COMMENTARY

Role of plectin in cytoskeleton organization and dynamics

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SUMMARY

Plectin and its isoforms are versatile cytoskeletal linker proteins of very large size (>500 kDa) that are abundantly expressed in a wide variety of mammalian tissues and cell types. Earlier studies indicated that plectin molecules were associated with and/or directly bound to subcomponents of all three major cytoskeletal filament networks, the subplasma membrane protein skeleton, and a variety of plasma membrane-cytoskeleton junctional complexes, including those found in epithelia, various types of muscle, and fibroblasts. In conjunction with biochemical data, this led to the concept that plectin plays an important role in cytoskeleton network organization, with consequences for viscoelastic properties of the cytoplasm and the mechanical integrity and resistance of cells and tissues. Several recent findings lent strong support to this concept. One was that a hereditary disease, epidermolysis bullosa simplex (EBS)-MD, characterized by severe skin blistering combined with muscular dystrophy, is caused by defects in the plectin gene. Another was the generation of plectin-deficient mice by targeted inactivation of the gene. Dying shortly after birth, these animals exhibited severe defects in skin, skeletal muscle and heart. Moreover, in vitro studies with cells derived from such animals unmasked an essential new role of plectin as regulator of cellular processes involving actin stress fibers dynamics. Comprehensive analyses of the gene locus in man, mouse, and rat point towards a complex gene expression machinery, comprising an unprecedented diversity of differentially spliced transcripts with distinct 5′ starting exons, probably regulated by different promoters. This could provide a basis for cell type-dependent and/or developmentally-controlled expression of plectin isoforms, exerting different functions through binding to distinct partners. Based on its versatile functions and structural diversification plectin emerges as a prototype cytolinker protein among a family of proteins sharing partial structural homology and functions.

Key words: Cytoskeleton, Linker protein, Cell stabilization/ morphogenesis

INTRODUCTION

The cytoskeleton is viewed as a complex network array of cytoplasmic fibers that determine and control viscoelastic properties and mechanical strength of cells, organize and give structure to their interior, and control many dynamic processes, such as intracellular trafficking, cell division, adhesion, and locomotion. Actin/myosin filaments, microtubules, and intermediate filaments, the three major protein fiber systems forming this skeleton, contribute to these functions to various degrees. The spatial organization of the cytoskeleton is strongly dependent on the kind of interactions cytoskeletal filaments engage in. Thus, filament binding proteins that interlink cytoskeletal filaments of the same or of different types or link them to other cellular constituents at junctional and anchoring sites are likely to play key roles in the functional specialization of cells involving morphogenetic events.

The most versatile cytoskeletal linker protein known to date is plectin, which was first isolated nearly 20 years ago (Pytela and Wiche, 1980). Based on the identification of intermediate filaments (IFs) as interacting partner, its presumptive structural relationship to high molecular mass microtubule-associated proteins (MAPs), and because of its immunolocalization within dense cytoplasmic network arrays of cultured cells, we postulated very early that plectin molecules might be involved in the organization and network formation of the cytoskeleton, hence its name (Wiche and Baker, 1982; Wiche et al., 1982). However, only recently have we begun to obtain a better understanding of the exact role of plectin molecules and their mechanisms of action. Several key observations and important research developments contributed to this progress. The cloning and sequencing of plectin cDNA, first reported for rat (Wiche et al., 1991), opened the door for molecular genetic studies and the analyses of gene structure and regulation. Of particular importance were the characterization of the exon-intron organization and the chromosomal localization of the human gene, both first reported by Liu et al. (1996) and independently confirmed by McLean et al. (1996). Other recent breakthroughs were the finding that the hereditary disease epidermolysis bullosa simplex (EBS)-MD, a severe skin blistering disease combined with muscular dystrophy, is

caused by defects in the plectin gene, as reported by several groups (Gache et al., 1996; Smith et al., 1996; McLean et al., 1996), and the generation of plectin (−/−) animals by targeted gene disruption in mice (Andrä et al., 1997).

In this review I intend to highlight recent developments in plectin-related research and, together with earlier findings, put them into perspective with plectin's proposed role as an essential linker and organizer protein of the cytoskeleton. For recent reviews on cytoskeletal cross-linkers see Bousquet and Coulombe (1996), Ruhrberg and Watt (1997), Fuchs and Cleveland (1998), and Houseweart and Cleveland (1998).

STRUCTURAL AND MOLECULAR PROPERTIES

Plectin's molecular mass initially was estimated as ~300,000 based on the protein's comigration with subcomponents of high molecular mass microtubule-associated proteins (MAPs) in SDS-PAGE (Pytela and Wiche, 1980). After the later cloning and sequencing of plectin cDNA this estimate had to be elevated to over 500,000. Actually, the precise size predictions for full length plectin isoforms vary from 507,000 to 527,000, depending on several putative first coding exons (see below).

Secondary structure predictions based on cDNA and deduced amino acid sequences (Wiche et al., 1991), as well as electron microscopy of purified plectin molecules (Foisner and Wiche, 1987), revealed a multidomain structure composed of a central ~200-nm-long α-helical coiled coil rod structure flanked by large globular domains. Both the microscopic dimensions of dumbbell-shaped plectin molecules and gel permeation HPLC data indicated a molecular mass of plectin molecules in solution of slightly over 1.1×10^6 (Foisner and Wiche, 1987; Weitzer and Wiche, 1987). Since all plectin isoforms thus far analyzed have a predicted molecular mass of over 500 kDa in their intact full length form, it follows that plectin in solution most probably is a dimeric molecule. A predominantly tetrameric form, previously favored on grounds of the apparently too low molecular mass estimates obtained by SDS-PAGE (Foisner and Wiche, 1987), appears now less likely.

As a prominent phosphoprotein, plectin was found to be an in vivo target of Ca^{2+}/calmodulin-dependent kinase and of protein kinases A and C (Herrmann and Wiche, 1983, 1987; Foisner et al., 1991). Furthermore, plectin has been identified as a substrate of p34[cdc2] kinase which phosphorylates plectin at a single site (threonine 4542) close to its carboxy terminus (Malecz et al., 1996).

EXPRESSION AND SUBCELLULAR LOCALIZATION

Plectin is a widespread if not ubiquitous protein of mammalian cells. Using antiserum (Wiche and Baker, 1982) and later a panel of mAbs (Foisner et al., 1994) raised to plectin purified from rat glioma C6 cells we have shown by immunoblotting and/or immunostaining that plectin is expressed in a variety of tissues and mammalian cell lines. In fact, except for certain neurons (Errante et al., 1994), mammalian cells devoid of plectin immunoreactivity have not been reported to date. It is possible, however, that even cell types that lack immunoreactivity with the existing antibodies express certain isoforms of plectin (see below) that either lack the epitopes recognized by the available

antibodies, for instance due to the expression of differentially spliced transcripts, or contain the protein in a form in which the epitopes of the antibodies tested were inaccessible. The widespread expression of plectin has been confirmed by RNase protection assays using cDNA representative of a variety of tissues and cell lines in combination with ribonucleotide probes corresponding to distinct coding regions of the gene (Elliott et al., 1997; and unpublished data).

Initial studies on the immunolocalization of plectin in cultured cells and tissues revealed a codistribution of the protein with different types of IFs and a prominent association with the plasma membrane attachment sites of both IFs and microfilaments (Wiche et al., 1983, 1984a). Particularly conspicuous was plectin's prominent association with the basal cell surface membrane of keratinocytes in the basal cell layer of stratified epithelium, including hemidesmosomal structures, with Z-lines of striated muscle and dense plaques of smooth muscle, intercalated discs of cardiac muscle, and focal adhesion contacts of cells in culture (Seifert et al., 1992). These studies lent strong support to our original proposal that plectin molecules play an important role as organizer and linker elements of the cytoskeleton. Furthermore, in several tissues plectin expression was found to be prominent in cells forming tissue layers at the interface between tissues and fluid-filled cavities, including the surfaces of kidney glomeruli, liver bile canaliculi, bladder urothelium, gut villi, ependymal layer lining the cavities of brain and spinal cord, and endothelial cells of blood vessels (Wiche et al., 1983; Errante et al., 1994; Yaoita et al., 1996).

MOLECULAR INTERACTIONS OF PLECTIN

Consistent with its cellular localization at sites of strategic importance for the positioning and organization of cytoarchitectural elements, plectin molecules have been shown to interact with a variety of cytoskeletal structures and proteins on the molecular level. In fact, none of the other cytolinker protein family members identified to date seem to exhibit such versatile binding activities as have been demonstrated for plectin.

Intermediate filaments

Plectin's interaction with IFs has been demonstrated in a number of ways including in situ localization of plectin with IFs of cultured cells using gold-immunoelectron microscopy (Foisner et al., 1988; Svitkina et al., 1996), and in vitro binding assays using purified proteins. The initial characterization of vimentin as a direct binding partner of plectin (Pytela and Wiche, 1980; Wiche et al., 1982) was later extended to other cytoplasmic IF subunit proteins, including GFAP, epidermal cytokeratins, neurofilament triplet proteins, and desmin (Foisner et al., 1988; and unpublished data), and the nuclear IF protein, lamin B (Foisner et al., 1991). After its initial allocation to the carboxy-terminal globular domain (Wiche et al., 1993), the IF binding site of plectin has recently been mapped to a stretch of ~50 amino acid residues linking the carboxy-terminal repeat domains 5 and 6 (Nikolic et al., 1996). A basic amino acid residue cluster within a typical bipartite nuclear localization sequence (NLS) motif was identified as an essential element of this site. Residing downstream and outside

of the highly conserved repeat 5 domain core region which, like all other carboxy-terminal repeat domains comprises multiple copies of a tandemly repeated 19 (38) amino acid residue-long sequence motif, plectin's IF binding site is part of one of the much less conserved linker regions between repeat domains which probably form looplike structures exposed to the outside of globular protein core structures.

There is evidence from both in vitro and in vivo experiments indicating that plectin-IF interactions are differentially regulated by phosphorylation involving different types of protein kinases (PK). Plectin's interaction with lamin B was found significantly decreased upon phosphorylation of either binding partner by cAMP-dependent protein kinase (PKA) or Ca^{2+}/phosphadidyl-dependent kinase (PKC), while its binding to vimentin was increased after PKA-, but decreased after PKC-phosphorylation (Foisner et al., 1991). The phosphorylation of plectin molecules may play also a specific role during mitosis. It has been shown that during M-phase plectin becomes a target of protein kinase $p34^{cdc2}$ and dissociates from vimentin IF structures (Foisner et al., 1996). Thus freed and accessible to other interaction partners, plectin's vimentin binding site containing the NLS sequence motif could then become a sequestering site for NLS binding proteins including lamin B. The function of the plectin-lamin B interaction may be to contribute to the disassembly of the nuclear matrix, or conversely, to promote nuclear reassembly after cell division.

Microtubules

Using both immunoblotting and gold-immunoelectron microscopy plectin has been shown to copurify with microtubules assembled in vitro from extracts of cultured rat glioma C6 cells (Koszka et al., 1985). It stayed associated with microtubules during repeated rounds of temperature-dependent assembly as well as after taxol-induced assembly, and ultrastructurally plectin molecules were arranged in patches along the surface of microtubules. Subsequently it was found that purified plectin specifically bound to high molecular mass

microtubule-associated proteins (MAP2, and MAP1 subtypes) from brain (Herrmann and Wiche, 1987). Since at least certain MAP1 subtypes are abundantly expressed in glioma C6 cells (Wiche et al., 1984b; Zauner et al., 1992), plectin's coassembly with microtubule polymers probably occurred indirectly via its binding to MAPs. However, a direct interaction of plectin with tubulin, particularly in cells that do not express neuronal MAPs, could not be ruled out. Convincing evidence that plectin-microtubule interactions exist on the cellular level was recently reported by Svitkina et al. (1996) who used immunogold electron microscopy of whole mount cytoskeletons to visualize plectin molecules as thin (2-3 nm) and up to 200 nm long filaments bridging microtubules with vimentin filaments.

Microfilaments

The immunolocalization of plectin at microfilament-plasma membrane junctions in a variety of tissues, most notable in all types of muscle, suggested early on that the protein might be involved in the organization of the actin-based cytoskeleton. Its conspicuous accumulation at focal contacts of cultured cells and its colocalization with their actin stress fibers, most noticeable in cells at stationary phase, strongly supported this view. The partial colocalization of plectin, microfilaments and intermediate filaments, as revealed in these studies, suggested that plectin molecules may mediate interactions between these two types of cytoskeletal network systems. This notion received further support through a study in which whole mount electron microscopy of glioma C6 cell clones in combination with immunogold labeling was used to demonstrate that plectin molecules formed thin (3 nm) filamentous structures linking IFs to actin filaments, aside from forming bridges between IFs themselves (Foisner et al., 1995).

cDNA sequencing (see below) revealed a highly conserved actin binding domain (ABD), in proximity of plectin's amino-terminal end (Fig. 1). Consisting of a pair of calponin-like (CH) subdomains (Goldsmith et al., 1997) plectin's ABD is of the type found in a large family of actin binding proteins,
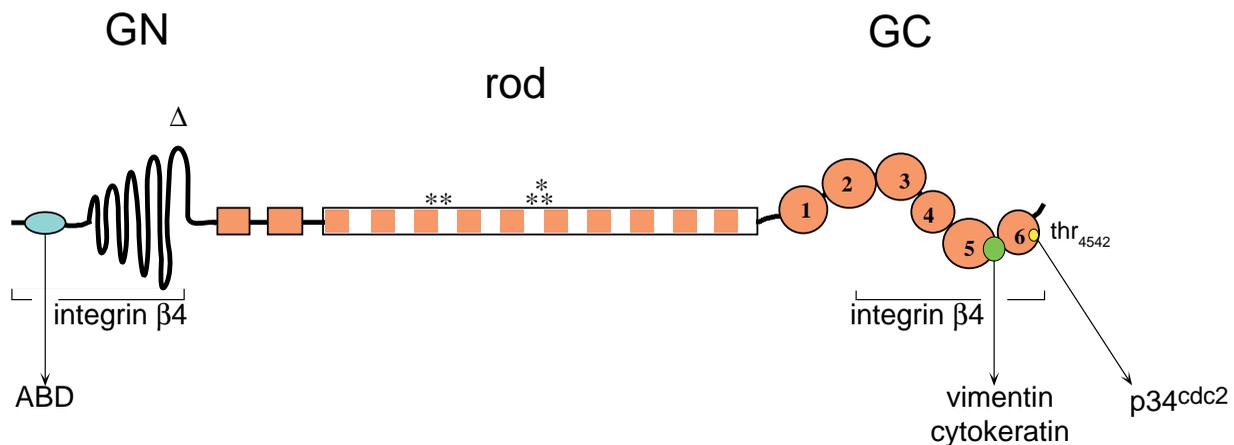

Fig. 1. Domain map of plectin. The tripartite structure of plectin molecules comprises a central rod flanked by globular amino-terminal domain (GN) and carboxy-terminal (GC) domains. The GC domain consists of 6 highly homologous repeat regions. Defined subdomains for binding to actin (ABD) and IFs (vimentin/cytokeratin), as well as a unique protein kinase $p34^{cdc2}$ phosphorylation site (thr^{4542}) are indicated. The GN and the GC domains both harbor binding sites for integrin β4. Asterisks (*) along rod indicate locations of mutations in the single exon encoding this central domain, which lead to premature stop codons and result in EBS-MD; Δ, location of one mutation consisting of a 9 bp deletion (3 amino acid residues) which results in an impaired plectin molecule and a EBS-MD phenotype.

including spectrin and dystrophin, and the neuronal form of BPAG1/dystonin (Elliott et al., 1997). Recently, the ABD of plectin was shown to be indeed functional, both in vitro, upon expression in recombinant form, and in vivo, when ectopically expressed in cells (K. Andrä et al., unpublished).

Subplasma membrane (cortical) cytoskeleton

Plectin has been shown to interact in vitro with the subplasma membrane skeleton proteins α-spectrin and its non-erythroid counterpart the 240 kDa chain of fodrin (Herrmann and Wiche, 1987). Purified plectin and fodrin exhibit binding affinities in the nM range (K_d ~10^{-8} M), exceptionally high for constituent proteins of the cytoskeleton (G. Weitzer et al., unpublished). Plectin colocalized with fodrin in a number of tissues examined, notably eye lens, muscle, and skin (unpublished results). In polarized MDCK cells, a model of simple epithelial cells, plectin was restricted to areas underlying the lateral plasma membrane, where it colocalized with fodrin. Interestingly, codistribution with fodrin and in vitro complex formation in cell lysates were dependent on the polarized state of the cell, indicating that plectin-membrane cytoskeleton interaction is important for the establishment and/or maintenance of epithelial cell polarization, probably via plectin-mediated IF attachment at the lateral cell membrane (Eger et al., 1997).

Desmosomes

The ultrastructural localization of plectin in simple epithelial cells (MDCK cells) and epithelial tissue (tongue) using both pre-embedding and post-embedding combined with gold labeling and cryo-sectioning techniques revealed a concentration of plectin along the surface of desmosomal plaque regions, in general agreement with its predominantly lateral membrane association. As determined morphometrically, epitopes residing in the center of plectin's rod domain were found between 50 and 125 nm (average distance: 90 nm) from the membrane, while desmoplakin molecules were found at distances between 25 and 50 nm. Considering the extraordinary length of individual plectin molecules (~200 nm) these data would be consistent with a model where plectin forms bridges between the bona fide desmosomal protein desmoplakin and cytoskeletal IF networks. This model is further supported by the demonstration of a direct interaction between plectin and desmoplakin in vitro (Eger et al., 1997).

Hemidesmosomes

The localization of plectin at hemidesmosomes (HDs) of human skin by peroxidase immunoelectron microscopy nearly 15 years ago (Wiche et al., 1984a) suggested early on that the protein might be involved in the attachment of IFs to this type of adhesion structure. Plectin's precise role in the formation and/or function of HDs, however, remained obscure for several years. Recent progress towards solving this question was made along two fronts, one of which was the plectin gene knockout in mice (Andrä et al., 1997). Although plectin (−/−) mice exhibited severe skin blistering caused by degeneration of keratinocytes, HDs observed in skin areas unaffected by blistering appeared ultrastructurally intact and numerous filaments were seen emanating from their cytoplasmic plate structures. However, compared to basal keratinocytes of

control mice, the keratin filaments appeared looser and less bundled, particularly at their insertion site into the inner plate structure. Moreover, a significant reduction in the number of HDs along the basal cell surface membrane of keratinocytes was noticed, and there was evidence for a blistering mechanism in which cell rupture could occur irregularly on both sides (apical and basal) of the hemidesmosomal inner plate structure, or within the plate itself. This indicated that plectin, albeit being dispensable for HD formation per se, was important for their stabilization and, consequently, most likely promotes formation of these junctional complexes.

Evidence in favor of a model where plectin serves as an essential linker and stabilizing element of HDs came also from biochemical studies showing that the protein directly interacts with the transmembrane HD-specific integrin receptor α6β4 via two separate segments of the cytoplasmic tail domain of the β4 subunit (Rezniczek et al., 1998). Furthermore, overexpression in cultured cells of integrin β4 polypeptides containing these binding sites, but lacking the remainder of the polypeptide chain, including its transmembrane spanning domain, led to bundling and collapse of IF network arrays, likely due to interference with IF-membrane attachment. Moreover, ectopic expression of similar integrin β4 polypeptides largely suppressed plectin's association with HD in cells (804G) capable of forming such structures in vitro. The biochemical data and the gene knockout data combined can be considered as strong evidence for plectin's essential role in interlinking integrin β4 subunits and cytokeratin filaments at the HD, and thus in maintaining the integrity and necessary strength of this vital cytoskeleton-extracellular matrix junction.

Due to the very large size (>500 kDa) of plectin molecules predicted on the basis of cDNA sequencing (see below), and their extended (~200 nm long) multi-domain structure, as visualized by electron microscopy (Foisner and Wiche, 1987), plectin molecules would have the dimensions to span the entire tripartite structure characteristic of HDs. In this way they could extend from the plasma membrane, the location of integrin β4, to the filament anchorage site at the HD inner plate structure and beyond. In fact, a recent gold immunoelectron microscopy localization of plectin molecules in basal keratinocytes using domain-specific antibodies (Rezniczek et al., 1998) revealed plectin-specific epitopes at the levels of cytokeratin bundles inserting into the inner plate structure as well as in basolateral regions of HDs, without any apparent orientation of plectin molecules. Based on this and the finding that plectin interacts with the cytoplasmic tail of integrin β4 via carboxy- as well as amino-terminal domains and the previous identification of a highly conserved IF (cytokeratin)-binding site in the carboxy-terminal globular domain of plectin molecules (Nikolic et al., 1996), the following scenario (Fig. 2) was suggested: randomly oriented plectin molecules act as a stabilizing clamp bridging plasma membrane-associated hemidesmosomal integrin receptor complexes and HD inner plate-anchored cytokeratin filament bundles. The presence of multiple plectin-binding sites on the integrin β4 subunit may enable plectin to further enhance the stability of HDs through interlinking of integrin β4 subunits. Self-interaction of integrin β4 subunit proteins, as demonstrated using recombinant proteins in conjunction with in vitro binding

Fig. 2. Schematic portrayal of plectin's putative role as a stabilizer of hemidesmosomes. Plectin molecules are depicted to connect the plasma membrane-associated plaque structure (location of integrin receptor α6β4) with cytokeratin filaments (anchored at the inner plate) in a clamp-like fashion via interaction sites located at their opposite ends. Note that plectin molecules in dimeric (two polypeptide chains arranged parallel to form a coiled coil rod; at flanks) as well as tetrameric forms (two dimers arranged anti-parallel; at center) could provide IF- and integrin β4-binding sites at opposite ends and would be able to bridge the entire cytoplasmic domain of the hemidesmosome via their ~200 nm long rod domains.
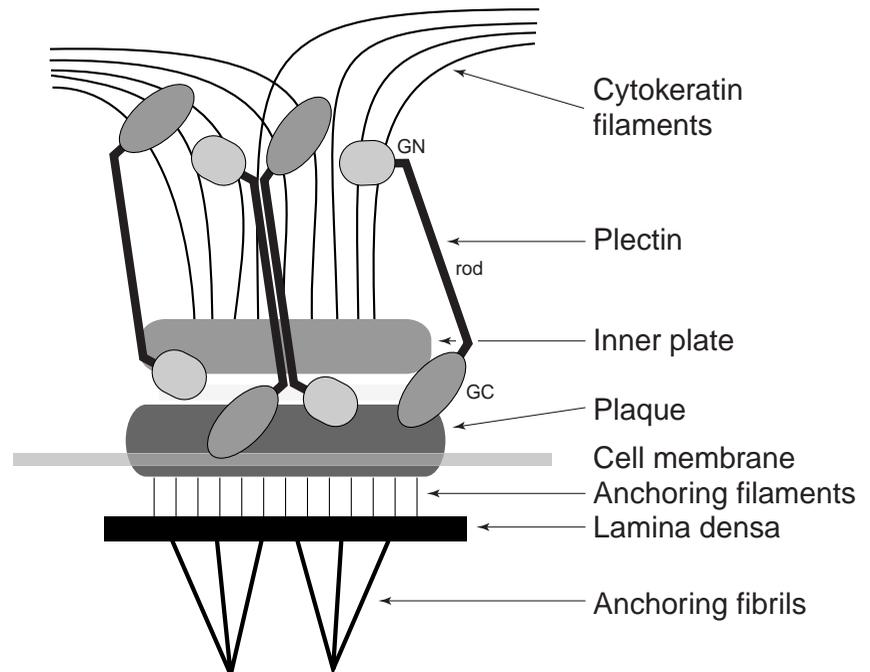
assays (Rezniczek et al., 1998), is likely to provide additional stabilization of this complex. Moreover, plectin and/or integrin β4 are likely to interact with other HD-associated proteins, such as BPAG1e, further enhancing the mechanical integrity of the adhesion complex.

Plectin's manifold molecular interactions and strategic cellular localization, together with the phenotypes of EBS-MD patients and plectin-deficient mice (see below), clearly confirmed the concept that plectin stabilizes cells mechanically by acting as a versatile linker and scaffolding protein of the cytoskeleton in different cell types (Fig. 3).

GENE ORGANIZATION AND ISOFORM DIVERSITY

The first plectin species cloned and sequenced was from rat (Wiche et al., 1991). The sequence of the human gene, its exon-intron organization and localization (q24) on chromosome 8 were reported five years later, first by Liu et al. (1996), and a few months later by McLean et al. (1996). The plectin gene locus has meanwhile also been studied in rat (Elliott et al., 1997) and mouse (Andrä et al., 1997; and unpublished results from our laboratory). In the first report on the human gene organization the coding sequence of plectin was found to contain 32 exons extending over 32 kb of the human genome. Most of the introns resided within a region encoding the amino-terminal domain of the molecule, whereas the entire central rod and carboxy-terminal domains were found to be encoded by single exons of unusual length, i.e. >3 kb and >6 kb, respectively. McLean et al. (1996) independently confirmed this analysis, except for reporting 33 exons, one of which was found to be noncoding and preceding the first coding exon. Moreover, the first coding exon reported by these authors differed in size and sequence from the one characterized by Liu et al. (1996) indicating isoform variation. In subsequent studies (Elliott et al., 1997; and unpublished results from this

laboratory) plectin transcripts with alternative first coding exons were identified, in a variety without precedent in the literature. Thus far, at least eight such exons, including those originally identified by Liu et al. (1996) and McLean et al. (1996), were found spread over ~35 kb of genomic DNA.

Alternatively spliced transcripts containing sequences corresponding to these exons have been identified in a variety of tissues by RNase protection assays. Their expression levels in different tissues were found not to be uniform, showing characteristic expression patterns in the various tissues examined. Some variants were found to be clearly dominant over others, for instance in skeletal muscle, heart, or brain. In all variants thus far identified the distinct first coding exons were found to have the same splicing acceptor, namely exon 2, which is the first of six exons encoding plectin's highly conserved ABD (see above). In addition, variants have been identified, that contain modified versions of the ABD, generated by differential splicing of exons encoding this domain. Another level of 5′ transcript complexity was detected upstream of certain exon 1 loci, where differential splicing of several different noncoding exons into the same first coding exon occurred. This included variants generated by internal splicing of one of the noncoding exons (unpublished data from this laboratory).

Such vast diversity of transcript 5′ termini exceeds that of dystrophin, for which six alternative first coding exons have been reported. The biological role of plectin's 5′ transcript complexity remains to be established. We consider it most likely that the various starting exons (coding or noncoding), similar to the case of dystrophin, are part of distinct promoters, which may be independently controlled by tissue-specific, development-, or environment-dependent transcriptional regulators. Additionally, distinct 5′ termini may affect differential targeting of plectin mRNAs to distinct subcellular locations. Also, it is still unclear whether the selection of different 5′ exons during transcription determines,
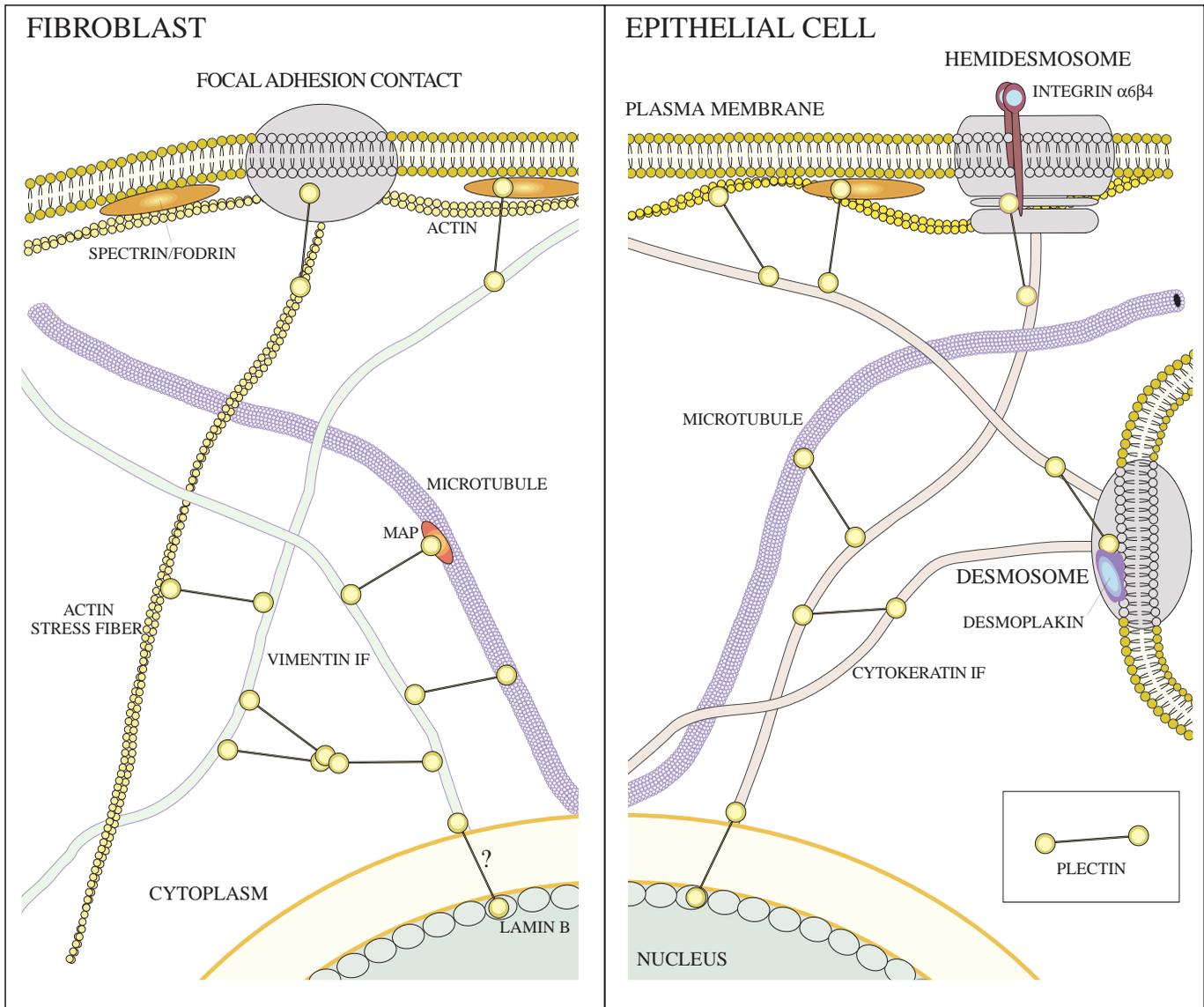
Fig. 3. Model outlining plectin functions in the organization and stabilization of cytoarchitecture in fibroblast and epithelial cells. Plectin molecules are depicted as mechanical linkers between IFs (vimentin, cytokeratins) and various cytoskeletal structures, including other filament networks (microtubules, actin stress fibers, peripheral actin filaments), the subplasma membrane protein skeleton (spectrin/fodrin), specialized transmembrane complexes (focal adhesion contacts, hemidesmosomes, desmosomes), and the nuclear lamina. Constituent proteins of plectin-associated structures that have been shown to specifically bind to plectin on the molecular level are indicated. Note, molecular binding domains thus far identified reside either on opposite (actin, IFs) or both ends (integrin receptor beta-4 subunit) of the plectin molecule (see Fig. 1), facilitating linking function. The evidence for network formation and long distance bridging through self-interaction of plectin molecules has been discussed previously (Wiche, 1989). The model shown does not imply that all interfaces of plectin molecules with the various binding partners necessarily have to be activated simultaneously and all the time. In fact, their differential regulation at distinct subcellular sites could allow local fine tuning of cellular viscoelastic properties and plasticity.

or favors, splicing events taking place further downstream leading to distinct isoform expression. However, with the exception of exon 31-less variants, which lack most, if not all, of plectin's rod domain (Elliott et al., 1997), such isoforms have yet to be found. On the protein level distinct amino-termini encoded by the various first coding exons may affect isoform functions, including subcellular localization, protein interactions, and modulation of functional domains, particularly the nearby actin binding domain encoded by succeeding exons 2-8.

RELATED PROTEINS

The structure of the carboxy-terminal domain of plectin is dominated by six highly homologous ~300 amino acid long repeats (see Fig. 1), which occur in lesser number also in desmoplakin (3 repeats; Green et al., 1990), the epithelial and neuronal isoforms of bullous pemphigoid antigen 1, BPAG1e and BPAG1n (also called dystonin) which have 2 repeats (Sawamura et al., 1991), and the more recently identified envoplakin (1 repeat; Ruhrberg et al., 1996). Additional

structures features shared by these proteins and plectin are the central α-helical rod and the amino-terminal globular domains. A recently identified protein called periplakin (Ruhrberg et al., 1997) contains an amino-terminal globular domain and a rod, but lacks a carboxy-terminal globular domain. Because of significant sequence homologies found in various parts of these molecules, in particular the amino- and carboxy-terminal domains, it is likely that these proteins represent a family of proteins that all arose (derive) from a common precursor gene. Whether this novel family of proteins will be called *plakins* (Uitto et al., 1996; Ruhrberg and Watt, 1997), inferring plaque or plate association (see Franke et al., 1982, where the name desmoplakin was coined), or should be given a more function-oriented name, such as cytolinker proteins or *cytolinkers,* based on their often used description as cytoskeletal linker or cross-linker proteins (see e.g. Houseweart and Cleveland, 1998; Yang et al., 1996), remains to be decided. The answers to questions whether the proteins IFAP300 (Yang et al., 1985), HD1 (Hieda et al., 1992), and 450 kDa human epidermal autoantigen (Fujiwara et al., 1996), belong to this protein family and to which extent these proteins are related to plectin, or to one of its isoforms, await the full molecular characterization including cloning and sequencing of the genes encoding these proteins. Until then, the use of double names, such as plectin/HD1, occasionally found in the literature, seems premature. Furthermore, a situation where highly similar gene products are derived from distinct genes, such as in the case of dystrophin and utrophin (for a review see Blake et al., 1996), may apply also to plectin and some of these related proteins.

PLECTIN DEFICIENCY IN MAN AND MOUSE

Based on plectin's prominence at plasma membrane junctional sites of IFs, in particular HDs of basal keratinocytes, an involvement of plectin in bullous skin diseases has been anticipated (Wiche et al., 1984a). Likewise, the high level expression of plectin in all types of muscle tissues and its conspicuous association with presumptive organizing centers of muscle cell cytoarchitecture, known for a long time (Wiche et al., 1983; Zernig and Wiche, 1985; see also Elliott et el., 1997), made us aware that muscle could also be affected by plectin deficiencies. Some of these expectations were indeed met, when in 1996 a number of groups reported that patients suffering from epidermolysis bullosa simplex (EBS)-MD, an autosomal recessive severe skin blistering disease combined with muscular dystrophy, lack plectin expression in skin and muscle tissues (Gache et al., 1996; Smith et al, 1996; McLean et al., 1996; Pulkkinen et al., 1996; Chavanas et al., 1996). Meanwhile it has been reported that some EBS-MD patients additionally suffer from inspiratory stridor and respiratory distress involving laryngeal obstruction and urethral strictures (Mellerio et al., 1997; Dang et al., 1998). In all the cases reported, the defect has been traced to homozygous mutations in the plectin gene leading to premature stop codons within the >3 kb-long exon encoding the rod domain of the protein, except for one case (Pulkkinen et al., 1996), in which a 9 bp deletion was found in a preceding exon (see also Fig. 1); the missing 3-amino acid sequence identified in this case must be central to plectin function. A form of hereditary (autosomal dominant) EBS (EBS-Ogna), linked to chromosome 8q24 and showing abnormalities in plectin expression (Koss-Harnes et al., 1997), is probably also caused by a plectin gene defect.

The skin and muscle phenotypes associated with EBS-MD, as far as documented, were also found in plectin-deficient mice (Andrä et al., 1997). However, the more extensive phenotypic analysis of plectin (−/−) mice opened up new perspectives regarding plectin's involvement in diseases other than those known as EBS-MD. For instance, focal lesions similar to those found in skeletal muscle of plectin (−/−) mice are typical of an autosomal recessive type of myopathy (minicore/multicore disease), which is characterized by multiple small randomly distributed areas in the muscle fibers with myofibrillar degenerative changes and, as reported for some cases, cardiomyopathies (Magliocco et al., 1989; Bertini et al., 1990). Since the genetic basis for this disease is unknown, it would be interesting to establish whether it can be mapped to the plectin gene locus on chromosome 8.

Pathological alterations of heart muscle observed in plectin (−/−) mice raise expectations that EBS-MD patients may have a similar, hitherto unnoticed phenotype. A partial disruption of intercalated discs, as observed in plectin-deficient mice, may reflect an increased fragility of plectinless desmosomes, which represent a major adhesive device along the intercalated disc structure of heart.

Although there is suggestive evidence for a possible functional role of plectin in neuronal tissue (Errante et al., 1994; Smith et al., 1996) no pathological alteration could be observed in the brain of plectin (−/−) mice. Considering that symptoms of neurodegeneration usually arise at an older age, the most likely explanation for the absence of any such alterations could be the early death of the animals. However, compensation for plectin deficiency through other proteins, that are functionally related would be another plausible explanation. A candidate would be dystonin, the neuronal isoform of BPAG1, which is structurally highly related to plectin (see above). In fact, ablation of the dystonin gene causes dystonia and sensory neuron degeneration in mice (Guo et al., 1995), and a partial deletion causes *dystonia musculorum*, a hereditary neurodegenerative disease in mice (Brown et al., 1995). Thus, dystonin may take over plectin's role in cells that normally express both proteins such as motor neurons. Furthermore, it would not be surprising if a demyelinating neuropathy found in gypsies, that has been mapped to chromosome 8q24 (Kalaydijeva et al., 1996), the gene locus of plectin (Liu et al., 1996), will be traced to defects in the plectin gene.

NEW FUNCTIONS REVEALED IN PLECTIN (−/−) CELLS

The phenotypes of EBS-MD patients and of plectin-deficient mice both lent strong support to the concept that plectin is a stabilizer of cells against mechanical stress due to its linker and scaffolding functions, as depicted in Fig. 3. However, the establishment of plectin (−/−) in vitro cell cultures from plectin-deficient mice has started to yield new insights into plectin functions that go beyond this concept. Cultivated plectin-negative dermal fibroblasts derived from 2-day-old plectin (−/−) mice displayed a dramatic increase in the number of actin stress fiber bundles and focal adhesion contacts,

compared to control cells (K. Andrä et al., unpublished). This phenomenon was particularly noticable within short periods (~2 hours) after seeding cells onto solid surfaces. Moreover, contrary to control cells, actin stress fibers of mutant cells were insensitive to extracellular stimuli activating rho, rac, and cdc42 GTPase signalling cascades, which control actin stress fiber (rho), lamellipodia (rac), and filipodia formation (cdc42). In addition, plectin (−/−) fibroblast cells showed significantly diminished motility in wound healing and filter penetration assays, and stronger adherence to substrata when exposed to defined fluid shear stress. Confirming these observations, plectin deficiency substantially diminished the ability of cultured astroglial cells to undergo dibutyryl-cAMP-induced differentiation, a process accompanied by dramatic morphological changes, involving actin-based mechanisms mediated by rho. These findings strongly support the idea that plectin not only provides cells with mechanical strength, but also plays an essential role as regulator of cellular processes linked to actin filament dynamics.

CONCLUSIONS AND PERSPECTIVES

Since its first description by Pytela and Wiche (1980) plectin has fulfilled much of the initial promise as a candidate organizer protein of the cytoskeleton. Early results demonstrated an unprecedented versatile binding ability to other cytoskeletal proteins, a widespread and abundant expression in cells and tissues, and a cellular localization at sites of strategic importance for cytoarchitecture integrity, such as cytoskeletal filament anchorage and junctional crossover sites. With the identification of a human disease (EBS-MD) caused by plectin gene defects and the phenotypic analyses of patients suffering from this disease and that of plectin null mice generated by targeted gene disruption, the search for plectin function, which began nearly 20 years ago, has reached decisive breakthroughs. These studies together with the identification of plectin's interaction partners at cytoskeleton-plasma membrane junctions, such as hemidesmosomal integrin β4, convincingly demonstrated that plectin plays a major role in the stabilization of cells and tissues against mechanical stress.

However, recent studies with cultured fibroblasts and astroglial cells derived from plectin null mice, which unmasked plectin as a regulator of actin filament dynamics, add a new perspective on putative functions of the protein. Considering its abundance and distribution throughout the cytoplasm along cytoplasmic IFs networks and in peripheral plasma membrane regions, a G-actin sequestering role of plectin seems likely. Through the binding of unpolymerized actin, plectin may counteract actin stress fiber formation and thus positively control cell motility requiring actin filament dynamics. It will be a challenge for future research to establish whether this mechanism is indeed the one operating in plectin-mediated actin cytoskeleton regulation, or whether alternative or additional mechanisms exist. New insights are also expected from studies of other types of plectin (−/−) cells where plectin may play a central role, such as myoblasts, Schwann cells, endothelial cells, keratinocytes, and others.

An additional important function of plectin could be to serve as a docking site for other cytoskeletal and/or soluble cytoplasmic proteins. Due to their large size, multiple binding abilities, and association with internal IFs and peripheral subplasma membrane structures and junctional complexes, plectin molecules may provide a structural platform for the localized assembly of multi-component protein machineries, including regulatory factors and molecules forming signalling cascades. Plectin's recently demonstrated direct interactions with the integrin receptor α6β4 can be considered as first evidence for a role of plectin in signal transduction. Studies focussing on this putative new role of plectin and on molecular mechanisms involved in plectin-mediated signalling are expected to intensify substantially in the future.

The recently discovered vast diversity of differentially spliced plectin transcripts, varying in 5′ end structure, and their differential expression in different tissues and cells types created new perspectives regarding both possible functions of expressed isoforms and regulation of gene expression. Future studies will have to explore whether such transcript diversity is a mere consequence of a complex gene regulatory machinery controlling the level and the timing of plectin expression in different tissues and cell types, or whether it sets up a basis for providing isoforms with different properties and functions. Considering the complexity of plectin functions emerging from recent studies both scenarios may come true. On the whole, like no other protein thus far analyzed, plectin seems to be fit for its proposed complex role as organizer of cytoarchitecture and regulator of cellular plasticity and morphogenesis. The years to come will tell.

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