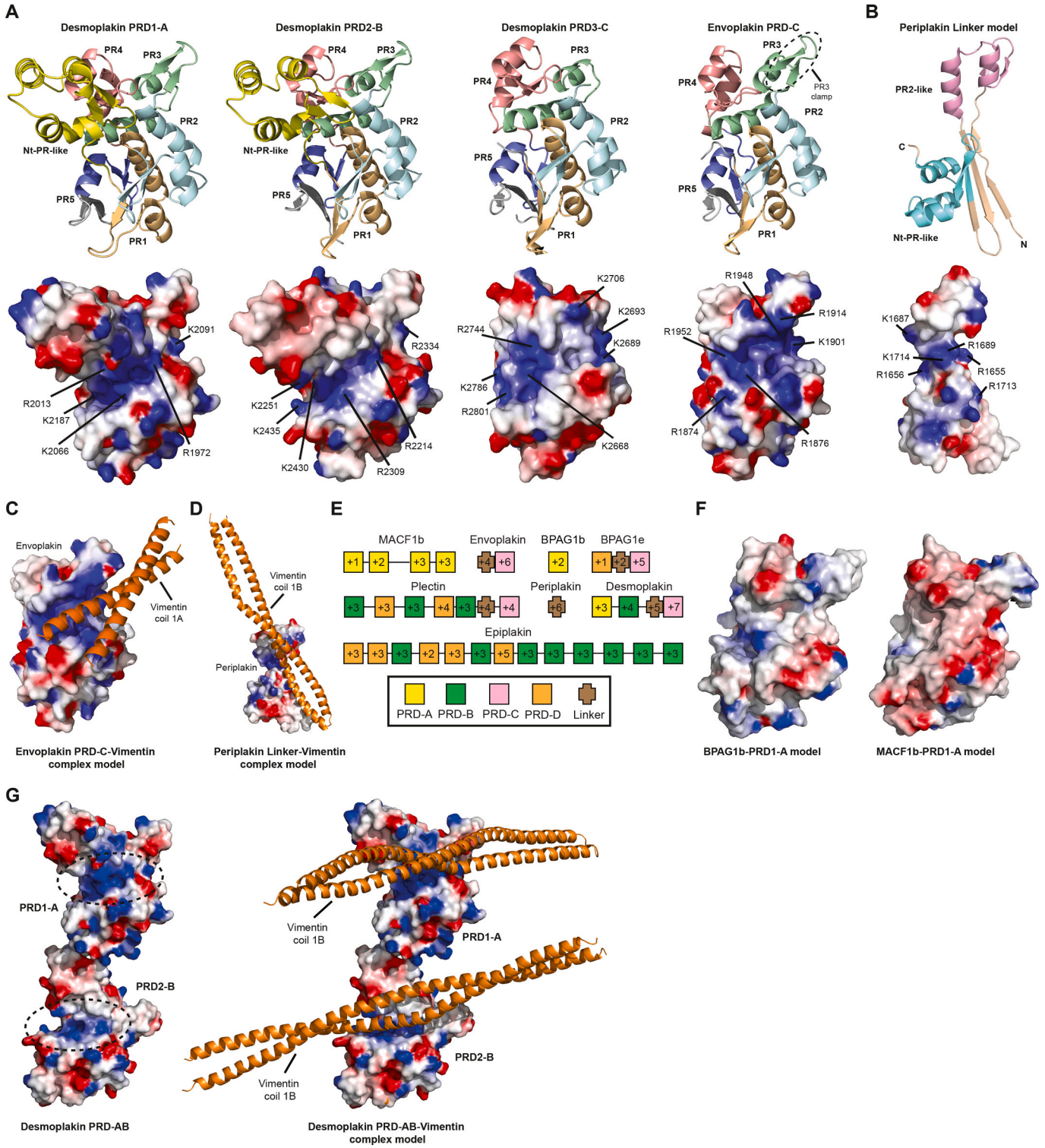


Fig. 2. Molecular basis of plakin PRD/linker module interactions with IFs. A) Ribbon representation and electrostatic surface potential of the desmoplakin PRD1-A (PDB: 5DZZ), PRD2-B (PDB: 1LM7) and PRD3-C (PDB: 1LM5) and envoplakin PRD-C (PDB: 4QMD). Plakin repeats 1–5 are coloured light orange, cyan, green, salmon and blue, respectively, with their PR motifs and secondary structural elements labelled. Desmoplakin PRD1-A and PRD2-B also possess an additional PR-like motif at their N-termini (yellow). PRD-C modules possess a unique clamp feature in PR3 which may assist in IF tethering (highlighted with black dashed lines in envoplakin PRD-C). The electrostatic surface potential of each PRD was calculated with Delphi and ranges from −7 (red) to +7 (blue) in units of kT/e. Key basic residues are highlighted. B) Ribbon representation and electrostatic surface potential of an I-TASSER derived periplakin linker model showing PR2-like (pink) and Nt PR-like (cyan) elements and highlighting the position of basic groove residues. C) Haddock-derived model of the envoplakin PRD bound to coil 1A of dimeric vimentin (orange). D) Haddock derived model of the periplakin linker complexed with coil 1B of dimeric vimentin (orange). E) Net positive charge distribution within the groove of human plakin PRDs and linker modules. F) Models of the first PRDs of BPAG1b and MACF1b showing electrostatic surface potential. G) Molecular surface representation of desmoplakin PRD-AB (PDB: 5DZZ) with their grooves indicated (left). Haddock-derived model of desmoplakin PRD-AB in complex with dimeric vimentin (PDB: 3UF1) fragments (right). The relative orientation of the grooves suggests that the tandem PRDs are unlikely to bind the same vimentin dimer.

architectures. The principal features of the vimentin binding groove of envoplakin are conserved, with broadly similar diameters, lengths and charge distributions present in the equivalent region of desmoplakin PRDs [9,27,28]. As keratin and desmin resemble vimentin in presenting poly-acidic motifs along their rod surfaces [24,30,31], all PRDs may engage IFs in comparable ways.

The molecular mechanism underpinning periplakin linker interactions with the cytoskeleton is emerging [29]. The periplakin linker encompasses a broad groove lined by six basic residues (Fig. 2D), of which three are relatively conserved across plakin linker modules. Charge reversal mutations of these residues, and of acidic residues (D176 and E187) in vimentin coil 1B, compromise their interactions [29]. Consistent with this, mutation of basic residues in the periplakin, desmoplakin and plectin linker modules disrupts targeting to IFs in cultured cells [29,32]. In addition, deletion of residues 1694 DWEEI 1698, present in the PR2-like motif of the periplakin linker and proximal to the groove, abolishes IF targeting in transfected cells [13], probably due to altered orientations of basic groove residues responsible for IF binding [29]. Overall, linker module-IF interactions are electrostatic in nature, mirroring the docking pose proposed for PRD-IF interfaces [9]. A universal cytoskeletal recognition mechanism for plakins is emerging in which electrostatic attraction mediated by respective basic grooves of series of PRD and linker modules provide the relevant avidity for stable and dynamic IF tethering.

The individual domains offer divergent functions, as a vimentin rod construct (residues 99–249) binds more tightly with the envoplakin PRD (K_D = 19 μM) compared to the periplakin linker (K_D = 71 μM) [9,29]. Interestingly, the desmoplakin linker shows no detectable binding to the same vimentin rod construct despite displaying a groove with comparable electro-positive surface distribution to the periplakin linker. Hence, in common with envoplakin PRD-IF interactions, the propensity for vimentin binding to the linker is not solely defined by basic character. However, increasing the basic character of the desmoplakin linker groove does enhance vimentin affinity, albeit not to the extent of the periplakin linker, indicating that charge is an important but not exclusive determinant [29]. Evolutionary pressure on periplakin, which does not possess a PRD, may have preserved its stronger linker-mediated interactions with IFs. By contrast, desmoplakin is comprised of three juxtaposed PRDs that offer a diverse range of IF binding surfaces. The envoplakin linker groove encompasses four positive residues, as opposed to six in the corresponding surface of periplakin (Fig. 2E), suggesting that it might bind more weakly to IFs. Hence envoplakin may rely on its PRD in combination with the periplakin linker for tighter IF binding in tissues where both are expressed. Multimerization of plakin proteins offers opportunities to multiplex and rigidify IF interactions, with the C-terminal binding domain generally offering the tightest interaction.

The basic grooves of both the envoplakin PRD and the periplakin linker module accommodate dimeric vimentin rods (Fig. 2C–D). Given that IFs are organised into higher order structures the question arises as to what stage of the IF assembly process does interaction with plakin proteins occur. We envisage two possibilities, either plakin proteins interact with IFs during assembly or some local untangling of IFs occurs after assembly allowing plakin protein access to dimeric rods. In either scenario plakin proteins are likely to be locked into place in fully assembled IFs, increasing the adhesive properties of the interaction.

5. Predicting plakin binding to IFs

To build a predictive basis for IF interactions, molecular models were generated for all human PRD and linker modules and the net positive charge distributions within their IF binding groove were determined (Fig. 2E). PRD-A grooves have the least basic character (+1 to +3), and so are likely to offer the weakest electrostatic-mediated IF binding. Interestingly, the BPAG1b and MACF1b isoforms exclusively contain PRD-A subtypes, which may explain their lack of binding to IFs [10,11]. The sole BPAG1b PRD demonstrates a low net groove charge of +2, and the N-terminal pair of PRD modules from MACF1b possess net groove charges of +1 and +2 respectively (Fig. 2E–F). Intriguingly, the latter mediate Golgi localization, although the mechanism by which this occurs is unknown [10]. MACF1b encompasses two additional PRD-A subtypes, with each demonstrating a net groove charge of +3. These then might be expected to have a moderate IF tethering potential. However it may be that nearby plakin domain or spectrin repeat region sterically preclude binding of IFs to these centrally positioned PRDs.

PRD-B domains contain moderately basic grooves (+3 to +4), whereas PRD-C modules offer highly electropositive grooves (+4 to +7). In addition PRD-C modules possess a unique PR3 feature comprised of an apparently dynamic β-hairpin incorporating a relatively conserved positively charged side chain that may assist IF tethering by clamping down and locking a filament into position [9]. PRD-Cs lie adjacent to linker modules and are found in those plakin proteins (desmoplakin, plectin and BPAG1e) that are present in cell junctions and are likely to bind most strongly to IFs. The linker domains connecting the PRD-B and C subtypes in these proteins have a net positive groove charge and are likely to add to the capacity of these proteins to bind IFs. The basic character of PRD-D modules varies considerably (+1 to +5) and these domains, primarily found in epiplakin, are distinguished by divergent PR4-PR5 sequences that cap their grooves.

The homologous PRD/linker modules bearing 1–7 basic determinants could offer graded filament affinities, providing a functional classification for all PRD/linker superfamily members based on electrostatic complementarity. Overall, there appears to be an inverse correlation between the net positive charges within PRDs/linker grooves and the number of PRD elements found in plakin proteins. For example, plakin proteins with either single PRDs and/or linkers such as envoplakin and periplakin exhibit the highest net positive groove charges. Basic character of the grooves is unlikely to be the sole determinant of binding affinity with steric fit and nonpolar contributions from the variety of available filaments and potential binding partners likely to be important. Basic residues within the Nt-PR-like motifs of PRDs -A/-B/-D [27,28] which border the positively charged groove may also contribute to IF interactions, as could the unstructured intervening segments between PRDs [12], underscoring the complexity of IF recognition by full length plakins. A spectrum of weak and strong ligands are also available given that IFs offer multiple potential binding sites with varying numbers of charged residues. Altogether this implies that PRD and linker modules are presented with a wealth of opportunities for flexibly sliding and locking onto filaments, facilitating the dynamic assembly and disassembly of junctional complexes between epithelial cell architectures.

6. The role of tandem PRDs

The precise function of plakins encompassing tandem PRDs is obscure. The most obvious possibility is that juxtaposed PRDs offer multivalent binding sites that provide high affinity plakin-IF attachments. Indeed, individual desmoplakin PRDs form weak interactions with vimentin IFs whilst exhibiting stronger co-operative binding when all three PRDs are present [27]. Furthermore, multiple C-terminal domains of plectin, including PRD5-B and PRD6-C, bind to keratin 5/keratin 14 in a concerted fashion to ensure efficient association with IF proteins [8]. Molecular studies of the desmoplakin C-terminal domains have provided the framework for studying IF interactions with multi-modular plakins [28]. Small angle X-ray scattering analysis reveals that the three desmoplakin PRDs and linker form an elongated 'beads on a string' structure [28]. The desmoplakin linker demonstrates a degree of flexibility suggesting that it may facilitate domain motions that provide appropriate geometric positioning of flanking PRDs and allowing dynamic recognition of targets. We envisage similar functions for plectin and BPAG1e linker modules.

An intriguing question is whether all four desmoplakin domains

engage the same IF rod. Molecular surface analysis of the desmoplakin PRD-AB structure [28] suggests that this is unlikely given the relative orientations of the IF binding grooves (Fig. 2G, left). Modelling indicates that individual vimentin dimers slot similarly into the desmoplakin PRD basic grooves with minimal structural rearrangement. The lowest energy complex models obtained comprise of each PRD element interacting with a separate vimentin coil 1 fragment encompassing residues L146-I249 (PDB: 3UF1) (Fig. 2G, right), consistent with previous functional studies [7]. This mode of docking suggests that each groove could accommodate adjacent rods, bringing them together within a fully assembled IF. By contrast, the envoplakin-periplakin heterodimer could conceivably bind to the same rod during IF assembly as the envoplakin PRD engages with coil 1A of vimentin [9], whereas the periplakin linker module interacts with coil 1B. Delineation of multivalent binding modes requires further analysis, but could involve sliding of binding grooves along filaments to secure adaptive attachments.

Development of comprehensive mechanistic insights into how plakins encompassing tandem PRDs engage IFs will require three-dimensional structures of entire IF building blocks. This has proven difficult as IF proteins form extended coiled-coils that associate into high-order complexes under wide-ranging ionic environments. Initially the "divide and conquer" approach was the only viable strategy for generating atomic resolution data on IFs, with fragments demonstrating monomeric, dimeric, or even tetrameric association states within their crystal lattices [33]. More recently the use of electron paramagnetic resonance spectroscopy in combination with fragmented structures and molecular modelling approaches have provided the first experimentally driven model of a complete IF rod domain [34]. We anticipate that the assembled model will not only provide a basis for exploring IF dynamics and assembly but will also illuminate ligand docking modes with multidomain plakin proteins and define how stable yet dynamic cytoskeletal attachments are formed.

7. Predicting pathogenic effects of disease-causing mutations

Numerous disease-associated mutations have been documented for the PRD/linker modules of desmoplakin and plectin (Fig. 3A) in the Human Gene Mutation Database [35]. Recent PRD and linker structures allow these to be mapped (Fig. 3B–D). A missense mutation R2366C in desmoplakin PRD2-B contributes to skin fragility/woolly hair syndrome [36], and diminishes IF binding [19]. The desmoplakin PRD2-B structure shows that R2366 mediates a salt bridge between E2290 and E2293, supporting the PRD fold. The desmoplakin PRD2-B model encompassing the R2366C mutation reveals that these interactions would be abolished, creating structural vulnerabilities (Fig. 3B). In addition, the thiol group of C2366 may be susceptible to oxidation which could lead to the formation of physiologically irrelevant disulphide-linked PRD-B dimers. Similarly, the homozygous missense mutation G2375R in desmoplakin PRD2-B results in ARVC with skin and hair abnormalities [37], and reduces IF binding [38]. Conserved G2375 residue at the end of PR3 of PRD2-B executes a sharp turn (Fig. 3C) that is likely to be conformationally critical (Fig. 1B), with an arginine here unlikely to be structurally tolerated due to sterically clashes with the N-terminal PR-like motif (Fig. 3C).

The majority of plectin pathogenic mutations identified are caused by frameshift and premature termination codon events in the rod domain. However, missense mutations in the PRD and linker regions have been identified (Fig. 3A). For example, the R3527C substitution in plectin PRD3-B results in EBS [39]. Analysis of the model reveals that R3527 mediates an ionic interaction with D3523 which contributes to stabilising the PR3 element (Fig. 3D). Introducing a cysteine at this position is likely to abolish this contact and adversely affect the PRD fold (Fig. 3D). These structural models provide the beginning of a predictive basis for how disease associated mutations affect PRD/linker stabilities and ligand interactions. These predictions are likely to improve following structural determination of plakin PRD/linker-IF complexes.

8. Impact of post translational modifications

There is increasing evidence to indicate that plakin–IF interactions are regulated by post translational modifications (PTMs) of residues emanating from PRD and linker modules. Plectin is phosphorylated at T4542 in PRD6-C by p34cdc2 kinase [40] and a highly reactive cysteine in PRD5-B serves as a nitrosylation target *in vitro* [41]. Mutation of plectin linker module residue S4645 compromises binding to IFs in transfected cells [42]. Potential phosphorylation sites in plakin PRDs and linkers were mapped using PhosphoSitePlus [43]. Residues that are phosphorylated include desmoplakin Y2275 and Y2316 in the first helices of the PR1 and PR2 motifs of PRD2-B, Y2720 and Y2731 in the second helix of PR3 of PRD3-C, and the corresponding plectin Y4517 and Y4529 residues in PRD6-C. Since these residues map distal to the basic groove, phosphorylation may induce long-distance conformational changes or destabilising effects that disrupt IF binding. The plectin linker encompasses two additional phosphorylation sites, T4267 and S4268, which given their location, are likely to utilise similar allosteric mechanisms for regulating IF tethering. By contrast, other linker domain residues subject to phosphorylation including S1657 in periplakin and Y1700 in envoplakin border the positively charged groove and may directly impact IF interactions.

The GSR region at the extreme C-terminus of plakin proteins confers an important role in modifying plakin protein-IF interactions. For example, phosphorylation of S2849 in the desmoplakin GSR reduces desmoplakin-IF interactions and promotes desmosome assembly [44]. Phosphorylation of the equivalent residue S4642 in plectin has a similar effect [45]. Furthermore, methylation of desmoplakin R2834 facilitates glycogen synthase kinase 3 (GSK3) recruitment, phosphorylation of nearby serine residues and decreases IF interactions, promoting assembly [46]. Interestingly, R2834 is mutated in ARVC and transgenic mice engineered to over-express human desmoplakin containing this mutation exhibit increased cardiac fibrosis, apoptosis and lipid accumulation, as well as ventricular enlargement and cardiac dysfunction [47]. Although a direct correlation between delays in desmosome assembly and ARVC pathogenesis remains to be established, it is interesting to note that GSK3β is mislocalised to the intercalated disk in ARVC patients and its pharmacological inhibition improves cardiac histology and function in ARVC models [48]. Since the GSR domains of desmoplakin and plectin are highly conserved these complex cytoskeletal regulations are likely to be more widespread.

Finally, IF networks are highly dynamic with individual filaments undergoing constant reshaping [49]. PTM events such as phosphorylation and glycosylation play a pivotal role in regulating IF dynamics [50] and this adds another layer of complexity to the already bewildering plakin-IF interaction landscape. Further studies are undoubtedly required to establish the precise interplay between PTMs in regulating IF assembly and plakin binding.

9. Plakin proteins as orchestrators of IF reorganisation

The role of plectin in organising IF architecture is well established [51]. It anchors IFs to junctional complexes, organelles and the nuclear envelope and engages microfilaments and microtubules. In its absence IF networks are aberrantly organised and can collapse, particularly in muscle cells [51]. Other plakin proteins are also critical for regulating IF networks. The conserved K1646 near the beginning of the periplakin linker is SUMOylated in response to stress-mediated reorganisation of keratin and regulates cytoskeletal reorganisation [52]. It is possible that following the covalent conjugation of the periplakin linker with SUMO-1, a globular protein with distinct electropositive surface patches, the linker module exhibits a greater capacity to accommodate negatively

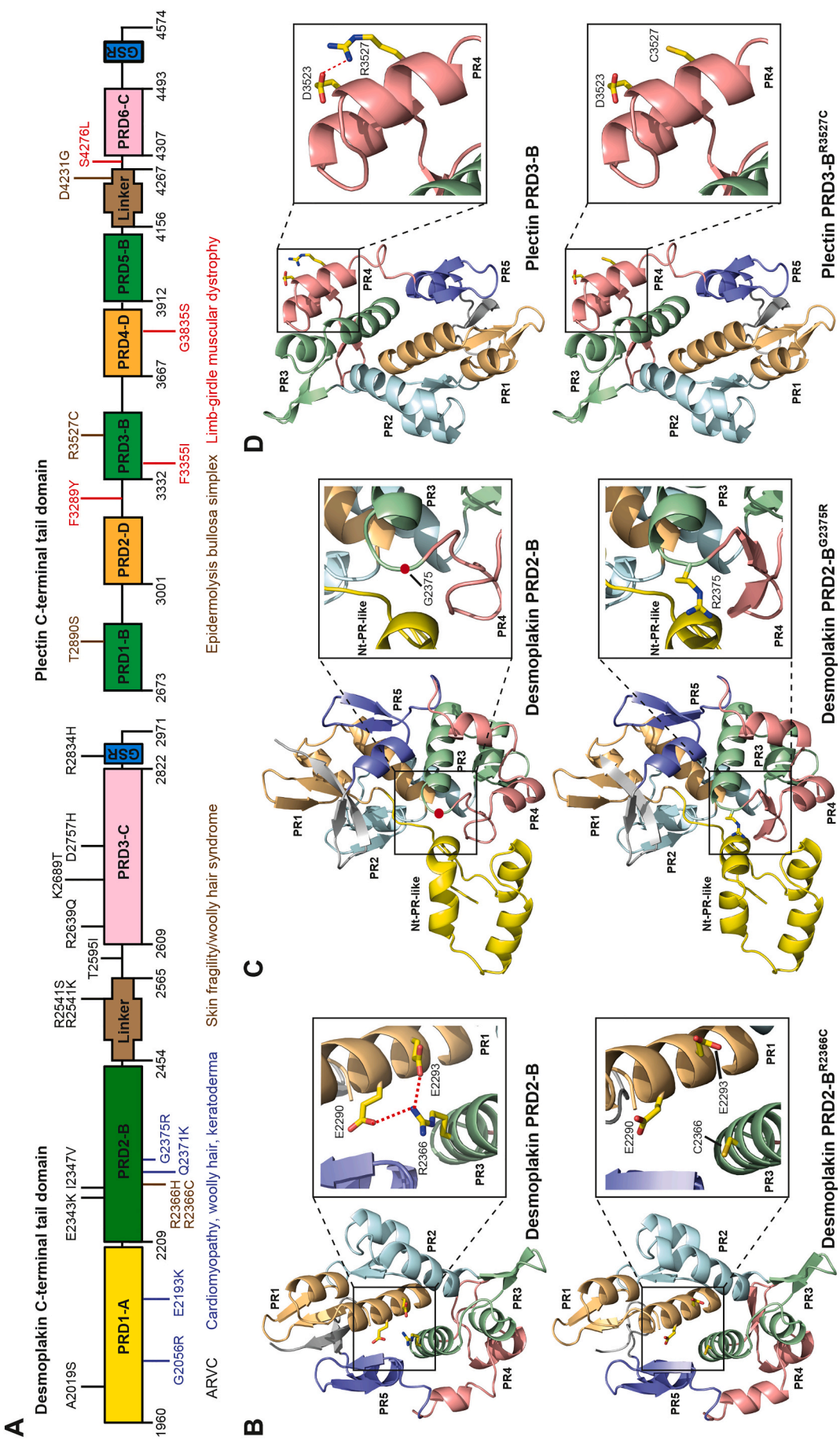


Fig. 3. Structural mapping of pathogenic-causing missense mutations to desmoplakin and plectin. A) Location of point mutations associated with human disease on the primary structure of desmoplakin and plectin. PRD modules are not drawn to scale. B–C) Mapping of ARVC-linked mutations R2366C and G2375R on the crystal structure of desmoplakin PRD-B. D) Mapping of EBS-linked mutation R2366C on the plectin PRD3-B model. Plakin repeats 1–5, Nt-PR-like patterns and PR2-like motifs are labelled and coloured as in Fig. 2A. Salt bridge interactions are highlighted (red dashed lines).

charged keratin rod elements. Epiplakin is highly expressed in the epidermis, but unlike other plakins, its loss does not result in skin fragility and blistering [53]. However, like periplakin, it assists in keratin IF reorganisation and ensures resistance to experimentally induced disruption [54–56]. Together, these reports suggest that plakin PRD and linker domains possess functions beyond simply tethering IFs to the membrane in order to maintain and regulate tissue integrity.

10. Conclusion

Substantial progress is being made in our understanding of the mechanistic basis of plakin protein-IF interactions based on recent structural and functional studies of PRD and linker modules, and IF rod domains. This has informed the development of models for mapping pathogenic mutations and accurately predicting their impact on structures. However, the molecular basis of how pathogenic mutations lead to disorders such as ARVC is far from established. Pathogenicity of mutations could result from reduced IF interactions, although additional effects could include altered protein stability, trafficking, ligand binding dynamics, intracellular signalling and cell adhesion. Despite the emerging picture about the physiological and pathological functions of PRD/linker module interactions, several questions remain unanswered. The molecular basis underpinning the specificity and differential binding of PRDs/linker module elements to IFs are obscure. How higher order IF structures modulate plakin interactions or drive disease progression remains to be characterised, necessitating structures of complexes. How multiple PTMs mediate the dynamic interplay of plakin-IF attachments remains elusive. Recent advances in proteomic approaches could contribute new insights into the plakin interactome including of the stages of IF reorganisation, presenting tantalizing avenues for future research.

Funding

This work was supported by the Wellcome Trust [grant numbers 099266/Z/12/Z to FM, 094303/Z/10/Z to MO]; the Campus Alberta Innovates Program [grant number RCP-12-002C to MO]; Alberta Prion Research Institute/Alberta Innovates Bio Solutions [grant number 201600018 to MO]; NSERC [grant number RGPIN-2018-04994 to MO].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A. Sonnenberg, R.K. Liem, Plakins in development and disease, Exp. Cell Res. 313 (10) (2007) 2189–2203.
[2] J.E. Bouameur, B. Favre, L. Borradori, Plakins, a versatile family of cytolinkers: roles in skin integrity and in human diseases, J Invest Dermatol 134 (4) (2014) 885–894.
[3] L. Hu, Z. Huang, Z. Wu, A. Ali, A. Qian, Mammalian plakins, giant cytolinkers: versatile biological functions and roles in cancer, Int. J. Mol. Sci. 19 (4) (2018).
[4] P.A. Coulombe, M.L. Kerns, E. Fuchs, Epidermolysis bullosa simplex: a paradigm for disorders of tissue fragility, J. Clin. Invest. 119 (7) (2009) 1784–1793.
[5] H. Herrmann, M. Haner, M. Brettel, S.A. Muller, K.N. Goldie, B. Fedtke, A. Lustig, W.W. Franke, U. Aebi, Structure and assembly properties of the intermediate filament protein vimentin: the role of its head, rod and tail domains, J. Mol. Biol. 264 (5) (1996) 933–953.
[6] R. Kirmse, S. Portet, N. Mucke, U. Aebi, H. Herrmann, J. Langowski, A quantitative kinetic model for the in vitro assembly of intermediate filaments from tetrameric vimentin, J. Biol. Chem. 282 (25) (2007) 18563–18572.
[7] B. Favre, N. Begre, J.E. Bouameur, P. Lingasamy, G.M. Conover, L. Fontao, L. Borradori, Desmoplakin interacts with the coil 1 of different types of intermediate filament proteins and displays high affinity for assembled intermediate filaments, PLoS One 13 (10) (2018) e0205038.
[8] J.E. Bouameur, B. Favre, L. Fontao, P. Lingasamy, N. Begre, L. Borradori, Interaction of plectin with keratins 5 and 14: dependence on several plectin domains and keratin quaternary structure, J Invest Dermatol 134 (11) (2014) 2776–2783.
[9] C. Fogl, F. Mohammed, C. Al-Jassar, M. Jeeves, T.J. Knowles, P. Rodriguez-Zamora, S.A. White, E. Odintsova, M. Overduin, M. Chidgey, Mechanism of intermediate filament recognition by plakin repeat domains revealed by envoplakin targeting of vimentin, Nat. Commun. 7 (2016) 10827.
[10] C.M. Lin, H.J. Chen, C.L. Leung, D.A. Parry, R.K. Liem, Microtubule actin cross-linking factor 1b: a novel plakin that localizes to the Golgi complex, J. Cell Sci. 118 (Pt 16) (2005) 3727–3738.
[11] M.F. Steiner-Champliaud, Y. Schneider, B. Favre, F. Paulhe, S. Praetzel-Wunder, G. Faulkner, P. Konieczny, M. Raith, G. Wiche, A. Adebola, R.K. Liem, L. Langbein, A. Sonnenberg, L. Fontao, L. Borradori, BPAG1 isoform-b: complex distribution pattern in striated and heart muscle and association with plectin and alpha-actinin, Exp. Cell Res. 316 (3) (2010) 297–313.
[12] W. Wang, H. Sumiyoshi, H. Yoshioka, S. Fujiwara, Interactions between epiplakin and intermediate filaments, J. Dermatol. 33 (8) (2006) 518–527.
[13] T. Karashima, F.M. Watt, Interaction of periplakin and envoplakin with intermediate filaments, J. Cell Sci. 115 (Pt 24) (2002) 5027–5037.
[14] S. Kazerounian, J. Uitto, S. Aho, Unique role for the periplakin tail in intermediate filament association: specific binding to keratin 8 and vimentin, Exp. Dermatol. 11 (5) (2002) 428–438.
[15] M.F. Jonkman, A.M. Pasmooij, S.G. Pasmans, M.P. van den Berg, H.J. Ter Horst, A. Timmer, H.H. Pas, Loss of desmoplakin tail causes lethal acantholytic epidermolysis bullosa, Am. J. Hum. Genet. 77 (4) (2005) 653–660.
[16] E.E. Norgett, S.J. Hatsell, L. Carvajal-Huerta, J.C. Cabezas, J. Common, P.E. Purkis, N. Whittock, I.M. Leigh, H.P. Stevens, D.P. Kelsell, Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma, Hum. Mol. Genet. 9 (18) (2000) 2761–2766.
[17] V.R. Iyer, A.J. Chin, Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), Am J Med Genet C Semin Med Genet 163C (3) (2013) 185–197.
[18] S. Mazurek, G.H. Kim, Genetic and epigenetic regulation of arrhythmogenic cardiomyopathy, Biochim. Biophys. Acta Mol. basis Dis. 1863 (8) (2017) 2064–2069.
[19] B. Favre, N. Begre, L. Marsili, J.P. van Tintelen, L. Borradori, Desmoplakin gene variants and risk for arrhythmogenic cardiomyopathy, Circ Genom Precis Med 11 (9) (2018) e002241.
[20] G. Walko, M.J. Castanon, G. Wiche, Molecular architecture and function of the hemidesmosome, Cell Tissue Res. 360 (3) (2015) 529–544.
[21] I. Szeverenyi, A.J. Cassidy, C.W. Chung, B.T. Lee, J.E. Common, S.C. Ogg, H. Chen, S.Y. Sim, W.L. Goh, K.W. Ng, J.A. Simpson, L.L. Chee, G.H. Eng, B. Li, D.P. Lunny, D. Chuon, A. Venkatesh, K.H. Khoo, W.H. McLean, Y.P. Lim, E.B. Lane, The Human Intermediate Filament Database: comprehensive information on a gene family involved in many human diseases, Hum. Mutat. 29 (3) (2008) 351–360.
[22] B. Cogne, J.E. Bouameur, G. Hayot, X. Latypova, S. Pattabiraman, A. Caillaud, K. Si-Tayeb, T. Besnard, S. Kury, C. Chariau, A. Gaignerie, L. David, P. Bordure, D. Kaganovich, S. Bezieau, C. Golzio, T.M. Magin, B. Isidor, A dominant vimentin variant causes a rare syndrome with premature aging, Eur. J. Hum. Genet. (2020), https://doi.org/10.1038/s41431-020-0583-2 Online ahead of print.
[23] D.M. Toivola, P. Boor, C. Alam, P. Strnad, Keratins in health and disease, Curr. Opin. Cell Biol. 32 (2015) 73–81.
[24] C.S. Clemen, H. Herrmann, S.V. Strelkov, R. Schroder, Desminopathies: pathology and mechanisms, Acta Neuropathol. 125 (1) (2013) 47–75.
[25] M.R. Taylor, D. Slavov, L. Ku, A. Di Lenarda, G. Sinagra, E. Carniel, K. Haubold, M.M. Boucek, D. Ferguson, S.L. Graw, X. Zhu, J. Cavanaugh, C.C. Sucharov, C.S. Long, M.R. Bristow, P. Lavori, L. Mestroni, R. Familial Cardiomyopathy, B.D. Bank, Prevalence of desmin mutations in dilated cardiomyopathy, Circulation 115 (10) (2007) 1244–1251.
[26] K.J. Green, D.A. Parry, P.M. Steinert, M.L. Virata, R.M. Wagner, B.D. Angst, L.A. Nilles, Structure of the human desmoplakins, Implications for function in the desmosomal plaque, J Biol Chem 265 (19) (1990) 11406–11407.
[27] H.J. Choi, S. Park-Snyder, L.T. Pascoe, K.J. Green, W.I. Weis, Structures of two intermediate filament-binding fragments of desmoplakin reveal a unique repeat motif structure, Nat. Struct. Biol. 9 (8) (2002) 612–620.
[28] H. Kang, T.M. Weiss, I. Bang, W.I. Weis, H.J. Choi, Structure of the intermediate filament-binding region of desmoplakin, PLoS One 11 (1) (2016) e0147641.
[29] E. Odintsova, F. Mohammed, C. Trieber, P. Rodriguez-Zamora, C. Al-Jassar, T.H. Huang, C. Fogl, T. Knowles, P. Sridhar, J. Kumar, M. Jeeves, M. Chidgey, M. Overduin, Binding of the periplakin linker requires vimentin acidic residues D176 and E187, Commun Biol 3 (1) (2020) 83.
[30] C.H. Lee, M.S. Kim, B.M. Chung, D.J. Leahy, P.A. Coulombe, Structural basis for heteromeric assembly and perinuclear organization of keratin filaments, Nat. Struct. Mol. Biol. 19 (7) (2012) 707–715.
[31] C.G. Bunick, L.M. Milstone, The X-ray crystal structure of the keratin 1-keratin 10 Helix 2B heterodimer reveals molecular surface properties and biochemical insights into human skin disease, J Invest Dermatol 137 (1) (2017) 142–150.
[32] B. Nikolic, E. Mac Nulty, B. Mir, G. Wiche, Basic amino acid residue cluster within nuclear targeting sequence motif is essential for cytoplasmic plectin-vimentin network junctions, J. Cell Biol. 134 (6) (1996) 1455–1467.
[33] A.A. Chernyatina, S. Nicolet, U. Aebi, H. Herrmann, S.V. Strelkov, Atomic structure of the vimentin central alpha-helical domain and its implications for intermediate filament assembly, Proc. Natl. Acad. Sci. U. S. A. 109 (34) (2012) 13620–13625.
[34] D.D. Gae, M.S. Budamagunta, J.F. Hess, R.M. McCarrick, G.A. Lorigan, P.G. FitzGerald, J.C. Voss, Completion of the vimentin rod domain structure using experimental restraints: a new tool for exploring intermediate filament assembly and mutations, Structure 27 (10) (2019) 1547–1560 e4.
[35] P.D. Stenson, M. Mort, E.V. Ball, K. Shaw, A. Phillips, D.N. Cooper, The Human Gene Mutation Database: building a comprehensive mutation repository for clinical

and molecular genetics, diagnostic testing and personalized genomic medicine, Hum. Genet. 133 (1) (2014) 1–9.
[36] N.V. Whittock, H. Wan, S.M. Morley, M.C. Garzon, L. Kristal, P. Hyde, W.H. McLean, L. Pulkkinen, J. Uitto, A.M. Christiano, R.A. Eady, J.A. McGrath, Compound heterozygosity for non-sense and mis-sense mutations in desmoplakin underlies skin fragility/woolly hair syndrome, J Invest Dermatol 118 (2) (2002) 232–238.
[37] R. Alcalai, S. Metzger, S. Rosenheck, V. Meiner, T. Chajek-Shaul, A recessive mutation in desmoplakin causes arrhythmogenic right ventricular dysplasia, skin disorder, and woolly hair, J. Am. Coll. Cardiol. 42 (2) (2003) 319–327.
[38] B. Favre, N. Begre, L. Borradori, A recessive mutation in the DSP gene linked to cardiomyopathy, skin fragility and hair defects impairs the binding of desmoplakin to epidermal keratins and the muscle-specific intermediate filament desmin, Br. J. Dermatol. 179 (3) (2018) 797–799.
[39] M.C. Bolling, J.D.H. Jongbloed, L.G. Boven, G.F.H. Diercks, F.J.D. Smith, W.H. Irwin McLean, M.F. Jonkman, Plectin mutations underlie epidermolysis bullosa simplex in 8% of patients, J Invest Dermatol 134 (1) (2014) 273–276.
[40] N. Malecz, R. Foisner, C. Stadler, G. Wiche, Identification of plectin as a substrate of p34cdc2 kinase and mapping of a single phosphorylation site, J. Biol. Chem. 271 (14) (1996) 8203–8208.
[41] R. Spurny, K. Abdoulrahman, L. Janda, D. Runzler, G. Kohler, M.J. Castanon, G. Wiche, Oxidation and nitrosylation of cysteines proximal to the intermediate filament (IF)-binding site of plectin: effects on structure and vimentin binding and involvement in IF collapse, J. Biol. Chem. 282 (11) (2007) 8175–8187.
[42] T. Karashima, D. Tsuruta, T. Hamada, N. Ishii, F. Ono, K. Hashikawa, B. Ohyama, Y. Natsuaki, S. Fukuda, H. Koga, R. Sogame, T. Nakama, T. Dainichi, T. Hashimoto, Interaction of plectin and intermediate filaments, J. Dermatol. Sci. 66 (1) (2012) 44–50.
[43] P.V. Hornbeck, B. Zhang, B. Murray, J.M. Kornhauser, V. Latham, E. Skrzypek, PhosphoSitePlus, 2014: mutations, PTMs and recalibrations, Nucleic Acids Res 43 (Database issue) (2015) D512–D520.
[44] L.M. Godsel, S.N. Hsieh, E.V. Amargo, A.E. Bass, L.T. Pascoe-McGillicuddy, A.C. Huen, M.E. Thorne, C.A. Gaudry, J.K. Park, K. Myung, R.D. Goldman, T.L. Chew, K.J. Green, Desmoplakin assembly dynamics in four dimensions: multiple phases differentially regulated by intermediate filaments and actin, J. Cell Biol. 171 (6) (2005) 1045–1059.
[45] J.E. Bouameur, Y. Schneider, N. Begre, R.P. Hobbs, P. Lingasamy, L. Fontao, K.J. Green, B. Favre, L. Borradori, Phosphorylation of serine 4,642 in the C-terminus of plectin by MNK2 and PKA modulates its interaction with intermediate filaments, J. Cell Sci. 126 (Pt 18) (2013) 4195–4207.
[46] L.V. Albrecht, L. Zhang, J. Shabanowitz, E. Purevjav, J.A. Towbin, D.F. Hunt, K.J. Green, GSK3- and PRMT-1-dependent modifications of desmoplakin control desmoplakin-cytoskeleton dynamics, J. Cell Biol. 208 (5) (2015) 597–612.
[47] Z. Yang, N.E. Bowles, S.E. Scherer, M.D. Taylor, D.L. Kearney, S. Ge, V.V. Nadvoretskiy, G. DeFreitas, B. Carabello, L.I. Brandon, L.M. Godsel, K.J. Green, J.E. Saffitz, H. Li, G.A. Danieli, H. Calkins, F. Marcus, J.A. Towbin, Desmosomal dysfunction due to mutations in desmoplakin causes arrhythmogenic right ventricular dysplasia/cardiomyopathy, Circ. Res. 99 (6) (2006) 646–655.
[48] S.P. Chelko, A. Asimaki, P. Andersen, D. Bedja, N. Amat-Alarcon, D. DeMazumder, R. Jasti, C.A. MacRae, R. Leber, A.G. Kleber, J.E. Saffitz, D.P. Judge, Central role for GSK3beta in the pathogenesis of arrhythmogenic cardiomyopathy, JCI insight 1 (5) (2016).
[49] A. Robert, C. Hookway, V.I. Gelfand, Intermediate filament dynamics: what we can see now and why it matters, Bioessays 38 (3) (2016) 232–243.
[50] N.T. Snider, M.B. Omary, Post-translational modifications of intermediate filament proteins: mechanisms and functions, Nat Rev Mol Cell Biol 15 (3) (2014) 163–177.
[51] L. Winter, G. Wiche, The many faces of plectin and plectinopathies: pathology and mechanisms, Acta Neuropathol. 125 (1) (2013) 77–93.
[52] M. Gujrati, R. Mittal, L. Ekal, R.K. Mishra, SUMOylation of periplakin is critical for efficient reorganization of keratin filament network, Mol. Biol. Cell 30 (3) (2019) 357–369.
[53] M. Goto, H. Sumiyoshi, T. Sakai, R. Fassler, S. Ohashi, E. Adachi, H. Yoshioka, S. Fujiwara, Elimination of epiplakin by gene targeting results in acceleration of keratinocyte migration in mice, Mol. Cell. Biol. 26 (2) (2006) 548–558.
[54] D. Spazierer, J. Raberger, K. Gross, P. Fuchs, G. Wiche, Stress-induced recruitment of epiplakin to keratin networks increases their resistance to hyperphosphorylation-induced disruption, J. Cell Sci. 121 (Pt 6) (2008) 825–833.
[55] K.L. Wogenstein, S. Szabo, M. Lunova, G. Wiche, J. Haybaeck, P. Strnad, P. Boor, M. Wagner, P. Fuchs, Epiplakin deficiency aggravates murine caerulein-induced acute pancreatitis and favors the formation of acinar keratin granules, PLoS One 9 (9) (2014) e108323.
[56] S. Szabo, K.L. Wogenstein, C.H. Osterreicher, N. Guldiken, Y. Chen, C. Doler, G. Wiche, P. Boor, J. Haybaeck, P. Strnad, P. Fuchs, Epiplakin attenuates experimental mouse liver injury by chaperoning keratin reorganization, J. Hepatol. 62 (6) (2015) 1357–1366.
[57] P. Bawono, J. Heringa, PRALINE: a versatile multiple sequence alignment toolkit, Methods Mol. Biol. 1079 (2014) 245–262.