INTRODUCTION

The membrane is a lipid bilayer that functions to divide and separate the cells and organelles. It undergoes many dynamic morphological changes during cellular processes such as endocytosis, exocytosis, vesicular transport, and morphogenesis. Growing evidence has demonstrated that actin cytoskeleton dynamics are involved in these processes. However, the interactions between microfilaments and membranes vary in different cell types and locations. Some cytoskeletal elements may interact with membranes directly. Transmembrane proteins can regulate membrane-cytoskeleton interactions directly or indirectly through adaptor proteins or adaptor complexes. Furthermore, some proteins have domains that can associate with the membrane, and domains that can interact with cytoskeletal components. These are the main types of membrane-cytoskeleton interactions (Doherty and McMahon, 2008). The extracellular matrix (ECM) of animals mainly consists of proteinaceous materials. However, the plant cell wall, which deviates plant cell shapes from spherical shapes, mainly consists of carbohydrates. This implies that there are differences in the intracellular interactions that occur in membrane-cytoskeleton of animal and plant. In mammals, cytoskeletal proteins that can function as adaptors, such as talin (Heise et al., 1991), vinculin (Geiger et al., 1980), and filamin (Dissel et al., 2001) bind the actin cytoskeleton to membranes; homologs of these proteins are absent from plants (Hussey et al., 2002). There are many plant-specific linker molecules. For example, myosin VIII binds directly or indirectly to plasma membrane-localized callose synthase complexes (Verma and Hong, 2001; Ostergaard et al., 2002) and it also binds to actin filaments in the cytoplasm, which implies that myosin VIII associates plasma membrane with actin filaments in plants. Moreover, a plant-specific Networked (NET) superfamily of actin-binding proteins is found in Arabidopsis. Members of the NET superfamily localize to the actin cytoskeleton and specify different membrane compartments. NET1A is located at the plasma membrane and binds directly to actin filaments through a novel actin-binding domain. The NET superfamily is grouped into four phylogenetic clades, and other members have functions at the tonoplast, nuclear membrane, and pollen tube plasma membrane, which suggest that this superfamily is involved in regulating actin-membrane interactions (Deeks et al., 2012).

A large amount of literature has fostered our current understanding of the membrane, the actin cytoskeleton, and of actin-binding proteins that mediate membrane and actin cytoskeleton components. Profilins are actin-binding proteins, and have the capacity to interact with three classes of ligands. In addition to G-actin, they also associate with poly-L-proline (PLP) which can interact with the binding cleft formed from the N-terminal and C-terminal helices of profilin (Meister et al., 1994; Mahoney et al., 1999) and phosphoinositides (Gibbons and Staiger, 2000; Jockusch et al., 2007) which offers the possibility that profilin interacts with the membrane. In recent years, much evidence has been verified that profilins can interact with membranes directly or indirectly. In this review, we will summarize recent findings and focus predominantly on the functions of profilins in the direct or indirect relationships among actin cytoskeleton, profilin and membranes in plant cells.

MULTIFUNCTIONAL PROFILINS

Genomic DNA sequences of putative profilins contain three exons; these may be separated by introns of different sizes (Huang et al., 1996), and are dispersed throughout the genome. Comparing the amino acid sequences of different profilins reveal that profilins have less than 25% identity across different kingdoms (Pollard and Quirk, 1994), but are highly conserved, with at
least 70% identity, across various plant species (Mittermann et al.,
1993; Vidal et al., 1995). This is consistent with the analysis
of the phylogenetic tree shown in Figure 1. Although the sec-
donary and tertiary structures of all profilins are well conserved
(Fedorov et al., 1997; Thorn et al., 1997; Lockshch et al., 2007),
the fact that many varieties of profilins isoforms exist in differ-
ent species, and even in the same organism, may indicate that
members of the profilin family have diverse functions. Plant pro-
filins are from multigene families and can be divided into two
major groups: the vegetative group, in which profilins exist exten-
sively and are constitutively expressed in all plant tissues, and
the reproductive group, where profilins are expressed in repro-
ductive tissues (Kandasamy et al., 2002). The Arabidopsis profilin
family includes five highly different isoforms: AtPRF1–AtPRF5;
seedlings. Pronp1 represents a unique profilin as it has activities
pollen, elongating pollen tubes, and the root hairs of developing
tobacco profilin gene, pronp1, is prominently expressed in mature
(Kandasamy et al., 2002). AtPRF1 has much higher affinities for
anther or in other organs using a non-radioactive labeling method
expressed only in pollen grains, and not in other parts of the
(Yu et al., 1998). RcPRO1, a
which suggests that actin filament formation is blocked in the
sieve-tube exudates, RcPRO1 has 15-fold molar excess to actin,
is expressed in epidermal, cortex, pith, and xylem tissue. In the
remodels following treatment with phospholipase C activator
mastopectan (Baluska et al., 2001). Therefore, profilins may be
a linker between the plasma membrane and actin cytoskele-
through the relationship between actin-binding proteins and PIP2,
action of the actin cytoskeleton with the plasma membrane is
famous plant pathogen Phytophthora infestans, profilin is expressed
and accumulates at the site of infection on the plasma mem-
brane in plants (Cvrekova et al., 2004). For exam-
ple, in Arabidopsis, formin homology 6 (AFH6) interacting with
profilin locates at the plasma membrane and is uniformly dis-
buted (Evrey et al., 2004). Furthermore, AFH1 and AFH5 are
reported to associate with the cell membrane (Banno and
Chua, 2000; Cheung and Wu, 2004; Ingouff et al., 2005). This
verifies that plant type I formins are likely to be membrane-
bound, with AFH8 being the exception, as it is targeted to the
nuclear envelope (Xoe et al., 2011). The site of the profilin bind-
ing FHI PLP tracks is on the opposite face of the actin binding
site of profilin (Schutt et al., 1993), and this explains why pro-
filin can bind PLP and actin simultaneously without mutual
influence (Tanaka and Shibata, 1985; Perelroizen et al., 1994).
Profilin has an indirect connection and possibly acts as a reg-
ulator in the linkage of the plasma membrane and the actin
cytoskeleton.

The plant cell is able to defend itself from infection by exoge-
nous fungi. During this process, the cytoskeleton reorganizes and
the papilla localizes at penetration sites, this leads to a thick cell
wall being formed to prevent pathogen ingress (Schneider, 2002).
Material is site-directed to arrive at positions around the fungal
infection structure beneath the cell wall, and the actin filament
and microtubule re-orientate their structures toward the pene-
tration site (Schmitzer, 2002; Takenos et al., 2003). In cultured
celery cells, undergoing attack from infection with the oomycete-
tous plant pathogen Phytophthora infestans, profilin is expressed
and accumulates at the site of infection on the plasma mem-
brane, and the actin cables focus at the penetration site where
Rop GTases also accumulate (Schutt et al., 2006). In addition,
in developing microspores and mature pollen of Zea mays, pro-
filin is associated with the plasma membrane (von Witsch et al.,
1998). Profilin accumulates in the tip zone near the plasma mem-
brane in root hairs of Arabidopsis (Braun et al., 1999; Baluska et al.,
2000). These results suggest that profilins play a role in both sig-
nal transduction and linkage between the plasma membrane and actin cytoskeleton

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FIGURE 1 | An unrooted phylogenetic tree of profilins. The plant genes are Arabidopsis thaliana AtPRF1–AtPRF5 (AT2G19760, AT2G19770, AT5G56600, AT4G29340, AT2G19770), Petroselinum crispum PcPRF1–PcPRF5 (AY900012-AY900016), Zea mays ZmPRF1–ZmPRF5 (X73279, X73280, X73281, AF023370, AY201459), Oryza sativa OsPRF1–OsPRF5 (LOC_Os10g17680, LOC_Os06g05880), Nicotiana tabacum NtPRO1 (AJ130969), tomato LePRO1 (U50195), Ricinus communis RcPRO1 (AF092547), Phaseolus vulgaris PvPRO1 (CAA57508), Lilium longiflorum LlPRO1 (AF200184), and selected fungal and metazoan sequences are included: Mus musculus MmPRO1–MmPRO4 (NP_035202, NP_062283, NP_083579, AK013595), Homo sapiens HsPRO1 and HsPRO4 (BC057828, BC029523), Caenorhabditis elegans CePRO1 (PFN-1, NP_014765), and Saccharomyces cerevisiae ScPRO1 (PFY1, NP_014765). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.
PROFILIN IS INVOLVED IN ORGANELLE LOCATION WITH THE ACTIN CYTOSKELETON

There is much evidence that, in various eukaryotic cells, the cytoskeleton is involved in organelle movements. In plant cells, the role of the actin cytoskeleton in organelle movements has been reported for movements of chloroplasts (Kandasamy and Meagher, 1999); the endoplasmic reticulum (ER; Boeving et al., 1998), and the Golgi apparatus (Boeving et al., 1998; Nebenfuhr et al., 1999).

In Arabidopsis, CHUP1 (Chloroplast unusual positioning 1) which is a 112 kDa protein that is closely related with chloroplast movement (Kasahara et al., 2002; Oikawa et al., 2003) is directly targeted to the outer envelope of the chloroplast; this is dependent on its N-terminus domain (Oikawa et al., 2003). In addition to the N-terminus domain, the CHUP1 protein has four other domains, including two leucine-zippers, an actin-like type-actin binding domain (Gimmey et al., 2002), and a proline-rich motif (PRM) that is similar to PRM1 identified as a profilin binding motif (Holt and Koffer, 2001). A fusion protein which includes GST and the actin binding domain of CHUP1 can bind F-actin in vitro (Oikawa et al., 2003). The *in vitro* biochemical analyzes revealed that CHUP1 interacts with profilin as a modulator of actin polymerization through the PRM of C-terminal part of CHUP1 (CHUP1-CT). The experiment of CHUP1-CT titrated to a mixture of profilin and actin confirmed that the trimeric complex of actin, profilin, and CHUP1-CT is more stable than the individual binary complex. Though CHUP1 can bind F-actin directly, profilin has been reported to enhance the connection between chloroplasts and actin filaments (Schmid von Braun and Schleiff, 2008).

Although profilin can bind to form, the type II formins do not contain the transmembrane domains present in type I formins (Cyrkova et al., 2004). In rice, like other plant type II formins, formin homology 5 (FHS) has a characteristic N-terminal phosphatase tensin (PTEN)-related domain that may interact with membranes (Cyrkova et al., 2004). The experiments of transiently expressing the PTEN-HFP fusion protein in tobacco (Nicotiana tabacum) cells and immunostaining analysis using rice leaf cells revealed that the PTEN-like domain of FHS is sufficient to confer localization of the protein to the chloroplast surface. This suggests that the PTEN domain of FHS may be a bridge between chloroplasts and the actin cytoskeleton (Zhang et al., 2011). Furthermore, FHS was capable of nucleating actin assembly from the actin/profilin complex in vitro biochemical analyzes (Yang et al., 2011; Zhang et al., 2011). Therefore, profilin is indirectly involved in the localization of chloroplast to the actin filaments. In Arabidopsis, observations of living cells in stable transgenic plants revealed that 35S:: GFP-AtPRF1 forms a filamentous network likely associated with actin filaments; this was verified by treatment with latrunculin A, and through a recovery experiment involving the removal of latrunculin A. Whereas, 35S:: GFP-APRF2 forms polygonal meshes resembling ER in the same latrunculin A treatment conditions (Wang et al., 2009). Furthermore, in plants, profilins possibly participate in the linkage of the nuclear envelope and the actin cytoskeleton during the interphase of Arabidopsis; this is because AtFHII locates primarily to the nuclear envelope at this stage (Xue et al., 2011).

PROFILIN IS INVOLVED IN VESICLE TRAFFICKING

Profilins are known to play an important role in endocytosis and membrane trafficking in lower eukaryotes (Wolfen et al., 2000; Pearson et al., 2003). In mammalian cells, profilins may also be involved in membrane trafficking. It has been reported that profilin 1 exists in budding Golgi vesicles, and that dynamin 2 recruitment to the Golgi is dependent on profilin 1 (Dong et al., 2000). Moreover, in mammalian cells, there are multiple phosphoinositide 3-kinases (PI3Ks), and these can be grouped into three main classes. Class I and II PI3Ks can induce receptor-dependent trafficking processes, such as phagocytosis. Class III PI3Ks, which represent the most ancient form of PI3Ks, and are the only ones conserved in lower eukaryotes, mammals, and plants. Class III PI3Ks mainly regulate receptor-independent trafficking events, such as endocytic membrane traffic (Lindmo and Stemmark, 2006). In animal cells, PI3Ks have been reported to play many different roles in vesicle trafficking, and inhibition of PI3Ks induces the inhibition of clathrin-dependent endocytosis (Martin et al., 1996; Sprio et al., 1996). In plant cells, Class III PI3K protein complexes may have a regulatory function during vesicle trafficking (Matsuoka et al., 1995; Kim et al., 2001; Jung et al., 2002). In *Phaseolus vulgaris*, in addition to the N- and C-terminal PLP-binding domain, profilin has a domain around Tyr72; this can recognize and bind PLP and PI3K (Aparicio-Fabre et al., 2006). Profilin can bind directly to Class III PI3Ks in a manner reliant upon the tyrosine phosphorylation status of the PLP domain in profilin. This interaction between profilin and Class III PI3Ks suggests that profilins may participate in membrane trafficking, and may act as a linker between the endocytic pathway and the actin reorganization dynamics (Aparicio-Fabre et al., 2006).

With advances in biotechnology, diverse pharmaceutical drugs have been used to study the interaction between vesicular trafficking and cytoskeleton. Brefeldin A (BFA) is a drug that inhibits the recycling of vesicular trafficking, and disrupts secretion in yeast, mammalian, and plant cells (Vogel et al., 1993; Samaj et al., 2004; Citterio et al., 2008; Robinson et al., 2008). In Arabidopsis roots, BFA-compartments can be formed due to the accumulation of trans-Golgi network (TGN) secretory and recycling vesicles, which gather together following BFA treatment (Geldner et al., 2005). During this process, profilin 2 is up-regulated and accumulates in the BFA compartments, which then interacts with the actin to remodel the actin cytoskeleton. This study suggested that profilin 2 may bridge vesicular trafficking to the actin cytoskeleton in a BFA-dependent manner (Takac et al., 2011). Table 1 lists the profilins cited in the present article and emphasizes some of their cellular functions. Therefore, the recently investigated interactions between membranes and the actin cytoskeleton have revealed profilins to be of particular interest, this is because they may act as linkers and regulate communication and cooperation between the two cellular members in plants. Currently available studies suggest that diverse interaction mechanisms are required to satisfy the different structural and dynamic requirements of particular systems. Future...
research is required to unravel how membrane-actin cytoskeleton interactions are regulated through profilins and their different ligands.

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REFERENCES
Aparicio-Fabre, R., Gauslin, G., Estrada, G., Ollaruez-Grajales, J., Garrella, G., and Sanchez, I. (2006). Profilin tyrosine phosphorylation in poly-L-proline-binding regions inhibits binding to phosphoinositide 3-kinase.

Caroni, P. (2001). Actin cytoskeleton regulation through modulation of PI(4,5)P(2).

Couchman, J. R., Voigt, S., Lim, S. T., Lim, Y., Oh, E. S., Prestwich, G. D., et al. (2002). Immunoglobulin-like GBF1 is a GTPase-activating protein for rabenosin.

Dong, J., Radau, B., Otto, A., Muller, E., Lindschau, C., and Westermann, P. (2000). Profilin I attached to the Golgi is required for the formation of constitutive transport vesicles at the trans-Golgi network.

Engler, J., et al. (2004). A superfamily of actin-binding proteins at the actin-membrane nexus of higher plants.

Favery, B., Chelysheva, L. A., Lebris, M., Jammes, F., Marmagne, A., De Almeida-Esclapez, M., et al. (2004). Arabidopsis formin AFPH6 in a plasma membrane-associated protein upregulated in giant cells induced by parasitic nematodes.

Fischer, A. A., Ball, T., Mahoney, N. M., Valenta, R., and Almos, S. C. (1997). The molecular basis for allergen cross-reactivity: crystal structure and IgE-epitope mapping of birch pollen profilin.

Fleischer, I. (1983). Confidence limits on phylogenies: An approach using the bootstrap.

Flenstra, J. (1983). Convergence limits on phylogenies: An approach using the bootstrap.

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Table 1 | Profilin's and its cellular functions in plant cells.

| Profilin involving in the cellular pathway | Profilins | Cells or ligands | Reference |
|---------------------------------------------|-----------|-----------------|----------|
| Plasma membrane-actin cytoskeleton interaction | ZmPRF1 | Root cells of maize PIP2 | Bakusa et al. (2001) |
| | AtPRF1 etc | Arabidopsis seed endosperm, root cells etc | Banno and Chua (2000); Cheung and Wu (1997) |
| | PfPRF1 | Cultured parsley cells | Schulte et al. (2008) |
| Organelles location with the actin cytoskeleton | PVF1 from Arabidopsis | NA | Okawa et al. (2000); Schmidt von Braun and Schneid (2008) |
| | | Rice leaf cells OsPRF1 | Zhang et al. (2011); Fang et al. (2011) |
| | | Arabidopsis epidermal cells, trichomes, stem epidermal cells ER | Wang et al. (2009) |
| | | Arabidopsis root cells AFPH6 | Xue et al. (2011) |
| Vesicle trafficking with the actin cytoskeleton | PV-PRF1 | Phaseolus vulgaris root nodules Class III | Aparicio-Fabre et al. (2006) |
| | | PisPa | Takai et al. (2011) |

NA, not available.
Mahoney, N. M., Rozwarski, D. A., Fedorov, E., Fedorov, A. A., and Kandasamy, M. K., McKinney, E. C., and Meagher, R. B. (2002). Plant profilin
Kandasamy, M. K., and Meagher, R. B. (1999). Actin-organelle interaction: movements. Cytoskeleton 42, 227–242. doi: 10.1080/10970189809551362
Holt, M. R., and Koffer, A. (2001). Cell motility: proline-rich proteins promote cytoskeleton. Trends Cell Biol. 11, 56–60. doi: 10.1016/S0962-8924(00)01766-6
Huang, S., McDowell, J. M., Weis, M. I., and Meagher, R. B. (1996). The Arabidopsis profilin family: evidence for an ancient split between constitutive and prol-
Geldner, N., Anders, N., Wolters, H., Keicher, J., Kornberger, W., Muller, P., et al. (2008). The Arabidopsis GOMA ARF-GEP mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Cell 132, 119–128. doi: 10.1016/j.cell.2007.12.017
Gibbons, R. J., and Staszel, C. J. (2001). "Profilin" in Actin: a Dynamic Framework for Multiple Plant Cell Functions, ed S. J. Staszel, F. Badaux, D. Vollmann, and P. Barlow (Dordrecht, The Netherlands: Kluwer Academic Publishers), 45–65.
Gimona, M., Djonovic-Carugo, K., Kranewitter, W. J., and Winder, S. J. (2002). Functional/plasticity of C-domain. FEBS Lett. 515, 98–106. doi: 10.1016/S0014-5793(02)00258-9
Goldschmidt-Clermont, P. J., MacKiey, L. M., Balaban, J. J., and Pollard, T. D. (2006). The Actin-binding protein profilin binds to PIP2 and inhibits its nucleotide exchange on phospholipid C. Science 312, 1575–1578. doi: 10.1126/science.1127979
Garcia, M. I., and Alche, J. D. (2012). Characterization of profilin polymor-
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