

# Plectin–intermediate filament partnership in skin, skeletal muscle, and peripheral nerve

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**Abstract** Plectin is a large, 500-kDa, intermediate filament (IF)-associated protein. It acts as a cytoskeletal crosslinker and signaling scaffold, affecting mechanical as well as dynamic properties of the cytoskeleton. As a member of the plakin family of cytolinker proteins, plectin has a multidomain structure that is responsible for its vast binding portfolio. It not only binds to all types of IFs, actin filaments and microtubules, but also to transmembrane receptors, proteins of the subplasma membrane protein skeleton, components of the nuclear envelope, and several kinases with known roles in migration, proliferation, and energy metabolism of cells. Due to alternative splicing, plectin is expressed as various isoforms with differing N-terminal heads that dictate their differential subcellular targeting. Through specific interactions with other proteins at their target sites and their ability to bind to all types of IFs, plectin molecules provide strategically located IF anchorage sites within the cytoplasm of cells. In this review, we will present an overview of the structural

features and functional properties of plectin and discuss recent progress in defining the role of its isoforms in stress-prone tissues and the implicated diseases, with focus on skin, skeletal muscle, and Schwann cells of peripheral nerve.

**Keywords** Plectin · Intermediate filaments · EBS · Skin · Skeletal muscle · Schwann cells

## Introduction

The cytolinker protein plectin, although able to interact with actin filaments and microtubules, is primarily an intermediate filament (IF)-binding protein. In this capacity, it anchors IFs to strategic cellular sites such as focal adhesions, desmosomes and hemidesmosomes (HDs), Z-disks and costameres, intercalated disks, as well as the nuclear, mitochondrial, and subplasma membrane skeleton, conferring mechanical stability to the IF network and thereby to the cell. Structurally, plectin bears no similarity to IF proteins except for possessing a central, in this case unusually long,  $\alpha$ -helical coiled coil rod domain that mediates dimerization and higher oligomer formation and is instrumental for networking and bridging different cellular structures. As plectin is expressed in practically all cell types, plectin mutations result in a pleiotropic phenotype affecting simultaneously several tissues primarily skin, muscle, and nerve.

Plectin, an IF-associated protein

Plectin was originally isolated from IF-enriched preparations of rat glioma C6 cells (Pytela and Wiche 1980). A few years later, a protein then named 300 K intermediate

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filament-associated protein (IFAP-300 K) was isolated from baby hamster kidney (BHK-21) cells based on its ability to codistribute with vimentin IFs (Lieska et al. 1985). In 1992, a protein referred to as HD-1 was identified as a major component of HDs isolated from bovine corneal epithelial cells (Hieda et al. 1992). With the advent of molecular cloning and sequencing, it was possible to show that the three proteins were identical (Clubb et al. 2000; Okumura et al. 1999; Wiche et al. 1991). In addition to its copurification and colocalization with IFs, plectin has been shown to directly bind to several IF subunit proteins including vimentin, glial fibrillary acid protein, neurofilaments (Foisner et al. 1988), desmin (Reipert et al. 1999), type I and II keratins (Geerts et al. 1999; Steinböck et al. 2000), and lamin B (Foisner et al. 1991). There is evidence that plectin-IF interactions are modulated by phosphorylation (Herrmann and Wiche 1983, 1987). For example, plectin dissociates from vimentin upon phosphorylation by protein kinase C (PKC), whereas binding to vimentin is enhanced by cAMP-dependent protein kinase (PKA) phosphorylation (Foisner et al. 1991). Likewise, plectin's association with lamin B is significantly decreased upon PKA- and PKC-phosphorylation of either binding partner (Foisner et al. 1991). During mitosis, plectin is phosphorylated by the cyclin-dependent kinase CDK1, triggering its dissociation from vimentin (Foisner et al. 1996). As the various plectin isoforms allocate IF-docking sites to different subcellular locations, plectin molecules exert a significant influence over the spatial and temporal distribution of IF networks (Wiche and Winter 2011; and see below).

#### Plectin: more than a crosslinker of the cytoskeleton

Besides its association with IFs, plectin has been shown to interact with the other two major cytoskeletal filament systems, actin-based microfilaments, and microtubules (MTs). Plectin interaction with the microfilament system was demonstrated by ultrastructural studies using immunogold-labeled antibodies to plectin. When whole mount electron microscopy was applied to glioma C6 cells, plectin was detected as thin (3-nm) filamentous structures that linked IFs (vimentin) to each other and also to actin filaments (Foisner et al. 1995). This association is consistent with plectin's occurrence at microfilament–plasma membrane junctions in all types of muscle (Wiche et al. 1983), its buildup at focal adhesions, and its colocalization with actin stress fibers (Andrä et al. 1998). Furthermore, plectin has a functional actin-binding domain (ABD; André et al. 1998) that is structurally similar to those of the spectrin/dystrophin superfamily of actin-binding proteins (Fontao et al. 2001, García-Alvarez et al. 2003, Sevcík et al. 2004). The binding affinity of plectin for actin is

comparable to that of other actin-binding proteins (Andrä et al. 1998) such as dystonin (Yang et al. 1996), tensin (Lo et al. 1994), and dystrophin (Jarrett and Foster 1995). The involvement and impact of plectin linking IFs to focal adhesions has recently been established (Bhattacharya et al. 2009; Burgstaller et al. 2010).

Plectin has been shown to bind to microtubule-associated proteins (MAPs; Herrmann and Wiche 1987), to copurify with MTs through repeated cycles of in vitro assembly/disassembly (Koszka et al. 1985), to form cross-bridges between IFs and MTs in fibroblasts depleted of the actin cytoskeleton (Svitkina et al. 1996), and to be part of the MT interactome during cell division (Gache et al. 2010; Mack and Compton 2001; Sauer et al. 2005). In a very recent report, it was shown that plectin binds to MAP2 via its SH3 domain. By seizing MAP2, plectin antagonizes MAP-mediated MT stabilization, and thus, destabilizes MTs (Valencia et al. 2013).

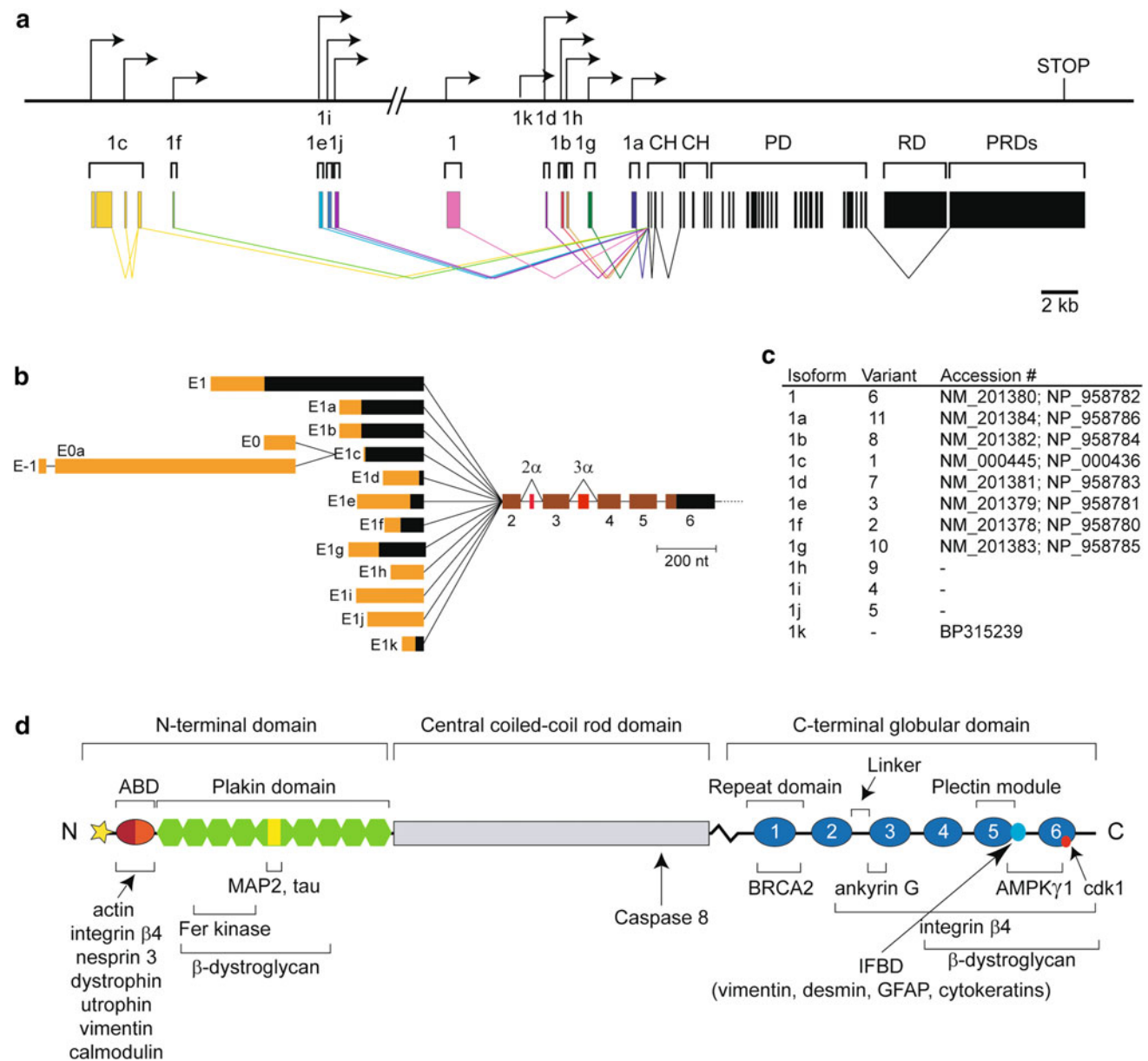
As plectin deficiency favors stress fiber formation and reduces MT dynamics, the binding of plectin to microfilaments and MTs cannot merely be seen as providing a structural scaffold but rather as contributing to their dynamic regulation.

#### Gene structure: alternative splicing as a source of functional diversity

Plectin has been cloned from a rat glioma C6 cell cDNA library (Wiche et al. 1991), a human placenta cDNA library (Liu et al. 1996), and a mouse skeletal muscle cDNA library (Fuchs et al. 1999). Likewise, plectin's gene locus has been analyzed in human (Liu et al. 1996; McLean et al. 1996), rat (Elliott et al. 1997), and mouse (Fuchs et al. 1999) genomes. The human plectin gene was mapped to 8q24 at the end of the long arm of chromosome 8 (Liu et al. 1996; McLean et al. 1996), and the mouse gene to a syntenic region on chromosome 15 (Fuchs et al. 1999); the rat gene is located on chromosome 7 (RefGene, GeneID: 64204). The gene spans over 62 kb and is split into 41 exons. The most striking feature is that multiple variable first exons are spliced into a common exon 2 (Fig. 1a, b). Nine of these exons (1-1g, 1k) are first coding exons (Fuchs et al. 1999; McInroy and Määttä 2011), while the coding regions of three others (1h-1j) start in exon 6, generating variants lacking most of plectin's (exons 2–8 encoded) ABD (Reznicek et al. 2003). Downstream of exon 2 there are 31 mostly constant exons, with exons 2–8, encoding the ABD (Liu et al. 1996; Sevcik et al. 2004). Two additional small exons, 2 $\alpha$  and 3 $\alpha$ , within the ABD-encoding genomic region are optionally spliced within exons 2 and 4. The following 22 exons, exons 9–30, encode the plakin domain (see below) of plectin, whereas single large exons (31 and

32) encode the central and C-terminal domains, respectively. Low level expression of a variant-lacking rod-encoding exon 31 has also been reported (Elliott et al. 1997; Fuchs et al. 2005).

The particular structure of the plectin gene has unveiled the mechanism for the generation of transcript variants differing only in their first coding exon, as each of the 12 variable exons is separately spliced into a common set of



**Fig. 1** Schematic representation of the plectin gene and the encoded protein. **a** Genomic organization of the plectin gene. Variable first coding exons are represented by colored boxes, constant exons by black boxes, introns by lines, and splicing by diagonal lines with the color code as for the exons; arrows indicate direction of transcription. **b** Transcripts generated by alternative splicing of the 5' end of the gene. Exons are indicated by boxes, orange areas within the boxes denote noncoding regions, black areas, coding regions. Brown boxes indicate coding sequences if preceded by one of the first coding exons (1-1g, 1k), or noncoding sequences if preceded by one of the noncoding exons (1h-1j). The black area within exon 6 denotes the first coding region of transcripts starting with exons 1h, 1i, and 1j. Two optionally spliced exons inserted between exons 2 and 4 are

shown in red. **c** Accession numbers of plectin transcript variants and isoforms. **d** Schematic representation of the protein. The N-terminal domain contains an actin-binding domain (ABD) consisting of two calponin homology domains (dark and light red), and a plakin domain comprising nine spectrin repeats (green) and one SH3 domain (yellow). The central rod domain is a 200-nm-long coiled coil. The C-terminal domain contains six plectin repeat domains, each consisting of a conserved region, referred to as module (blue), and a linker region, one of which harbors the IF-binding domain (IFBD, light blue). The different N termini of the isoforms are indicated by a star. Positions of the mapped binding regions to various proteins are indicated below the scheme

downstream exons. A similar gene structure has been found only in a few other genes (Zhang et al. 2004). Thus, most plectin isoforms differ just in their short N-terminal sequences (Fig. 1b). These short sequences are of crucial importance, however, as they determine the cellular localization of the isoforms and confer their tissue-specific expression (Reznicek et al. 2003). Plectin isoform 1 (P1), for example, is targeted to the nucleus/ER membrane (Reznicek et al. 2003), plectin 1a (P1a) to HDs (Andrä et al. 2003), P1b to mitochondria (Winter et al. 2008), P1c to MTs (Valencia et al. 2013), P1d to Z-disks (Konieczny et al. 2008), and P1f to focal adhesions and costameres (Burgstaller et al. 2010, Konieczny et al. 2008).

### Expression and cellular localization

Plectin is expressed in a wide variety of tissues and cell types, and it is particularly abundant in tissues subjected to great mechanical stress, such as stratified and simple epithelia, skeletal and heart muscle, and blood vessels (Wiche et al. 1983, 1984). At the cellular level, plectin is found at plasma membrane attachment sites of IFs and microfilaments, for instance, Z-disks in stratified muscle, dense plaques in smooth muscle, intercalated disks in cardiac muscle, HDs in the basal cell layer of stratified epithelia, desmosomes, and focal adhesions. Furthermore, plectin accumulates in cells forming tissue layers at the interface between tissues and fluid-filled cavities such as kidney glomeruli, liver bile canaliculi, bladder urothelium, gut villi, ependymal cell layers lining the cavities of brain and spinal cord, glial-endothelial interfaces, and endothelial cells of blood vessels (Errante et al. 1994; Lie et al. 1998; Wiche et al. 1983; Yaoita et al. 1996). Plectin is also a major component of trabecular meshwork cells of the eye which control intraocular pressure (Inoue et al. 2010).

### Protein structure and binding partners

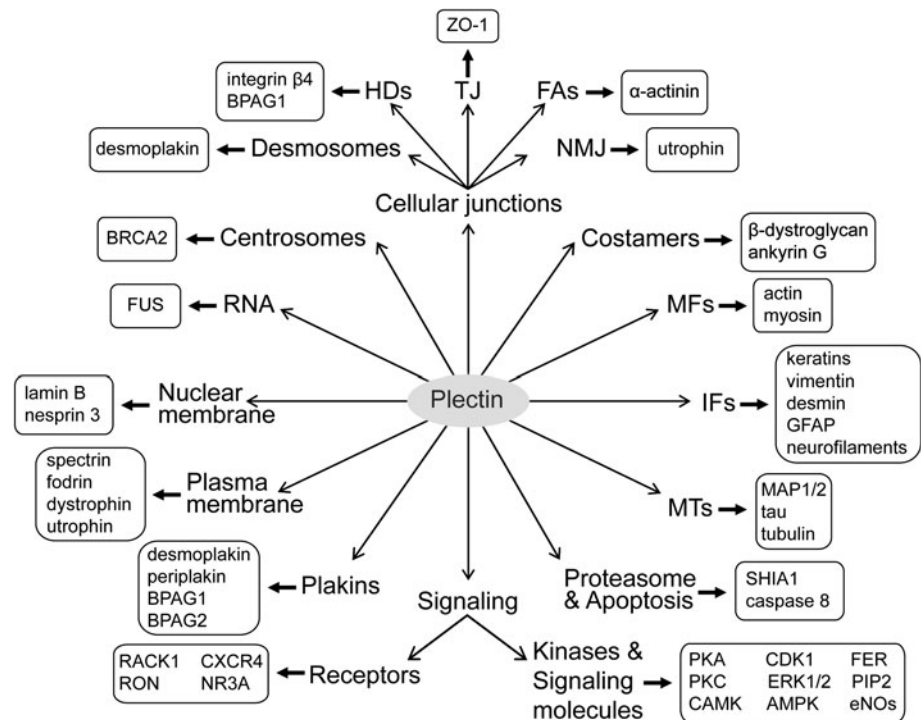
Full-length plectin is expressed as a 499–533-kDa protein depending on the particular plectin isoform. Using rotary shadowing electron microscopy, plectin has been visualized as a dumbbell-like structure comprising a central 200-nm-long rod domain flanked by two large globular domains (Foisner and Wiche 1987). This structure has been confirmed by secondary structure predictions based on plectin's amino acid sequence (Wiche et al. 1991). Functionally, plectin is a multidomain protein with similarities to other cytoskeletal proteins of the plakin family (Sonnenberg and Liem 2007). The amino-terminal domain harbors a conventional ABD preceding the plakin domain (Fig. 1d). The ABD is composed of two calponin homology domains closely resembling the ABD of fimbrin (Sevcík et al. 2004), whereas the plakin domain comprises

nine spectrin repeats with one Src-homology 3 (SH3) domain inserted in repeat 5 (Sonnenberg et al. 2007). The central rod domain contains an almost continuous 1127 residue long, mainly  $\alpha$ -helical coiled coil showing long stretches of heptads repeats with a staggered charge periodicity of 10.4 for both acidic and basic residues (Wiche et al. 1991). The C-terminal domain contains six plectin repeat domains (PRDs) and a short terminal tail. Each PRD is built up of a conserved core region and short linker sequences connecting the cores (Janda et al. 2001, Wiche et al. 1991). The PRDs are most likely tightly packed through interlinkage by hydrogen bonds and disulfide bridges (Spurny et al. 2007), forming a compact C-terminal globular domain. The C-terminal tail represents a 70 residues long serine-rich region.

As a protein composed of multiple functional domains, plectin is able to interact with a vast array of different proteins via one or more of those domains (Figs. 1d, 2). Thereby it can act as a mechanical reinforcement or a scaffold for signaling molecules, or both. Plectin binds to vimentin (Nikolic et al. 1996), desmin (Favre et al. 2011; Reipert et al. 1999), glial fibrillary acidic protein (Foisner et al. 1988), lamin B (Foisner et al. 1991), and cytokeratins (Steinböck et al. 2000) via an IF-binding site located in the linker region between PRDs 5 and 6. The IF-binding site has been further narrowed down to a stretch of  $\sim 50$  amino acids containing a basic residue cluster typically found in nuclear localization signals (Nikolic et al. 1996). The ABD of plectin turned out to be multifunctional, binding not only to actin (Fuchs et al. 1999) but also to integrin  $\beta 4$  via the first pair of fibronectin type III repeats (Geerts et al. 1999; Reznicek et al. 1998), vimentin (Sevcik et al. 2004), nesprin 3 (Wilhelmsen et al. 2005), the EF-ZZ domain of dystrophin and utrophin (Reznicek et al. 2007), and calmodulin (Kostan et al. 2009). Binding of F-actin and integrin  $\beta 4$  to the ABD of plectin has been shown to be mutually exclusive (Geerts et al. 1999; Litjens et al. 2003). Additional binding sites for integrin  $\beta 4$  and  $\beta$ -dystroglycan have been mapped to the plakin and C-terminal repeat domains of plectin (Koster et al. 2004; Reznicek et al. 1998, 2007). The plakin domain also serves as a binding site for the hemidesmosomal transmembrane protein, bullous pemphigoid antigen 180 (BPAG2; Koster et al. 2003), and the nonreceptor tyrosine kinase Fer (Lunter and Wiche 2002). Furthermore, the SH3 domain embedded in the plakin domain serves as a docking site for MAP2 (Valencia et al. 2013). Regarding the rod domain, there is evidence that it mediates plectin self-association via coiled coil interactions (Foisner and Wiche 1987; Walko et al. 2011). It also recruits caspase 8 that cleaves plectin toward the end of the rod (Asp 2395) in the early stages of apoptosis (Stegh et al. 2000). Plectin is rich in cysteine residues (34 in humans) half of which are spread over its C-terminal



**Fig. 2** Schematic representation of proteins known to interact directly with plectin. Plectin is shown in the center with the interacting proteins grouped by their cellular location or functional categories. Abbreviations (clockwise from top): *HDs* hemidesmosomes, *TJ* tight junctions, *FAs* focal adhesions, *NMJ* neuromuscular junction, *MFs* microfilaments, *IFs* intermediate filaments, and *MTs* microtubules. For details see text



globular domain. One of these, a highly reactive cysteine present in PRD5, has been shown to serve as a target for nitrosylation *in vitro* and presumably is also the one responsible for plectin's nitrosylation observed *in vivo* (Spurny et al. 2007). The phosphorylation site for mitotic CDK1 has been mapped to threonine 4542 (Swiss-Prot: P30427) at the end of PRD 6 (Malecz et al. 1996), and a high-affinity binding site for the ubiquitin ligase Shia E3 to residues 95–117 of plectin isoform 1 (House et al. 2003).

Many other interaction partners have been identified, among them the membrane skeleton proteins fodrin and  $\alpha$ -spectrin (Brown et al. 2001; Herrmann and Wiche, 1987), desmoplakin (Eger et al. 1997), bullous pemphigoid antigen (BPAG) 1 and 2 (Koster et al. 2003; Steiner-Champlaud et al. 2010), the outer nuclear membrane protein nesprin 3 (Wilhelmsen et al. 2005), the tight junction protein ZO-1 (zona occludens-1; Chen et al. 2006), and skeletal muscle-specific ankyrins (Maiweilidan et al. 2011). The breast cancer susceptibility gene product, BRCA2, which associates with the centrosome during S and early M phase, interacts with plectin forming a complex that controls centrosome localization (Niwa et al. 2009). Furthermore, plectin regulates FUS (fused in sarcoma), an RNA-binding protein involved in transcription, splicing, and mRNA transport, by sequestering it in the cytoplasm (Thomsen et al. 2012).

Lately, it has become clear that plectin, in addition to structurally reinforcing the cytoskeleton, acts as a scaffold for molecules and proteins involved in signaling, by positioning them at specific sites within the cells. Examples are

the signaling molecule phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>; involved in the interaction of plectin with actin; Andrä et al. 1998), the nonreceptor tyrosine kinase Fer (requires plectin to turn down its autophosphorylation; Lunter and Wiche 2002), the receptor for activated C kinase 1 (RACK1; when bound to plectin downregulates PKC signaling during the initial stages of cell adhesion; Osmanagic-Myers and Wiche 2004), the  $\gamma$ -subunit of AMP-activated protein kinase (AMPK; associates with Z-disk-bound plectin in differentiated myofibers; Gregor et al. 2006), components of the MAP kinase Erk 1/2 signaling pathway (required for controlled cell migration; Osmanagic-Myers et al. 2006), the chemokine receptor CXCR4 (plays an important role in stromal-derived factor-1 signaling and trafficking, and in HIV-1 infection; Ding et al. 2008), the RON receptor (a member of the Met proto-oncogen family frequently overexpressed in pancreatic cancer; Yu et al. 2012), and the NR3a subunit of the glutamate receptor (Eriksson et al. 2007).

#### Plectin and human diseases

In 1996, several groups reported that mutations in the plectin gene (*PLEC*) were the cause of autosomal recessive forms of the skin disease epidermolysis bullosa simplex associated with muscular dystrophy (EBS-MD; OMIM # 226670). Patients with EBS-MD lack plectin expression in their cells due to premature stop codons coupled with nonsense-mediated mRNA decay. In the meantime, other forms of EBS have been ascribed to mutations in the

plectin gene, such as EBS with pyloric atresia (EBS-PA; OMIM #612138) and EBS-MD with myasthenic syndrome (EBS-MD-MyS). The first and thus far only autosomal dominant plectin mutation, known as EBS-Ogna (OMIM #131950), was reported in 2002. Carriers of this mutation suffer from a generalized skin fragility but do not show a muscular phenotype. More recently, also patients, that show only a muscular phenotype without skin abnormalities, have been identified; these patients carry a mutation in the first coding exon of isoform P1f (Gundesli et al. 2010). More details about phenotypes caused by plectin mutations will be given below.

A few recent reports have implicated plectin upregulation in several types of epithelial cancers (Katada et al. 2012; Bausch et al. 2009; Lee et al. 2004; Pawar et al. 2011). Furthermore, plectin has been identified in a phage display screen for biomarkers of pancreatic ductal adenocarcinoma (Kelly et al. 2008). In this type of malignant cells, plectin translocates to the cell membrane, whereas in non-tumor cells, plectin is restricted to the cytoplasmic compartment; concurrently, expression of plectin increases with tumor progression and metastasis (Bausch et al. 2009, Bausch et al. 2011). Due to the specificity of plectin for premalignant and high grade pancreatic lesions, plectin is being developed as an in vivo imaging biomarker for pancreatic cancer (Bausch et al. 2011; Konkalmatt et al. 2013).

## Plectin in skin

### Expression and subcellular localization

The analysis of plectin transcripts in murine skin revealed expression of all eight plectin isoforms starting with alternative first coding exons (Fuchs et al. 1999). Immunofluorescence microscopy of skin sections showed that P1a expression was restricted to the basal (proliferating) keratinocyte cell layer of interfollicular epidermis, whereas P1c was found in basal as well as suprabasal (differentiating) keratinocyte cell layers (Ackerl et al. 2007; Andrä et al. 2003; Walko et al. 2011) (Fig. 3a). Plectin, presumably P1a, is also expressed in the outer root sheath of hair follicles (Ackerl et al. 2007; Joubert et al. 2003). Coexpression of P1a and P1c in basal keratinocyte cell cultures has been demonstrated on both, mRNA and protein levels (Andrä et al. 2003; Kostan et al. 2009; Walko et al. 2011), with P1a being expressed at highest level (Andrä et al. 2003). Similar to the in vivo situation, expression of P1c was maintained when cultured keratinocyte monolayers were induced to stratify and undergo terminal differentiation, whereas expression of P1a was down-regulated (Kostan et al. 2009). The most prominently expressed isoforms in epidermal

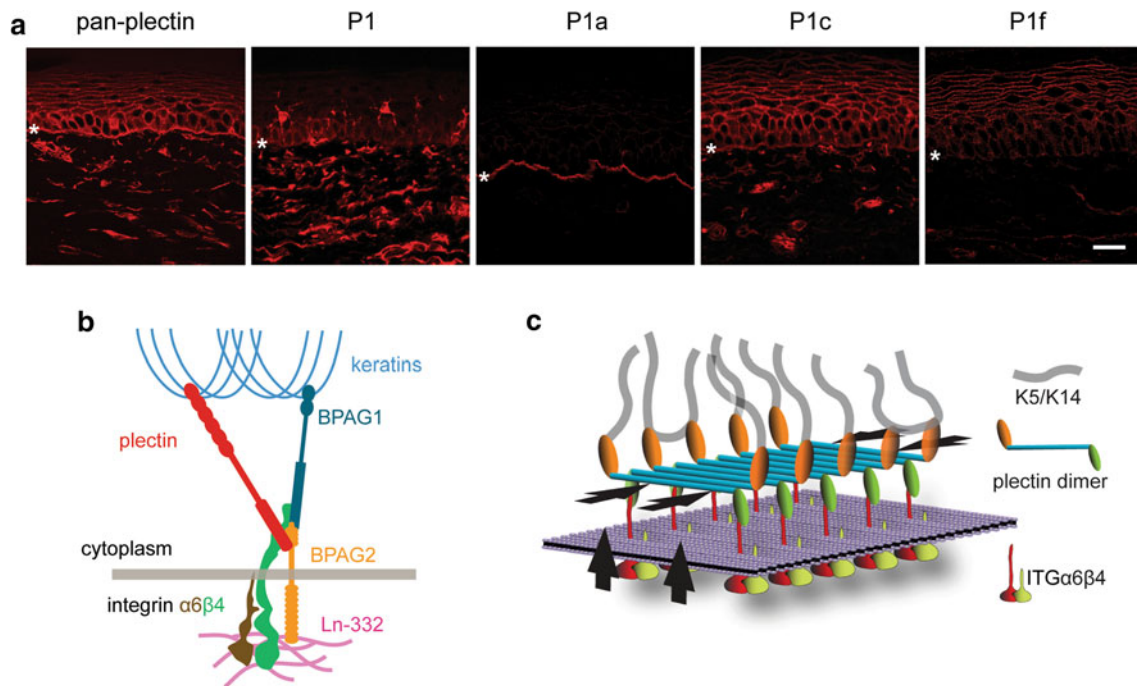
cells other than keratinocytes are P1 (Langerhans cells and Thy 1.2-expressing T lymphocytes; Abrahamsberg et al. 2005) and P1c (melanocytes; unpublished observations of the authors). In dermal fibroblasts, expression of plectin isoforms P1, P1b, P1c, and P1f has been demonstrated (Abrahamsberg et al. 2005; Burgstaller et al. 2010; Winter et al. 2008), and, upon skin wounding, also the macrophages and T lymphocytes infiltrating the dermis are P1 and P1f positive (Abrahamsberg et al. 2005). In addition to full-length plectin isoforms, variants lacking the central rod domain have been detected in cultured keratinocytes and/or dermal fibroblasts on mRNA as well as protein levels (Koster et al. 2004; Natsuga et al. 2010a, b).

### Specific functions of isoforms

#### *P1a: key element for assembly and homeostasis of HDs*

Based on plectin's prominence at plasma membrane junctional sites of keratin IFs in the epidermis (Wiche et al. 1983, 1984), it had been anticipated early on that plectin could be a component of HDs and desmosomes. HDs are specialized IF-associated multiprotein complexes that provide stable adhesion of basal epithelial cells to the underlying basement membrane in stratified as well as certain complex and simple epithelia (Margadant et al. 2008), while desmosomal complexes link IFs to sites of intercellular adhesion. In contrast to HDs, desmosome-like structures are also found in non-epithelial tissues, such as heart and skeletal muscle (Delmar and McKenna 2010, Green and Simpson 2007). The high molecular mass IF-associated proteins, HD1 and IFAP300, later identified as orthologs of plectin, had in fact been characterized in early studies as component of HDs and desmosomes in bovine tongue tissue and in a rat epithelial cell line (Hieda et al. 1992; Skalli et al. 1994). The first evidence for isoform P1a being a hemidesmosomal protein was provided by immunogold electron microscopy of rat skin applying isoform-specific antibodies (see Reznicek et al. 1998, Fig. 9), before a series of subsequent studies clearly established P1a as the plectin isoform specifically associating with HDs (Andrä et al. 2003; Kostan et al. 2009).

Studies in cultured keratinocytes have demonstrated that the association of integrin  $\alpha 6\beta 4$  with P1a via the integrin  $\beta 4$  subunit and their clustering is crucial for the formation of HDs. This initial P1a/integrin  $\beta 4$  association fosters the recruitment of BPAG2 and BPAG1e molecules to the complex and the anchorage of the keratin IF network through P1a and BPAG1e follows (Hopkinson and Jones 2000; Koster et al. 2003; Litjens et al. 2006). Additional stabilization of HDs against mechanical stress appears to be mediated by lateral interaction of P1a molecules via their rod domains (Walko et al. 2011). This notion is also



**Fig. 3** Expression of plectin isoforms in mouse skin and schematic models of HDs. **a** Cryosections of adult mouse skin were subjected to immunofluorescence microscopy using pan-plectin and isoform-specific antibodies as indicated. Asterisks indicate basal cell membrane separating epidermis (top) from dermis (bottom). Note, *P1*-specific antibodies strongly stained dermal fibroblasts and epidermal dendritic cells, but failed to stain the upper epidermal layers; staining of basal epidermal keratinocytes was negligible. *P1a* immunoreactivity was restricted to the basal membrane of basal keratinocytes, while *P1c*-specific signals were more prominent in cells of the upper epidermal layers. Anti-*P1f* antibodies showed specific but relatively faint peripheral staining of upper layer keratinocytes. Bar 20  $\mu\text{m}$ . **b** Schematic drawing of the major components of skin HDs. The

transmembrane domain proteins integrin  $\alpha 6 \beta 6$  and *BPAG2* bind to laminin 322 (*Ln-322*), a major component of the extracellular matrix, and to the cytoplasmic proteins plectin and *BPAG1*, which in turn connect to the keratin IF network. **c** Hypothetical model for HD stabilization through plectin self-association. *P1a* clusters integrin  $\alpha 6 \beta 4$  molecules at the basal cell surface and strengthens the HD through its association with keratin K5/14 filaments generating a vertical force component (arrows pointing upwards). Lateral association of dimeric *P1a* proteins via their rod domains generates an additional (horizontal) force component that exerts cohesion and compaction (opposing arrows). (**b** and **c** are modified from Rezniczek et al. 2010 and Walko et al. 2011, respectively)

supported by the fact that rodless isoforms can only partially substitute for full-length *P1a* in maintaining HD function in skin of EBS-MD patients (Koster et al. 2004; Natsuga et al. 2010a, b). Lateral aggregation of *P1a* molecules in HDs also appears to protect *P1a* from degradation via calpains, a family of calcium-dependent proteases that could be involved in HD destruction to mediate migration and terminal differentiation (Kostan et al. 2009; Walko et al. 2011). The importance of the integrin subunit  $\beta 4$ -*P1a* interaction is confirmed in vivo by the much reduced level or complete absence of HDs in mice deficient in either plectin, or integrin subunits  $\alpha 6$  and  $\beta 4$ , which are born with large patches of detached epidermis and generally die within a few hours after birth (Andrä et al. 1997; Dowling et al. 1996; Georges-Labouesse et al. 1996; van der Neut et al. 1996). Similarly, the anchorage of keratin IFs at HDs was found to be compromised in mice carrying null alleles of either plectin or *BPAG1e* (Andrä et al. 1997; Guo et al. 1995), or in knock-in mice carrying the dominant negative Oгна mutation (Walko et al. 2011). Furthermore,

underscoring the importance of *P1a* for HD function, skin blistering due to compromised assembly and function of HDs was observed in EBS-Oгна mice (having reduced *P1a* levels), but not in mice that were deficient in *P1c* (Fuchs et al. 2009; Walko et al. 2011).

Studies of adult mice carrying conditional deletions of either plectin or the integrin subunit  $\alpha 6$  in the epidermis indicated that in vivo HDs are very stable structures, exhibiting a very low turnover (Ackerl et al. 2007; Niculescu et al. 2011). However, under certain circumstances, such as wound healing or carcinoma invasion, HDs in migrating and proliferating keratinocytes turn into highly dynamic structures, similar to keratinocytes cultivated ex vivo where HDs assemble and disassemble continuously (Geuijen and Sonnenberg 2002; Ozawa et al. 2010). The mechanism of HD disassembly has been addressed in several studies. It was shown that *P1a* binds to integrin subunit  $\beta 4$  via multiple interaction domains including its N-terminal ABD (de Pereda et al. 2009; Koster et al. 2004; Rezniczek et al. 1998). Growth factor-induced elevations of



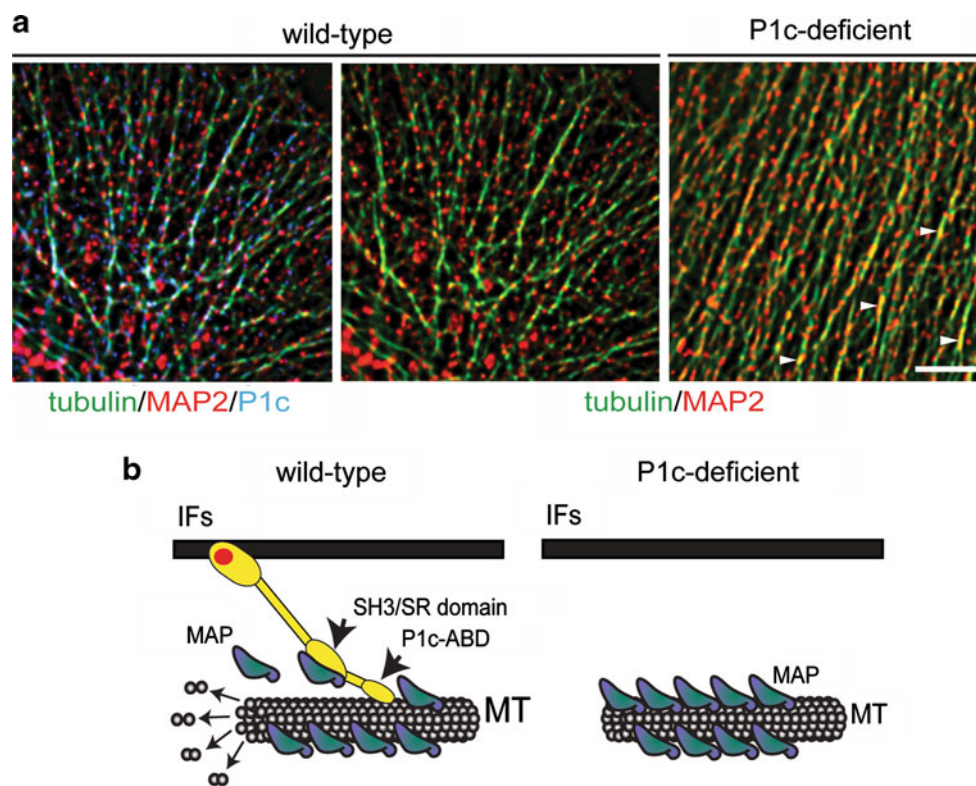
intracellular  $\text{Ca}^{2+}$  levels lead to the activation of  $\text{PKC}\alpha$  that phosphorylates the integrin  $\beta 4$  subunit on serine and threonine residues located in domains mediating the interaction with plectin (Frijns et al. 2012; Margadant et al. 2008; Rabinovitz et al. 2004), while  $\text{Ca}^{2+}$ /calmodulin interacts with P1a's ABD (Kostan et al. 2009). Both events cause the dissociation of P1a from membrane-bound integrin followed by a redistribution of both proteins and disassembly of HDs (see Kostan et al. 2009, Fig. 9).

#### *P1c: regulator of MT dynamics in keratinocytes*

The immunolocalization of P1c, the second major isoform of plectin expressed in basal keratinocytes, reveals partial colocalization with MTs, mostly in a dotted pattern (Fig. 4a). Unexpectedly, and contrary to the function of other cytolinker proteins previously analyzed, P1c has recently been shown to act as a MT destabilizer (Valencia et al. 2013). Compared to wild-type cells, MTs in P1c-deficient keratinocytes were found to be more resistant toward nocodazole-induced disassembly and to display

increased acetylation of tubulin, a modification indicating reduced dynamic behavior. In addition, live imaging of MTs in P1c-deficient as well as in plectin-null cells revealed decreased MT dynamics. Accordingly, increased MT stability in keratinocytes due to P1c deficiency led to changes in a number of cellular properties and functions known to be MT dependent, including altered cell shape, increased velocity but loss of directionality of migration, smaller-sized focal adhesions, higher glucose uptake due to increased transfer of glucose transporters to the plasma membrane, and mitotic spindle aberrations combined with reduced growth rates of cells.

A mechanism proposed for microtubule destabilization through P1c, based on *ex vivo* and *in vitro* assays, is depicted by the cartoon shown in Fig. 4b. According to this model, P1c is targeted to MTs via its isoform-specific N-terminal sequence (including the ABD), while one of its further downstream molecular domains, the SH3-like domain embedded within its spectrin repeats (plakin domain), interacts with MT-associated proteins (MAPs), known stabilizers of MT polymers. Since the binding of



**Fig. 4** *P1c*: a MT destabilizer. **a** Deconvolved immunofluorescence microscopy images of cytoplasmic regions of wild-type (*left and middle panel*) and *P1c*-deficient (*right panel*) keratinocytes. Antibodies used are indicated; merged triple- or double-channel images are shown. Note *dotted* decoration of MTs by both, *P1c* and *MAP2*, in wild-type cells, whereas in *P1c*-deficient cells, MTs were decorated with *MAPs* over much longer distances. **b** Proposed mechanism of *P1c*-mediated MT destabilization. *P1c*, anchored to *IFs* via its

C-terminal IF-docking site (*red sphere*), binds to MTs via its isoform-specific N-terminal sequence (including the *ABD*), while the *SH3* domain located within the spectrin repeats (*SH3/SR*) binds to *MAPs*. Interference with the MT-stabilizing function of *MAPs* ensures a dynamic MT network in wild-type cells (*left panel*), whereas in *P1c*-deficient cells, more *MAPs* can bind along MTs leading to their stabilization (*right panel*). (**a** and **b** correspond to parts of Fig. 9 in Valencia et al. 2013)



plectin's SH3 domain antagonizes the MT-stabilizing and assembly promoting function of MAPs, P1c interaction with MTs leads to MT destabilization, which in turn favors a dynamic state of the MT network in cells. On the other hand, when P1c is absent, such as in P1c-deficient cells, more MAPs are bound along MTs (Fig. 4a) leading to the stabilization of the polymer (Fig. 4b). Based on experiments in which truncated forms of P1c were expressed into P1c-deficient keratinocytes to assess their potential for rescuing MT deficits, it was concluded that normal MT properties could be restored in P1c-deficient keratinocytes only if P1c variants were used that contained the C-terminal IF-binding domain of the molecule and thus were able to associate with the keratin IF network (Valencia et al. 2013). Hence, beyond revealing an unexpected and novel function of P1c and plectin in general, this study opened an intriguing new perspective on how the cytoskeleton regulates its dynamics by uncovering the potential of IFs to destabilize MTs via an associated cytolinker protein, thereby stimulating MT dynamics.

*P1: the missing link to the nucleus of skin cells and a role in migration?*

Plectin has been shown to bind to the outer nuclear envelope protein nesprin 3 $\alpha$ , thereby linking IFs to the nucleus in keratinocytes, fibroblasts, and other cell types (Ketema et al. 2007; Wilhelmsen et al. 2005). It remains to be established which plectin isoform is associated with nesprin 3 $\alpha$ . P1 would definitively be a good candidate, as studies in muscle tissue indicated that P1 is a major linker component between IFs and the nuclear/ER membrane (Konieczny et al. 2008; Rezniczek et al. 2007). Indeed, P1 transcripts have been identified in cultured keratinocytes (Andrä et al. 2003).

The analysis of primary dermal fibroblasts derived from isoform P1-deficient mice revealed abnormalities in the actin cytoskeleton and an impaired migration potential of the cells (Abrahamsberg et al. 2005), reminiscent of plectin-null fibroblasts (Andrä et al. 1998). Along these lines, in skin wound healing experiments carried out with P1-deficient mice, reduced leukocyte infiltration was observed during in vivo wound healing, and P1-deficient T cells isolated from lymph nodes of these mice showed diminished chemotactic migration ex vivo (Abrahamsberg et al. 2005). While these findings demonstrate that P1 is involved in the regulation of leukocyte recruitment during skin wound healing, the underlying mechanism remains to be determined. Nuclear positioning via P1-IF networking could possibly play a role.

Special functions of other isoforms expressed in epidermal and/or dermal skin cells, particularly of P1f, have not been analyzed. Based on P1f's predominantly

peripheral localization in keratinocytes (Fig. 3a), fibroblasts, and myofibers (see below), a role of this isoform in actin-network regulation (Andrä et al. 1998), in addition to its IF-anchoring function, is not unexpected.

Human skin disease phenotypes caused by plectin mutations

In 1996, several groups reported that patients suffering from the skin blistering disease epidermolysis bullosa simplex with muscular dystrophy, lacked plectin expression in skin and muscle tissues due to defects in the plectin gene (Chavanas et al. 1996; Gache et al. 1996; McLean et al. 1996; Pulkkinen et al. 1996; Smith et al. 1996). Since then, mutations within the plectin gene were identified in an increasing number of EBS variants with skin involvement. These variants include EBS-MD, EBS-MD-MyS, EBS-PA, and EBS-Ogna (reviewed in Rezniczek et al. 2010; Winter and Wiche 2013). In all plectin-associated EBS variants, skin blistering is caused by trauma-induced rupture of basal epidermal keratinocytes in their cytoplasm, usually at a level just above the basal cell membrane-anchored HDs. Cell integrity in the suprabasal keratinocyte cell layers is generally not affected, and cell–cell interactions are preserved.

Until now, there are reports of 30 independent cases of EBS-MD, in which the diagnosis was confirmed by identifying the mutation (see Table 1 in Winter and Wiche 2013; and additional cases reported in Charlesworth et al. 2013). At the molecular level, EBS-MD is frequently associated with nonsense, insertion, or deletion mutations, resulting in premature stop codons within exon 31, the exon that encodes plectin's coiled coil rod domain. This predicts truncated polypeptides and mRNA downregulation through nonsense-mediated mRNA decay. As processing of the rodless variant of plectin remains unaltered by mutations in exon 31 and as the expression of the rodless isoform has been detected in cells of EBS-MD patients (Koster et al. 2004; Natsuga et al. 2010a, b), it has been postulated that rodless plectin could partially rescue some basic functions of plectin in association with its N- and C-terminal domains. EBS-MD was further found to be caused by several in-frame insertion and deletion mutations in exons located up- or downstream of exon 31 (Charlesworth et al. 2013; Winter and Wiche 2013). It is likely that these mutations affect specific protein–protein interactions relevant for the proper functioning of plectin in HDs. In general, clinical phenotypes of affected individuals vary considerably with respect to severity of skin blistering. In addition to congenital skin blistering, deformation of the nails and tooth decay were observed in a number of EBS-MD patients, and in some cases, erosive lesions on the oral or laryngeal mucosa, hoarseness, respiratory complications

during infancy, and urethral strictures have been reported (Babic et al. 2010; Charlesworth et al. 2013; Kunz et al. 2000; Schara et al. 2004).

EBS with pyloric atresia (EBS-PA) is one of the most severe EBS variants. It manifests as severe generalized neonatal skin blistering and gastric abnormalities (pyloric or duodenal atresia) and frequently leads to early postnatal demise of affected individuals. Eleven patients with EBS-PA have been reported so far carrying homozygous or compound heterozygous mutations (Charlesworth et al. 2013; Winter and Wiche 2013). The majority (9) of EBS-PA-associated mutations have been localized in exons encoding the N-terminal region of plectin, some (6) were found in exon 32 encoding the C-terminal domain, and only one (situated at the very distal portion of exon 31) affects the rod domain of plectin. This specific localization pattern of mutations suggests that the EBS-PA phenotype could result from genetic mutations specifically affecting the N- and C-terminal portions of plectin that are harboring the integrin  $\beta 4$ - and IF-binding sites. Moreover, since these mutations are mostly outside of the rod, premature termination will lead to loss of expression of both, full-length and rodless, plectin isoforms (Charlesworth et al. 2003; Natsuga et al. 2010a), and thus to a more severe phenotype. Interestingly, a first case of non-lethal EBS-PA improving with age has recently been reported (Charlesworth et al. 2013). This clinical phenotype was found to be caused by two compound heterozygous mutations resulting in premature termination codons within exons 30 and 32. Considering that one of the mutations allows the synthesis of a truncated version of the plectin molecule missing only its most C-terminal part (PRD 6 and tail) and that low but detectable expression of plectin has been demonstrated in the patient's epidermis, it has been proposed that the truncated protein, which still harbors the critical residues for integrin  $\alpha 6\beta 4$ - and IF-binding, could partially compensate for the loss of plectin expression from the other allele (Charlesworth et al. 2013).

EBS-MD-MyS is a rare EBS variant that has been diagnosed in three patients with compound heterozygous PLEC mutations (Banwell et al. 1999; Forrest et al. 2010; Selcen et al. 2011), and additionally in one patient with a homozygous insertion mutation in the gene encoding the acetylcholine receptor subunit CHRNE (Maselli et al. 2010). Three of the patients revealed marked skin blistering from birth, whereas one exhibited only sparse signs of epidermolysis bullosa (Banwell et al. 1999; Forrest et al. 2010; Selcen et al. 2011).

Besides the recessive forms of plectin-linked EBS, there is one autosomal dominant form designated EBS-Ogna. This rare mutation was originally identified in a kindred near the small Norwegian town of Ogna (hence its name) and later in four unrelated German families and two Dutch

individuals (Bolling 2010a; Gedde-Dahl 1971; Kiritsi et al. 2013; Koss-Harnes et al. 2002). EBS-Ogna is caused by a heterozygous missense mutation, R2000 W, within plectin's rod-encoding exon 31 (Kiritsi et al. 2013; Koss-Harnes et al. 2002). In contrast to the other more severe plectin-associated EBS variants, EBS-Ogna has a mild course with localized acral skin blistering and formation of erosions, which predominate with increasing age (Kiritsi et al. 2013; Koss-Harnes et al. 2002). Other skin-related symptoms encompass posttraumatic violaceous macules, hypopigmented macules, mild focal palmoplantar keratoderma, and pitting of the teeth (Kiritsi et al. 2013; Koss-Harnes et al. 2002). EBS-Ogna patients do not develop MD, nor do they show signs of PA. The molecular mechanisms underlying the unique skin phenotype have been shown to involve the isoform-specific proteolytic degradation of P1a in HDs of basal keratinocytes (Walko et al. 2011). Besides being the only mutation with an autosomal dominant type of inheritance reported thus far, the Ogna mutation is emerging as the most frequent mutation of plectin described in families of different ethnic origins, suggesting that it may represent a hotspot for mutations within the plectin gene.

#### Mouse models for plectin-related skin blistering diseases

To mimic the clinical features of plectin-associated skin blistering diseases in an animal model, several transgenic mouse lines have been generated. The first was a plectin-null mouse (Andrä et al. 1997), followed by a conditional knockout mouse line, where the ablation of plectin was restricted to keratin 5-expressing epithelia (K5-Cre/cKO; Ackerl et al. 2007). Within 1 to 2 days after birth (occasionally even at birth), plectin-null mice begin to show signs of gross skin blistering, especially at fore- and hindlimbs and, in some cases, around the mouth and nasal cavities (Andrä et al. 1997). In K5-Cre/cKO mice, skin blistering develops with a similar onset, body distribution, and severity (Ackerl et al. 2007). In some cases, plectin-null and K5-Cre/cKO mice are born with denuded paws (authors' unpublished observations), resembling aplasia cutis congenita, similar to the most severe cases reported for plectin-associated EBS-PA (Charlesworth et al. 2003, 2013; Nakamura et al. 2005; Pfendner and Uitto 2005). In plectin-null mice, blisters on the lower extremities are often hemorrhagic (Andrä et al. 1997), which, however, is not observed in K5-Cre/cKO mice (authors' unpublished observations). This type of bleeding might indicate a possible perturbation of vascular endothelial cells, which normally express plectin at relatively high levels (Errante et al. 1994; Wiche et al. 1983). Epidermal desmosomes and consequently cell-cell interactions were found to be

unaffected by keratinocyte-specific deletion of plectin (Ackerl et al. 2007). Inspection of the upper gastrointestinal tract of newborn K5-Cre/cKO mice after at least one nursing revealed the presence of multiple blisters on their palates and in some cases also on their tongues but not in the esophagus. Thus, plectin knockout mice seem to die from malnutrition as a consequence of blistering of the oral epithelia and possibly obstruction of the oral cavity by especially large blisters or cellular debris (Ackerl et al. 2007). Recently, a mouse line carrying the EBS-Ogna mutation has been generated (Walko et al. 2011). EBS-Ogna mice mimic the human disease by displaying mild skin fragility but no extracutaneous pathologies. The skin phenotype of EBS-Ogna mice reflects sparse and dysfunctional HDs, and insufficient protein levels of (HD-associated) P1a, whereas P1c is maintained at normal levels (Walko et al. 2011). Noteworthy, HD deficiency in basal keratinocytes of the epidermis could be partially restored by treating mice with calpain inhibitors, setting the stage for further exploration of treatment strategies for the disease.

## Plectin in skeletal muscle

### Expression and subcellular localization

The original immunolocalization of plectin in mammalian tissues and isolated cardiomyocytes showed a prominent association with desmin at Z-disks of skeletal muscle, dense plaques of smooth muscle, and Z-disks and intercalated disks of cardiac muscle (Wiche et al. 1983; Zernig and Wiche 1985). Using immunogold labeling, a number of studies extended these findings to the ultrastructural level. It was shown that plectin-positive filamentous structures radiating from Z-disks were linked to desmin IFs generating lateral linkages among adjacent Z-disks (Hijikata et al. 1999; Reipert et al. 1999; Schröder et al. 1999) and that similar bridges were connecting Z-disks of peripheral myofibrils to sarcolemmal densities presumed to represent costameric remnants (Hijikata et al. 2003; Reipert et al. 1999; Reznicek et al. 2007; Schröder et al. 1999). Furthermore, plectin was shown to be associated with desmin IFs linking myofibrils to mitochondria at the level of the Z-disk and along the entire length of the sarcomere (Reipert et al. 1999). These studies strongly suggested that the desmin IF network in partnership with plectin was forming an extensively connected three-dimensional intermyofibrillar and subplasma membrane protein scaffold that physically linked the contractile apparatus to internal organelles and to junctional complexes at the sarcolemma, thus providing a mechanical continuum between internal myofiber structures and the extracellular matrix surrounding the fiber.

### Specific functions of isoforms

In skeletal muscle tissue, all eight of the originally identified transcript variants containing alternatively spliced first coding exons were found to be expressed, although for some of the first exons, very weak signals were obtained by RNase protection assays (Fuchs et al. 1999). Moreover, a tissue-specific dominance of isoform P1d was detected, which was most prominent in skeletal muscle and heart. Beyond P1d, the muscle-specific set of major isoforms comprises P1, P1b, and P1f, with relative transcript levels of >10:4:3:1 for P1d, P1f, P1b, and P1, respectively (Fuchs et al. 1999). Visualization of GFP fusion proteins upon forced expression in cells (Reznicek et al. 2003, 2007), and immunofluorescence microscopy of teased muscle fibers using isoform-specific antibodies (Konieczny et al. 2008), demonstrated that the various isoforms were targeted to distinct subcellular locations, where, as it turned out, they are fulfilling distinct functions (Fig. 5a; see also Fig. 8 in Konieczny et al. 2008).

#### *P1d: the Z-disk anchor protein of desmin filaments*

P1d is the only isoform of plectin that is specifically expressed in skeletal and cardiac muscle, but not in other tissues (Fuchs et al. 1999). When GFP fusion proteins of P1d were expressed in differentiated myoblasts, they were found to be targeted exclusively to Z-disks (Reznicek et al. 2007). Furthermore, when cryosections of skeletal muscle from isoform-specific P1d knockout mice were immunolabeled using antibodies recognizing all isoforms (anti-pan-plectin), only the sarcolemma and dotted remnants in the interior of fibers were stained, contrasting the highly ordered cross-striated staining pattern typical of wild-type tissue. Similarly, in fibers of P1d-deficient muscle, plectin signals were preserved in peripheral, perinuclear, and costameric regions, where P1 and P1f were still present. Moreover, in P1d-deficient muscle tissue and teased muscle fibers, aggregates of desmin IFs were observed in the interior but not peripheral areas of the fiber. While these observations clearly show that P1d is responsible for the recruitment and anchorage of desmin IFs to Z-disks, the question how P1d itself is specifically targeted to Z-disk structures is still unsolved. It will be a challenging task to answer this question considering that the isoform-specific sequence of P1d preceding its ABD (common to all isoforms) comprises only 5 amino acid residues.

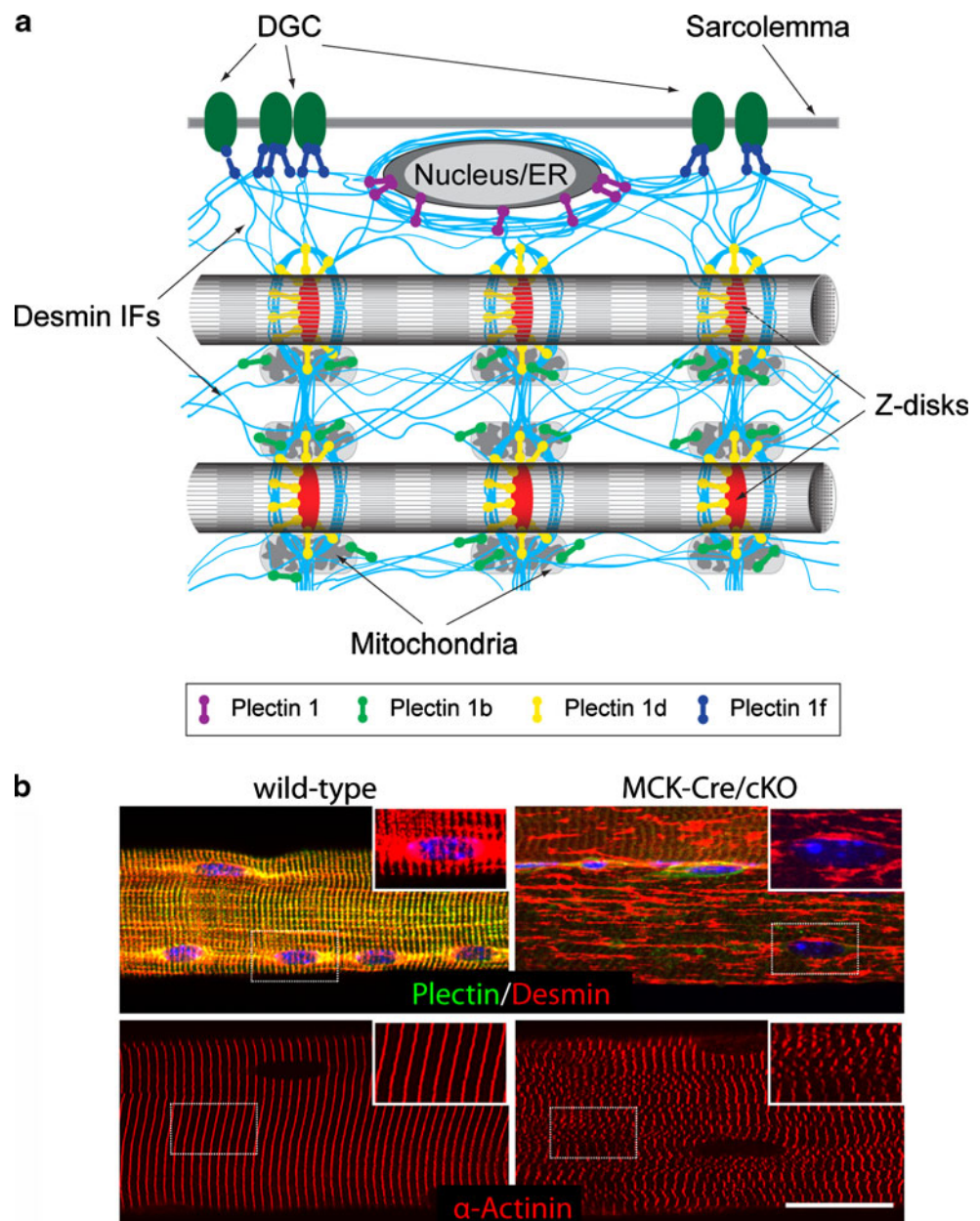
#### *P1f: plugging the contractile apparatus to the sarcolemma*

In fibroblasts, P1f was shown to be an integral component of mature, focal adhesion-evolved fibrillar adhesions, which are characterized by their robust, fibronectin fibril-aligned,



**Fig. 5** Plectin in skeletal muscle. **a** Schematic representation of plectin's isoform diversity and specific functions. The four major isoforms expressed in muscle, *P1d* (yellow), *P1f* (dark blue), *P1b* (green), and *P1* (purple) are site-specifically bound via their N-terminal molecular domains (not indicated) to Z-disks (*P1d*), the costameric/transmembrane dystrophin-glycoprotein-complex (DGC) (*P1f*), mitochondrial organelles (*P1b*), and the nuclear/ER membrane system (*P1*). The various isoforms recruit (via their C-terminal IF-binding domain) the desmin IF network (blue lines) to their specific locations, thereby creating a densely interwoven resilient filamentous network that mechanically integrates the contractile apparatus with cytoplasmic organelles, the sarcolemma, and the extracellular compartment.

**b** Immunofluorescence microscopy of teased muscle fibers from wild-type and conditional muscle-restricted knockout (*MCK-Cre/cKO*) mice. Visualized proteins are indicated; myonuclei are stained in blue. Note massive formation of desmin-positive protein aggregates and misaligned and disoriented Z-disks, both hallmarks of myofibrillar myopathies, in specimens of mutant mice (corresponds to Fig. 2D in Konieczny et al. 2008)



elongated structure, and central subcellular localization. P1f-mediated vimentin IF network formation at these sites creates a resilient cage-like core structure of IFs that encases and positions the nucleus while being stably connected to the exterior of the cell (Burgstaller et al. 2010). The situation in muscle fibers seems to be similar. The forced expression of GFP-labeled P1f in differentiated myotubes showed that the fusion protein was targeting exclusively to the sarcolemma where it associated with the dystrophin-glycoprotein complex (DGC; Reznicek et al. 2007). In fact, plectin binds directly to dystrophin and the transmembrane laminin receptor  $\beta$ -dystroglycan, two of the major DGC constituent proteins, via two independent binding sites located in its N-terminal portion (see Fig. 5),

leaving its C-terminal IF-binding site freely accessible (Reznicek et al. 2007). In this way, P1f can act as a well-anchored docking site for desmin IF networks that link costameres (and associated structures in the extracellular compartment) with peripheral myofibrils, mitochondria, and myonuclei (see Fig. 5a). Based, among others, on the strikingly similar expression patterns of P1f and dystrophin during differentiation of myoblasts to myotubes, a role of P1f in the formation and maturation of costameres has been suggested (Reznicek et al. 2007), reminiscent of the transformation of focal adhesions to fibrillary adhesion in fibroblasts. In certain types of mature muscle fibers, such as fast type 2A fibers of quadriceps muscle from adult mice, P1f could additionally be found at Z-disks, suggesting a



certain degree of interchangeability of plectin isoforms once IF-docking sites have been formed and become functional (Rezniczek et al. 2007).

What seems to be most crucial for myofiber integrity is the structural axis generated by P1d and P1f that interlinks sarcomeres at their Z-disks and connects them with the plasma membrane. Disruption of one (P1d) or both (P1f and P1d) of these IF-linking elements inevitably leads to the loss of muscle fiber integrity. In fact, as demonstrated by isoform-specific gene targeting, elimination of P1d alone leads to the formation of aggregates in the interior of the fiber, but not in its peripheral sarcolemmal regions. Despite that in this case, the remaining P1f was able to preserve costameric IF structures; isoform P1d knockout mice showed a similarly severe muscle phenotype as the conditional knockout mice (lacking all isoforms), or desmin-null mice (Konieczny et al. 2008).

#### *P1b: integrating mitochondria into the desmin IF network*

Colocalizing with mitochondria, P1b acts as a direct linker between the organelle and the desmin network. This linkage has been demonstrated for IFs of the vimentin and desmin type, but most likely occurs also with other types of IFs. The exon 1b-encoded sequence of this isoform serves as a mitochondrial targeting and anchoring signal, inserting the protein into the outer mitochondrial membrane via its N-terminal part. The bulk of the protein remains in the cytosol, where it interacts via its C-terminal high-affinity binding site with IFs recruiting them to the organelle (Winter et al. 2008). In fact, the loss of P1b leads to marked shape changes of mitochondrial networks, manifesting as elongation of mitochondrial networks in P1b-deficient fibroblasts and myoblasts (Winter et al. 2008). Moreover, in muscle tissue that is deficient in P1d (but not P1b), association of residual plectin with mitochondria and colocalization of collapsed desmin IFs with mitochondrial aggregates were observed; whereas in plectin-null muscle, similar to P1b-deficient muscle, this was not the case (Konieczny et al. 2008). The underlying mechanism of mitochondrial network deregulation through P1b deficiency and its biological significance remain to be determined. However, there is evidence that mitochondrion-bound plectin, in addition to having a positioning effect, serves as a scaffolding platform for signaling complexes (Winter et al. 2008).

#### *P1: linking desmin IFs to the nuclear/ER membrane system*

In muscle fibers, contrary to other isoforms, P1 localizes primarily to the outer nuclear/ER membrane system (Rezniczek et al. 2007, Konieczny et al. 2008). Exon 1 encodes a putative nuclear localization signal (NLS), and

N-terminal fragments of P1 up to a certain length exhibit nuclear localization (Rezniczek et al. 2003). Thus, the NLS domain is likely to play a role in recruiting P1 to the nuclear/ER membrane, where upon binding to other local interaction partners, it forms a recruitment platform for IFs. One of these putative proteins has been identified as nesprin-3, a KASH domain-containing protein that interacts with plectin's ABD (Ketema et al. 2007). As nesprin-3 is an outer nuclear membrane protein, it provides via plectin a direct link between the nuclear envelope and the cytoplasmic IF network (Ketema and Sonnenberg 2011). Other nuclear/ER membrane-associated proteins that bind directly either to the isoform-specific sequence or to other molecular domains of P1 are expected to be found. In addition to the nuclear/ER membrane system, P1 showed partial association with the sarcolemma in certain types of fully differentiated muscle fibers (Rezniczek et al. 2007). Thus, like P1f, P1 may show spatial interchangeability with other isoforms at mature or late stages of myofiber development.

The isoform-specific N-terminal sequence of P1 (180 amino acid residues) is the longest of all known plectin isoforms. One of its specific features is that it contains a binding site of unusual high activity for the substrate-binding site of Siah, a ring domain protein functioning in E3 ubiquitin ligase complexes that mediate degradation of a wide range of cellular proteins. It has therefore been suggested that P1 could serve as a dominant negative regulator of Siah proteins (House et al. 2003).

#### Skeletal muscle phenotypes of plectinopathy patients

Plectin has been found to be prominently expressed in muscle cells early on, but its importance for the structure and function of muscle became clearly evident only when patients with defects in the plectin gene were found to suffer, in addition to skin blistering, from late onset muscular dystrophy (for a recent review see Winter and Wiche 2013). The clinical phenotypes of patients vary considerably with respect to onset and progression of muscular symptoms as well as severity of skin blistering. Skeletal muscle weakness usually starts in the second decade of life and shows relatively slow disease progression leading to a loss of ambulation after 10–20 years by affecting facial, extraocular, limb, and trunk muscles. Moreover, patients with cardiac pathology have been reported that display asymptomatic left ventricular hypertrophy (Schröder et al. 2002) and adult-onset biventricular dilated cardiomyopathy (Bolling et al. 2010b). Similar to skin, immunofluorescence microscopy of skeletal muscle tissue of EBS-MD patients revealed either drastically reduced or completely absent plectin-specific staining (McMillan et al. 2007). While antibodies recognizing epitopes in the central rod domain

of plectin in general lack immunoreactivity, antibodies directed against plectin's C- or N-terminal regions often revealed specific signals, indicating the expression of rodless or truncated plectin versions, which might attenuate the clinical phenotypes (Koster et al. 2004; Natsuga et al. 2010a, b). Immunolabeling for desmin usually reveals the formation of cytoplasmic and subsarcolemmal desmin-positive protein aggregates. Moreover, ultrastructural analyses of skeletal muscles showed disarrayed myofibrils and loss of sarcomeric organization, accompanied by pathological changes of membranous organelles and neuromuscular endplates (Bauer et al. 2001; McMillan et al. 2007; Schröder et al. 2002). Therefore, most plectinopathies can be assigned to the group of myofibrillar myopathies (MFMs), an expanding group of clinically and genetically heterogeneous muscle diseases characterized by misfolded proteins and pathological desmin-positive protein aggregates, myofibrillar degeneration, and mitochondrial abnormalities (Ferrer and Olive 2008; Schröder and Schoser 2009). EBA-PA cases do not display signs of muscular pathology; however, onset of MD is to be expected if patients survive long enough (Charlesworth et al. 2013, Winter and Wiche 2013).

Muscular symptoms are displayed also by patients suffering from EBS-MD-MyS or LGMD2Q. EBS-MD-MyS patients show first signs of muscle weakness accompanied by myasthenic symptoms within the first decade of life. In fact, defects in neuromuscular transmission, as demonstrated by a pathological decremental response in neuromuscular transmission studies, were observed in all cases. Recently, a homozygous 9-bp deletion in the isoform-specific sequence of P1f was found to cause limb-girdle muscular dystrophy type Q2 (LGMD2Q), characterized by a muscular pathology without any skin involvement (Gundesli et al. 2010). In this case, the probands suffered from early-onset limb-girdle syndrome with loss of ambulation in their late twenties. As distinct isoforms perform different functions, mutations in other isoforms can be expected to be identified in the future and should be considered in cases of undiagnosed muscular dystrophies.

#### Mouse models for plectin-related muscular dystrophies

Plectin-null mice, similar to plectinopathy patients, show abnormalities reminiscent of minicore myopathies manifesting in skeletal muscle as focal loss of myofibrils and disruption of sarcomeres affecting Z-lines and adjacent myofibrils, Z-band streaming, and increased amounts of necrotic fibers (Andrä et al. 1997), and in heart as disintegration of intercalated disks. However, as these mice die within 2–3 days after birth, they are of limited use as animal models for plectin-related muscular dystrophies. To overcome this problem, a conditional, striated muscle-

restricted plectin knockout mouse line (MCK-Cre/cKO) was generated. Closely mimicking the human disease, these mice show progressive degenerative alterations, including aggregation and partial loss of desmin IFs, aggregation and dysfunction of mitochondria, and a partial detachment of the contractile apparatus from the sarcolemma (Konieczny et al. 2008). The analysis of these mice showed that the disruption of myofibrils, and particular of Z-disks, most likely occurs as a consequence of disruptive contractions of myofibrils. Due to the loss of its anchorage via plectin and the resulting defective lateral alignment of myofibrils, the desmin IF network can no longer keep sarcomeres in register. The additional formation of dysfunctional protein (desmin) aggregates in subplasma membrane and interior cellular compartments contributes to the deleterious phenotypes of plectin-deficient myofibers. Mutant mice, mimicking the complex disease pattern of human plectinopathies, and ex vivo myocyte cell cultures derived from them, thus provide powerful tools for elucidating functional and molecular consequences of plectin gene defects leading to skeletal muscle pathology.

For three of the four major plectin isoforms expressed in muscle, P1d, P1b, and P1, isoform-specific knockout mouse lines were generated, which lack just one of these isoforms, while expressing all others. Some of their phenotypes have been described above. In addition, a double knockout mouse line, deficient in P1d and desmin has been generated (Konieczny et al. 2008), and a muscle-restricted conditional plectin knockout line lacking dystrophin in addition to plectin has recently become available (Raith et al. 2013). The phenotypic analysis of the latter unveiled a new facet of plectin's ever increasing functional repertoire by establishing a mechanistic link between sarcolemma-associated plectin, its microtubule-destabilizing action, and glucose transporter-mediated glucose uptake of muscle fibers (Raith et al. 2013).

#### Plectin in Schwann cells of peripheral nerve

Similar to skin and muscle tissues, plectin shows widespread occurrence in neural cells and tissues (Errante et al. 1994), and neurodegeneration has been reported in some cases of EBS-MD (Smith et al. 1996, Bauer et al. 2001). Plectin has also been implied in Alexander disease, a neurological disorder caused by mutations in GFAP (Tian et al. 2006). The phenotypic analysis of isoform P1c-specific knockout mice revealed slightly reduced neurofilament densities in some of their axons and significantly reduced nerve conduction velocity in sciatic nerve (Fuchs et al. 2009). Whether these alterations are mechanistically linked remains to be shown. With respect to the diversity and specific functions of isoforms, the best studied neural

cell system are myelinating Schwann cells of the peripheral nerve.

#### Expression and isoform diversity

Immunolabeling of murine sciatic nerve using isoform-specific antibodies revealed expression of P1 and P1c in the cytoplasm and at the periphery of axons as well as in the cytoplasm of myelinating Schwann cells (Fig. 6a; see also Fuchs et al. 2009; Walko et al. 2013). In addition, relatively strong P1-specific labeling of some neurofilament-negative cells, probably representing endoneural fibroblasts was noticed. P1a, the predominantly in stratified epithelial tissues-expressed isoform, could not be detected in axons or myelinating Schwann cells, but was found to be expressed in putative endoneural fibroblasts; in these cells, also P1f, one of the major isoforms of dermal fibroblasts (Burgstaller et al. 2010) was detected. Faint, dotted signals of P1f were also observed in axons, whereas myelinating Schwann cells were found to be P1f negative. When the expression of isoforms in cultures of primary Schwann cells and nerve fibroblasts was assessed by immunoblotting of cell lysates, the expression of full-length versions of isoforms P1, P1c, and P1f was demonstrated for both types of cells. In addition, in Schwann cell lysates, the corresponding rodless isoform variants lacking the central rod domain were detected at relatively high levels, whereas in nerve fibroblast samples, a rodless version was found only in the case of P1 (Walko et al., 2013). Consistent with its in situ visualization in tissues (Fig. 6a), P1a was detectable in lysates of nerve fibroblast, but not Schwann cell cultures (Walko et al. 2013).

#### Subcellular distribution

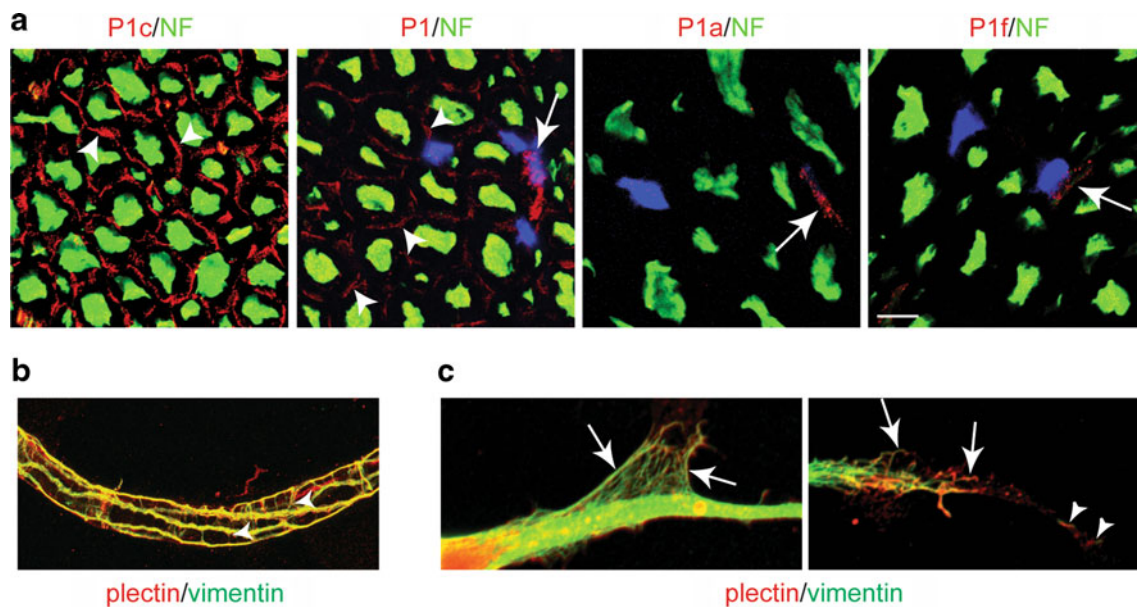
Confocal immunofluorescence microscopy of teased sciatic nerve fibers using antibodies that do not discriminate between isoforms revealed codistribution of plectin and vimentin IFs in longitudinal Cajal bands and transversing trabeculae (Fig. 6b; Walko et al. 2013) which constitute the cytoplasm of myelinating Schwann cells (Court et al. 2004). In cultured Schwann cells, plectin was found to colocalize with the vimentin IF network as well as with vimentin-positive short filamentous structures (Fig. 6c) that most likely were representing filament precursors, as previously reported for fibroblast cells (Spurny et al. 2008). Coimmunoprecipitation and immunolocalization experiments revealed that plectin present in Cajal bands formed a tight complex not only with vimentin but also with  $\beta$ -dystroglycan, the core component of the DGC (Walko et al. 2013). Thus, by anchoring vimentin IFs to dystroglycan, plectin fulfills in myelinating Schwann cells a similar function as in skeletal muscle where it

anchors the desmin network to the DGC (Rezniczek et al. 2007).

#### Specific functions of isoforms

As recently shown, the binding of plectin to  $\beta$ -dystroglycan and the ensuing recruitment of the vimentin IF network to the abaxonal Schwann cell membrane contribute to the stabilization of the dystroglycan complex (Walko et al. 2013). The Schwann cell-specific ablation of plectin in a protein zero (P0)-Cre/conditional plectin knockout mouse line (P0-Cre/cKO) was found to lead to faulty vimentin IF-dystroglycan complex formation, leading to an aggravation of aging-related destabilization of the myelin sheath (Walko et al. 2013). In contrast to skeletal muscle, where the loss of plectin causes massive aggregation of desmin IFs (Konieczny et al. 2008), in myelinating Schwann cells, the impact of plectin deficiency on the organization of the vimentin IF system was detectable just as a decrease in the vimentin IF network density in a fraction of nerve fibers characterized by large caliber axons (Walko et al. 2013). One possible explanation for this difference could be that peripheral nerves in general are more protected and thus less exposed to mechanical stress compared to skeletal muscle fibers. The isoform of plectin acting as the specific interaction partner of  $\beta$ -dystroglycan in differentiating Schwann cells has still to be identified. Given the absence of P1f in this type of cells (see Fig. 6a), the strongest candidate for this function is P1, which has previously been shown to interact with the DGC component  $\alpha$ -dystrobrevin-1 (Hijikata et al. 2008). In this context, it is noteworthy that albeit the P1a-specific binding partner integrin  $\beta$ 4 is expressed in myelinating Schwann cells (Nodari et al. 2008), cell-extracellular matrix junctions similar to HDs are not formed by this type of cell, at least not at its fully differentiated state, due to the lack of isoform P1a expression.

Concerning potential roles of other Schwann cell-expressed plectin isoforms, it is of interest that P1f is abundantly expressed in ex vivo cultures of Schwann cells, in contrast to fully differentiated (myelinating) tissue cells. Given the established role of this isoform in regulating focal adhesion turnover and cell shape in fibroblasts (Burgstaller et al. 2010), its expression in ex vivo cell cultures would be consistent with a role in cell migration, a feature presumably required by precursor and regenerating, but not fully differentiated Schwann cells. The role of P1c, the other major plectin isoform identified in Schwann cells, presently is unknown. Whether it performs MT-related functions in Schwann cells, as it does in keratinocytes, remains to be tested. Even if no alterations in the organization of MTs were observed in Cajal bands of sciatic nerves from myelinating Schwann cell-specific knockout



**Fig. 6** Plectin in peripheral nerve. **a** Frozen sciatic nerve cross sections were immunolabeled using antibodies specific to *P1c*, *P1*, *P1a*, and *P1f* as indicated; sections were double labeled using antibodies to neurofilament (*NF*) M chain to specifically label axons; nuclei were labeled with Hoechst dye (*blue*). *Arrowheads*, myelinating Schwann cells; *arrows*, neurofilament-negative/plectin-positive cells. *Bar* 10  $\mu\text{m}$ . **b** and **c** Association of plectin with vimentin IFs in Schwann cells. Teased sciatic nerve fibers (**b**) or cultured primary

Schwann cells (**c**) were double immunolabeled using antibodies to plectin (not discriminating between isoforms) and vimentin. *Bars* 20  $\mu\text{m}$  (**b**), 10  $\mu\text{m}$  (**c**). Note codistribution of plectin and vimentin in Cajal bands and transverse trabeculae (*arrowheads* in **b**), and in radial and axial lamellipodial protrusions (*arrows* in **c**, *left* and *right* panel, respectively). Also note colocalization of plectin with short filamentous vimentin *IF* intermediates (*arrowheads*). (Reprinted in modified form from Walko et al. 2013)

mice (Walko et al. 2013), *P1c* could still play a role in the regulation of MT-dependent transport processes in the cytoplasm of these cells. Of note, in contrast to isoform *P1c* knockout mice, a defect in motor nerve conduction velocity of sciatic nerve could not be detected in conditional, Schwann cell-specific plectin knockout (*P0-Cre/cKO*) mice (authors' unpublished data), suggesting that this phenotype is specifically related to axonal *P1c*.

### Perspective

Plectin has emerged as a preeminent cytoskeletal linker protein controlling IF functions. Studies on skin, skeletal muscle fiber, and Schwann cells, the systems discussed in this review article, clearly showed that distinct cell types express specific sets of plectin isoforms that are targeted to distinct cellular locations. By recruiting IF networks to their target sites, plectin isoforms control interior cytoarchitecture as well as cell shape, with in part dramatic effects on mechanical resilience as well as an array of other basic cellular function, among them proliferation, metabolism, migration, and signal transduction. In light of plectin's isoform diversity and IF partnership in practically all mammalian cell types, the challenge for future relevant research lays in the analysis of isoform-specific functions and their mechanisms in systems other than the highly

stress-prone tissues discussed above. It will be of particular interest to unravel the roles of plectin isoforms in the development and function of the brain and of motor neurons in the peripheral nervous system, as for both cases, there is evidence for important physiological roles of plectin, based on phenotypic analyses of plectinopathy patients as well as plectin knockout mice; and a similar situation pertains to the vascular system.

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