Update on Cytoskeleton-Plasma Membrane-Cell Wall Continuum

Cytoskeleton-Plasma Membrane-Cell Wall Continuum in Plants. Emerging Links Revisited¹

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Eukaryotic cells typically deviate from spherical shapes due to complex interactions between elements of their cytoskeleton and the extracellular matrix (ECM). Communication between the cytoskeleton and ECM is one of the most characteristic features of cellular mechanics and allows cells to respond effectively to various signals, especially mechanical stimuli. Cells devoid of the ECM and/or cytoskeleton inevitably lose their polar shapes returning to the default, preferentially spherical, shape (for plant cells, see Smith, 2001; Baluška et al., 2003b). This loss of cellular polarization prevents cells from their cell-to-cell interactions and communication. This is true for both animal and plant cells, even when modes of ECM-cytoskeleton and cell-to-cell interactions are based on contrasting principles in these two types of multicellular organisms (Baluška et al., 2003b). Multicellularity in plants and animals has evolved independently. Animals and humans are truly multicellular organisms as their inherently motile cells aggregate together and typically can associate with a wide range of cell types (Critchley, 2000; Geiger and Bershadsky, 2001). On the other hand, plants are supracellular organisms because their immobile cells divide via the phragmoplast-based incomplete cytokinesis that results in the formation of cytoplasmic cell-to-cell channels known as plasmodesmata (Lucas et al., 1993). Consequently, plant cells are not fully separated, and both the plasma membrane and endoplasmic reticulum traverse cellular borders through plasmodesmata. These membranes are physically interlinked into continuous membraneous system essentially spanning the whole plant (Lucas et al., 1993). The ECM of plant cells, better known as cell walls, is integrated into the apoplast—a structurally coherent superstructure extending throughout the plant body (Wojtaszek, 2001). Cell walls represent an inherent part of plant cells (Wojtaszek, 2000) although plant protoplasts can rapidly and reversibly retract from the cell wall during osmotic stress-induced plasmolysis (Oparka, 1994; Lang-Pauluzzi and Gunning, 2000). In contrast, most animal and human cells are truly “naked” and interact with the ECM in its vicinity that is often produced by other cells, occasionally at discrete domains known as focal adhesions (Critchley, 2000) or tight adherens junctions and desmosomes in the case of cell-to-cell contacts (Geiger and Bershadsky, 2001).

Entering the post-genomic era, contemporary cell biology is becoming aware of epigenetic mechanisms of cell differentiation (Goldman, 2003; Orlando, 2003). In particular, one of the new priorities in the cell biology would be to understand how mechanical forces regulate and integrate cellular activities (Ingber, 2003a, 2003b). It is well known that mechanical forces are rapidly, and almost without loss of information, translated into biochemical messages (Riveline et al., 2001; Ingber, 2003a, 2003b). Moreover, mechanical forces play essential roles in the control of cellular behavior (Janmey, 1998; Geiger et al., 2001; Gillespie and Walker, 2001; Riveline et al., 2001; Ingber, 2003a, 2003b; Ponta et al., 2003; Rorth, 2003). Generally, cellular structure is inherently endowed with information that can be both stored and handed over to other structures via templating processes (for plant cells, see Baluška et al., 1997, 2003b). Although available data are rather scarce for higher plants, and critical linker molecules between the cytoskeleton and ECM are still missing (Pont-Lezica et al., 1993; Wyatt and Carpita, 1993; Fowler and Quatrano, 1997; Miller et al., 1997a, 1997b; Kohorn, 2000; Heath, 2001), it is increasingly obvious that cell shape and mechanical forces dictate several processes among which the control of division planes is one of the most obvious (Lintilhac and Vesecky, 1984; Lynch and Lintilhac, 1997; Green, 1999; Sipiczki et al., 2000; Smith, 2001). The integrated cytoskeleton and its dynamic adhesion to the plasma membrane allow these complex interactions between mechanical forces and chemical signals (Critchley, 2000; Geiger and Bershadsky, 2001; Sheetz, 2001). What is still unknown in plants is the molecular nature of linkers between elements of the cytoskeleton and components of the cell wall. Available Arabidopsis Genome Database

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make it clear that plants lack true homologs of classical adhesion proteins of animal cells including integrin, talin, vinculin, filamin, α-actinin, and tensin (Hussey et al., 2002). In this Update, we provide a brief survey of linker molecules in animal cells and highlight emerging plant-specific linkers. We hope that our Update will stimulate more activity in this exciting and very important area of research.

LINKER MOLECULES BETWEEN THE CYTOSKELETON AND ECM IN ANIMAL CELLS

Integrins

Physical coupling between cytoskeleton and ECM is relatively well understood in animal cells. Several molecules are well known to act as linkers between elements of the cytoskeleton and ECM components. The most famous and best-understood are the integrins, which communicate signals between fibronectin, vitronectin, laminin, and other RGD-containing ECM proteins and the actin cytoskeleton within the cytoplasm. Integrins allow bidirectional signaling in all multicellular eukaryotes with the exception of plants and fungi (Burke, 1999; Hynes, 1999, 2002; Geiger et al., 2001; Miranti and Brugge, 2002; Schwartz and Ginsberg, 2002). Surprisingly, Drosophila sp. lacks classical integrin ECM ligands, such as fibronectin and vitronectin, but there are other RGD-containing ECM proteins in insects that interact with integrins (Hynes and Zhao, 2000). At the cytoplasmic side, integrins bind directly with several actin-binding proteins such as talin, vinculin, filamin, paxillin, α-actinin, and tensin (Calderwood et al., 2000; Critchley, 2000; Geiger et al., 2001; Hynes, 2002; Miranti and Brugge, 2002; Schwartz and Ginsberg, 2002; Sakai et al., 2003). These actin-binding proteins then recruit the next cohort of actin-binding proteins such as profilin, VASP, and Arps, which drive local actin polymerization (Critchley, 2000; Craig and Chen, 2003; Sakai et al., 2003). After receiving inputs at the ECM-plasma membrane interface, integrins convey this information downstream via several signal transducers including members of the Ras family of small GTPases and mitogen-activated protein kinases (MAPKs; Juliano, 2002; Schwartz and Ginsberg, 2002). Additionally, integrins signal into the cell interior via focal adhesion kinase, p21-activated kinase, phosphatidylinositol 3-kinase, integrin-linked kinase, Tyr kinase γ-Src, adenylyl cyclase, protein kinase A, LIM-kinase, and protein kinase C (Geiger et al., 2001; Wu and Dedhar, 2001; Zamir and Geiger, 2001a, 2001b; Juliano, 2002; Inger, 2003b; Sakai et al., 2003).

Cadherins

Cadherins are calcium-dependent transmembrane adhesion molecules that play a key role in the maintenance of tissue architecture (Adams and Nelson, 1998) due to their homophilic cell-to-cell interactions and abundant interactions with the dynamic actin cytoskeleton (Kovacs et al., 2002b). Cadherins accumulate at specialized cell-to-cell adhesion domains known as tight junctions, adherens junctions, and desmosomes (Adams and Nelson, 1998). Cadherins interact with the actin cytoskeleton and many signaling molecules like Rac/Rho families of small GTPases and PI 3-kinase suggesting that cadherins are, in fact, adhesion-activated-signaling receptors (Noren et al., 2001; Braga, 2002; Kovacs et al., 2002a). Several other signaling molecules interact with cadherins including Tyr kinases and phosphatases, lipid kinases, heterotrimeric GTPases, and MAPKs (Pece and Gutkind, 2000).

Immunoglobulin Superfamily, Selectins, and Dystrophin-Glycoprotein Complexes

Like cadherins, these three less studied groups of cell adhesion receptors also perform homophilic and heterophilic interactions to hold adjacent cells together. Similarly to integrins and cadherins, they link ECM with the actin cytoskeleton and employ MAPK cascades for signal transduction (Hynes, 1999; Juliano, 2002; McEver, 2002; Michele and Campbell, 2003).

Hyaluronan Receptors CD44 and RHAMM

CD44 and RHAMM are transmembrane glycoproteins present in most vertebrate cells. At the ECM side, they interact with hyaluronan, but also with collagen, laminin, and fibronectin. Within the cytoplasm, these transmembrane proteins interact with the actin cytoskeleton (Culty et al., 1992; Lokeshwar et al., 1994; Entwistle et al., 1996; Föger et al., 2000; Ponta et al., 2003). Hyaluronan receptors are linked to the actin cytoskeleton via actin-binding proteins including those of the band 4.1 superfamily, ankyrin, and proteins of the ERM family counting gzerin, radixin, and moesin (Bretscher et al., 2002; Legg et al., 2002; Ponta et al., 2003). Interestingly, CD44 and RHAMM act as receptors for endocytosis-mediated internalization of hyaluronan (Culty et al., 1992; Turley et al., 2002; Ponta et al., 2003). These ECM-cytoskeleton linkers signal into the cytoplasm via Tyr kinases, MAPKs, Rac1, PI 3-kinase, and protein kinase C (Oliferenko et al., 2000; Legg et al., 2002; Turley et al., 2002; Ponta et al., 2003).

Tetraspanin Proteins

Transmembrane proteins of the tetraspanin family, with large extracellular loops and cytoskeleton interacting cytoplasmic tails, emerge as a new candidate for the ECM-cytoskeleton linker in multicellular organisms (Lagaudrière-Gesbert et al., 1998; Boucheix and Rubinstein, 2001; Hemler, 2001; Stipp et al.,
Besides several actin-binding proteins, PIP2 let of the plasma membrane (Nebl et al., 2000; Caroni et al., 2002). Interestingly, one class of tetrasin proteins known as the secretory carrier membrane proteins, which is important for the synaptic vesicle trafficking, is present also in higher plants, but it is absent from unicellular eukaryotes (Fernández-Chacón and Südhof, 2000; Hubbard et al., 2000).

**UNIQUE ROLE OF THE ACTIN CYTOSKELETON IN ECM-CYTOSKELETON COMMUNICATION**

A recurring theme common to ECM-cytoskeleton linkers of animal cells is that these mechanotransducing transmembrane molecules communicate and interact preferentially with the actin cytoskeleton on the cytoplasmic side of the plasma membrane (Lagaudriére-Gesbert et al., 1998; Critchley, 2000; Ponta et al., 2003; Sakai et al., 2003; Stipp et al., 2003). Inherent association of the actin cytoskeleton with the plasma membrane is due to interactions among actin-binding proteins and phosphatidylinositol-bisphosphate (PIP2), which localizes to the inner leaflet of the plasma membrane (Nebel et al., 2000; Caroni, 2001). Besides several actin-binding proteins, PIP2 binds also to transmembrane adhesion proteins like CD44, ICAMs, and syndecan-4 (Heiska et al., 1998; Couchman et al., 2002). Thus, it is not surprising that adhesion of the actin cytoskeleton to the plasma membrane is dependent on PIP2 (Raucher et al., 2000). PIP2 was localized to discrete domains at the plasma membrane of maize (Zea mays) root cells. These resemble profilin-enriched domains and, intriguingly, the phospholipase C activator mastoparan induced redistribution of PIP2 and remodeling of the actin cytoskeleton (Baluška et al., 2001d).

Generally, the actin cytoskeleton has been optimized during eukaryotic evolution for acting as a structural scaffold for diverse signaling complexes (Juliano, 2002). Recent data from plants support the concept whereby the dynamic actin cytoskeleton is closely linked to the signaling cascades initiated at the plasma membrane (Meagher et al., 1999; Volkmann and Baluška, 1999; Staiger, 2000; Staiger et al., 2000; McCurdy et al., 2001; Hussey et al., 2002; Šamaj et al., 2002).

**EMERGING LINKER MOLECULES BETWEEN THE CYTOSKELETON AND CELL WALLS IN PLANTS**

Plant cells and, probably fungal cells, are unique in the whole eukaryotic superkingdom in terms of the linker molecules between the cytoskeleton and components of the ECM/cell walls. Despite the presence of proteins immunologically related to both integrins and cadherins (Kaminsky and Heath, 1994; Katembe et al., 1997; Barthou et al., 1998; Canut et al., 1998; Faik et al., 1998; Kiba et al., 1998; Baluška et al., 1999b; Labouré et al., 1999; Laval et al., 1999; Nagpal and Quatrano, 1999; Swatzell et al., 1999; Sun et al., 2000), higher plants seem to lack true homologs of these proteins (Arabidopsis Genome Initiative, 2000). This situation might be surprising in the face of rather well-conserved nature of the actin cytoskeleton (Meagher et al., 1999; Staiger et al., 2000; McCurdy et al., 2001; Hussey et al., 2002). One could argue that the different organization of adhesion sites in plants is due to the unique molecular nature of plant cell walls. Generally, the animal ECM is proteinaceous, and protein fibrils are the prime mechanical devices. In contrast, carbohydrates are the major building blocks of plant cell walls. This implies a different type of intermolecular interaction for which integrins and/or cadherins might not be adapted. However, Drosophila sp. lacks vitronectin and fibronectin and still uses other RGD-containing ECM proteins to link integrins with the cytoskeleton (Hynes and Zhao, 2000). Although higher plant cells also seem to use RGD-containing proteins to connect their cell walls with the plasma membrane (Schindler et al., 1989; Katembe et al., 1997; Barthou et al., 1998; Canut et al., 1998; Faik et al., 1998; Kiba et al., 1998; Labouré et al., 1999; Laval et al., 1999; Sun et al., 2000; Mellersh and Heath, 2001), they seem to lack the true homologs of integrins.

One hypothesis to explain the absence of integrins, cadherins, and other animal-type linkers in plants is that plant cells, especially root cells, are often exposed to hyperosmotic stress, which necessitates rapid and reversible retractions of their protoplasts from the cell walls, the so-called plasmolytic cycle (Oparka and Crawford, 1994; Lang-Pauluzzi and Gunning, 2000; Komis et al., 2003). To maintain mechanical integrity, osmotically stressed plant cells must retract their protoplasts from their cell walls almost immediately. Integrin- and cadherin-based adhesion complexes are apparently too complex to disintegrate rapidly. Moreover, the Arabidopsis genome lacks not only genes for integrins, but also genes for actin-associated proteins acting as critical linkers between integrins and the actin cytoskeleton including talin, vinculin, filamin, α-actinin, and tensin (Hussey et al., 2002). Therefore, plant cells may have designed other molecules and used other principles for the very dynamic interactions between cytoskeleton and cell walls. Bruce Kohorn discussed putative plant-specific linker molecules, focusing on the four most appealing candidates: cell wall- associated kinases (WAKs), arabinogalactan proteins (AGPs), pectins, and cellulose synthases (Kohorn, 2000). Progress made during the last three years has resulted in additional candidates including formins,
plant-specific myosins of the class VIII, phospholipase D, and callose synthases.

WAKs and Cell Wall Pectins

Among the emerging plant-specific linkers of the cytoskeleton with plant cell walls, the WAKs appear to be the most attractive candidate because they have, in addition to cell wall and transmembrane domains, a cytoplasmic Ser/Thr protein kinase domain (He et al., 1996, 1999; Kohorn, 2000, 2001; Anderson et al., 2001; Wagner and Kohorn, 2001; Lally et al., 2001). In contrast to the cytoplasmic and transmembrane domains, which are well-conserved, the extracellular domains of WAKs are the most variable among the five Arabidopsis WAK isoforms (WAK1–5) and contain motifs typical for animal proteins such as epidermal growth factor repeats, tenasin-like, collagen-like, and neurexin-like sequences (He et al., 1999; Anderson et al., 2001). So far, the functional significance of these motifs remains unknown, although epidermal growth factor repeats suggest calcium-mediated dimerization of WAKs. Interestingly, the phosphorylated version of WAK1 was found to be firmly bound to plasma membrane-associated cell wall pectins (Kohorn, 2000, 2001; Anderson et al., 2001; Wagner and Kohorn, 2001; Cosgrove, 2000). Plasma membrane-associated pectins have adhesive properties (Mollet et al., 2000; Iwai et al., 2002; Lord and Mollet, 2002) and undergo endocytosis in meristematic root cells (Baluška et al., 2002; Yu et al., 2002). Intriguingly, depriving cells of boron, which cross-links RGII pectins in cell walls, results in inhibition of endocytosis of cell wall pectins (Yu et al., 2002). In addition, boron deprivation exerts immediate impacts on the cytoskeleton (Yu et al., 2001, 2003). Similarly, aluminum binds cell wall pectins (Horst et al., 1999), affects the cytoskeleton (Sivaguru et al., 1999), and induces rapid expression of WAKs (Sivaguru et al., 2003). Obviously, both aluminum and boron bind cell wall pectins and affect events at the cell wall-cytoskeleton interface (Horst et al., 1999; Yu et al., 2002). All this suggests that complex interactions between pectins, boron, and the cytoskeleton are important for the assembly of the cell wall-cytoskeleton continuum as well as for its maintenance via signal-mediated processes.

An intriguing possibility is that WAKs act as receptors for endocytosis of adhesive cell wall pectins. Interestingly, WAKs released from cell walls after pectinase treatments are still in a complex with cell wall pectins, or their fragments, because antibodies against pectins detect released WAKs on western blots (Anderson et al., 2001; Wagner and Kohorn, 2001). This situation is analogous to endocytosis of hyaluronan via CD44/RHAMM adhesion receptors in animal cells (Culty et al., 1992; Turley et al., 2002; Ponta et al., 2003). Plant pectins resemble hyaluronan in many aspects: Both are abundant components of the ECM having structural as well as signaling functions; they both perform endocytic internalization; and their smaller fragments, internalized presumably via endocytosis, have important signaling roles at the plasma membrane and within the cytoplasm (Van Cutsem and Messiah, 1994; Thain et al., 1995; Lee and Spicer, 2000; Baluška et al., 2002).

Besides cell wall pectins, WAK1 also binds to a glycin-rich cell wall protein AtGRP3 (Park et al., 2001) and to the 2C-type protein phosphatase KAPP in the cytoplasm forming an approximately 500-kD signalosome complex (Anderson et al., 2001). There are some additional data showing that glycine-rich proteins (GRPs) could be bound to pectins. Thus, pectinase activity could release WAKs directly or through release of GRPs from complexes. Studies using transgenic plants revealed that WAKs are essential for plant cell elongation (Lally et al., 2001; Wagner and Kohorn, 2001). In addition to the WAKs, recent database searches identified a large family of WAK-like kinases that might also be relevant for interactions between cell walls and the cytoskeleton (Verica and He, 2002).

AGPs

Another emerging candidate for signaling-mediated interactions between the cell wall and cytoskeleton of plant cells are the AGPs, which are predicted to have both adhesive and signaling properties (Schultz et al., 1998, 2000; Šamaj et al., 1998a, 1998b, 1999, 2000; Majewska-Sawka and Nothnagel, 2000). Interestingly, AGPs bind to cell wall pectins (Nothnagel, 1997), and they might interact also with WAKs because they seem to localize to the same domains at the plasma membrane of BY-2 protoplasts (Gens et al., 2000). Although AGPs do not span the plasma membrane, classical AGPs contain carboxy-terminal glycosyl phosphatidylinositol (GPI) anchors that keep them in tight association with the external leaflet of the plasma membrane and allow interference with signaling (Schultz et al., 1998, 2000; Oxley and Bacic, 1999; Svetek et al., 1999; Shi et al., 2003). These GPI anchors can be cleaved by phospholipase C in a signal-mediated manner (Sherrier et al., 1999; Borner et al., 2002) allowing controlled release of AGPs from the plasma membrane into the cell walls and the surrounding medium.

AGPs can be precipitated with Yariv reagent, which specifically binds the carbohydrate moiety of AGPs. Yariv reagent induces depolarization and ballooning of cells in roots of Arabidopsis (Willats and Knox, 1996). Moreover, Yariv also inhibits plant cell growth and can even induce programmed cell death implicating AGPs in diverse activities of plant cells (Gao and Showalter, 1999; Majewska-Sawka and Nothnagel, 2000). Interestingly, SOS5 is a plasma membrane-associated AGP protein that contains two-fasciclin-like domains and a C-terminal GPI an-
null
for polar transport of auxin (Baluška et al., 2003a, 2003b).

FUTURE PROSPECTS

WAKs interacting with pectins emerge as the most attractive candidate for the plant-specific cytoskeleton-cell wall linker. Unfortunately, nothing is known about possible interactions of WAKs with the cytoskeleton. WAKs belong to the large family of receptor-like protein kinases (RLKs) that are very abundant in plants. For instance, 2.5% of the Arabidopsis genome is represented by RLK genes (Shiu and Bleecker, 2001). RLKs can be classified according to predicted extracellular domains. Another interesting class of RLKs, with 38 putative genes, consists of lectin receptor protein kinases, which are predicted to bind cell wall lectins (Hervé et al., 1999; Shiu and Bleecker, 2001). Interestingly, lectin receptor protein kinases can be activated by pectin oligomers (Riou et al., 2002).

It is becoming increasingly clear that adhesion domains perform mechanosensory functions in eukaryotic cells (Geiger and Bershadsky, 2001; Rivelino et al., 2001). Physical state and local mechanical properties of the ECM are effectively sensed by plasma membrane-spanning linker molecules accumulated at these adhesion domains. Linker molecules then process this information and signal it, via diverse signal-transducing molecules associated with the dynamic cytoskeleton, preferentially actin-based, further down into the cytoplasm and toward the nucleus (Katz et al., 2000; Ingber, 2003a, 2003b). This allows the orchestration of diverse cellular activities according to physical properties of the ECM.

Mechanosensing properties of adhesion domains are extremely appealing especially for higher plants, which are known to be very sensitive toward mechanical signals (Bögre et al., 1996; Monshausen and Sievers, 1998). Retracting protoplasts of plasmolyzing cells are connected to cellular peripheries via Hechtian strands. These adhesive structures are often anchored at plasmodesmata and pit-fields that are enriched with callose, plant-specific myosin of the class VIII, and calreticulin (Baluška et al., 1999a, 1999b, 2001a). Also, there are reports showing that mechanical stress affects gating of plasmodesmata (Oparka and Prior, 1992). All of these data converge toward a model according to which positive feedback loops between integrated cytoskeleton and biochemical/mechanical signals at specialized adhesion domains of eukaryotic cells drive cell growth, differentiation, and cell-to-cell communication not only in animals and humans, but also in supracellular plants.

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LITERATURE CITED


Figure 1. Schematic presentation of emerging linkers between the cytoskeleton and cell walls in plant cells. We depict here idealized cross-wall of a root cell with one plasmodesm (PD) traversed by an element of endoplasmic reticulum (ER). Adhesive pectins (in orange color) in the cell wall (CW) are known to line the outer face of the plasma membrane (PM) and to accomplish endocytosis-driven recycling (orange circles). Pectins can directly interact with WAKs, other receptor-like kinases, and arabinogalactan-proteins. Putative linkers are numbered (see below) and are suggested to interact, directly or via unknown adaptor molecules, either with actin filaments (green lines) or with microtubules (black lines). Unconventional myosin of the class VIII (red circles) accumulates within plasmodesmata and may serve as an adaptor between the actin filaments and callose synthase. Putative linkers between the plant cytoskeleton and cell wall include: 1, WAK; 2, arabinogalactan-protein (AGP); 3, callose synthase; 4, plant-specific myosin of the class VIII; 5, other receptor-like kinase; 6, formin; 7, cellulose synthase; 8, phospholipase D. The question marks refer to hypothetical interactions.


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Riveline D, Zamir E, Balaban NQ, Schwarz US, Ishizaki T, Narumiya S, Kam Z, Geiger B, Bershadsky AD (2001) Focal contacts as mechanosen-

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