Pollen tube targeting and axon guidance: parallels in tip growth mechanisms

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The growth of pollen tubes to plant egg cells and the guidance of axons to neural synapses are classic examples of targeted cell growth. Despite the evolutionary time that separates animals and plants, axon and pollen tube guidance share remarkable mechanistic similarities. In both instances, extracellular cues are transduced by intracellular signal-transduction pathways that culminate in directed tip growth. Do the mechanistic similarities extend to the molecular level? Here, we address this question by a comprehensive review of the molecules and pathways involved in pollen tube targeting and axon guidance. The emerging scenario is that similar intracellular molecules are recruited to control tip growth, while different extracellular molecules mediate guidance through the distinct plant and animal extracellular matrices.

Both higher plants and animals depend on targeted growth for their development: for example, plants require the precise guidance of pollen tubes to the eggs, and animals rely on the proper growth of axons to form an intricate array of synaptic connections. Despite their structural and physical differences, pollen tubes and axons accomplish a similar feat: they reach distant targets with remarkable and reproducible accuracy, recognizing long- and short-range guidance cues and interacting with varied cells and tissues in their path. Although more than 600 million years[1] of evolution separate plants from animals, the emerging scenario is that both of these targeted growth processes rely on similar intracellular molecules to transduce guidance signals. By contrast, they recruit different extracellular molecules to mediate guidance through the distinct environments of the plant and animal extracellular matrices. Here, we review recent studies on pollen tube growth and point out relevant similarities and differences in comparison with axon guidance, suggesting fertile areas for future investigations.

Reproduction in flowering plants is achieved by pollination, followed by fertilization. Pollination initiates when pollen grains from the male reproductive organs (anthers) are deposited on the stigma of the female reproductive organs (pistils; Fig. 1a). The various aspects of pollination and fertilization have been reviewed recently[2–4]. On the stigma, pollen absorbs water and forms a pollen tube – a long polar process that carries all of the cellular contents including the sperm. The pollen tube invades the pistil and migrates past several different cell types: first growing between the walls of the stigmatic cells, then travelling through an extracellular matrix (ECM) in the transmitting tissue of the style and finally arriving at the ovary, where it targets an ovule that contains an egg. In many plant species, a single pollen tube is guided towards each ovule micropyle where the tube bursts and delivers the sperm (Fig. 1b).

During the development of a nervous system, neurons send out axons that extend at their tips, or growth cones. After elongating along specific paths, the axon forms a specialized junction called a synapse with the target nerve or muscle cell (Fig. 1c). The elongation of axon growth cones is strikingly similar to the extension of pollen tubes[5,6]. In both cases, the growing tips are influenced by long- and short-range guidance cues that are presented by the target cell or by cells in the path leading to the target. Proteins in the growing cell perceive such signals, activating transduction pathways that target the cytoskeleton. Subsequent changes in cyto-architecture mediate downstream events, such as cell shape changes and exocytosis, and culminate in directed tip growth (Fig. 2). Despite similarities in their directed growth, pollen tubes and axons differ in at least one important aspect – the entire cytoplasm, including the nucleus, is transferred to the growing tip of the pollen tube, and new walls within the tube sever the connection to the evacuated pollen grain. By contrast, a neuron retains its nucleus in the cell body, and the growing tip of an axon consequently remains functionally connected to the rest of the cell, maintaining communication over long distances.

Diffusible guidance cues: long-range signals

Diffusible chemicals, secreted either by the target cell or by cells in the path to the target, need to fulfil the following criteria to be considered guidance cues: they must (1) form a concentration gradient in a direction that determines the orientation of growth; (2) elicit a specific response; (3) remain stable over a defined period of time; and (4) vary in effectiveness with distance to the target. Netrins are diffusible attractive cues for axon growth that meet these criteria; similarly, secreted semaphorins belong to the group of repulsive cues[7] (Table 1). In plants, an early hypothesis proposed that a single chemical guidance cue, in a continuous gradient emanating from the ovule, is responsible for proper pollen tube growth[8]. Recent models, however, predict that the maximum distance over which pollen

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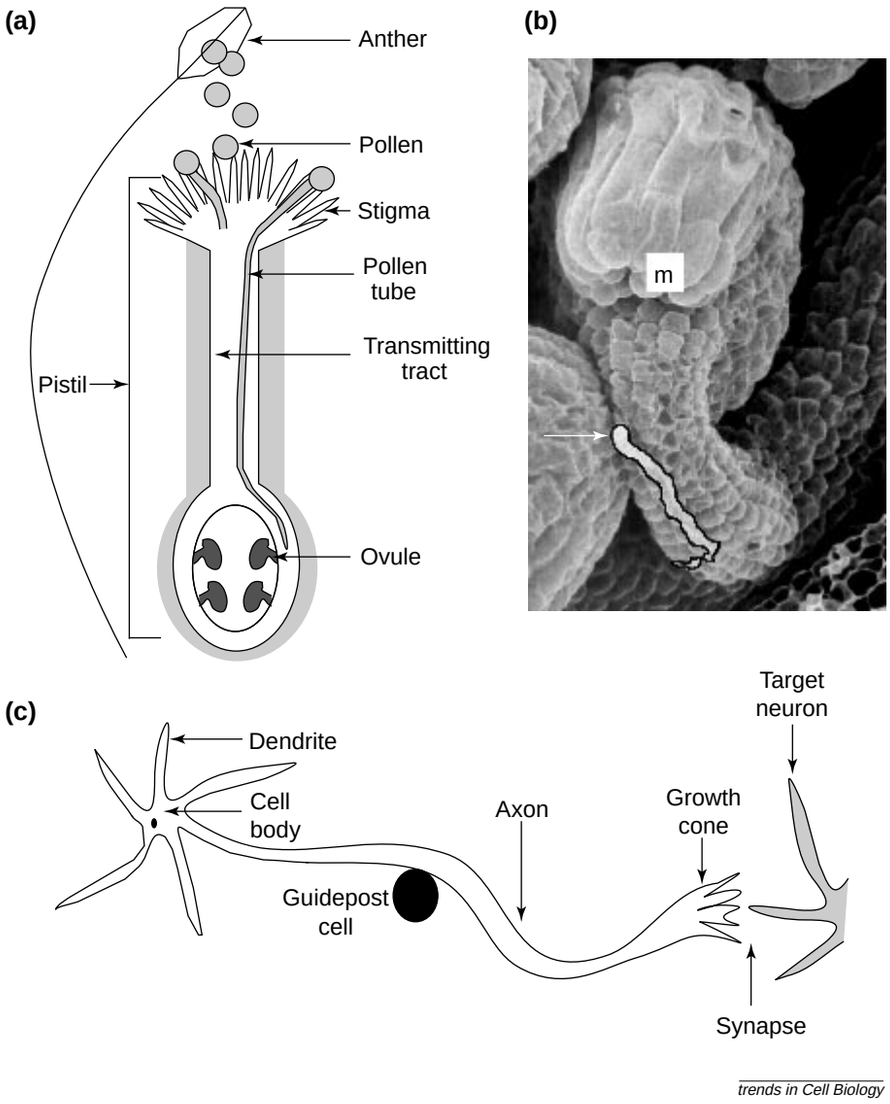

FIGURE 1

Targeted cell growth in pollination and axon guidance. (a) Pollen grains from anthers are deposited on the pistil, where they initiate a pollen tube. Sperm cells are carried within the tube to an egg cell inside an ovule. (b) Scanning electron micrograph of a pollen tube (outlined for emphasis, white arrow) approaching an ovule micropyle (m). (c) An axon extends from a neuron cell body, expanding at the growth cone and utilizing guidance cues to form a synapse with target cells.

tubes can be guided by diffusible chemicals is 1–9 mm – far less than the distance travelled by pollen tubes in many species[9]. Thus, it is more likely that different chemical cues from multiple intermediate targets guide pollen tubes to the ovules. This scenario is similar to that observed in axons, where signals can control guidance over a theoretical maximum distance of 2.5 mm[9]; guidance to distant targets is consequently mediated by multiple guidepost cells[10] (Fig. 1c).

Diffusible small molecules that direct pollen tube growth have not been identified. As described below, genetic studies have nonetheless strongly implicated the egg and surrounding haploid cells[11–13], as well as diploid pistil tissues[14], as the source for such signals. Furthermore, an *in vitro* system that reproduces guidance to haploid germ cells has been developed in *Torenia fournieri*[15]. Activation of this guidance system required interactions with the pistil – only pollen tubes that grew through a stigma and transmitting tract were attracted. Despite the mechanistic similarities, a survey of the nearly complete *Arabidopsis* genome, as well as the expressed sequence tag (EST) databases from maize, tomato and rice, did not reveal any close relatives of netrins or semaphorins, although there are numerous homologues in nematodes, flies and mammals. Elucidating the cues that direct pollen tubes promises to reveal an exciting class of novel guidance molecules, providing a new resource for understanding targeted cell growth.

Contact-mediated guidance cues: short-range signals

ECM molecules play a crucial role by providing local guidance through contact-mediated adhesion. In animals, ECM adhesion glycoproteins such as collagen, laminins, vitronectin, fibronectin and heparin-binding growth-associated molecule promote guided axon growth, while tenascin and aggrecans inhibit growth[10,16,17] (Table 1). Some ECM molecules can also bind to soluble guidance molecules and limit their diffusion; this effectively sharpens concentration gradients and extends the range over which diffusible signals can act[18]. In plants, the ECM is represented by the cell wall, a protein- and carbohydrate-rich matrix that surrounds all plant cells. Antibodies raised against vitronectin and fibronectin have identified candidate homologues in plants[19], but definitive proof that these proteins are functional homologues and insights into their adhesion roles in pollen tube growth have not been obtained. In addition, sequence-based homology searches of the genomes of *Arabidopsis* and other plants have not revealed any proteins similar to animal ECM guidance molecules such as collagen, laminins, vitronectin, fibronectin or tenascin. One possibility is that plants might recruit different kinds of ECM molecules for contact-mediated guidance. In support of this, a lipid-transfer protein and a stylar protein localized to the transmitting tract ECM of lily were found to be crucial for proper pollen tube adhesion to the pistil cells[20] (Table 1).

Collagen belongs to a group of proteins that are rich in hydroxyproline, an unusual amino acid resulting from the posttranslational modification of proline. In plants, hydroxyproline-rich glycoproteins (HRGPs) might represent the functional equivalent of collagen. Plant HRGPs consist of four subclasses: cell wall extensin, proline-rich proteins, arabinogalactan proteins (AGPs) and lectins[21]. AGPs play a crucial role in cell–cell signalling and cell recognition during pollination. Support for this hypothesis comes from

observations that phenylglycosides that specifically bind and precipitate AGPs also inhibit pollen tube growth when injected into transmitting tissues[22]. Furthermore, the AGPs TTS1 and TTS2 from *Nicotiana tabacum* and NaTTS from *N. alata* are localized to the transmitting tract ECM and stimulate pollen tube growth *in vitro*[23,24] (Table 1). Transgenic tobacco plants expressing antisense *TTS1* and *TTS2* mRNA are sterile[24,25]. These TTS proteins display a gradient of increasing glycosylation that correlates with the direction of pollen tube growth; both *in vitro* and *in vivo* studies indicate that the TTS proteins[24] and another AGP, NaPRP5 (Ref. 26), are incorporated into pollen tubes, where they are deglycosylated (Table 1).

In animal systems, target cells, as well as cells leading to the target, often employ proteinaceous cell-adhesion molecules (CAMs) to guide migrating axons. Members of this group include the immunoglobulin (Ig) and cadherin superfamilies, which are contact attractants that promote adhesion, and Ephrin (Eph) ligands and membrane-bound semaphorins, which function as contact repellants[10]. A search of the plant genome databases did not identify any gene with high sequence similarities to these animal cell-surface adhesion proteins. By contrast, homologues of a novel *Drosophila* cell-adhesion protein, Pollux, which is expressed abundantly along axon pathways of the central nervous system, have been found in *Arabidopsis* and rice[27]. Pollux does not fall into any of the previously characterized CAM or ECM gene families; it nonetheless contains several adhesion motifs, including a site that mediates integrin recognition and/or attachment[27].

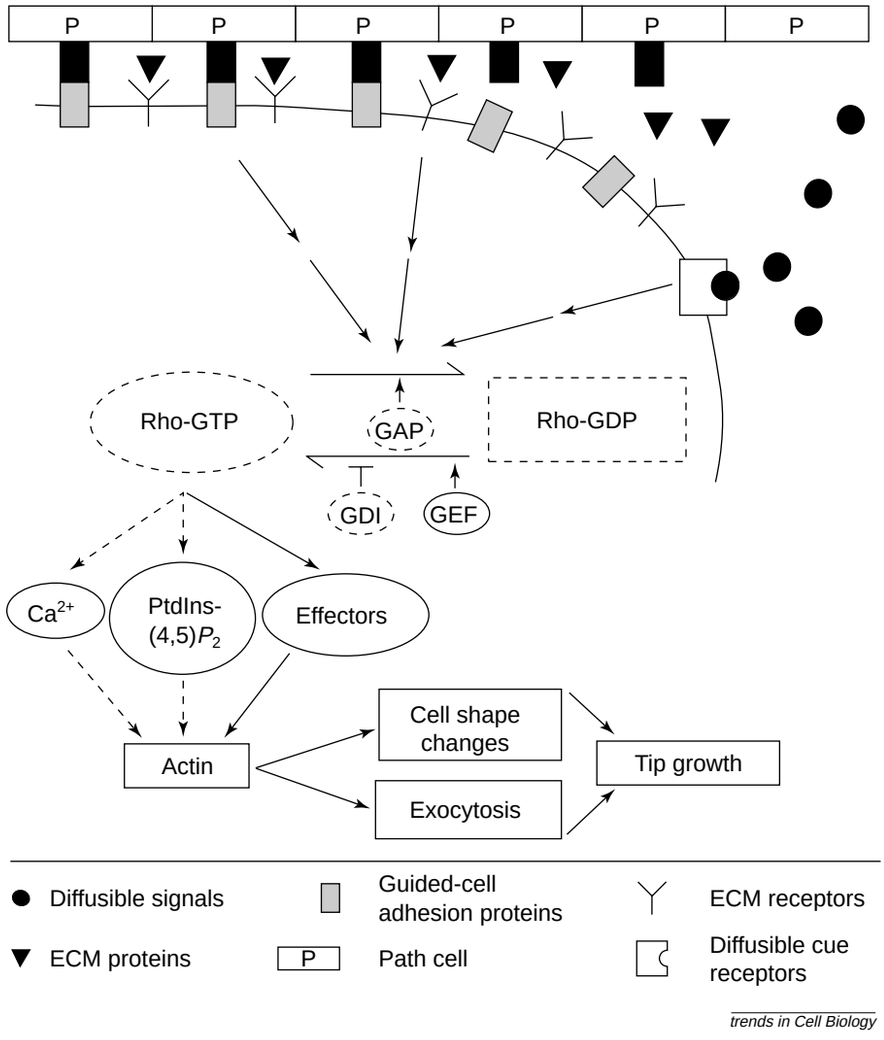

FIGURE 2

Signal-transduction pathways involved in tip growth. Extracellular guidance cues are provided by diffusible signals (filled circles), extracellular matrix (ECM) proteins (filled triangles) and cell-adhesion proteins on the guided cell (grey rectangles) and on cells in its path (P, black rectangles). Receptors for ECM proteins (Y) and for diffusible cues (open rectangle) are also indicated. Signals from the membrane are to upstream regulators [GTPase-activating protein (GAP), guanine nucleotide dissociation inhibitor (GDI) and GDP–GTP exchange factor (GEF)] of Rho GTPases. Activated Rho influences the actin cytoskeleton through phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], calcium (Ca^{2+}) and other effector proteins. Actin cytoskeleton rearrangements result in downstream events (cell shape changes and directed exocytosis) that culminate in targeted tip growth. Components and processes identified in plants are indicated by dashed lines.

Signal perception by the guided cell

Axons perceive long-range diffusible guidance molecules through surface receptors, such as the *C. elegans* netrin receptors UNC-40 and UNC-5, or the mammalian semaphorin receptor, neuropilin[7]. Short-range ECM guidance signals are perceived by axons through surface transmembrane proteins, including integrins and glycoproteins[10] (Table 1). Integrins have an extracellular N-terminal domain that binds to ECM molecules, and a cytoplasmic C-terminal domain that interacts with components of signal-transduction pathways as well as with the cytoskeletal apparatus[28]. Interestingly, pollen tube migration might be directed in a similar manner; although their role in pollination has not been tested, two proteins with integrin domains, Wak1 and At14a, localize to the *Arabidopsis* cell wall and plasma membrane, respectively[29,30]. Axon cell-surface glycoproteins, such as the members of the syndecan and glypican family, interact with cell-surface adhesion molecules to mediate axon growth and pathfinding[16]. Similarly, a maize glycoprotein found in the inner wall of pollen tubes, Pex1, might be required for adhesion to the transmitting tract cells[31] (Table 1).

The contact-dependent repulsive effect of ephrins on axon growth is mediated by the ephrin (Eph) ligand binding to receptor protein tyrosine kinases[7,10]. Although the analysis of such ligand–receptor guidance systems in pollen is not complete, receptor-like protein kinases have been identified in *Arabidopsis* and tomato[32,33]. Of these, the tomato protein LePRK2, which localizes to the plasma membrane of growing pollen tubes, has been implicated in transducing extracellular signals from the pistil tissues[33]. Biochemical identification of the long-range guidance signals for

TABLE 1 – MOLECULES MEDIATING AXON GUIDANCE AND POLLEN TUBE TARGETING

Signal type	Axons[b]	Pollen tubes
Guidance signals		
Long-range		
Chemoattractants	Netrins, UNC6	Not identified
Chemorepellants	Secreted semaphorins	Not identified
Short-range attractants		
ECM glycoproteins	Laminin, vitronectin, fibronectin, HB-GAM[c], collagen	Lipid-transfer protein, stylar pectin, TTS, NaTTS, NaPRP5
CAMs	Immunoglobulin (Ig) CAMs, cadherin CAMs	Not identified
Short-range repellants		
ECM proteins	Tenascin, aggrecans	Not identified
CAMs	Ephrins, membrane-bound semaphorins	Not identified
Other-short range cues[a]		
CAMs	Pollux	Pollux homologues
Cell-surface glycoproteins		NaPRP4
Signal perception		
Long-range		
Chemoattractant receptors	UNC40, DCC, Frazzled, UNC5	Not identified
Chemorepellant receptors	Neuropilin	Not identified
Short-range		
ECM receptors	Integrin	Wak1[d], At14a[d]
Cell-surface glycoproteins	Syndecans, glypicans	Pex1
CAM receptors	Ephrin RPTK[e]	LePRK2

[a]Attractive or repulsive nature not determined.
[b]Only some of the known molecules are listed.
[c]Heparin-binding growth-associated molecule.
[d]Activity in pollen tubes not yet determined.
[e]Receptor protein tyrosine kinases.

pollen tubes would greatly facilitate the characterization of additional receptors. However, genetic screens for mutants with defective pollen tube guidance might prove to be more successful as they have the potential to uncover receptors in the absence of any information on the guidance cues.

In addition to protein signals, axons and pollen tubes also respond to electrical fields: growing cells exhibit endogenous electric fields, and the application of external fields can influence their direction and rate of growth[34,35]. The entry of ions such as H^+, K^+ and Ca^{2+} at the tip of the pollen tube and the exit of ions through the pollen grain generates an endogenous electrical field. This tip-focused ion flux has led to the hypothesis that electrical fields maintain growth at the tip of pollen tubes[36]. In addition to this long-range electrical field, a short-range current loop has recently been discovered[37]. This is generated by the entry of protons at the apex of the pollen tube tip and exit at its base and is probably mediated by the precise localization of ion transporters[37]. In axons, external electric fields combined with the application of guidance signals can orient growth at the tip[34]; such synergistic effects have yet to be demonstrated in pollen tubes.

Signal transduction: the role of Rho GTPases

Rho-like GTPases are part of the Ras family of small GTP-binding proteins. In human fibroblasts and neurons, this subfamily comprises three members: Rho, Rac and CDC42. These GTPases are crucial members of signalling pathways that transduce extracellular stimuli to mediate a myriad of downstream events including actin cytoskeleton reorganization and exocytosis[38]. GTPases act as molecular switches: they are active when bound to GTP and inactive when the bound GTP is hydrolysed to GDP by their intrinsic GTPase activity. Although there are no reports of bona fide Rho homologues in plants[39], indirect evidence for a Rho activity exists: injection of C3 exotoxin, which inhibits Rho but not CDC42 or Rac, arrests cytoplasmic streaming and pollen tube growth[40].

Interestingly, plants have an additional subfamily of GTPases that are closely related to animal Rac. These proteins are referred to as Rop (Rho of plants) in *Arabidopsis* and pea[41] or as At-Rac (*Arabidopsis thaliana* Rac)[42]. The Rop and At-Rac GTPases have been implicated in several stages of plant development, including pollination[39]. Pollen tube growth was severely inhibited when polyclonal antibodies against Rop1 were injected into pea pollen tubes[40] or when dominant-negative mutants of Rop were expressed in pollen tubes[43]. These observations are strikingly similar to the axon migration defects found in *mig-2* mutants, which are defective in the *C. elegans* Rho-like GTPase[44].

TABLE 2 – ROLE OF OVULE TISSUES IN POLLEN TUBE GUIDANCE[a]

Genotype	Pollen tube guidance	Funiculus (2n)	Outer integument (2n)	Inner integument (2n)	Embryo sac (n)
Wild type	Normal	+	+	+	+
ant	Defective	+/−	−	−	−
bel1	Defective	+/−	+/−	+/−	−
sin1	Defective	+	+/−	+/−	−
tso	Defective	+	+/−	+/−	−
ino	Defective	+	−	+	+

[a]Symbols: +, structure present; +/−, aberrant structure; −, structure absent.

Upstream regulators of Rho-like GTPases control transduction of external stimuli, and their roles in neuronal growth cones have been well characterized[7,45]. Positive upstream regulators include guanine nucleotide exchange factors (GEFs), which stimulate the exchange of GDP for GTP and thus favour the accumulation of active forms (Fig. 2). At least two negative upstream regulators contribute to the accumulation of inactive GDP bound forms: GTPase-activating proteins (GAPs) and guanine nucleotide disassociation inhibitors (GDIs; Fig. 2)[46]. Cell migration and axon path finding defects in *C. elegans* result from mutations in the Rac activator UNC-73 (Ref. 47). Although there are no reports of pollen-specific regulators of Rop GTPases, the interplay between GAP and Rho GTPase observed in animals seems to be conserved. Three GAP-like proteins that bind to *Lotus japonicus* Rac1 and Rac2 have been isolated[48]. Surprisingly, while GAP- and GDI-like proteins have been found in the *Arabidopsis* genome, no proteins with homology to animal GEFs have been described, leading to the suggestion that plants instead use non-conventional GEFs[39].

Activated Rho-like GTPases interact with downstream effector proteins to relay external stimuli to the actin cytoskeleton. One such effector is phosphatidylinositol monophosphate kinase, which is required for the synthesis of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2]. In animal cells, PtdIns(4,5)P_2 controls axon tip growth by regulating the activity of the actin-binding proteins profilin, gelsolin and vinculin[49]. PtdIns(4,5)P_2 is also required for regulated exocytosis, thereby facilitating tip growth[50]. A similar mechanism seems to be conserved in plants: At-Rac2, which plays a central role in maintaining the polarized growth of pollen tubes, associates with and regulates phosphatidylinositol monophosphate kinase activity[42].

Signal transduction: the role of calcium

Calcium has been implicated in the regulation of several axon growth cone behaviours including axonogenesis, axon growth, axon turning and rearrangements of the actin cytoskeleton[51]. Similarly, several lines of evidence confirm that Ca^{2+} plays a central role in ensuring proper pollen tube guidance[2,52]. Growing pollen tubes actively take up Ca^{2+}, and inhibiting this uptake results in growth arrest. Calcium levels within pollen tubes are influenced by both influx at the pollen tube tip and release from internal stores[2,52]. Ratiometric Ca^{2+} imaging shows a gradient in pollen tubes, with the highest concentrations at the growing tip. Only growing pollen tubes exhibit this gradient; polarized growth is lost when the gradient is abolished[2,52]. Intriguingly, the cytosolic Ca^{2+} levels are not static, their fluctuations occur immediately after each periodic cycle of pollen tube growth[53]. This observation has led to the proposal that growth oscillations give rise to Ca^{2+} oscillations, and hence secretion, which in turn is essential for the subsequent growth oscillations[53]. In addition, a positive correlation between Ca^{2+} levels and the direction of pollen tube growth has been found: high Ca^{2+} levels mark the direction in which pollen tubes turn[54]. There is a strikingly similar correlation between high Ca^{2+} levels and the orientation of growing axons[55], raising the possibility that the pathways by which Ca^{2+} influences tip growth are similar.

The Ca^{2+} gradient in the growing pollen tube tip mediates directional secretion of vesicles (exocytosis) and hence the extension of plasma membrane. Several lines of evidence suggest that the Rop1 GTPase, which is localized at the tip of the pollen tube, plays a central role in maintaining this gradient in *Arabidopsis*. Injection of antibodies against Rop1 into pollen tubes, as well as expression of dominant-negative forms of Rop1, diffused the tip-focused gradient and eliminated pollen tube growth, an effect that was partially relieved by the addition of high concentrations of extracellular Ca^{2+} (Ref. 41). It remains to be seen whether axons display a similar interaction between Rho-like GTPases and Ca^{2+}. Increasing the level of intracellular Ca^{2+} triggers exocytosis in neuronal cells, a process that is regulated by protein kinase C (PKC)[56]. A recent study localized a Ca^{2+}-dependent PKC-like activity in pollen tube tips[57]; importantly, spatial redistribution of this kinase activity resulted in reorientation of pollen tube growth.

Actin cytoskeleton changes in response to external stimuli

One target of the extracellular guidance signals is the actin cytoskeleton, which affects downstream processes such as cell shape changes and exocytosis. A role for the actin cytoskeleton in the maintenance of pollen tube growth is well established: inhibition of actin polymerization by latrunculin B or other inhibitors arrests pollen tube growth[58]. In pollen tubes, cortical actin cables are oriented longitudinally and contain only a few fine filamentous structures in the tip[59]. This cytoarchitecture is probably necessary for the maintenance of directed exocytosis and pollen tube growth. Tobacco pollen tubes transiently expressing a constitutively active form of At-Rac2 GTPase were depolarized and exhibited extensive cortical actin cables in an aberrant spiral pattern (Fig. 3). Expression of a dominant-negative form of At-Rac2 had the opposite effect, resulting in the reduction of actin bundling and the inhibition

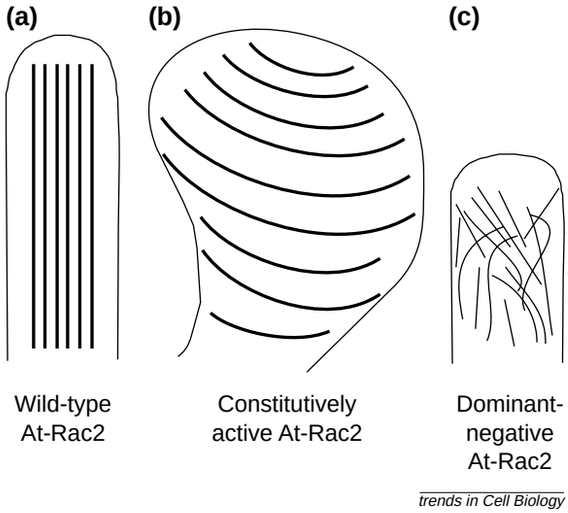

FIGURE 3

Altered expression of At-Rac2 causes rearrangements of the actin cytoskeleton in pollen tubes[42]. Constitutively active At-Rac2 depolarizes growth and causes actin filaments to become helical (b); a dominant-negative form of At-Rac2 inhibits growth and reduces filament bundling and organization (c) compared with wild-type At-Rac2 (a).

of pollen tube growth[42], observations reminiscent of a loss of Rho GTPase activity in animal cells (Fig. 3).

Mutants defective in pollen tube targeting

The recent identification of several mutants defective in pollen tube guidance offers great promise for the elucidation of guidance molecules, potentially bridging the gap between the understanding of intracellular control of pollen tube polarity and the extracellular signals for guidance. What is the source of the guidance cues? Significant insights into this question have come from the analysis of ovule development mutants. In *bel1* and *sin1* (Ref. 11), *ant9* (Ref. 60), *tso1* (Ref. 61) and plants with reduced levels of the ethylene-forming enzyme ACC oxidase[62], ovule development is abnormal and pollen tube targeting is defective (Table 2). These studies demonstrate that ovules are necessary for pollen tube guidance; however, they are unable to determine whether guidance cue(s) arise from diploid ovule tissues or from haploid germ cells, both of which are defective in the mutants. In an attempt to distinguish between these possibilities, Ray *et al*. generated pistils in which half the ovules contained apparently normal diploid tissues yet lacked a haploid female embryo sac[12]. Pollen tubes correctly targeted the normal, but not the aberrant, ovules. Similarly, *maa1* and *maa3* mutants have highly specific alterations during late stages of embryo sac development (the two polar nuclei fail to fuse; Fig. 4) and fail to attract pollen tubes[13]. These studies provide firm evidence that the haploid embryo sac plays a crucial role in pollen tube guidance, either directly, by secreting attractive signals, or indirectly, by stimulating surrounding diploid tissues to release guidance cues. Diploid tissues have been implicated in pollen tube guidance even when the embryo sac is normal: the *pop2 pop3* mutant has a viable and functional embryo sac, yet pollen tubes do not adhere to the funiculus[14]. Similarly, *inner-no-outer* (*ino*) mutant ovules, which have apparently normal haploid embryo sacs but lack the diploid outer integument, are defective in pollen tube targeting[63].

The growth of a pollen tube can be broadly classified into four phases[11]:

- growth on the stigma,
- growth between cells of the transmitting tissue,
- emergence into the ovary, and
- growth along the ovule funiculus to reach the micropyle.

The *pop2 pop3* and the *maa* mutants (Fig. 4) reveal that the fourth phase can be further divided into two distinct stages: pollen tubes first adhere to and grow up the funiculus and subsequently they are guided to the micropyle. In *maa* mutants, the pollen tubes adhere and grow up on the funiculus but fail

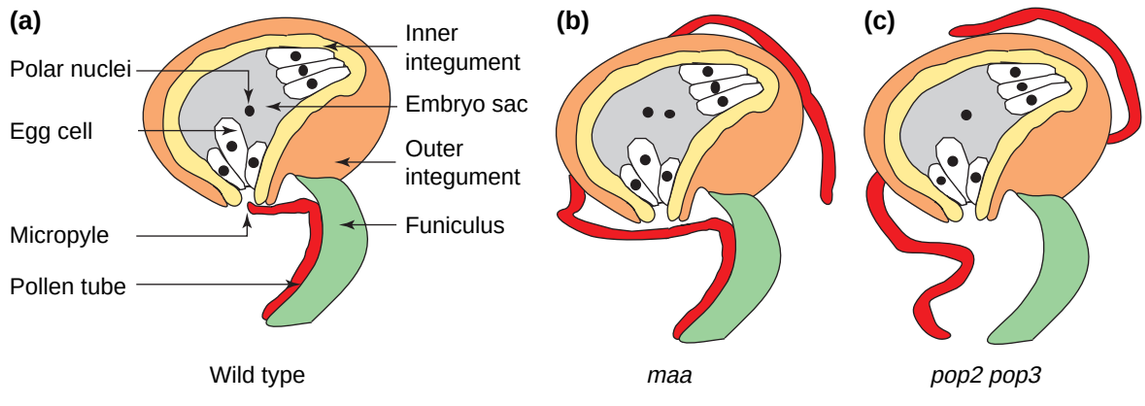

FIGURE 4

Arabidopsis mutants that affect the final stages of pollen tube growth. (a) In wild-type ovules, the egg cell resides within the embryo sac, which is surrounded by two layers of diploid tissues – the inner and outer integuments. All of these structures are attached to the ovary by the funiculus. Pollen tubes adhere to the funiculus and enter an opening between the two integuments, the micropyle, to reach an egg cell. (b) The *maa* mutants are defective in the micropylar guidance phase, whereas both funicular and micropylar guidance are defective in the *pop2 pop3* mutant (c).

to enter the micropyle, indicating that distinct cues are essential for micropylar entry[13]. However, in *pop2 pop3* mutants, guidance is defective at an even earlier step: pollen tubes fail to adhere to the funiculus and, even when close to the micropyle, fail to enter, indicating that adhesion to the funiculus is a prerequisite for subsequent micropylar entry[14].

Concluding remarks

The many mechanistic similarities with axon guidance offer an intellectual frame work for studies of pollen tube targeting. Although this analogy assumes similarities, there might be important differences between the two systems; in particular, extracellular interactions in animals and plants might rely on distinct components of the ECM. In recent years, the understanding of the intracellular signalling pathways required for pollen tube guidance has improved considerably; the identification and functional characterization of extracellular signalling molecules and their receptors remain exciting challenges for the future.

Biochemical studies in mammals, coupled with genetic screens in *Drosophila* and *C. elegans*, have been instrumental in defining the controls on axon growth. The availability of *Arabidopsis* mutants defective in pollen tube guidance, coupled with pioneering biochemical work in other plants, is likely to result in a similar synergy. With the upcoming completion of the *Arabidopsis* genome sequence, and the accessible collection of insertional mutations in nearly every *Arabidopsis* gene[64], research on targeted plant cell growth is entering an exciting phase.

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Aggresomes, inclusion bodies and protein aggregation

Ron R. Kopito

Intracellular and extracellular accumulation of aggregated protein are linked to many diseases, including ageing-related neurodegeneration and systemic amyloidosis. Cells avoid accumulating potentially toxic aggregates by mechanisms including the suppression of aggregate formation by molecular chaperones and the degradation of misfolded proteins by proteasomes. Once formed, aggregates tend to be refractory to proteolysis and to accumulate in inclusion bodies. This accumulation has been assumed to be a diffusion-limited process, but recent studies suggest that, in animal cells, aggregated proteins are specifically delivered to inclusion bodies by dynein-dependent retrograde transport on microtubules. This microtubule-dependent inclusion body is called an aggresome.

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Protein aggregation is an inevitable consequence of cellular existence. Protein aggregates are oligomeric complexes of non-native conformers that arise from non-native interactions among structured, kinetically trapped intermediates in protein folding or assembly[1–3] (Fig. 1). Protein aggregation is facilitated by partial unfolding during thermal or oxidative stress and by alterations in primary structure caused by mutation, RNA modification or translational misincorporation[2,4]. Protein aggregates can be either structured (e.g. amyloid; Fig. 1a) or amorphous (Fig. 1b). In either case, they tend to be insoluble and metabolically stable under physiological conditions. Their accumulation is tightly linked to neuronal degeneration or organ failure in many 'protein deposition' diseases.

The fact that protein aggregates do not accumulate in unstressed cells, despite their continued production, is due in part to the existence of cellular 'quality control' machinery. This suppresses the formation of aggregates by ensuring the fidelity of transcription and translation, by chaperoning nascent or unfolded proteins, and by selectively degrading improperly folded polypeptides before they can aggregate[5,6]. The prominence of protein aggregates in intracellular and extracellular lesions associated with cell death in many degenerative diseases (e.g. amyloid diseases, Alzheimer's disease, Parkinson's disease, Huntington's disease and alcoholic liver disease[7]) underscores the physiological importance of these quality control pathways and suggests that the failure of these systems to control aggregation might have catastrophic consequences for the organism. However, protein aggregation need not be pathogenic. For example, in yeast, cytoplasmic inheritance of aggregated prion proteins underlies the propagation of stable epigenetic traits that are not associated with any known pathology[8].

Although considerable research has addressed the mechanisms by which cells avoid forming protein aggregates, the way that cells deal with proteins once they have aggregated is relatively unexplored. This article focuses on the cellular machinery that directs aggregated proteins into inclusion bodies and how this process might help to protect cells from potentially toxic protein aggregates.

Protein misfolding: a fact of life

The yield of folded protein obtained from *in vitro* refolding studies is typically low, owing largely to competition for folding intermediates by thermodynamically stable, 'dead end' conformations. These so-called 'off pathway' (off the folding pathway) conformers generally lead to the formation of insoluble aggregates (Fig. 1). All cellular folding compartments therefore contain abundant molecular chaperones that help to minimize the contribution of off-pathway reactions during protein folding

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