Actin fringes of polar cell growth

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Abstract

The eukaryotic actin cytoskeleton is a highly dynamic framework that is involved in many biological processes, such as cell growth, division, morphology, and motility. G-actin polymerizes into microfilaments that associate into bundles, patches, and networks, which, in turn, organize into higher order structures that are fundamental for the course of important physiological events. Actin rings are an example for such higher order actin entities, but this term represents an actually diverse set of subcellular structures that are involved in various processes. This review especially sheds light on a crucial type of non-constricting ring-like actin networks, and categorizes them under the term ‘actin fringe’. These ‘actin fringes’ are visualized as highly dynamic and yet steady structures in the tip of various polarized growing cells. The present comprehensive overview compares the actin fringe characteristics of rapidly elongating pollen tubes with several related actin arrays in other cell types of diverse species. The current state of knowledge about various actin fringe functions is summarized, and the key role of this structure in the polar growth process is discussed.

Key words: Actin cytoskeleton, actin fringe, actin network, actin ring, endomembranes, microfilaments, polar cell growth, pollen tubes, tip growth, vesicular transport.

Introduction

Actin is one of the most abundant proteins in the cytoplasm of eukaryotes and its amino acid sequence has been highly conserved during evolution (Mishra et al., 2014). It is a multifaceted protein that polymerizes into microfilaments (F-actin) creating diverse cellular arrays that are subject to a dynamic balance of structural assembly and disassembly (Holmes et al., 1990; Higaki et al., 2007). Bundling and branching of microfilaments through actin-binding proteins, mediating cross-linking and nucleation, is essential for spatial construction of higher order structures (McCurdy et al., 2001; Staiger and Blanchoin, 2006; Henty-Ridilla et al., 2013; Smith et al., 2013). The accurate set-up of such greater actin cytoskeleton units is fundamental for the correct sequence of many cellular processes. In the course of evolution the actin microfilament bundles have differentiated and spatially assembled into linear, patch, network, or ring-like arrays, that perform different cellular tasks such as structural organization and stabilization of cell shape, as well as movement and localization of membrane compartments and organelles (Wada and Suetsugu, 2004; Pollard and Cooper, 2009; Geitmann and Nebenführ, 2015). Cell morphogenesis and growth are essential cellular processes that require the creation of highly specialized macromolecular actin configurations (Smith and Oppenheimer, 2005; Hussey et al., 2006). Unfortunately, still too little is known about specialized actin structures and associated protein networks involved in polarized cell growth. Filamentous growing plant pollen tubes, for example, exhibit large longitudinal bundles of actin filaments that extend through...
their shank, acting as a backbone for cytoplasmic streaming (Lancelle and Hepler, 1992). Besides that, an exceptional and yet still under-specified ring-like actin structure has been identified in pollen tube tips that seems to be involved in polar cell growth and is termed the ‘actin ring’ or ‘actin fringe’ (Kost et al., 1998; Lovy-Wheeler et al., 2005).

Plant pollen tubes are specialized cells that undergo extremely rapid unidirectional tip growth to deliver sperm cells in their cytoplasm to the ovule for subsequent fertilization of the female gametes. To perform its role this cell type utilizes to the clarification of its function.

Actin fringe structure in angiosperm pollen tubes

The actin network of angiosperm pollen tubes can be spatially differentiated into specific structures according to their localization in the shank, subapical, and apical area. A variety of methods have been used to detect and visualize the actin framework in pollen tubes of several plant species. All used detection methods provide a basic description and spatial classification of the actin cytoskeleton into a shank area with distinct longitudinal microfilaments, and a subapical to apical area with a variable clustered actin structure or fine actin filaments (Figs 1A, B and 2A). More precisely, a compact but very dynamic actin array fills the subapex of healthy growing pollen tubes, while the extreme apex appears to be largely devoid of F-actin and harbours at the most very fine filaments (Figs 1A, B and 2A). Especially remarkable is the fact that results characterizing microfilament organization in the subapical and apical region significantly vary between different detection procedures or experimental conditions. Approximately 5 μm behind the apex is a particularly striking actin structure consisting of densely arranged microfilaments that has been consistently identified with various laser scanning confocal microscopy detection techniques (Fig. 1A, B asterisk). The spatial character of this subapical actin array is variably described as a fringe, ring, band, collar, mesh, funnel, or basket (Table 1).

Initially this subapical ‘actin ring’ or ‘actin fringe’ was identified via expression of a green fluorescent protein (GFP)–mTalin fusion protein in tobacco pollen tubes (Table 1C). This structure was confirmed by subsequent studies via fluorophore coupled phalloidin staining, in chemically fixed and even in living pollen tubes, despite the known toxicity of phalloidin (Table 1A). Similarly, anti-actin and anti-ADF immunolabelling of rapid-freeze fixed pollen tubes revealed distinct longitudinally oriented cortical microfilaments forming a dense ring-like array (Table 1B). In contrast, the use of a broad range of GFP-labelled actin-binding proteins (ABPs) enabled live cell imaging of a subapical ring-like network formed by highly dynamic interlaced actin filaments (Table 1C). At this point in time, the actin-marker Lifeact, a 17-amino-acid peptide that is derived from the yeast actin-binding protein ABP-140 (Riedl et al., 2008), is referred to as being the most authentic marker because it is regarded as labelling the actin cytoskeleton without interfering with cellular functions in live pollen tubes of Nicotiana, Lilium, or Arabidopsis (Table 1C2 and Fig. 1A).

Complexity of detection

It is striking that by comparison all mentioned detection techniques identified different three-dimensional structures of the same subapical actin fringe entity. On the one hand, phalloidin staining and immunolabelling visualized ring-like palisades of massive longitudinal microfilament bundles in the subapical cortex area, while on the other hand live cell imaging methods, utilizing various actin-binding GFP fusion proteins, uncovered a dynamic fine meshwork also extending...
into the central cytoplasm. This indicates that every individual marker very likely identifies only specific parts of the actin cytoskeleton and none detects the complete cellular actin entity (Wilsen et al., 2006).

Moreover, some labelling substances might affect the natural F-actin integrity. For example, the observation that phalloidin visualizes massive bundles in the subapical cortex of pollen tubes corresponds to its known F-actin stabilizing effect (Cooper, 1987), which means that it partially produces an unnatural over-representation of distinct microfilament bundles. Besides that, chemical and cryofixation of fast growing cellular systems remains difficult, and such highly dynamic actin networks presumably represent the fixed state not exactly their natural form in living cells. In addition, live cell imaging utilizing ABPs raises particular problems as well. Given the observation that NtADF1 overexpression interferes with tobacco pollen tube growth (Chen et al., 2002), and that GFP-mTalin causes severe defects in actin organization and growth of root hairs (Ketelaar et al., 2004), one has to consider that ectopic expression of ABPs impairs the natural organization and functions of the actin network. Even Lifeact has to be utilized carefully as studies indicate that expression levels are critical, notably higher levels reducing actin dynamics, affecting growth rate, and inducing abnormal morphologies (Vidali et al., 2009a; Bergs et al., 2016; Courtemanche et al., 2016).

All this points to the significant experimental issues that have to be resolved to clarify the precise fringe configuration, since all the various culture conditions and detection techniques influence the delicate natural equilibrium of the highly dynamic actin order in the pollen tube tip. This impedes experimental data acquisition, and results in a manifold appearance of the actin fringe and fine accessory filaments. In summary, despite variant data, all applied detection methods have consistently identified a ring-like structure that is formed by an extremely dynamic network of short irregular actin filaments.

Refined fringe structure

A special focus should be placed on the fact that this dynamic subapical F-actin network represents a peripheral area in pollen tube tips that defines the edge of the ‘clear zone’, where vesicles accumulate. This organelle-free zone spans from the apical dome to about 5–10 μm distal from the tube apex and constitutes an inverted vesicular cone in the tube tip (Fig. 1A, B; Lovy-Wheeler et al., 2005). Phalloidin staining and immunolabelling essentially demonstrate a subapical collar of longitudinally oriented cortical actin filaments (Lovy-Wheeler et al., 2005, 2006; Wilsen et al., 2006; Dong et al., 2012), whereas an approximately cone-shaped cytoplasmic meshwork was detected by GFP-labelled ABPs and sporadically by phalloidin as well (Geitmann et al., 2000; Vidali et al., 2001; Wilsen et al., 2006; Dong et al., 2012; Stephan et al., 2014).

Combining both observations leads to a three-dimensional model that depicts the actin fringe as a highly dynamic funnel-like structure (Fig. 1B, C, asterisk). This subapical funnel-like structure borders the inverted vesicular cone and seems to be composed of a much denser collection of microfilaments in its cortical collar part than in its basal core (Fig. 1B, C, asterisk). Vidali et al. (2001) provide a similar description of a ‘funnel-like structure’ at the base of the clear zone, and state that this ‘structure is internal and does not appear to constitute a cortical ring’. Three-dimensional z-stack reconstructions of apical actin filaments labelled by rhodamine phalloidin in cryofixed

Fig. 1. Actin fringe, trans Golgi network (TGN), and apical vesicle accumulation zone in the tip region of growing angiosperm pollen tubes. (A) Single confocal optical sections of growing pollen tubes. The actin cytoskeleton was visualized by YFP:lifeact. Vesicles of the apical ‘clear zone’ were labelled with the endocytic styryl dye FM4-64. The YFP:RISAP-specific TGN colocalizes with the F-actin fringe in the subapical region. Asterisks: pollen tube actin fringe; arrowheads: actin microfilament deficient region located basally of the actin fringe; scale bar: 20 μm. (Reproduced from Stephan et al. 2014; Copyright American Society of Plant Biologists; www.plantcell.org.) (B) Schematic cross-sectional view of the angiosperm pollen tube. Coloured three-dimensional forms summarize the subcellular distribution and represent the characteristic organization of F-actin (blue), apical vesicles (red), and the RISAP-specific TGN (green) in the normally growing pollen tube tip. Asterisks: pollen tube actin fringe; arrowheads: actin microfilament deficient region located basally of the actin fringe. (C) Overlays of schematic cross-sectional views of pollen tubes from (B). Instead of a complete triple combination, two separate overlays are presented for better visual clarity, and thus only two coloured structures were combined in one tube.
Lilium longiflorum pollen tubes support this spatial conception of a funnel-like configuration (Kroeger et al., 2009).

Zones of sparse F-actin surround the fringe

Interestingly this striking subapical structure is enclosed on its apical as well as basal side by two areas that are sparsely filled with F-actin (Fig.1A, B arrowheads) (Lovy-Wheeler et al., 2006; Wilsen et al., 2006; Dong et al., 2012; Stephan et al., 2014). While the documented shortage of distinct microfilaments in the apical area (clear zone) seems obvious and is a matter of intense discussion, another area extending about 5 μm distal from the actin fringe also appears to be lacking especially large thick actin bundles as well, which by contrast can be found further down in the tube shank. Yet, this aforesaid F-actin bundle-deficient area, which borders the fringe basally, remains unnoticed and undiscussed in literature to this day, despite adequate visual indications (Fig. 1A, B arrow heads) (Lovy-Wheeler et al., 2006; Wilsen et al., 2006; Dong et al., 2012; Su et al., 2012; Stephan et al., 2014).

In contrast, fine apical filaments extending from the fringe through the ‘clear zone’ to the apical plasma membrane have been a matter of controversial debate because their quantity and organization were not consistently characterized. Early experiments identified a dense network of delicate longitudinally aligned actin filaments extending directly to the apex amidst the vesicles of the inverted cone (Tiwari and Polito, 1988; Derksen et al., 1995), whereas immunogold labelled F-actin constitutes a few fine filaments or bundles, which are loosely arranged and do not extend completely to the pollen tube apex (Miller et al., 1996). More recent studies showed scattered apical actin and detected a few delicate bundles extending throughout the clear zone to the extreme apex (Kost et al., 1998; Fu et al., 2001; Vidali et al., 2009a), and beyond that propose a coordinated assembly of apical filaments regulated by...
fimbrins, villins, and formins, which anchor the filaments to the plasma membrane directly at the tip (Cheung et al., 2010; Dong et al., 2012; Qu et al., 2013; Zhang et al., 2016). However, too little evidence exist and much more experimentation is needed to study in detail the exact organization of these apparent highly dynamic filaments and to develop a clear picture of the potential role in maintenance of the apical growth area.

The subapical fringe and the apical filaments exhibit variable spatial composition that correlates with pollen tube growth rate (Dong et al., 2012). Externally induced reduction of growth coincides with a displacement of the fringe filaments into the apical clear zone, and when elongation completely stops, a dynamic actin meshwork is localized at the extreme apex (Dong et al., 2012). Likewise, influences on the dynamic integrity of the actin fringe affect pollen tube growth rate, indicating that growth and fringe configuration might be interdependent. Moreover, rearrangement and asymmetrical accumulation of the actin fringe material seems to precede a reorientation of growth direction, which appears to be aligned towards the higher actin filament density (Dong et al., 2012). Consequently this implies a substantial role for the fringe in determination of tube orientation and alignment of the growth focus.

Equivalent actin structures in other cell types

The subapical actin fringe has been demonstrated to be essential for pollen tube tip growth of many plant species representing monocotyledons and dicotyledons, such as *Nicotiana tabacum, Lilium longiflorum, Lilium formosanum, Arabidopsis, Papaver rhoes and Zea mays* (Kost et al., 1998; Gibbon et al., 1999; Geitmann et al., 2000; Kroeger et al., 2009; Lovy-Wheeler et al., 2005; Wilsen et al., 2006). Does this ring-like actin fringe represent a particular structure that is limited to angiosperm pollen tubes or have other eukaryotic cells comparable actin configurations with similar function?

With the evolutionary occurrence of diverse eukaryotic cell types the actin cytoskeleton has differentiated into various specific structures such as longitudinal filament bundles, meshworks, arcs, patches, arrays, collars, and rings. Among this great diversity, the actin ring represents an outstanding type of configuration that has been identified in various cell types. However, this general nomenclature for a variety of ring-like structures appears confusing because rather different actin features are superficially termed rings. These manifold types of actin rings can be distinguished through their organization and function.

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**Table 1. List of ‘actin fringe’ studies with applied detection methods, organisms used, and different fringe designations**

<table>
<thead>
<tr>
<th>Year</th>
<th>Detection method</th>
<th>Reference</th>
<th>Name</th>
<th>Species</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>1999</td>
<td>Phallolid</td>
<td>Collar</td>
<td>Zm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mesh/collar</td>
<td>Pr</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Funnel</td>
<td>Li</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li</td>
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<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li</td>
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<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li</td>
</tr>
<tr>
<td>B</td>
<td>2005</td>
<td>Anti-actin antibody</td>
<td>Lovy-Wheeler et al. (2005)</td>
<td>Lf, Li, Nt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Collar/fringe</td>
<td>Lf, Li, Nt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Lf</td>
</tr>
<tr>
<td>B</td>
<td>2006</td>
<td>Anti-ADF antibody</td>
<td>Lovy-Wheeler et al. (2006)</td>
<td>Fringe</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li</td>
</tr>
<tr>
<td>C</td>
<td>1998</td>
<td>GFP-mTalin</td>
<td>Kost et al. (1998)</td>
<td>Ring</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Band/collar/ring</td>
<td>Nt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li, Nt</td>
</tr>
<tr>
<td>C</td>
<td>2002</td>
<td>GFP-NiADF1</td>
<td>Cher et al. (2002), Cheung et al. (2008)</td>
<td>Basket/mesh</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Basket/mesh</td>
<td>Nt, Li</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Lf, Nt</td>
</tr>
<tr>
<td>C</td>
<td>2008</td>
<td>NiPLIM2b-GFP</td>
<td>Cheung et al. (2008)</td>
<td>Ring/collar</td>
</tr>
<tr>
<td>C</td>
<td>2009</td>
<td>YFP-mTalin</td>
<td>Zhang et al. (2009, 2010)</td>
<td>Ring/collar</td>
</tr>
<tr>
<td>C2</td>
<td>2009</td>
<td>Lifeact-mEGFP</td>
<td>Vidali et al. (2009a)</td>
<td>Ring</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fringe</td>
<td>Li, Lf</td>
</tr>
<tr>
<td>C3</td>
<td>2012</td>
<td>GFP-LfIM1</td>
<td>Su et al. (2012)</td>
<td>Fringe</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n.a.</td>
<td>At</td>
</tr>
<tr>
<td>C4</td>
<td>2013</td>
<td>VLNL2-GFP</td>
<td>Qu et al. (2013)</td>
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<tr>
<td>C2</td>
<td>2013</td>
<td>Lifeact-EGFP</td>
<td>Qu et al. (2013)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>n.a.</td>
<td>At</td>
</tr>
<tr>
<td>C2</td>
<td>2014</td>
<td>YFP-Lifeact</td>
<td>Stephan et al. (2014)</td>
<td>Fringe</td>
</tr>
</tbody>
</table>
One type comprises a structurally and functionally rather inhomogeneous set of non-constricting actin rings that mainly perform stabilizing roles in a multitude of inter- and intracellular events, for instance support of the axonal shape or establishment of cell–cell contacts (Engl et al., 2014; Ganguly et al., 2015).

A second type is implicated in the process of cell separation and comprises all contractile actomyosin rings that are essential for cytokinesis at the end of mitosis in various eukaryotes from the animal kingdom, or for septation of fungal hyphae (Cheffings et al., 2016; Meitinger and Palani, 2016). These actomyosin rings execute their constricting function through participation of myosin motor proteins that move anti-parallel actin filaments, but also through depolymerization of cross-linked filaments (Zumdieck et al., 2007; Mendes Pinto et al., 2012). In particular the differences in spatial microfilament organization between the cytokinetic ring and the subapical actin fringe demonstrate their diverse cellular roles. The cytokinetic ring is composed of multiple circularly bundled actin fibres, which are cross-linked by myosins and arranged in a massive cortical ring aligned with the equatorial cell division plane. In contrast, the very dynamic subapical fringe is formed by short fibres, which are rather longitudinally aligned in a funnel-like network extending between cortex and the central cytoplasm (Fig. 1B). This third type is especially discussed in this review and represents three-dimensional framework structures that establish a boundary zone in the subapex of tip-growing cells, and therefore the designation ‘fringe’ seems to outline most precisely its actual character. The following comprehensive survey reveals clear commonalities in shape, composition, and localization between fringes of diverse phylogenetic species.

**Gymnosperm pollen tubes**

It has been demonstrated that microfilaments constitute actin fringes with similar characteristics in pollen tubes of various angiosperm species, and therefore structural comparison with F-actin networks in pollen tubes of plants representing primary steps of evolution might give insights about the emergence of the actin fringe. Male gametophytes of gymnosperms represent an earlier evolutionary level in development of plant sexual reproduction, and thus pollen tubes of *Ginkgo biloba* and *Pinophyta* (conifers) hold the potential to provide information about comparatively primordial forms of tip actin structures.

Interestingly, the subcellular organization of organelles and cytoskeleton in pollen tubes clearly differs between angiosperms and gymnosperms, which is reflected in corresponding variations in their growth velocities, with angiosperm tubes growing much faster by comparison (Table 2) (Fernando et al., 2005; Williams, 2012). Pollen tubes of the gymnosperm *Pinus sylvestris* exhibit no distinct organelle zonation resulting in a general distribution of dictyosomes and trans Golgi network (TGN) including the tip region (De Win et al., 1996). Moreover secretory vesicles were found to be less abundant in gymnosperm tubes and did not organize an apical inverted cone as described for angiosperms, and yet a ‘clear zone’ (hyaline cap) lacking amyloplasts also extends about 30 μm into the subapex (De Win et al., 1996; Wang et al., 2005). In pollen tubes of *Pinus* and *Picea* actin filaments of variable thickness exhibit an axial oriented distribution throughout the tube, including an actin network at the tip (De Win et al., 1996; Lazzaro, 1996; Fernando et al., 2005; Chen et al., 2007; Lazzaro et al., 2013). Unfortunately the few reports highlighting the actin cytoskeleton in the tip of gymnosperm pollen tubes present varying results, which might be caused by artefact-creating fixation and staining procedures. Remarkably the apical actin network in *Picea abies* tubes forms a thick cortical layer in the tip enclosing an actin-depleted core region reaching 30 μm back from the apex to a subapical demarcation (Lazzaro, 1996). Likewise other reports corroborate that microfilament bundles in *Pinus densiflora* and *Picea abies* pollen tubes do not extend to the extreme apex (Terasaka and Niitsu, 1994; Anderhag et al., 2000). On the other hand thick axial F-actin bundles in the shank and a denser radial star-like network, reaching from the subapical centre to the tip, were reported in pollen tubes of the ‘living fossil’ *Ginkgo biloba* (Liu et al., 2005) and also in *Picea abies* (Lazzaro et al., 2013) (Fig. 2B).

Nevertheless, in consideration of all observations, the following potential zonation of the gymnosperm pollen tube actin cytoskeleton seems to be appropriate: (i) massive longitudinal microfilament bundles extending through the shank; (ii) a demarcation of clustered F-actin in the subapical area; and (iii) a few delicate microfilaments reaching to the apex. Altogether this roughly resembles the organization in angiosperm pollen tubes, even though a well-defined actin fringe is missing. Yet, the radial star-like network located at the subapical centre could possibly be considered as a more basic evolutionary progenitor structure (Fig. 2B) (Liu et al., 2005; Lazzaro et al., 2013), which also might be substantiated by the reduced vesicle accumulation zone in comparison with angiosperms. Both features seem to be underdeveloped in gymnosperm tubes and are thus consistent with the significantly lower growth rate (Table 2). This highlights a role for subapical actin arrays in the formation of distinct apical vesicle clusters, and consequently in the set-up of apical growth areas, already as crude precursor forms in gymnosperm pollen tubes.

**Root hairs**

In elongating late bulges and short root hairs of *Vicia sativa*, fluorescein–phalloidin labelling visualized subapical accumulations of actin filaments as a radial collar, and showed hair tips that are devoid of actin bundles (Fig. 2D) (Miller et al., 1999). Longitudinal massive microfilament bundles traversing the shank cytoplasm of polar growing root hairs fray out into a dense actin network of delicate bundles in the subapical region (Fig. 2C, D). It should be emphasized that this actin fringe in root hair tips appears as a spatial boundary structure at the subapex spreading from the cortex throughout the cytoplasm, even though it has a slight cortical tendency. In young elongating root hairs (or late growing bulges) the subapical actin fringe and the large apical zone that is devoid of actin filaments highly resemble their counterparts in pollen tubes (Fig. 2D). On the other
Hand, longer hairs while they still grow exhibit a denser subapical actin network shifted towards the apex, and thus a smaller actin-devoid apical cleft of 5 μm in diameter (Fig. 2C). Both features appear similar to the characteristics observed in slow growing pollen tubes (Dong et al., 2012) and also in some slow growing fungal hyphae (e.g. Aspergillus nidulans) (Table 2; Torralba et al., 1998; Schultzhaus et al., 2016). Additionally GFP–Talin labelled massive apical F-actin caps in growing Arabidopsis root hair tips (Baluska et al., 2000), yet in this case an apical actin cleft and a filamentous composition of the cap have not been specified, probably due to technical reasons.

Fern protonemata

Rhodamine–phalloidin staining showed a transversal cortical ring-like structure in the subapical region of fern protonemal cells of Adiantum capillus-veneris and Pteris vittata (Fig. 2E) (Kadota and Wada, 1989; Kagawa et al., 1992), which corresponds to the actin fringe in angiosperm pollen tubes. However, the transverse alignment of the subapical microfilaments in fern protonemata highly resembles the actin fringe described in rhizoids of brown algae (see below) (Fig. 2E, H) (Kadota and Wada, 1989; Karyophyllis et al., 2000a). Similar to pollen tubes (Fig. 2A), moss caulonema (Fig. 2F), and algal rhizoids (Fig. 2H), this subapical fringe network in fern protonemata (Fig. 2E) is proximally and distally surrounded by areas spanning ~5 μm, which are deficient in thick F-actin bundles but exhibit fine short actin filaments (Kadota and Wada, 1989). Remarkably, in plasmolysed fern protonemata the protoplasm remains attached exclusively to the subapical cell wall, also showing a distinct subapical actin fringe, and both fringe and subapical cortical junction disintegrate upon cytochalasin B treatment (Kagawa et al., 1992). These observations suggest a connection between the subapical actin fringe, the plasma membrane, and the cell wall in this area, which supports a role for fringes as a structural reinforcement of the cortex, or in positioning of cell wall connected membrane complexes, e.g. cell wall synthesizing enzyme complexes.

Table 2. Range of growth rates for several tubular elongating cell types of diverse species

<table>
<thead>
<tr>
<th>Growth rate</th>
<th>Species</th>
<th>Conditions</th>
<th>Reference</th>
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<tr>
<td>3.0–49.8 μm s⁻¹</td>
<td>Conospermum sp.</td>
<td>in vitro</td>
<td>Stone et al. (2004)</td>
</tr>
<tr>
<td>3.8 μm s⁻¹</td>
<td>Nicotiana tabacum</td>
<td>in vitro</td>
<td>Stephan et al. (2014)</td>
</tr>
<tr>
<td>2.7 μm s⁻¹</td>
<td>Zea mays</td>
<td>in vivo (in style)</td>
<td>Mascarenhas (1993)</td>
</tr>
<tr>
<td>0.1–0.33 μm s⁻¹</td>
<td>Lilium longiflorum</td>
<td>in vitro</td>
<td>Dong et al. (2012), Li et al. (1996), Messerli et al. (2000), Parton et al. (2001), Zerzour et al. (2009)</td>
</tr>
<tr>
<td>0.25 μm s⁻¹</td>
<td>Ornithogalum virens</td>
<td>in vitro liquid</td>
<td>Stepka et al. (2000)</td>
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<tr>
<td>0.23 μm s⁻¹</td>
<td>Lilium longiflorum</td>
<td>in vivo (in style)</td>
<td>Jauh and Lord (1995)</td>
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<td>0.16 μm s⁻¹</td>
<td>Alopecurus pratensis</td>
<td>in vitro liquid</td>
<td>Heslop-Harrison and Heslop-Harrison (1984)</td>
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<tr>
<td>0.05–0.15 μm s⁻¹</td>
<td>Ornithogalum virens</td>
<td>in vitro solid</td>
<td>Stepka et al. (2000)</td>
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<td>0.08–0.14 μm s⁻¹</td>
<td>Arabidopsis thaliana</td>
<td>in vivo</td>
<td>Moulene et al. (2002), Wilhelm and Preuss (1996)</td>
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<tr>
<td>0.025 μm s⁻¹</td>
<td>Arabidopsis thaliana</td>
<td>in vitro liquid</td>
<td>Park and Nebenführ (2013)</td>
</tr>
<tr>
<td>0.01–0.014 μm s⁻¹</td>
<td>Aspergillus nidulans</td>
<td>in vitro liquid</td>
<td>Chebi et al. (2012)</td>
</tr>
<tr>
<td>0.01–0.014 μm s⁻¹</td>
<td>Aspergillus goyyspii</td>
<td>in vitro liquid</td>
<td>Kaminskyj et al. (1992)</td>
</tr>
<tr>
<td>13 μm min⁻¹</td>
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<td>in vitro liquid</td>
<td>Karyophyllis et al. (2008)</td>
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<td>3.5–5.0 μm min⁻¹</td>
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<td>in vitro solid</td>
<td>Rodrigues de Melo et al. (2006)</td>
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<tr>
<td>2.5 μm min⁻¹</td>
<td>Candida albicans</td>
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<td>Heslop-Harrison and Heslop-Harrison (1984)</td>
</tr>
<tr>
<td>0.51–0.64 μm min⁻¹</td>
<td>Aspergillus nidulans</td>
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<td>Horio and Oakley (2005), Schultzhaus et al. (2016)</td>
</tr>
<tr>
<td>Root hairs</td>
<td>Acidobasidium thaliana</td>
<td>in vitro</td>
<td>Dolan et al. (1994), Lee et al. (2008)</td>
</tr>
<tr>
<td>1.7 μm min⁻¹</td>
<td>Nitella spec.</td>
<td>in vitro</td>
<td>Chen (1973)</td>
</tr>
<tr>
<td>0.44–0.72 μm min⁻¹</td>
<td>Chara contraria</td>
<td>in vitro solid</td>
<td>Kiss (1994)</td>
</tr>
<tr>
<td>Moss protonemata</td>
<td>Physcomitrium turbinatum</td>
<td>caulonema in vitro</td>
<td>Jensen (1981)</td>
</tr>
<tr>
<td>0.46 μm min⁻¹</td>
<td>Physcomitrella patens</td>
<td>caulonema in vitro</td>
<td>Menand et al. (2007)</td>
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<tr>
<td>0.33 μm min⁻¹</td>
<td>Physcomitrella patens</td>
<td>chloronema in vitro</td>
<td>Menand et al. (2007)</td>
</tr>
<tr>
<td>Gymnosperm pollen tubes</td>
<td>Picea abies</td>
<td>in vitro</td>
<td>Anderhag et al. (2000), Lazzaro et al. (2005)</td>
</tr>
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<td>0.18–0.33 μm min⁻¹</td>
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<td>Wang et al. (2005)</td>
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<tr>
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<td>in vitro</td>
<td>Hao et al. (2005)</td>
</tr>
<tr>
<td>0.02 μm min⁻¹</td>
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<td>De Win et al. (1996)</td>
</tr>
<tr>
<td>Fern rhizoids</td>
<td>Dryopteris affinis</td>
<td>in vitro liquid</td>
<td>Parton et al. (2000)</td>
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Moss protonemata

The apical cells of bryophytal protonemata perform highly polarized cell growth (Menand et al., 2007) and exhibit a clear three-dimensional filamentous actin network in their growing tips. Ring-like configured microfilament networks in the subapex of apical protonemata cells of the model bryophyte Physcomitrella patens have been identified by phalloidin labelling in fixed cells and by live-cell imaging via Lifeact-mEGFP (Vidali et al., 2007, 2009a, b) (Fig. 2F). The general organization of these microfilaments and especially their distinct longitudinal alignment corresponds strikingly to the one described for pollen tubes (Fig. 2A, F). Also in caulonemal cells of Ceratodon purpureus rhodamine–phalloidin staining visualized highly filamentous bundles of actin forming a collar structure in the subapex, with delicate filaments extending into the apical region (Meske and Hartmann, 1995). Particularly interesting is that this subapical actin fringe reorients towards the light source, anticipating the subsequent change of caulonemal growth direction.

Interestingly an apical actin focal point was observed in caulonemal cells of Physcomitrella patens (Vidali et al., 2009a), which appears similar to the F-actin accumulation at the tip-localized Spitzenkörper of fungal hyphae (see below, e.g. Neurospora crassa, Allomyces macrogynus; Srinivasan et al., 1996; Berepiki et al., 2010). Furthermore, dense apical actin arrays were also reported in protonemata tips of Physcomitrella patens and Funaria hygrometrica, with diffuse F-actin strands extending radially into the shank (Fig. 2G; Quader and Schnepf, 1989; Finka et al., 2007, 2008; Vidali et al., 2009a). Similar to fern protonemata and some fungal hyphae, the observed variations in tip actin of bryophyte protonemata are most likely explained by different cellular growth modes. It is therefore necessary to perform detailed studies precisely relating actin organization to growth velocity. Especially in this case a clear distinction of cell types seems important, as bryophyte protonemata are classified into rapid growing caulonemal cells and more differentiated chloronemal cells harbouring numerous large chloroplasts. The cellular growth mode and developmental state appears to be the explanation for the observed differences, because fast growing caulonemal cells have a subapical actin fringe and a distinct ‘clear zone’ (Fig. 2F), whereas slow growing chloronemal cells exhibit an apical actin cap (Fig. 2G) (Vidali et al., 2009a). Yet, even in chloronemal cells the actin fringe has been shown, albeit much closer to the apex compared with caulonemal cells (Vidali et al., 2007), which again emphasizes the requirement to establish experimentally a clear relation between actin fringe appearance and cellular growth velocity.

Algal rhizoids

In living young zygotes of fucoïd brown algae (Fucus, Pelvetia) F-actin accumulates as a cortical patch at the prospective germination site and marks the pole of growth axis in response to the light vector (Alessa and Kropf, 1999; Pu et al., 2000; Hable et al., 2003). Noteworthy is the fact that the cortical actin patch at the shaded pole transforms into a ring directly before germination, and moreover in the growing tubular rhizoid this ring is localized subapically 5 μm behind the elongating tip (Fig. 2H, I) (Alessa and Kropf, 1999; Pu et al., 2000; Hable et al., 2003). Especially, observations in polar growing cells of Sphacelaria rigidula demonstrate that subapical localization and structure of this actin band is strikingly similar to the actin fringe of fern protonemata (Fig. 2E, H) (Karyophyllis et al., 2000a, b). The implemented detection techniques visualized slight differences in the overall actin filament alignment between the cortical band of rhizoids and the fringe of pollen tubes. Microfilaments of the pollen tube actin fringe web appear to be primarily oriented parallel to the tube axis (Fig. 2A), whereas in algal rhizoids and fern protonemata the superordinate orientation of most filaments in the actin band seems to be directed transversely to the axis (Fig. 2H, I) (Karyophyllis et al., 2000b). Yet, it cannot be excluded that this observation of a divergent filament orientation in algal rhizoids might be a fixation artefact.

A diffuse fluorescence of low intensity also confirmed delicate short actin filaments in the apical dome, which is in accordance with the suggested fine apical filaments in pollen tubes. Interestingly, algal rhizoids also exhibit an unnoticed F-actin bundle deficient area extending ~5 μm basally to the fringe similar to pollen tubes (Figs 1A, B and Fig. 2A, H, arrowheads; Karyophyllis et al., 2000b).

Cellular axis development and polarized growth of fucales are affected by inhibitors of F-actin organization, as application of latrunculin B and cytochalasin D in Fucus and Pelvetia zygotes affects formation of polarized cortical actin networks at the rhizoid tip and inhibits photopolarization (Brawley and Robinson, 1985; Kropf et al., 1989; Kropf et al., 1995; Hable and Kropf, 1998; Pu et al., 2000). Additionally, these cortical F-actin accumulation sites have been implicated in directed cell expansion through localized secretion (Shaw and Quatrano, 1996; Belanger and Quatrano, 2000). More recent studies on gametophytes of Macrocystis and branching thallus cells of Splachnidium and Choristocarpus show that radial circular actin arrays are configured at cortical growth sites and most notably associate with HDEL markers labelling the endoplasmatic reticulum (Varvarigos et al., 2004, 2007). Hence, similar to pollen tubes (Fu et al., 2001), the tip-localized actin cytoskeleton of algal zygotes has been implicated in polarized cell growth processes regulated by fucoïd Rho of plants (ROPs) (Fd-RAC1) (Fowler et al., 2004).

Fungal hyphae

Prior to germination of fungal conidia their actin patches and cables were found to accumulate in the cell periphery polarized at the site of germ tube outgrowth, and subsequently upon tube emergence a dense actin meshwork at the tip and longitudinal filaments in the germ tube were observed (Harris et al., 1994; Torralba et al., 1998; Berepiki et al., 2010), which resembles the actin distribution described for polar growing yeast cells (Adams and Pringle, 1984; Moseley and Goode, 2006; Mishra et al., 2014).

However, the organization of the actin cytoskeleton in mature hyphae of filamentous fungi is considerably
Actin fringes of polar growth in diverse cells

different from germ tubes. Through immunogold transition electron microscopy and immunofluorescence microscopy the F-actin network was shown to be localized in peripheral plagues in tip-growing hyphae of Aspergillus nidulans and Neurospora crassa; most notably F-actin accumulated as an intensely stained diffuse subapical band with aggregating patches, and as a cluster in the apical cytoplasm at the Spitzenkörper (Fig. 2J) (Harris et al., 1994; McGoldrick et al., 1995; Srinivasan et al., 1996; Torralba et al., 1998; Heath et al., 2000; Cheng et al., 2001; Araujo-Bazán et al., 2008). Among immunofluorescence studies the observed appearance of the subapical actin fringe ranges from dense aggregated patches, over a diffuse fringe with individual patches, to a rather homogeneous fringe. More recent studies utilizing several GFP–actin markers detected similar organized cortical actin patches in the hyphal tube of N. crassa and A. nidulans and confirmed their accumulation as a dense subapical actin-collar of patches 2–5 μm below the apex (Fig. 2J); in addition a dense amorphous web of actin cables (a subapical actin web) was observed 18 μm distal from the apex (Taheri-Talesh et al., 2008; Upadhyay and Shaw, 2008; Berepiki et al., 2010; Bergs et al., 2016; Schultzhaus et al., 2016). Obviously, this filamentous subapical actin web clearly corresponds to the subapical actin fringe of pollen tubes, although hyphal tips generally present a higher proportion of patches and less distinct cables. Moreover, the evidence of a dynamic polymerizing and depolymerizing apical actin array in hyphae supports the existence of the much discussed delicate apical F-actin in pollen tubes (Schultzhaus et al., 2016).

Regarding structural correlations between the aforementioned ascomycota and other fungal phyla, observations from divers fungi, such as the zygomycetes Neozygites sp. (Butt and Heath, 1988), the arbuscular mycorrhizal fungus Glomus mossae (Aström et al., 1994), the pucciniomycete Uromyces phaseoli (Hoch and Staples, 1983), and the agaricomycete Schizophyllum commune (Raudaskoski et al., 1991; Weber et al., 2005), show a divergent configuration of clustered actin plaques in the apical region. A comparison of several independent studies demonstrates consistently that dense fibrillar actin caps extend from the apical plasma membrane to the subapex in hyphae of the oomycete Saprolegnia ferrax (Heath, 1987; Jackson and Heath, 1990; Kaminskyj and Heath, 1994; Gupta and Heath, 1997; Heath et al., 2000) (Fig. 2K). A comparative study covering simultaneously ascomycetes and homobasidiomycetes, confirmed an abundant microfilament accumulation in the apical to subapical area of hyphae, and yet structure, distribution, and density of F-actin varied between different fungal taxa (Salo et al., 1989).

In summary, fungal hyphae show either of two tip actin organizations: some fungal taxa have subapical actin fringes in combination with more or less distinctive Spitzenkörper actin (Fig. 2J), while others, by contrast, exhibit exclusively apical actin caps (Fig. 2K). Why is the organization of tip actin different between fungal taxa? Remarkably, fungal hyphae that display very distinct labelled F-actin at their Spitzenkörper (Srinivasan et al., 1996; Berepiki et al., 2010) have less intensely stained subapical actin fringes and vice versa (Torralba et al., 1998; Schultzhaus et al., 2016). This demonstrates an interdependency between these two cytoskeleton substructures and might indicate that the fringe and the Spitzenkörper actin are two manifestations of the same F-actin entity. Interestingly, some fungal hyphae generate actin caps that have apical actin clefts, which predict growth direction (Jackson and Heath, 1993a), and as a consequence resemble actin fringes. Accordingly, application of latrunculin B initially disrupts the dense apical actin cap of Saprolegnia ferrax, thereby enhancing the manifestation of a typical fringe structure (Gupta and Heath, 1997). This demonstrates the susceptibility of the apical network, even in those cells having a distinct apical actin cap. Those caps could be considered as fringe variations that were shifted apically and merged with compacted apical F-actin.

Divergences in the organization of tip actin among individual fungal taxa might reflect evolutionary adaptations to different conditions in their specific growth substrates. Correspondingly, the configuration of microfilaments in the tip has been reported to depend also on the hyphal growth mode and the rigidity of the growth medium. Invasive hyphae of Neurospora crassa have an actin-depleted zone at their apex that is related to a high agar concentration (Suei and Garrill, 2008). These invasive hyphae exhibit a subapical fringe while non-invasive hyphae have, in contrast, a dense actin network shifted to the extreme apex (Suei and Garrill, 2008). Similarly, normal growing pollen tubes exhibit a subapical fringe, whereas this actin network is delocalized to the apex in relation to growth reduction (Dong et al., 2012). It should be highlighted finally that fungal hyphae thus have, in comparison with tip-growing plant cells, a higher tendency for microfilament accumulation at the apex.

Latrunculin and cytochalasin affect actin fringes

All the aforementioned delicate subapical structures in various tip-growing plant and fungal cells seem to be generally composed of short interlaced F-actin that differs from the long, massive, and axially oriented actin cables in the shank, which are associated with cytoplasmic streaming throughout the tubular cell body. The structural and functional differences between the F-actin subpopulations in tip and shank arise in response to drugs. Treatment with the actin-depolymizing pharmacological drugs cytochalasin and latrunculin demonstrates that an intact actin cytoskeleton is essential for normal polarized growth and hence for the shape of specialized cells, such as pollen tubes (Miller et al., 1999; Vidali et al., 2001; Chen et al., 2007; Cárdenas et al., 2008), root hairs (Miller et al., 1999; Ketelaar et al., 2003), moss caulonemal cells (Finka et al., 2007; Vidali et al., 2009a), fern protonemata (Kagawa et al., 1992; Karyophyllis et al., 2006b), algal rhizoids (Hable and Kropf, 1998; Pu et al., 2000), and fungal hyphae (Harris et al., 1994; Srinivasan et al., 1996; Torralba et al., 1998; Sharpless and Harris, 2002; Taheri-Talesh et al., 2008; Berepiki et al., 2010; Schultzhaus et al., 2016). The reviewed cell types react to increasing concentrations of cytochalasin or latrunculin with inhibition of tip
growth, but, remarkably, individual substructures of the actin cytoskeleton responded differently to the same drug. Massive F-actin in the tube shank was nearly insensitive to drug treatment, whereas delicate network structures of the subapical fringe and the fine apical filaments in the tip of pollen tubes (Gibbon et al., 1999; Vidali et al., 2001; Cardenas et al., 2008; Stephan et al., 2014), moss protonemata (Vidali et al., 2009a), root hairs (Miller et al., 1999; Ketelaar et al., 2003), and fungal hyphae (Berepiki et al., 2010) were severely affected and rapidly disappeared already at low concentrations and short incubation times. Correspondingly, cytoplasmic streaming responded also less sensitively to the drug than tip growth. Latrunculin and cytochalasin bind to actin and prevent the polymerization and assembly of microfilaments, and therefore the abovementioned observations imply that massive cables in the shank might exist with longer turnover rates and thus be more stable than the highly dynamic fringe or delicate apical F-actin. Interestingly, immunofluorescence and phalloidin staining of tip actin was more intense in slow-growing than in fast-growing fungal hyphae, indicating that in this case the stability of the apical array is higher (Salo et al., 1989), which points to a relation between turnover rates of the fine apical microfilaments and the growth rate of elongating cells. All these observations demonstrate that the delicate microfilament structures adjoining the apical growth zone have essential functions in the growth process that clearly differ from mere cytoplasmic streaming. Consequently, this implies that the diverse actin cytoskeleton substructures within one cell (apical filaments, subapical fringe, shank cables) represent differently composed protein complexes (actin, ABPs, and regulators) which are separately modulated for their individual functions and stability, thus responding unequally to drug treatment.

Role of the actin fringe

Early findings allowed the interpretation that the cortically visualized actin fringe might represent clustered microfilament attachment sites at the plasma membrane of pollen tubes (Miller et al., 1996), such as already analogously hypothesized for the actin patches involved in polarized cell growth of yeast (Yang and Pon, 2002; Huckaba et al., 2004; Moseley and Goode, 2006). In accordance therewith, novel studies provide evidence that actin bundles are aligned from the pollen tube centre outwards to the cortex, expanding directly to the subapical cell membrane (Miller et al., 1996; Cheung et al., 2010; Qu et al., 2013; Zhang et al., 2016). In S. cerevisiae actin patches are generally considered to be involved in endocytosis, yet their association with post-Golgi vesicles also suggests a connection to exocytosis (Schott et al., 2002; Bretscher, 2003; Huckaba et al., 2004; Moseley and Goode, 2006) and moreover, recent results directly link polarized exocytosis to compensatory endocytosis induced by cortical actin polymerization via SEC4 (Jose et al., 2015; Johansen et al., 2016). The yeast actin cables provide routes for myosin-dependent traffic of post-Golgi secretory vesicles to a dense apical array of cortical actin patches, which represent the primary site of exocytosis required for polarized cell growth (Bretscher, 2003; Moseley and Goode, 2006; Gao and Bretscher, 2009; Jose et al., 2015).

Endomembrane guidance

If the visualized cortical actin array in pollen tubes were the cell membrane anchoring site for actin cables of the fringe network, one potential explanation might be that these attachment sites play a role in guiding the cables and thus in directional exchange of vesicles especially with the subapical area of the cell membrane. Notably, the high density of subapical microfilaments, potentially leading to the cortex, might then in turn imply that the plasma membrane annulus, which adjoins the actin fringe, were the main site of plasma membrane turnover. However, the share of fringe filaments extending to the subapical plasma membrane is unknown, and thus also the quantity of microfilaments that are involved in membrane exchange with the cell cortex at the flanks. In addition, the exact locations of the primary plasma membrane sites, which are subject to the major part of all membrane transport processes in the pollen tube tip, finally need to be unequivocally determined. It should also be considered that the exact sites of exo- and endocytosis are currently a matter of intense debate, and it is still unclear if both processes might be balanced at the subapical membrane zone. In contrast to the view that the apex is the primary site of vesicular secretion (Lancelle and Hepler, 1992; McKenna et al., 2009; Rojas et al., 2011; Bloch et al., 2016), Zonia and Munnik (2008) present a model in which exocytosis occurs mainly in the subapical area, spanning 3–10 μm distal to the apex, which coincides with the position of the actin fringe. Interestingly, also Geitmann and Dumais (2009) as well as Bove et al. (2008) argue that the rate of exocytosis and cell wall expansion appears maximal in an annular region below the apex and not at the apical pole, where endocytosis is accordingly predominant. These abovementioned contradictory views could possibly indicate that endo- and exocytotic processes actually occur balanced all over the plasma membrane at the tip. However, in the case of having separate endo- and exocytic zones within the pollen tube tip, this would also shed light on the widely discussed apical actin filaments. Microfilaments should then, by implication, reach to the plasma membrane of the apical dome, because it would be crucially important for vesicular traffic to follow organized vectorized routes. Either way, the actin fringe potentially operates as the main coordinator of an exo- and endocytotic harmony in the whole pollen tube tip. One simple concept could explain the cortical part of the fringe as a framework generating an annular endocytic zone at the subapical flanks, whereas the central funnel part of the fringe acts as the origin for an exocytic vesicular cone at the apex. A more complex view would additionally imply specific F-actin transport routes within the ‘clear zone’ to defined areas of endo- and exocytosis at the tip dome.

In this regard, research on polar growth of fungal hyphae provides a substantial informative input as well. Zones of exo- and endocytosis in hyphal tips do not overlap, and the
Actin fringes of polar growth in diverse cells

actin collar of patches at the subapical plasma membrane has been characterized as a major site of endocytic recycling, whereas exocytosis occurs associated with the Spitzenkörper at the apical dome (Araujo-Bazán et al., 2008; Taheri-Talesh et al., 2008; Upadhyay and Shaw, 2008; Hervás-Aguilar and Peñalva, 2010; Caballero-Lima et al., 2013), though for maintenance of hyphal growth a tight coupling of both spatially divided processes is required. Moreover, the subcellular position of the endocytic zone seems to be linked to growth mode, because actin patches are shifted further away from the apex in faster growing hyphae compared with slow-growing (Knechtle et al., 2006; Köhli et al., 2008). Similarly, in growing plant root hairs the highest concentration of early endosomal markers is localized to the subapical region, coinciding with the actin network there (Miller et al., 1999; von Wangenheim et al., 2015). Beyond that, the tip-localized actin cytoskeleton of root hairs plays a role in spatial regulation of the exocytotic area; in detail, the actin network apparently sets the focus of exocytosis to the apical plasma membrane (Ketelaar et al., 2003).

Considering similarities in the tip organization between pollen tubes and fungal hyphae raises the provocative question of whether, from an evolutionary point of view, the subapical actin fringe of pollen tubes represents a sophisticated unified structure. In particular, when compared with fungal hyphae, this would involve fusion of the subapical endocytic collar of patches with the microfilaments of the exocytotic Spitzenkörper. Did evolution reduce distances in endomembrane traffic to speed up polarized growth for pollen tubes? Is the subapical actin fringe a framework for a unified endomembrane centre organizing exo- and endocytotic processes in the pollen tube tip, thus creating the large apical vesicle accumulation zone?

Membrane transport processes via exocytotic and endocytotic vesicles are related to the TGN and linked to establishment and maintenance of cell polarity (Geldner and Robatzek, 2008; Viotti et al., 2010; Contento and Bassham, 2012). The TGN supplies intermediate organizing compartments for storage and sorting of vesicular cargo, subsequently directing the transport of material to designated target organelles or the apical plasma membrane (Viotti et al., 2010). A study provides a link between the actin fringe and tip-localized membrane transport in pollen tubes by drawing attention to a myosin receptor (NtRISAP) that binds to the globular tail domain of myosin XI (Stephan et al., 2014). This novel Rac/ROP effector is associated with the subapical actin fringe and the subapical TGN compartment (Fig. 1; Stephan et al., 2014), where endo- and exocytotic events apparently converge as initially described by Dettmer et al. (2006). Consequently the actin fringe and myosins cooperate to position the TGN subapically in the tip (Fig. 1), and vesicles arising from the TGN potentially traffic along fine actin cables, presumably by myosin XI, to the plasma membrane at the apex. Yet it still requires unequivocal verification that microfilaments provide tracks for vesicular transport between the actin fringe region and the apical plasma membrane. In this respect it is particularly striking that a class XI myosin motor of Arabidopsis thaliana has lately been described as accumulating in the subapex of growing root hairs facilitating cellular elongation (Park and Nebenführ, 2013; Madison et al., 2015). More precisely, this study shows for root hairs that the subapical actin network is essential for myosin XI localization and that myosin XI bidirectionally moves in the tip. Furthermore this report connects myosin XI to ROP-related membrane transport; in particular it suggested a role for MYOXI in partitioning of ROP2 between cytosol and the plasma membrane (Park and Nebenführ, 2013). In accordance with a role for the fungal Spitzenkörper in apical secretion, a fungal myosin I (MYOa) has been found to enrich in the apex of Aspergillus nidulans and to play an essential role in polarized growth and apical secretion (McGoldrick et al., 1995). Also GFP–tropomyosin was reported to localize at the apical Spitzenkörper and with actin filaments in the subapical region (Taheri-Talesh et al., 2008). Likewise, myosin XI of the bryophyte Physcomitrella patens associates with endomembrane clusters in the protonemal tip, and moreover, has been related to organization and polymerization of tip actin (Furt et al., 2013).

In Brefeldin A-treated pollen tubes, the formation of a star-like subapical actin aggregate is initiated, which localizes with BFA-induced membrane aggregates and with a similarly formed cluster of the myosin receptor NtRISAP in the core of the tip (Hörmanseder et al., 2005; Rounds et al., 2014; Stephan et al., 2014). Interestingly, fine actin cables emanate from this radial actin aggregate extending to the apex (Hörmanseder et al., 2005). This supports a connection between tip-localized endomembrane compartments and subapical actin arrays, and moreover, suggests that tip-directed actin filaments correlate with membrane transport routes to the apex. The abovementioned observations thus converge in a model in which the subapical fringe may serve as a scaffold organizing and holding vesicles available for the release into the inverted cone for apical secretion at the tip, which was already proposed by Miller et al. (1999).

Zoned localization

Interestingly the subapical position of the actin fringe corresponds to the region where a thinner and pectin-rich cell wall of the pollen tube apical dome converts to a thicker, stiffer and more differentiated cell wall of the shank region. In a region between 3 and 10 µm from the apex the statical characteristics of the cell wall are changed through several processes comprising pectin esterification, callose and cellulose deposition. Thus the actin fringe might influence morphogenic factors by spatially controlling plasma membrane constituents that regulate cell wall composition, which is also suggested for fungal tip growth (Chebli et al., 2012; Caballero-Lima et al., 2013).

Physical filtering

Apart from that, also mere physical roles such as a filtering function for the actin fringe were proposed, implying that this actin-mesh acts as a physical barrier through sorting of organelles that are larger than secretory vesicles, thus excluding
these from the apical clear zone (Kost et al., 1998). Despite the absence of deeper analysis, this might be partially applicable, considering that cytoplasmic streaming of organelles extends to the extreme apex upon latrunculin-dependent degradation of the fringe (Cárdenas et al., 2008).

Structural support

In eukaryotic cells microfilaments generate a framework that supports the plasma membrane and defines the cell shape, and therefore another possible role for the cortical actin fringe might be that it functionally provides a reinforcing palisade for structural support, consequently increasing cellular stiffness. This direct morphogenetic role might accord with a model in which the internal turgor pressure of the cell contributes to the polar cell growth process (Zerzour et al., 2009; Winship et al., 2011; Zonia and Munnik, 2011). In this case, the mechanical properties of the apical plasma membrane and cell wall are crucial, whereby additional positioning of cell wall affecting enzymes such as pectin methyl esterase by the actin fringe would influence the rate and direction of cellular expansion (McKenna et al., 2009; Zerzour et al., 2009; Rounds et al., 2014). It seems questionable whether forces exerted by the cytoskeletal structures solely might be able to compensate turgor pressure in the tip region, but they are likely to have a supportive part. The apical actin cap in Saproplegnia ferax hyphae has been suggested to be a structural enhancement that regulates and assists the role of turgor in polarized cell growth (Gupta and Heath, 1997). More precisely it seems to retain or promote cellular expansion in response to high or low turgor conditions. Studies utilizing F-actin depolymerizing drugs like cytochalasin D or latrunculin B reported tip swelling of pollen tubes and fungal hyphae, in accordance with a role for tip actin in balancing directed cell extension with turgor (Gupta and Heath, 1997; Torralba et al., 1998; Zerzour et al., 2009; Heath et al., 2000).

Force generation

An additional task of the tip structures might be that actin fibres themselves act directly as the producer of a driving force in the apical growth process, namely by their filament assembly. This is reminiscent to the mechanical effect produced by actin polymerization in the self-propelling mechanism of the intracellular pathogens Listeria monocytogenes (Jasmin and Crevenna, 2016) and Shigella flexneri (Agaisse, 2016). Moreover an arrangement of polymerizing actin filaments in meshworks or parallel bundles has been described as acting in the protrusion of the leading edge in motile cells, such as in lamellipodia or in filopodia (Footer et al., 2007). In this context Vidali et al. (2001) argue that tip growth in pollen tubes requires actin assembly in a process that is independent of cytoplasmic streaming. Latrunculin B treatment revealed that in particular the highly dynamic actin filaments of the pollen tube tip are required for polar growth, but it cannot be excluded that their results just reflect experimental inhibition of actin-dependent secretion at the tip (Gibbon et al., 1999; Vidali et al., 2001). Similar observations were also made concerning effects of cytochalasin D on distinct actin networks in outgrowing bulges and tips of young root hairs (Miller et al., 1999; Baluska et al., 2000). Vazquez et al. (2014) visualized a high density of microfilament plus ends in close proximity to the apical plasma membrane of root hairs, and they further-more state that actin polymerization regulates polar cell growth. Regarding the issue of actin polymerization directly driving tube elongation, few data have been collected and those actually do not allow the drawing of final conclusions, because they are incapable of unequivocally differentiating between all F-actin-involving processes. Most interestingly, however, application of Yariv phenylglycoside ([b-D-Glc]) arrested pollen tube growth, while the clear zone remained intact and apical secretion continued (Roy et al., 1999). [b-D-Glc] induces an elevated apical Ca²⁺ gradient that promotes exocytosis, and coincidently microfilament fragmentation as well. An apical directed Ca²⁺ gradient in the tip is essential for pollen tube and root hair growth, and presumably inhibits F-actin polymerization corresponding to the reported tip-focused G-actin gradient (Cárdenas et al., 2005; Lazzaro et al., 2005; Ivano et al., 2009; Steinhorst and Kudla, 2013). Tip-focused actin-binding proteins might participate, such as LdABP41, which severs F-actin into short actin filaments in a Ca²⁺-sensitive manner (Fan et al., 2004). Tip Ca²⁺ potentially coordinates actin-dependent apical exocytosis with actin filament assembly. In particular the results of Roy et al. (1999) imply that elongation requires processes separate from exocytosis at the tip, possibly including expanding forces based on actin polymerization. In the light of this, issues remain unanswered, such as, would the documented quantity of delicate microfilaments in the apical actin-deficient zone be sufficient to promote elongation? Could forces produced by myosin–actin interactions add up to pure polymerization?

Based on what is known about the participation of motor proteins in cellular movement and elongation of animal species, another view would additionally include that a myosin driven axial shift of microfilaments possibly contributes to apical growth of plant cells. Yet, a participation of myosins in the establishment of the fringe, or a direct contribution to the polar growth process of pollen tubes, has not been analysed in-depth. In protomenal cells of Physcomitrella patens myosin XI seems to be essential for the distinct axial orientation of F-actin bundles, for the establishment of a subapical actin fringe, and eventually for cellular polarization (Vidali et al., 2010). Furt et al. (2013) report that myosin XI associates with a vesicular cluster containing F-actin nucleators, and moreover, they suggest that this myosin XI-enriched compartment organizes the actin polymerization essential for protomenal tip growth. Accordingly, Park and Nebenführ (2013) demonstrate that the relationship between myosin motors and actin filaments in Arabidopsis root hairs is interdependent, and as a consequence, myosins move and organize their own cytoskeletal tracks, which was recently also validated for pollen tubes (Stephan et al., 2014; Madison et al., 2015).

Conclusions and perspectives

In summary, the character of the actin fringe is probably rather complex and multifunctional, comprising several proposed tasks, such as providing (i) the central framework...
for the organization of endomembrane compartments and
maintenance of focused exo- and endocytosis; (ii) microfila-
ment tracks and attachment sites to membrane; (iii) a physi-
cal organelle exclusion barrier; (iv) structural support for the
plasma membrane and regulation of turgor driven extension;
(v) zoned localization of plasma membrane constituents
e.g. enzymes regulating cell wall extension); and (vi) force
for physical cell elongation by displacement and polymeri-
zation of actin filaments. Furthermore, in conjunction with
all processes the actin fringe orientation in the tip appears
to be a critical factor determining growth direction (Jackson
and Heath, 1993a; Meske and Hartmann, 1995; Dong et al.,
2012).

Altogether the reviewed actin fringes play an essential role
in cellular polarization, as a crucial scaffolding and highly
adapted integrating centre that serves as point of origin
and conductor for a multitude of processes participating in directed
cell elongation. In spite of this, actin fringes exhibit variations
in their structures, which are represented by three subclasses:
(i) a subapical localized network of microfilaments that are
primarily oriented longitudinally to the tube axis (Fig. 2A–D,
F); (ii) a subapical localized network of microfilaments that
are primarily aligned transversely to the tube axis (Fig. 2E, H,
I); and (iii) a dense apical array of microfilaments with fibrillar
extensions (Fig. 2G, K). This less frequent apical actin cap of
subclass (iii) exist in some fungal hyphae (Butt and Heath,
1988; Heath et al., 2000), moss protonemata (Finka et al.,
2007, 2008; Vidali et al., 2009a), and fern rhizoids (Kadota
and Wada, 1989). Why are hyphae and protonemata hetero-
genous cell types that have subapical fringes as well as apical
caps? Subapical or apical actin structures seem to respectively
emerge in correlation with the growth mode and elongation
rate of cells (Table 2; Fig. 2), with rapidly growing tips present-
subapical fringes. Accordingly, slow growing chloro-
nemata of bryophytes exhibit apical actin arrays, for instance.
However, a precise species-wide correlation between the form
of appearance and the growth rate has not been unequivocally
demonstrated, because in contrast, some fast elongat-
ing fungal hyphae (Saprolegnia ferrax) form apical arrays in
response to environmental turgor conditions. Remarkably,
in pollen tubes and root hairs ceasing their elongation, the
subapical actin fringe network is correspondingly relocalized
to the extreme apex (Miller et al., 1999; Dong et al., 2012),
which supports the essential correlation between growth rate
and position of the fringe in the tip. Likewise, in slowly grow-
ing hyphae of Phytophthora infestans, F-actin accumulations
were located more closely to the apex than in rapidly elongat-
ing hyphae (Meijer et al., 2014).

With their configuration and function adapted to tip
growth, the subapical actin fringes constitute a specialized
subtype in the large variety of ring-like arrays of the actin
cytoskeleton. Currently available data implicate that this
type of non-constricting actin ring is particularly limited
to tip-growing cells of plant and fungi. Additional cells of
diverse species also produce polar growing cell protrusions,
for example pseudopodia in amoebozoan cell locomotion
(e.g. the slime mould Dictyostelium discoideum; Noegel and
Schleicher, 2000; Yumura et al., 2013), lamellipodia, and
filopodia of macrophages or neuronal axons (Mallavarapu
and Mitchison, 1999; Ganguly et al., 2015). These tip-grow-
ing cells exhibit long actin cables in their cytoplasm and
amorphous F-actin accumulations at their apex. The am-
orphous actin clusters in their growth cones generate the pro-
trusive force for elongation through actin filament dynamics
and actin–myosin interactions, and provide also membrane
transport routes to the apical plasma membrane, which appear
in parts as conceptually similar to the actin fringe and
yet direct structural conformance is minimal (Yumura et al.,
2013; Ganguly et al., 2015). Recently a sequence of cortical
actin rings throughout the neuronal axon have been dis-
covered that probably have a role in structural support of this
cell protrusion, but not directly in the tip growth process (Xu
et al., 2013; Roy, 2016).

The actin fringe of angiosperm pollen tubes seems to rep-
resent the most complex and highest evolved version through-
out the eukaryotic phyla, which obviously relates to the
extreme high growth rate. In an attempt to explain the appear-
ance and character of actin fringes, one might speculate that
the structure relates to the ‘evolutionary age’ of the respective
tubular cell species, and above all, to the growth rate (Table 2),
whereby evolutionarily older cells grow slower and have under-
developed primordial fringes. This seems partially applicable,
although the correlation between fringe form, ‘evolutionary
age’ of the cell-type, and growth rate is apparently not close.
Yet, exact evaluation is impaired by technical limitations of
visualizing the actin cytoskeleton, and by a lack of compre-
hensive and comparative data directly relating fringe appear-
ance to cellular growth rate. Tip-localized actin arrays might
possibly date back to one shared evolutionary precursor struc-
ture, but too little is known about their molecular composition
in particular, which complicates final conclusions about their
evolutionary origin. In this regard, the radial circular F-actin
patterns that were found at the germination site of fungal
cysts, in the tips of hyphal branches, in hyphal tips recovering
from latrunculin B treatment (Bachewich and Heath, 1998),
as well as similarly in the cortex of algal rhizoids (Varvarigos
et al., 2004, 2007) could represent elementary precursor forms
in the evolutionary development of the massive and more
complex subapical actin fringe in fast elongating cells. In the
history of the earth various filamentous polarized growing
cells evolved with the appearance of fungi, streptophyte algae,
sporophytes and gametophytes. Considering the similarities
between all their actin fringes, it seems rather unlikely that
such structures occurred so numerously independently dur-
ing evolution. Consequently, the putative predecessor might
possibly be found somewhere near the evolutionary origin of
plants and fungi, because actin structures of polarized animal
cells are apparently different.

To definitely reveal the origin of the actin fringe it will
be required to characterize in-depth its molecular set-up,
particularly the ABPs such as nucleating, severing and capping
proteins, as well as regulatory pathways that are specific
for this structure. In this context, the question also arises
how this subapical F-actin entity is maintained independent
of other actin structures in the cell. The molecular mecha-
nism underlying the configuration process is unknown.
Fringe organization

F-actin

Formin

Villin

Myosin


tropomyosin

actin variants

Endomembrane organization

TGN

secretory vesicles

NtRISAP

AIMYOKIX

PpMYO1

PpMYO2

AIMYOKIX

PpMYO1

PpMYO2

Fig. 3. Schematic representation of the molecular network regulating the F-actin fringe. F-actin constitutes the actin fringe, which is an essential subapical structure for the proper course of polar cell growth. In this diagram molecular components of the fringe network are depicted that are involved in maintenance of its structure and function. The blue area marks actin-binding proteins involved in filament organization and set-up of the subapical actin fringe. The red area marks actin- and membrane-associated proteins involved in organization and set-up of the subapical endomembrane system, which is particularly represented by the trans Golgi network (TGN) and apical secretory vesicles. Green identifies regulatory RAC/ROP GTPases and associated effectors, which are suggested to control actin fringe formation and subapical to apical endomembrane dynamics. Black lines represent the interplay between F-actin and actin-binding proteins constituting the actin fringe, which for its part is involved in TGN localization and endomembrane dynamics in the tip via MYO1 and RISAP. Asterisks: proteins specific for the actin fringe; question marks: putative proteins involved in fringe filament composition, yet not experimentally verified.

Composition of actin filaments potentially contributes to the fringe properties, but involvement of actin variants, actin modifications or tropomyosin still needs to be clarified (Taheri-Talesh et al., 2008; Gunning et al., 2015). What are key regulators that specifically control the fringe? To date several studies reveal some parts of a molecular network comprising Rho family GTPase ROP1 (Fu et al., 2001; Gu et al., 2005), GTPase effectors RIC1/3/4 (Gu et al., 2005; Lee et al., 2008; Zhou et al., 2015), formin (Cheung et al., 2010), fimbrins FIM1/5 (Wu et al., 2010; Su et al., 2012; Zhang et al., 2016), villins VLN2/5 (Qu et al., 2013), LIM1 (Wang et al., 2008), AGC kinases (Zhang et al., 2009), CROLIN1 (Jia et al., 2013), ABP41 (Fan et al., 2004), RISAP (Stephan et al., 2014), and MYO1 (Stephan et al., 2014; Madison et al., 2015), which all are involved in polarization via regulation of F-actin dynamics and associated membrane transport processes in the pollen tube tip. The abovementioned formins, fimbrins, villins, and myosins hold the potential to specifically configure the fringe (Fig. 3), whereas other isoforms in contrast promote the generation of longitudinal actin cables in the shank (Ye et al., 2009), or induce apical plasma membrane-associated F-actin formation (Cheung and Wu, 2004). Some ABPs supposedly act under control of Rac/ROP GTPases and associated effectors such as RICs (Fig. 3), but data are currently insufficient and the fringe network is far from being understood. Modulation of the actin cytoskeleton and associated endomembranes in the tip presumably occurs also in response to intracellular gradients such as Ca2+ and pH. Tip-localized pH and Ca2+ gradients have been reported in pollen tubes (Feijo et al., 1999; Lovy-Wheeler et al., 2006; Cárdenas et al., 2008), root hairs (Felle et al., 1996; Cárdenas et al., 1999), algal rhizoids (Brownlee and Pulsford, 1988; Kropf et al., 1995), and fungal hyphae (Jackson and Heath, 1993b; Jolicoeur et al., 1998). Hence, actin cytoskeleton remodelling might additionally be achieved over gradient-regulated ABPs such as profilin, gelsolin, actin depolymerizing factor ADF, and ARP2/3 complex.

Building on this, however, a lot of intense experimentation is further required to characterize the complete set of actin-binding proteins and regulatory networks that are involved in creation of the higher order actin structures regulating polar cell growth.

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Actin fringes of polar growth in diverse cells


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