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-amino butyric 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 no surprise 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
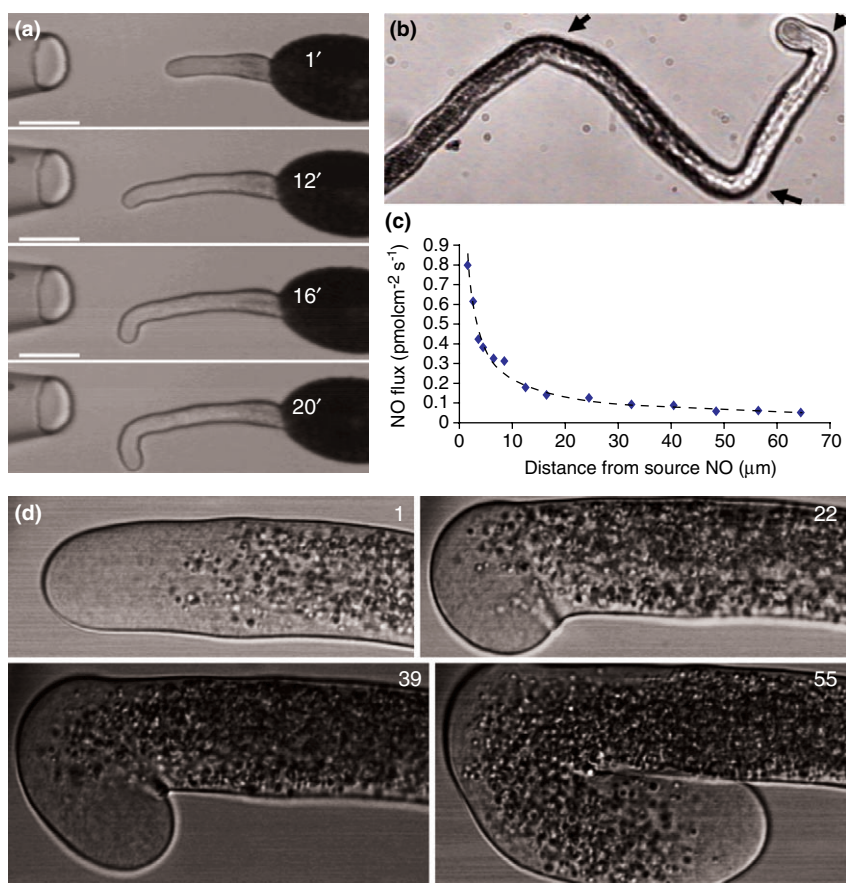
Figure 1

(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 = 30μm). (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 oxireductase 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

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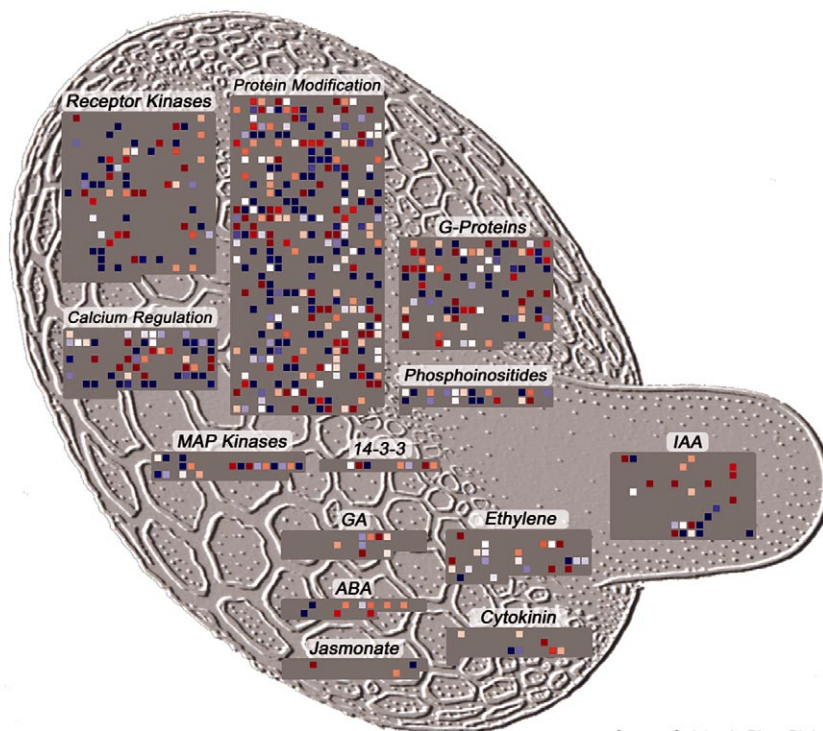
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Figure 2

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.

been shown to play fundamental roles in root-hair [18•] and pollen-tube development [19•,20•]. An elegant set of experiments that involved osmotic manipulations of tobacco pollen signals established several phosphotadylinositols as downstream effectors of the phosphatidic acid signal. This link builds up a scenario in which phospholipid signalling is likely to play a central role in many of the transduction pathways within tip-growing cells [20•].

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.]

Functional class	Fold change	Selectively expressed in pollen?	Probe set	AGI ID	Gene annotation	Pollen Expression value	Call	Leaf Expression value	Call	Seedling Expression value	Call	Silique Expression value	Call
Receptor kinase	249	Yes	246106_at	**AT5G28680**	*CRPK1L*	9135	P	22	A	27	A	30	A
Receptor kinase	143	Yes	257119_at	**AT3G20190**	LRR III	11290	P	42	A	43	A	62	A
Calcium regulation	195	Yes	263450_at	**AT2G31500**	*CPK24*	11605	P	37	A	34	A	59	A
Calcium regulation	126	Yes	250308_at	**AT5G12180**	*CPK17*	7078	P	31	A	33	A	40	A
Protein modification	176	Yes	264284_at	**AT1G61860**	*RLCKVII*	12224	P	43	A	40	A	61	A
Protein modification	175	Yes	258600_at	**AT3G02810**	*RLCKVII*	7676	P	28	A	27	A	28	A
G-protein	79	Yes	262742_at	**AT1G28550**	*AtRABA1i*	3154	P	33	A	33	A	30	A
G-protein	60	No	257951_at	**AT3G21700**	SGP1 monomeric G-protein related	8541	P	105	P	131	P	106	P
MAPK	8	No	266348_at	**AT2G01450**	*MPK17*	9958	P	982	P	1039	P	1051	P
MAPK	6	No	249239_at	**AT5G41990**	*ZIK6*	3363	P	325	P	604	P	620	P
Phospho-inositide	74	Yes	259425_at	**AT1G01460**	4,5 PIP kinase-related	6800	P	63	A	64	A	61	A
Phospho-inositide	19	Yes	251711_at	**AT3G56960**	*AtPIP5K1*	1446	P	52	A	45	A	64	P
14-3-3 protein	29	Yes	261015_at	**AT1G26480**	14-3-3 protein GF14 iota (*grf12*)	1177	P	25	A	28	A	23	A
Hormone metabolism (auxin)	229	Yes	263144_at	**AT1G54070**	Similar to auxin-repressed protein	13841	P	40	A	38	A	59	A
Hormone metabolism (auxin)	192	Yes	257121_at	**AT3G20220**	Putative auxin-induced protein	13022	P	39	A	38	A	63	A
Hormone metabolism (ethylene)	9	No	254434_at	**AT4G20880**	*ERT2*	3440	P	319	P	272	P	392	P
Hormone metabolism (ethylene)	3	No	257981_at	**AT3G20770**	*EIN3*	3236	P	869	P	743	P	703	P
Hormone metabolism (cytokinin)	31	No	245477_at	**AT4G16110**	Response regulator *ARR2*	353	P	129	P	193	P	143	P
Hormone metabolism (cytokinin)	2	No	257492_at	**AT1G49190**	Response regulator *ARR19*	1453	P	68	A	45	A	62	P
Hormone metabolism (ABA)	11	Yes	254668_at	**AT4G18350**	Putative neoxanthin cleavage enzyme (*NC1*)(*NCED1*)	251	P	50	A	58	A	48	A
Hormone metabolism (ABA)	4	Yes	248227_at	**AT5G53820**	ABA-inducible protein-like	931	P	25	A	25	A	18	A

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 they all have an expression value in all tissues?**]. The genes **AT5G28680** and **AT1G54070** belong to the 25 most-upregulated genes in pollen. *ARR2, AUXIN RESPONSE REGULATOR2; AtPIP5K1, XXX; AtRABA1i , XXX; CPK24, XXX;* CRPK1L, *xxx; EIN3, ETHYLENE INSENSITIVE3; ERT3, XXX;* GF14 iota, *XXXX; grf12, XXXX;* LRR III, LEUCINE-RICH RECEPTOR III; *NC1, NEOXANTHIN CLEAVAGE1; NCED1, XXX; RLCKVII, XXX; SGP1, XXX; ZIK6, XXX.* **[Please define all abbreviations that are not mentioned elsewhere in the review.]**

217

218 vegetative tissues). When looking at genes whose
219 transcripts are enriched in pollen, however, this number
220 goes up to 26%, making signalling genes the most
221 prominent class by far (JD Becker *et al.*, unpublished;

222

223 Table 1, Figure 2). These numbers have not yet been
224 backed up by data from root hairs. Comparison of the
225 pollen and root-hair transcriptomes could, however,
226 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 a 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 biophysical 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 [Please cite [24] in correct order.] [25,26]. Calcium ions have received particular attention [27,28], mostly because of the so-called 'Ca^{2+} signature' but probably also because of the existence of Ca^{2+} switches [29]. Recent genetic evidence showed that Ca^{2+}-ATPases are fundamental for pollen-tube growth [30$^\bullet$]. 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
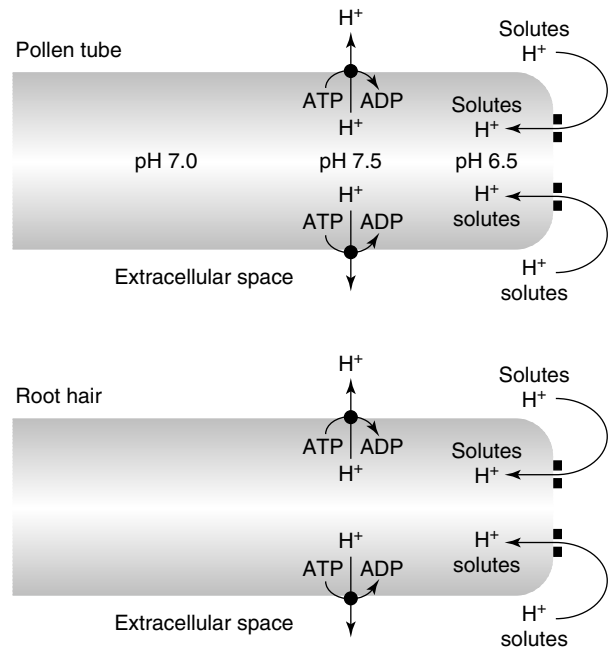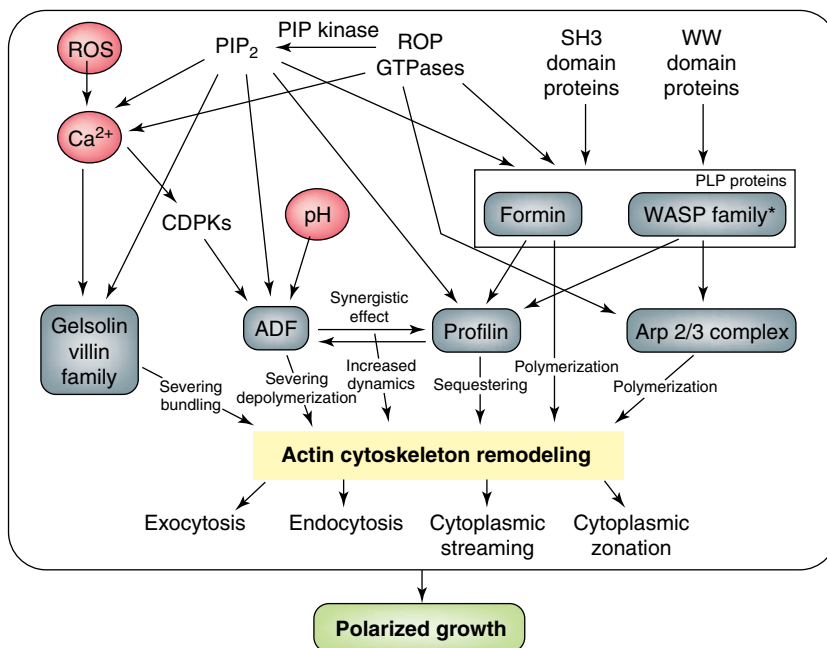

Figure 3

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 (adapted from [36]).

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. 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 alkalinization seems to underlie both the abscisic acid- or methyl-jasmonate-induced formation of reactive oxygen species (ROS) and stomatal closure [38]. Alkalinization 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].

322

323
324 **Figure 4**
325 Signalling pathways to the actin cytoskeleton. The major signalling pathways known to have a connection to the actin cytoskeleton involve the
326 action of ROS, ROP GTPases and PIP2 **[Please define PIP2.]**, but the majority of the effectors of these pathways and the interconnections
327 between them remain unknown. The actin-binding proteins represented in this scheme are the best characterized in plants, but others (e.g.
328 capping protein [CP] and AIP1) are starting to be characterized. The activity of these proteins must be tightly regulated for polarised growth to
329 occur in an effective way. In plants, profilin and ADF are a fulcral point in the regulation of actin dynamics. They act synergistically to increase actin
330 filament dynamics. ADF promotes the generation of new barbed-ends, and profilin delivers the monomers to the uncapped barbed-ends for
331 polymerisation. It is important to highlight the fact that, in pollen tubes, only formins have been described as actin-filament nucleators; whereas, in
332 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
333 conserved in plants are formins. Hence, a partner other than the WASP family of proteins must regulate the Arp2/3 complex. CDPKs, calcium-
334 dependent protein kinases.

335
336 ## And life met oxygen

337 2.5 billion years ago life met oxygen. A new aerobic
338 environment directed the evolution of biochemical
339 pathways towards the use of ROS. One of the ROS
340 generation systems described in plants is dependent on
341 NADPH-oxidase activity. Its activation requires the
342 participation of the small cytosolic GTPase Rac2 (see
343 Yang, this issue). The cytoplasmic amino-terminal
344 region of this GTPase contains two putative EF-hand
345 motifs, suggesting a that it is regulated by Ca^{2+}
346 ions [41,42]. Ten putative genes encode GTPases in
347 *Arabidopsis* and some of these genes function in abscisic
348 acid (ABA) signalling [43•]. A new exciting area in ROS
349 signalling was opened up by the discovery of the *root-
350 hair defective2* (*rhd2*) *Arabidopsis* mutant, which has a
351 defect in one catalytic subunit of the NADPH-oxidase.
352 In root hairs, as in pollen tubes, the maintenance of Ca^{2+}
353 transport across the membrane and the presence of a
354 tip-focused $[Ca^{2+}]$ gradient are fundamental **[For what?
355 Please clarify.]**. The *rhd2*-phenotype is characterised by
356 short root hairs and stunted roots, and no $[Ca^{2+}]$ gradient
357 could be observed in the root hairs of these mutants
358 [44]. Because *rhd2* mutants show defects in the steady
359 tip-focused $[Ca^{2+}]$ gradient, it was hypothesised that
360 ROS are required to stimulate Ca^{2+} influx during root-
361 hair elongation. This was shown through an elegant
362 experiment in which root-hair spheroplasts were

363 released by laser microsurgery from the apices of young
364 root hairs and *rdh2* bulges. Using patch-clamp and
365 indirect ROS imaging, Foreman *et al.* [45••] were able to
366 observe the activation of hyperpolarization-activated
367 Ca^{2+} channels by ROS. Thus, ROS appear to act
368 upstream of $[Ca^{2+}]$ in the signalling cascade, triggering a
369 $[Ca^{2+}]$ rise and a putative subsequent modulation of
370 actin dynamics that underlies polarised growth.

371 ROS have also been implicated in the curling response
372 that occurs during the *Rhizobium*–legume symbiosis
373 [46]. In *Medicago truncatula*, the nodulation (Nod)-factor
374 response interfered with the elicitation of H_2O_2 efflux;
375 instead of the oxidative burst found in plant defence
376 responses, ROS production decreases in the presence of
377 a symbiotic signal [46,47]. Finally, ROS have been
378 described as having a mechanistic role in *Fucus* rhizoid
379 development [48]. Hyper-osmotic treatment of *Fucus
380 rhizoids* induces a $[Ca^{2+}]$ wave and peripheral ROS
381 production. Inhibition of the NADPH-oxidase blocked
382 this $[Ca^{2+}]$ wave. Further it was shown that increased
383 cytosolic $[Ca^{2+}]$ was sufficient to induce ROS production
384 in mitochondria. This growing body of evidence to
385 describe the signalling links that occur after ROS
386 activation of Ca^{2+} channels have recently been
387 promoted to a general theory of polar growth, hormone
388 transduction, stress signalling and hypothetically
389 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 candidates for promoter elements that are 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^{2+} 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 formins) 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 formins 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. Arp2/3 is involved in the polymerisation of branched networks of actin filaments in animal cells and yeast. In root hairs, Arp2/3 has a crucial role because these cells become sinuous in *Arp2* (*wurm*) and *Arp3* (*distorted1*) *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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Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest

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