Signalling by tips
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New molecules, including protein kinases, lipids and molecules that have neurotransmitter activities in animals, continue to be described as important players in tip-growing cells. Transcriptomics is beginning to show that the largest single class of genes expressed in pollen tubes encode signal transducers, reflecting the necessity to decode all of the different pathways that are associated with tip growth. Many of these pathways may use common intracellular second messengers, with ions and reactive oxygen species emerging as two major common denominators in many of the processes involved in tip growth. These second messengers might influence the actin cytoskeleton through known interactions with actin-binding proteins. In turn, changes in the dynamic properties of the cytoskeleton would define the basic polarity events needed to shape and modify tip-growing cells.

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Abbreviations
ABA abscisic acid
ADF actin-depolymerising factor
AIP1 ACTIN-INTERACTING PROTEIN1
GABA γ-aminobutyric acid
Lat52 [Please define.]
LePRK Lycopersicon esculentum POLLEN RECEPTOR KINASE
MAPK mitogen-activated protein kinase
NO nitric oxide
pop2 [Please define.]
rhd2 root-hair defective2
ROS reactive oxygen species
SAGE serial analysis of gene expression
WASP Wiskott-Aldrich syndrome protein

Introduction
Tip-growing cells undergo an extreme type of polarised growth. Their growth is based on the occurrence of elongation exclusively at the apex, which is fuelled by newly synthesised membrane delivered by vectorial exocytosis. Tip-growing cells are probably the fastest linearly growing cells in nature. Furthermore, they have been perfected by evolution as machines that sense subtle extracellular signals and environmental changes, and that develop by changing their growth axis accordingly. In plants, there are two highly differentiated types of tip-growing cells: root hairs and pollen tubes. Root hairs have to sense the soil environment and grow so as to maximise water and ion uptake; they also respond to biotic stimuli, which may result in the establishment of sophisticated symbioses. Pollen tubes, on the other hand, have to communicate their ‘self’ properties (i.e. information about species and individuality) to the external stigma cells. These cells continuously interact with the female tissues to scout and find the right path into the open ovary cavity, until they reach the micropyle’s tiny opening and deliver sperm.

The biological functions of both of these cell types imply an innate capacity to communicate with and to decode signals from their environment. It is not surprising that signalling is likely to play a central role in defining these cell types. Many groups have focussed on signalling within tip-growing cells and have produced a significant body of information [1–5]. In this review, we highlight some recent developments in our understanding of signalling in apically growing plant cells.

Re-staging a classic with new actors
Tip-growing cells were identified some time ago as a good system in which to investigate known signalling molecules and mechanisms, and in which to discover new ones [5]. Besides the huge amount of information compiled on the self-incompatibility system, which is beyond the scope of this review, a paradigmatic view of the sophisticated signalling system within the pollen-tube has been uncovered through the description of the LePRK pollen receptor kinase signalling complex. In mature pollen, LePRK2 and LePRK1 are bound to each other in a complex, and the secreted protein Lat52 is associated with the LePRK2 [6]. In the presence of style extract, however, LePRK2 is de-phosphorylated and both LePRK1 and Lat52 are released. These observations suggest a model in which pistil ligands induce the dissociation of the complex and the release of the partners, including cytoplasmic partners that transduce the signal to the pollen tube [7]. Recently, new interactors of LePRK1 and LePRK2 have been described, namely LeSHY and LeSTIG [Please define the abbreviations LeSHY and LeSTIG.] [8]. Exogenous LeSTIG abolished the interaction between Lat52 and LePRK2, and promoted pollen-tube growth in vitro. These findings are consistent with model that LePRK1 and LePRK2 might interact with different
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Figure 1

(a) Time-lapse sequence of a *Lilium longiflorum* (lily) pollen-tube growing facing an extra-cellular NO point-source [SNAP [Please define SNAP.] on agarose [shown on the left of the image]]. The growth of the pollen tube slows as it moves into the NO gradient, but the direction of growth was unchanged for about 12 min. A new growth axis then starts to be defined, forming a sharp right-angle from the original axis (97.7° ± 3.6, \(n = 28\)). The pollen tube then regains its normal growth rate (after 16–20 min) [Bar = 30 µm]. (b) Lily pollen tube showing three consecutive re-orientation responses, which were induced by moving the same source to the locations marked with arrows. The growth axis moved reproducibly by right angles after each challenge by the NO source facing the pollen-tube tip. (c) Artificial NO-source measurements obtained by using a vibrating self-referenced polarographic probe to NO. The graph shows a typical exponential NO-gradient decay from the point source at different step distances. (d) Time-lapse sequence of a pollen tube being challenged with a diluted NO artificial source in the presence of sildenafil citrate (Viagra™) (numbers at the right-hand upper corner represent minutes after detection of the response). When these diluted sources are used, most pollen tubes do not show any response, often running into the pipette. For this experiment, pollen tubes were first incubated on standard medium and challenged with the diluted NO source. Despite the lower amount of NO used, reverse re-orientation angles were observed in the presence of sildenafil citrate (109.8° ± 9.8, \(n = 9\)) showing a sensitisation effect, from unresponsive to peak response (adapted from [10]).

Ligands at different stages of the growth of the pollen tube through the style, but unexpected molecules have also entered the scene. Two molecules that have neurotransmitter properties in animals were recently found to be involved in pollen-tube growth. γ-amino butyric acid (GABA) was shown genetically to be involved in the growth and guidance mechanisms of *Arabidopsis* pollen tubes [9]; the pollen tubes of *pop2* mutants are strongly impaired in their capacity to grow both in *vivo* and *in vitro* in the presence of GABA. *POP2* was convincingly demonstrated to encode a transaminase that is involved in the degradation of GABA. We have recently demonstrated a new role for nitric oxide (NO) in the regulation of pollen-tube growth in *Lilium longiflorum*, especially in the re-orientation response (Figure 1). NO may be involved in finding a suitable path for the pollen tube, possibly through a cGMP transduction pathway [10]. NO is a ubiquitous signalling molecule in animals [11], and growing evidence points to its widespread production and effects in plants [12,13]. Evidence has been found recently for enzymatic synthesis of NO in plants, involving both the constitutive enzyme *Arabidopsis thaliana* NO SYNTHASE1 (AtNOS1) [14] and/or inducible NO-synthase enzymes [15]. Nitrate reductase and xanthine oxidoreductase are also generally accepted to produce NO in plants [16]. Because of the largely diffusible and reactive properties of NO, its seems that the first reported role for NO in a tip-growing cell [10] is likely to be just one of several significant roles for NO in these cells.

Lipid signalling also stages a major entrance in tip-growing cells [17]. Of special notice, phosphatidic acid and phospholipases (e.g. phospholipase D [PLD]) have
Gene expression data in pollen relative to vegetative tissues (i.e. leaves, seedlings and siliques) are depicted using the MAPMAN tool \[24\] to display the genomic dataset derived from work by JD Becker (unpublished). Genes are symbolised by colour-encoded boxes (red, down-regulation; blue, upregulation; grey, absent call in pollen). Many genes in the classes 'protein modification' (protein kinases), 'receptor kinases', 'G-proteins' (GTPases and GTP-binding proteins) and 'calcium regulation' (calmodulins and calcium-dependent protein kinases) are enriched in pollen or even selectively expressed (see Table 1). These genes are probably involved in integrating signals from the female tissue with pollen-tube germination and growth processes, thus leading to a successful fertilisation. By contrast, genes that are involved in 'hormone metabolism' are in general downregulated in pollen, with a few exceptions mainly in auxin-induced proteins. Thus, the responses of pollen tubes to hormones might be either negligible or restricted to very specific responses.

The involvement of such a diversity of molecules in signalling in tip-growing cells is not surprising, and may well be necessary to provide specificity in many of the responses that these cells have to perform. The use of common fundamental molecules, although evolutionarily sensible, make sit less probable that a single molecule could convey all the information necessary for any given response. The diversity of signalling molecules within tip-growing cells also implies, however, that these cells employ sophisticated signalling mechanisms.

What the genes have to say

It is generally accepted that microsporogenesis involves the accumulation of significant levels of long-lived mRNA molecules within mature pollen; these mRNAs drive germination and early tube growth [5]. Thus, studies of the pollen transcriptome could presumably be used to define the genetic fingerprint needed for tip growth.

The importance of signalling processes in pollen relative to that in other tissues can be inferred from three recent studies of the pollen transcriptome of *Arabidopsis*. Two groups used Affymetrix 8K *Arabidopsis* GeneChips (covering about 8000 genes) to compare the transcriptome of highly purified, cell-sorted pollen grains with those of four vegetative tissues [21\*] or of non-sorted pollen grains with those of four developmental stages of the sporophyte [22\*]. In another approach, serial analysis of gene expression (SAGE) was used to profile the transcriptome of pollen under normal and chilling conditions [23\*]. Customised normalisation protocols were used to correct for the much lower number of genes called present in pollen (less than half than that in vegetative tissues). The GeneChip analysis revealed that as many as 25% of the genes that were identified as selectively expressed in pollen could be classified as being involved in signalling [21\*], whereas the SAGE analysis attributed 23% as members of this class [23\*]. A more recent analysis using the Affymetrix 24K *Arabidopsis* GeneChip revealed that 16% of the 6587 genes that were expressed in pollen were involved in signalling (as compared with 12% in
Table 1. [Please provide a title for this table.]

<table>
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<th>Functional class</th>
<th>Fold change</th>
<th>Selectively expressed in pollen?</th>
<th>Probe set</th>
<th>AGI ID</th>
<th>Gene annotation</th>
<th>Pollen expression value</th>
<th>Leaf expression value</th>
<th>Seedling expression value</th>
<th>Silique expression value</th>
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<td>Receptor kinase</td>
<td>249</td>
<td>Yes</td>
<td>246106_at</td>
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<td>P</td>
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<td>Receptor kinase</td>
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<td>Yes</td>
<td>257119_at</td>
<td>AT3G20190</td>
<td>LRR III</td>
<td>11290</td>
<td>P</td>
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<td>Calcium regulation</td>
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<td>Yes</td>
<td>263450_at</td>
<td>AT2G31500</td>
<td>CPK24</td>
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<td>P</td>
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<td>264284_at</td>
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<td>RLCKVII</td>
<td>12224</td>
<td>P</td>
<td>43</td>
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<td>Protein modification</td>
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<td>Yes</td>
<td>258600_at</td>
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<td>7876</td>
<td>P</td>
<td>28</td>
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<td>G-protein</td>
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<td>Yes</td>
<td>262742_at</td>
<td>AT1G28550</td>
<td>AtrABA1i</td>
<td>3154</td>
<td>P</td>
<td>33</td>
<td>A</td>
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<tr>
<td>G-protein</td>
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<td>No</td>
<td>257951_at</td>
<td>AT3G21700</td>
<td>G-protein related</td>
<td>8541</td>
<td>P</td>
<td>105</td>
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<td>MAPK</td>
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<td>266348_at</td>
<td>AT2G01450</td>
<td>MPK17</td>
<td>9958</td>
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<td>982</td>
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<td>6</td>
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<td>249239_at</td>
<td>AT5G41990</td>
<td>ZIK6</td>
<td>3363</td>
<td>P</td>
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<td>Phosphoinoside</td>
<td>74</td>
<td>Yes</td>
<td>259425_at</td>
<td>AT1G01460</td>
<td>4,5 PIP kinase-related</td>
<td>6800</td>
<td>P</td>
<td>63</td>
<td>A</td>
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<td>261015_at</td>
<td>AT1G26480</td>
<td>14-3-3 protein</td>
<td>1177</td>
<td>P</td>
<td>25</td>
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<td>229</td>
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<td>263144_at</td>
<td>AT1G54070</td>
<td>Similar to auxin-repressed protein</td>
<td>13841</td>
<td>P</td>
<td>40</td>
<td>A</td>
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<td>257121_at</td>
<td>AT3G20220</td>
<td>Putative auxin-induced protein</td>
<td>13022</td>
<td>P</td>
<td>39</td>
<td>A</td>
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<td>Hormone metabolism (ethylene)</td>
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<td>No</td>
<td>254434_at</td>
<td>AT4G20880</td>
<td>ERT2</td>
<td>3440</td>
<td>P</td>
<td>319</td>
<td>P</td>
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<td>AT3G20770</td>
<td>EIN3</td>
<td>3236</td>
<td>P</td>
<td>869</td>
<td>P</td>
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<td>AT4G16110</td>
<td>Response regulator ARR2</td>
<td>353</td>
<td>P</td>
<td>129</td>
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<td>Hormone metabolism (cytokinin)</td>
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<td>No</td>
<td>257492_at</td>
<td>AT1G49190</td>
<td>Response regulator ARR19</td>
<td>1453</td>
<td>P</td>
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<td>Hormone metabolism (ABA)</td>
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<td>Yes</td>
<td>254668_at</td>
<td>AT4G18350</td>
<td>Putative neoxanthin cleavage enzyme (NC1)(NCED1)</td>
<td>251</td>
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<td>A</td>
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<td>Yes</td>
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<td>AT5G53820</td>
<td>ABA-inducible protein-like</td>
<td>931</td>
<td>P</td>
<td>25</td>
<td>A</td>
</tr>
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</table>

Examples of genes that are most upregulated in pollen relative to vegetative tissues (i.e. leaves, seedlings and siliques) are listed for most of the functional classes shown in Figure 1. The second column contains the lower confidence bound of the fold change in gene expression (an average of the comparisons of pollen to the three vegetative tissues). The fourth and the fifth columns give the Affymetrix probe set and the TAIR locus (AGI ID) assigned to this probe set. In the following columns the expression value ([How is this expression value derived?] of the gene (weighted average of duplicates) and its detection call (present [P] or absent [A]) are given for the respective cell type or tissue.

Could you please clarify how the presence or absence of an detection call is determined. Why aren't all of the genes present since the functional classes shown in Figure 1. The second column contains the lower confidence bound of the fold change in gene expression (an average of the comparisons of pollen to the three vegetative tissues). The fourth and the fifth columns give the Affymetrix probe set and the TAIR locus (AGI ID) assigned to this probe set. In the following columns the expression value ([How is this expression value derived?] of the gene (weighted average of duplicates) and its detection call (present [P] or absent [A]) are given for the respective cell type or tissue.

[Please define all abbreviations that are not mentioned elsewhere in the review.]

217 222 vegetative tissues). When looking at genes whose transcripts are enriched in pollen, however, this number goes up to 26%, making signalling genes the most prominent class by far (JD Becker et al., unpublished; Table 1, Figure 2). These numbers have not yet been backed up by data from root hairs. Comparison of the pollen and root-hair transcriptomes could, however, allow a better comparison of the signalling pathways in...
these tip-growing cells and help to identify the fundamental signalling processes that underlie tip growth in plant cells.

Nevertheless, the obvious complexity deployed in the signalling pathways of tip-growing cells (Figure 2) makes it difficult to comprehend how these pathways are integrated and coordinated to produce a specific phenotype. Although probably a reductionist thought, it could well be that a great deal of this integration, especially in space and time, is based on smaller diffusible entities that affect multiple levels of the canonical signalling pathways by direct biochemical or physical actions. Hence, we now explore emerging evidence of the involvement of two such groups: free ions and radicals.

**Enter the ions!**

Certain ions have long been known to encode information, acting as second messengers in important signalling pathways. Recent genetic evidence showed that Ca\(^{2+}\) switches [29]. Recent genetic evidence showed that Ca\(^{2+}\)-ATPases are fundamental for pollen-tube growth [30]. Potassium ions also seem to play a role in this process [31] and chloride appears to be linked to the phosphatidylinositol signalling pathway, which is also involved in tip growth [32]. Moreover, life as we know it occurs in aqueous media. Since water spontaneously ionises, cells live in a ‘proton world’ and any change in pH will have an impact on a variety of molecules in different ways. Therefore, the most sophisticated information on Ca\(^{2+}\) and other ions is of limited value as long as the pH condition of the cell is not also determined [33].

A great deal of controversy still exists regarding a possible role for pH as a signal messenger. This controversy is mainly due to the extremely high conductivity of protons and the presumed consequent dissipation of any transiently formed gradient. It is also true, however, that this same property makes protons ideal candidates for encoding/decoding signals that operate with very short time frames, which are difficult to resolve with the techniques used at present. Conceivably, self-sustained proton waves could underlie fast calcium waves, which are known to propagate in a variety of cell types [34]. Pollen tubes have been successfully used as a model system for studies of ion dynamics in tip growth [26]. Among other ion fluxes, pollen tubes have been shown to contain a tip-focused pH gradient, with an acidic tip being associated with growth and a constitutive subapical alkaline region [35].

Both pollen tubes and root hairs have been shown to display ‘short-circuits’ of extracellular proton fluxes around their tips, a result hypothesised to reflect a polarised distribution of proton pumps ([36]; Figure 3). This model has recently been confirmed using molecular techniques for pollen tubes (AC Certal et al., unpublished), reinforcing the idea that proton dynamics may be an endogenous mechanism for determining and maintaining the polarity axis in these fast growing cells. The existence of this closed loop of proton fluxes could conceptually constitute a powerful sensor of the external milieu if intracellular mechanisms exist to decode small flux variations and to transform them into signalling information (adapted from [36]).

**Figure 3**

A model for proton gradients in pollen tubes and root hairs. The existence of ‘short-circuits’ of extracellular proton fluxes around the tips of pollen tubes and root hairs, a result hypothesised to reflect a polarised distribution of proton pumps, could conceptually constitute a powerful sensor of the external milieu if intracellular mechanisms exist to decode small flux variations and to transform them into signalling information. Acidification of the cell wall, a mechanism that may be conveyed through ethylene, also seems to initiate root hairs [37]. Interestingly, new evidence is starting to reveal that pH may also play an important role in the cascade of events that lead to oxidative burst in guard cells. In these cells, an alkalization seems to underlie both the abscisic acid- or methyl-jasmonate-induced formation of reactive oxygen species (ROS) and stomatal closure [38]. Alkalization is also involved upstream of the cytoskeleton in the signalling cascade that leads to the gravitropic response in roots [39]. In short, there is accumulating evidence for the existence of a proton signature. This signature would act as a signalling mechanism that underlies the development of tip-growing and possibly other kinds of plant cells, and there is no shortage of distinct physico-chemical properties that are associated with protons to test these assumptions [40].
Figure 4

Signalling pathways to the actin cytoskeleton. The major signalling pathways known to have a connection to the actin cytoskeleton involve the action of ROS, ROP GTPases and PIP2 [Please define PIP2.], but the majority of the effectors of these pathways and the interconnections between them remain unknown. The actin-binding proteins represented in this scheme are the best characterized in plants, but others (e.g. capping protein [CP] and AIP1) are starting to be characterized. The activity of these proteins must be tightly regulated for polarised growth to occur in an effective way. In plants, profilin and ADF are a focal point in the regulation of actin dynamics. They act synergistically to increase actin filament dynamics. ADF promotes the generation of new barbed-ends, and profilin delivers the monomers to the uncapped barbed-ends for polymerisation. It is important to highlight the fact that, in pollen tubes, only formins have been described as actin-filament nucleators; whereas, in root hairs, the Arp2/3 complex seems to be responsible for the same function. The only members of the poly-L-profilin-binding (PLP) proteins to be conserved in plants are formins. Hence, a partner other than the WASP family of proteins must regulate the Arp2/3 complex. CDPKs, calcium-dependent protein kinases.

And life met oxygen

2.5 billion years ago life met oxygen. A new aerobic environment directed the evolution of biochemical pathways towards the use of ROS. One of the ROS generation systems described in plants is dependent on NADPH-oxidase activity. Its activation requires the participation of the small cytosolic GTPase Rac2 (see Yang, this issue). The cytoplasmic amino-terminal region of this GTPase contains two putative EF-hand motifs, suggesting a that it is regulated by Ca²⁺ ions [41,42]. Ten putative genes encode GTPases in Arabidopsis and some of these genes function in abscisic acid (ABA) signalling [43]. A new exciting area in ROS signalling was opened up by the discovery of the root-hair defective2 (rhd2) Arabidopsis mutant, which has a defect in one catalytic subunit of the NADPH-oxidase. In root hairs, as in pollen tubes, the maintenance of Ca²⁺ transport across the membrane and the presence of a tip-focused [Ca²⁺] gradient are fundamental [For what? Please clarify.]. The rhd2-phenotype is characterised by short root hairs and stunted roots, and no [Ca²⁺] gradient could be observed in the root hairs of these mutants [44]. Because rhd2 mutants show defects in the steady tip-focused [Ca²⁺] gradient, it was hypothesised that ROS are required to stimulate Ca²⁺ influx during root-hair elongation. This was shown through an elegant experiment in which root-hair spheroplasts were released by laser microsurgery from the apices of young root hairs and rhd2 bulges. Using patch-clamp and indirect ROS imaging, Foreman et al. [45] were able to observe the activation of hyperpolarization-activated Ca²⁺ channels by ROS. Thus, ROS appear to act upstream of [Ca²⁺] in the signalling cascade, triggering a [Ca²⁺] rise and a putative subsequent modulation of actin dynamics that underlies polarised growth.

ROS have also been implicated in the curling response that occurs during the Rhizobium–legume symbiosis [46]. In Medicago truncatula, the nodulation (Nod)-factor response interfered with the elicitation of H₂O₂ efflux; instead of the oxidative burst found in plant defence responses, ROS production decreases in the presence of a symbiotic signal [46,47]. Finally, ROS have been described as having a mechanistic role in Fucus rhizoid development [48]. Hyper-osmotic treatment of Fucus rhizoids induces a [Ca²⁺] wave and peripheral ROS production. Inhibition of the NADPH-oxidase blocked this [Ca²⁺] wave. Further it was shown that increased cytosolic [Ca²⁺] was sufficient to induce ROS production in mitochondria. This growing body of evidence to describe the signalling links that occur after ROS activation of Ca²⁺ channels have recently been promoted to a general theory of polar growth, hormone transduction, stress signalling and hypothetically mechanotransduction [49]. Direct gene activation is, for
the moment, excluded from these generalisations because no transcription factor or promoter element that is redox sensitive is yet known in plants [41]. Nevertheless, transcriptional activation in eukaryotic cells does seem to be influenced by redox status. Indeed, redox status is known to regulate the expression of several plant genes, and there are several DNA-binding factors that may act as redox-response elements [41,50].

Indirect effects of ROS on the activity of a transcription factor activity have also been reported through the activation of mitogen-activated protein kinase (MAPK) [51].

**Dynamic skeletons: where all things come together?**

ROP GTPases (Yang, this issue), ionic gradients [52], lipids [17,53,54], and cyclic nucleotide levels [55], [10°]

all participate in signalling pathways that are known to affect the cytoskeleton. Actin-binding proteins are believed to integrate this information and to transduce it to alterations in the cytoskeleton [56]. For example, actin-depolymerising factor (ADF) and profilin act synergistically to affect actin dynamics: ADF generates more filament ends for polymerisation through its severing activity and by enhancing the dissociation of G-actin from slow-growing ends; profilins bind to G-actin and thus are incorporated in the free barbed end. Both ADF and profilin respond to ionic conditions.

The actin-severing activity of ADF is pH dependent, whereas profilin’s activity is Ca° dependent. Mechanisms for the regulation of ADF also include inhibition by both phosphorylation by a calmodulin-like domain protein kinase and membrane lipid binding. ADF is involved in the regulation of pollen-tube growth and uses the same signalling pathway as Rac/Rop GTPase [57].

Poly-l-profilin-binding (PLP) proteins (i.e. Wiskott–Aldrich syndrome protein [WASP], VASP [Please define this abbreviation] and forms) play a very important role in the signalling pathway cascades that affect the cytoskeleton in animal and yeast cells. These proteins are known to respond to Rho GTPases and to SH3- and WW-domain proteins, and to induce actin filament remodelling and nucleation. Formin overexpression in pollen tubes was recently shown to stimulate the production of supernumerary actin cables from the plasma membrane [58°]. Furthermore, overexpression of the formin AFH1 from Arabidopsis resulted in the formation of pollen tubes that had increased diameter, tip expansion and growth arrest, suggesting that forms are involved in the regulation of polarised growth. By contrast, low levels of AFH1 result in the production of pollen tubes with normal morphology and stimulate growth. Arabidopsis mutants Arp2 (wurm) and Arp3 (distorted) Arabidopsis mutants [59]. The same result was obtained by the mutation of the small subunit of the Arp2/3 complex (producing crooked mutants) [60]. Arp2/3 may also be involved in endocytosis as recently shown in yeast [61]. The best-characterized activators of the Arp2/3 complex are members of the WASP and contractin protein families. Because WASP and contractin proteins have still not been identified in plants, it remains to be established if this is also the case or if new effectors are to be found in plants.

One of the most-studied signalling pathways in root hairs is elicited by Nod factors, which are lipochito-oligosaccharides produced by the bacterium Rhizobium spp. Upon rhizobial infection, the responses of root hairs include swelling, membrane depolarisation, oscillations in calcium concentrations [62], cell-wall loosening, alterations in root-hair growth and the expression of host nodulation genes. The cytoskeleton is one of the targets of this system [63,64]. Recently, ACTIN-INTERACTING PROTEIN1 (AIP1) was suggested to be essential for the organisation of the actin cytoskeleton in plant cells [65]. As well as being a co-operator with the ADF protein, AIP1 has a capping activity, which enhances its activity. Cell expansion is compromised in plants in which AIP1 is silenced by RNA interference (RNAi) These plants showed thick actin bundles in all of the cell-types analysed, including root hairs (pollen was not studied). Hence, it seems that all of the proteins that coordinate the dynamics of the actin cytoskeleton must be tightly regulated in order for polarised and directional growth to take place. Conceivably, these proteins could be the major computational integrator of all of the diverse signalling machineries that contribute to tip growth (Figure 4).

**Conclusions**

New molecules continue to be described as important players in tip-growing cells. These include protein kinases, lipids, and molecules that have neurotransmitter activities in animals. Transcriptomics has shown that genes that are involved in signal transduction form the largest single class of genes that are more-represented in pollen tubes than in non-tip-growing cells, reflecting their capacity to decode all of the different contributing pathways. Many of these pathways may use common intracellular second messengers, and ions and ROS are emerging as two major common denominators in many of the processes involved in tip growth. Ultimately, the second messengers should influence the actin cytoskeleton through known interactions with actin-binding proteins.

In turn, changes in the dynamics properties of the cytoskeleton define the basic polarity events needed to shape and modify tip-growing cells.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
**of outstanding interest
8. The interaction between LePRK2 and Lat52 identifies the possibility of an autocrine signalling system. The LePRK signalling pathway is atypical, but not unique, when compared with other signalling systems in plants and animals. The binding of a ligand to the extracellular domain of LePRK triggers receptor dephosphorylation and LePRK complex dissociation. Subsequently, receptor auto-phosphorylation and complex assembly usually takes place. The authors also present some evidence to support a model for pollen–pistil interactions, in which different pistil ligands subsequently bind to pollen receptors along the style.
11. The authors use genetic and cellular approaches to demonstrate that the neurotransmitter GABA may have a role in pollen–stigma communication. pop2, which has abnormalities in pollen directional guidance, was shown to be a GABA-degrading transaminase. Its function may involve sensing external GABA from the female tissues.
13. In this work, the authors show that pollen tubes have a negative tropic response to external gradients of NO in vitro. The re-orientation response is downregulated by cGMP, and a new candidate molecule for in-vivo guidance of pollen tubes is hypothesised.


38. Suahida D, Raghavendra AS, Kwak JM, Vaasasseur A: Plant Ca⁺⁺ ATPase is not involved in polarity directly. Nevertheless, the data show that overall [Ca⁺⁺] homeostasis may be a critical aspect of tip growth.


42. The exploration of NADPH oxidase Arabidopsis mutants provides a genetic demonstration of the links between ABA and ROS signaling.


45. In this work, the authors show that the Arabidopsis rhd2 is defective in one catalytic subunit of a NADPH-oxidase that is responsible for ROS generation. They prove that ROS stimulate the activity of a plasma membrane hyperpolarization-activated Ca²⁺ channels. This shows the importance of ROS and NADPH oxidases in maintaining the tip-high [Ca⁺⁺] gradient during root-hair development.


The authors demonstrate that formins are involved in the process of actin nucleation in pollen, as well as in the regulation of polarised growth. They provide some evidence on the possible regulation of membrane structure by actin filaments. Formins are proposed as new components of the signalling crosstalk between pollen and the female tissues.


