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Minireview

Osmotic stress activates distinct lipid and MAPK signalling pathways in plants

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Abstract Plants are continuously exposed to all kinds of water stress such as drought and salinity. In order to survive and adapt, they have developed survival strategies that have been well studied, but little is known about the early mechanisms by which the osmotic stress is perceived and transduced into these responses. During the last few years, however, a variety of reports suggest that specific lipid and MAPK pathways are involved. This review briefly summarises them and presents a model showing that osmotic stress is transmitted by multiple signalling pathways.

Key words: Osmotic stress; Plant signal transduction; Phospholipase; Phosphatidic acid; Phosphoinositide; Diacylglycerol pyrophosphate

1. Introduction

Osmotic stress, in the form of drought, freezing temperatures or salt-contaminated soils, is a major limiting factor for plant growth and the colonisation of land. To survive these conditions, plants respond and adapt using a range of biochemical and developmental changes including the synthesis of stress hormones like abscisic acid (ABA) and the synthesis of proteins that prevent denaturation and oxidative damage [1,2]. Osmotic pressure and turgor are quickly regulated by modifying ionic fluxes and, over a longer period, via the synthesis of osmolytes such as sugar and amino acid derivatives [1,3].

In contrast to what is known on the longer timescale, relatively little is known about the primary signalling events. Nonetheless, one of the early responses to both salinity and drought is a rapid increase in cytosolic free Ca^{2+} concentration [4–6]. Although Ca^{2+} signals can be cell specific and differ in kinetics and magnitude dependent on the nature of the stress, producing a so-called 'Ca^{2+} signature', it should be clear that Ca^{2+} is not the only signalling event that determines the 'signature' [7–9].

During the last few years, extensive evidence has shown that plant cells contain a variety of phospholipid-based signalling pathways [10–12]. These include phospholipase C (PLC), D (PLD), A_2 (PLA_2) and novel pathways involving the formation of diacylglycerol pyrophosphate (DGPP) and phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$). What is striking is that they can all be activated by osmotic stress but that the stress level determines which combination is activated. In a similar way, protein kinases and especially mitogen-activated protein kinase (MAPK) pathways are also invoked. Therefore in this review we summarise recent data and fit it into a model showing that osmotic stress is signalled via an array of pathways.

2. Osmotic stress-induced lipid signalling

2.1. Phospholipase C and PI(4,5)P_2 formation

PLC signalling represents the paradigm for phospholipid-based signal transduction. Upon activation, PLC hydrolyses the minor lipid $PI(4,5)P_2$ into two second messengers: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases Ca^{2+} from intracellular stores whereas DAG activates certain members of the PKC super-family. In combination, these lipid-derived second messengers trigger a host of biochemical reactions in probably every mammalian cell.

In plants, all components of the PLC signalling cascade have been shown to be present except PKC (reviewed in [10,12]). Since DAG formed on signalling is rapidly phosphorylated to phosphatidic acid (PA), this led to the speculation that not DAG but PA is the lipid signal produced [10]. In support, the evidence that PA is rapidly formed as a biologically active lipid under a variety of stress conditions, has now become convincing [12]. This suggests that DAG kinase (DGK) should receive more attention as an important component of the PLC signalling cascade.

That osmotic stress activates the PLC pathway has long been presumed but only recently established. Earlier work showed that the level of polyphosphoinositides (PPIs) rapidly changed upon stimulation [13–16], but as we now know, these lipid molecules do much more than act as precursors for IP_3 (see below). Nonetheless, it has recently been shown for var-

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Abbreviations: DAG, diacylglycerol; DGPP, diacylglycerol pyrophosphate; IP_3, inositol 1,4,5-trisphosphate; L-PA, lyso-phosphatidic acid; MAPK, mitogen-activated protein kinase; PA, phosphatidic acid; PI3K, phosphoinositide 3-kinase; PIP, phosphatidylinositol phosphate; PIP_2, phosphatidylinositol bisphosphate; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D

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ious plant systems, that IP_3 levels rapidly increase upon hyperosmotic stress [16–19] and that the DAG formed is concomitantly converted to PA [20]. Not surprisingly then, putative inhibitors of PLC, i.e. neomycin and U73122, have been shown to affect the osmotic stress-induced calcium signal, to inhibit the increase in $InsP_3$ and to block the expression of some dehydration-induced genes [4,18]. It is tempting to believe that the IP_3 produced during stimulation is responsible for the rise in intracellular Ca^{2+} [4–6], especially since osmotic stress has been reported to enhance the competence of vacuoles to respond to IP_3 [21], but that remains to be established.

Another typical response to osmotic stress seems to be the accumulation of phosphatidylinositol bisphosphate (PIP_2) [15,16,19]. As mentioned above, not all PIP_2 made in response to stimulation is meant to be hydrolyzed by PLC. In mammalian cells, it also functions as a localised membrane-docking site that recruits and/or activates proteins into functional complexes involved in processes such as signal transduction, cytoskeletal rearrangements, and membrane trafficking. Proteins bind PIP_2 via pleckstrin homology (PH) domains, CalB/C2 domains and via lysine–arginine-rich (KR) regions [22–24]. Presumably, osmotic stress-induced PIP_2 formation in plants has a similar function. A few plant PH domains have already been described [25–28] and importantly, by fusing a PH domain with green fluorescent protein (GFP) [24,29], they can be used to localise PIP_2 in plant cells. This promises to be a powerful new technique for monitoring lipid signalling in living cells, for different GFP chimeras can be made using other lipid binding domains, e.g. domains specific for $PI(3,5)P_2$ and PA [30,31].

Several genes encoding components of the PLC system are strongly expressed in response to drought and/or salt stress. These include PLC, phosphatidylinositol phosphate (PIP) kinase and DGK [32–34]. This may represent a 'priming' response, sensitising the cell to further osmotic stress, but as pointed out by Hirt [35], increased expression has seldom been correlated with increased enzyme activity. Moreover, osmotic stress induces the expression of numerous genes so it may be part of a general response [36].

Under drying conditions, the plant must prevent water loss via transpiration and must therefore control stomatal aperture. There is evidence that PLC is involved in the closing mechanism. Micro-injecting caged IP_3 into guard cells and releasing it by photolysis, elevates Ca^{2+} levels and activates stomatal closure via the reversible inactivation of K^+-channels [37–40]. ABA, the phytohormone produced during water stress, also induces stomatal closure and this can be blocked by a PLC-inhibitor [41]. ABA is also reported to increase the levels of IP_3 and Ca^{2+} and to trigger small changes in the ^{32}P-labelling of PPI and PA [42–44]. Together these data suggest that ABA activates PLC. Nonetheless, such results could not always be confirmed [12,18,45]. This could be due to differences in the biological systems, but a very different explanation has recently been presented. Brearley's lab have questioned the role of IP_3 in raising Ca^{2+} concentrations by showing that IP_6 does the trick [45]. They also showed that the effects of micro-injected IP_3 are probably due to conversion to IP_6. One can also question whether ABA treatment results in IP_3 increases. First, the response was very small, increasing only 20–40%. Second, the radio-ligand IP_3 assay kit is not very specific and may well measure molecules other than the $I(1,4,5)P_3$ isomer that releases calcium. For example, $I(1,3,4,)P_3$ is present in 9-fold higher concentrations and also increased on ABA treatment [43].

2.2. Phospholipase D

It is clear that PA is not only generated through the PLC/DGK pathway but also via the activation of PLD [12]. One can distinguish between the two by using a 'differential $^{32}P_i$-labelling protocol' [12,46] and by PLD's unique ability to transphosphatidylate primary alcohols such as ethanol or 1-butanol in living cells [12,46,47]. The subsequent formation of phosphatidylethanol or phosphatidylbutanol is a relative but specific measure of PLD activity. In this way osmotic stress was shown to activate PLD in suspensions of *Chlamydomonas*, tomato and alfalfa cells [20], and dehydration was shown to activate PLD in the resurrection plant *Craterostigma plantagineum* and *Arabidopsis* [20,48,49]. Extractable PLD activity also increased during drought stress in cow pea and by using drought-resistant and drought-sensitive cultivars, the increase was found to be much higher in the drought-sensitive cultivar [50]. Since the latter results reflected increases in PLD mRNA levels, PLD activity could be regulated at the transcriptional level. Part of this response could have been via ABA, for this hormone is synthesised during water stress and it induces PLD expression in a similar manner [48,51,52]. However, whether these results represent PLD operating as a signalling enzyme or an enzyme involved in the adaptation response, e.g. remodelling the membrane, is still unclear. In this respect the timing of the different events is important. For example, salt and water stress activate PLD within minutes, which is likely to reflect signalling rather than a change in PLD gene expression, that occurs only after hours of osmotic stress. Similarly, ABA has been shown to activate PLD within minutes in leaf guard cells and in barley aleurone [53–55]. Moreover, extracellular addition of PA evoked ABA responses, supporting its role as a signal. In a complementary manner, when primary alcohols competed with water for transphosphatidylation and specifically inhibited the production of PA by PLD, ABA-induced responses were reduced. Those PA-related effects not only suggest that PA is needed for the responses, but raise the possibility that other alcohol-inhibitory effects could also be due to reductions in PLD-generated PA. The importance of PA as a second messenger in plants, particularly when subjected to different abiotic and biotic stresses, has been highlighted by a review on the subject [12].

Recently, the lipid sphingosine-1-phosphate (S1P) was implicated in drought signalling [9]. Leaf concentrations increased 1.3–2.4-fold after 11 days of water abstinence and when epidermal peels were treated with S1P, Ca^{2+} oscillations were triggered and stomatal closure induced. The report is reminiscent of that from Gilroy's lab on PA [55], especially since these lipids have the same head group and only differ in their lipid moieties. However, in contrast to S1P, PA did not increase the cytosolic Ca^{2+} concentration [55], even though it has been shown to do so in numerous animal cell systems (see [10]). Considering that IP_3 and cyclic ADP ribose have already been invoked in regulating Ca^{2+} levels, these new results add another level of complexity to stomatal guard cell signalling but at least underscore the important contribution that lipid signalling makes.

2.3. PA kinase and DGPP

If PA functions as a signalling molecule, it is important to

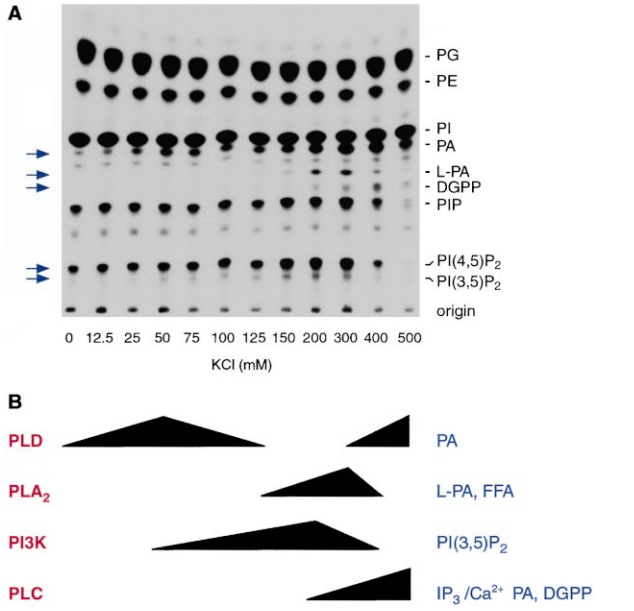

Fig. 1. Osmotic stress activates distinct lipid signalling pathways. A: $^{32}P_i$-labelled *Chlamydomonas* cells were treated for 5 min with different concentrations of KCl after which the lipids were extracted, separated by TLC and autoradiographed (for details, see [66]). B: Schematic representation of the different phospholipid signalling pathways that are activated. The interpretation is based on the autoradiograph in 'A' but also on other analyses.

down-regulate its level after stimulation. In this respect, the discovery of a PAK that converts PA into the novel phospholipid DGPP during signalling, was an important discovery [56]. Initially, the enzyme was an in vitro activity described by Wissing and co-workers who extracted it from many plants and tissues [57]. Currently, DGPP formation is seen as a common response to a variety of plant signals, including osmotic stress (Fig. 1) [10,12,20,46,56,58–61]. These include hyperosmotically stressed alga and suspension-cultured plant cells but also dehydrated intact resurrection plants [15,20].

It is unlikely that DGPP's only function is to attenuate PA levels, for in animal cells it has been shown to induce several inflammatory responses in macrophages, activating a MAPK pathway and a PLA_2 [62]. Of course, if DGPP is itself a signal, then it also needs to be down-regulated. In yeast, a DGPP phosphatase is responsible for that [63,64] and recently, two *Arabidopsis* homologues were found [65]. Interestingly, their expression levels were strongly up-regulated by various stresses known to activate DGPP formation, including mastoparan, radiation and a pathogenic elicitor. Unfortunately, water stress was not tested. The enzyme dephosphorylates both PA and DGPP, although one of the two isozymes clearly preferred DGPP [63,65]. Such dual specificity enzymes allow the cell to metabolise DGPP to DAG without accumulating 'active' PA.

2.4. Phospholipase A_2

PLA_2 hydrolyses phospholipids at the *sn*-2 position, generating lyso-phospholipids and free fatty acids. Osmotic stress rapidly stimulates activity. For example, in the alga *Chlamydomonas*, high concentrations of NaCl and other osmolytes were very effective in inducing the synthesis of lyso-phosphatidic acid (L-PA) in a time- and dose-dependent way [66]. A transient peak of L-PA was formed as PA was generated by activation of both the PLC/DGK and PLD pathways. Since it could be blocked by PLA_2 inhibitors, a PLA_2 was clearly implicated. Earlier, an L-PA response was observed in the halo-tolerant alga *Dunaliella salina* when the NaCl concentration in the growth medium was raised from 1.71 to 3.42 M [13]. In animal systems, L-PA is an important signalling molecule [67] but in plants this must still be shown. Nonetheless, lyso-phospholipids, and especially lyso-phosphatidylcholine, have been shown to affect protein kinase activity and H^+-ATPase pumping in plants, having dramatic consequences for the intracellular pH [10]. A similar role has been proposed for the free fatty acids produced by PLA_2. In addition, if C18:2 and C18:3 fatty acids are released, they can be metabolised via the octadecanoid pathway to compounds like jasmonic acid [10,68]. Indeed, osmotic stress activates a 6–10-fold increase in the concentration of jasmonic acid in *Chlamydomonas* (Dr. W. Dathe, personal communication).

2.5. Phosphoinositide 3-kinase

The final lipid signalling pathway that will be discussed is the so-called PI3K pathway, although this reflects more the increase in D3-phosphorylated inositol lipids than the activation of a PI3K. More specifically, we recently discovered that plant cells rapidly convert phosphatidylinositol 3-phosphate (PI3P) to the novel PIP_2 isomer $PI(3,5)P_2$ when subjected to water stress [11]. This was found in cell cultures of *Chlamydomonas*, tomato, and alfalfa and also in pea leaves and *Arabidopsis* plants, although it was not observed in cell suspensions of the latter [11,15,19]. The increase in $PI(3,5)P_2$ can be dramatic, fast and transient. In yeast, where $PI(3,5)P_2$ signalling was discovered [69], it is made in response to severe osmotic stress e.g. 1 M NaCl or 1.5 M sorbitol. In comparison, plants respond to much lower concentrations, ranging from 50 to 300 mM NaCl (see for example Fig. 1) or for other compounds in the same osmolar range [11].

The enzyme that makes $PI(3,5)P_2$ in *Saccharomyces cerevisiae* is called Fab1p [70–72]. Homologues are present in plants and our lab is currently cloning and characterising two cDNAs from tomato (Meijer and Munnik, unpublished). Fab1p contains a FYVE domain that specifically binds to PtdIns3P and thereby locates it to endocytic and vacuolar compartments. Yeast Fab1 mutants have abnormally large vacuoles that cannot divide or turnover. Therefore $PI(3,5)P_2$ seems to be crucial for vacuolar scissions (discussed in [11]). When plant and yeast cells are dehydrated, the water reservoir in the vacuole can compensate the water deficit in the cytosol, but its volume is consequently reduced while its surface area is unchanged. Fragmenting the vacuole easily solves the problem, and the more vesicles formed, the smaller the volume, while maintaining the membrane area. The formation of $PI(3,5)P_2$ could therefore help compensate a water deficit by promoting vacuole vesiculation. Indeed, osmo-stressed *Nicotiana tabacum* and *Schizosaccharomyces pombe* fragment their vacuoles [1,73], although the two processes have yet to be causally related.

Fab1 mutants have another interesting phenotype, their vacuolar pH is neutral rather than acidic. This suggests that $PI(3,5)P_2$ regulates vacuolar H^+-ATPase activity, perhaps by directly activating the pump. Yeast and plant cells can compensate a water deficit by accumulating osmotically active ions from the apoplast and, during a period of adaptation,

they synthesise and accumulate organic osmolytes [1]. The driving force for accumulation in the vacuole is the proton gradient over the tonoplast, generated by H^+-ATPases and H^+-PPases. This accounts for the electrogenic uptake of anions and, via H^+-antiporters, for the uptake of cations and sugars. Thus $PI(3,5)P_2$ synthesis during osmo-stress could stimulate the proton gradient and the accumulation of osmolytes, representing a signalling mechanism intrinsic to vacuolated cells, that helps the protoplast maintain turgor pressure and growth under desiccating conditions.

3. Osmotic stress-activated protein kinases

The involvement of MAPK pathways in osmotic stress signalling has been presumed for years, because their mRNA levels are up-regulated upon salt and drought stress [74], yet proof that they are activated was only produced 2 years ago. In alfalfa cells, osmotic stress led to the rapid activation of two protein kinases that phosphorylated myelin basic protein in an in-gel assay [75]. One kinase was activated at moderate concentrations, responding in a dose-dependent way, peaking at 500 mM NaCl, whereas the other was only activated at very high concentration, starting at 500 mM NaCl (see also Fig. 2). Both kinases were activated within minutes. Immunological studies identified the first as SIMK, the stress-induced MAPK from alfalfa. The second is still unknown but is likely to be a homologue of the *Arabidopsis* serine/threonine kinase 1 (ASK1), a member of the sucrose non-fermenting 1 (SNF1) kinase family that was found in tobacco [76]. There, the salicylic acid-induced protein kinase (SIPK) was identified as the osmotic stress-activated MAPK [76]. Besides salt, the enzymes are also activated by osmolytes such as sorbitol, suggesting that activation represents a general signalling response. Meanwhile various stress-activated protein kinases have been characterised in plant systems [75–80] and recently the first MAPK kinase that activates SIMK was identified [81].

In *Arabidopsis*, the salt-overlay-sensitive (*sos*) mutants are involved in salt-signalling [2]. SOS2 is a protein kinase that physically interacts with and is activated by the calcium binding protein SOS3 [77]. Thus an osmo-stress-induced increase in cytosolic Ca^{2+} [4] is translated into higher SOS2 kinase activity. This in turn is transduced into up-regulation of SOS1, a putative Na^+/H^+ antiporter. However, it must be emphasised that these components are only involved in the pathway leading to Na^+ tolerance, for the mutants are not sensitive to other forms of osmotic stress [2].

4. Perspectives: cross-talk and integration of signalling pathways

In considering the signalling pathways activated by hyperosmotic stress, we have so far ignored the question of how the signal is instigated, simply because only one potential osmotic stress receptor has so far been identified [82]. It is a histidine kinase in *Arabidopsis* referred to as *AtHK1* that can rescue osmo-sensor *SLN1* knockouts in yeast. Its expression in *Arabidopsis* is up-regulated by osmo-stress. We predict that it is the first of many that will be discovered, because yeast has at least three, SLN1 and SHO1 that operate between 100 and 600 mM NaCl [83], and feed into the HOG pathway, a MAPK cascade, while a third receptor must be assumed to explain the activation of $PI(3,5)P_2$ synthesis at NaCl concentrations above 0.9 M [69]. Since we know from animal cells that individual receptors can make use of several effector enzymes, one can expect osmo-signalling to be complex, in keeping with the growing complexity reported in this review.

Interestingly there is order in the complexity. This is best illustrated with data for *Chlamydomonas*, where cells were prelabelled with $^{32}P_i$ and then treated for just 5 min with concentrations of KCl ranging up to 500 mM. When the lipids were extracted, separated and an autoradiogram made from the TLC, the result was as shown in Fig. 1A. The lipid patterns illustrate that specific signalling pathways are activated over discrete ranges of KCl concentration (schematically represented in Fig. 1B). The clearest examples are for L-PA and $PI(3,5)P_2$ formation but, at lower concentrations for example, PLD is activated. It accounts for the initial peak in PA formation centred around 50 mM, but was measured more definitively on another TLC system via PLD's transphosphatidylation activity (data not shown). At very high concentrations, PLD and PLC/DGK were activated. Some of this signalling may reflect ionic stress as well as osmostress, but the picture clearly illustrates that a single stress factor is translated into different signals in a dose-dependent manner. This is not just true for lipid signals but also for protein kinase signalling, illustrated here for alfalfa cells treated with a range of NaCl concentrations (Fig. 2, adapted from [75]). These data mean that each stress level produces its own unique combination of signals (signal signature) that activates the appropriate graded response. The fact that different salt ranges activate different pathways supports the concept that stress is detected by different receptors responding over those limited ranges, in a manner similar to the osmosensors in yeast.

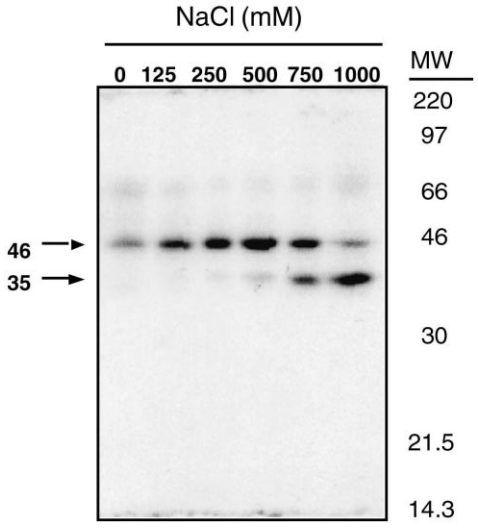

Fig. 2. In-gel protein kinase assay. Osmotic stress activates distinct protein kinase pathways. In-gel assay of protein kinase activity in osmotically stressed alfalfa cells. Suspension-cultured cells were treated with different concentrations of NaCl for 15 min, after which proteins were extracted and separated on a SDS–PAGE gel containing myelin basic protein. After protein renaturation, a kinase reaction was carried out in the gel using [γ-^{32}P]ATP, to reveal the presence of two active protein kinases: one at intermediate NaCl concentrations, identified as the MAPK 'SIMK', and one at high concentrations whose identity is still unknown but is likely to be a SNF1 homologue [75,76]. Reprinted with permission from [75].

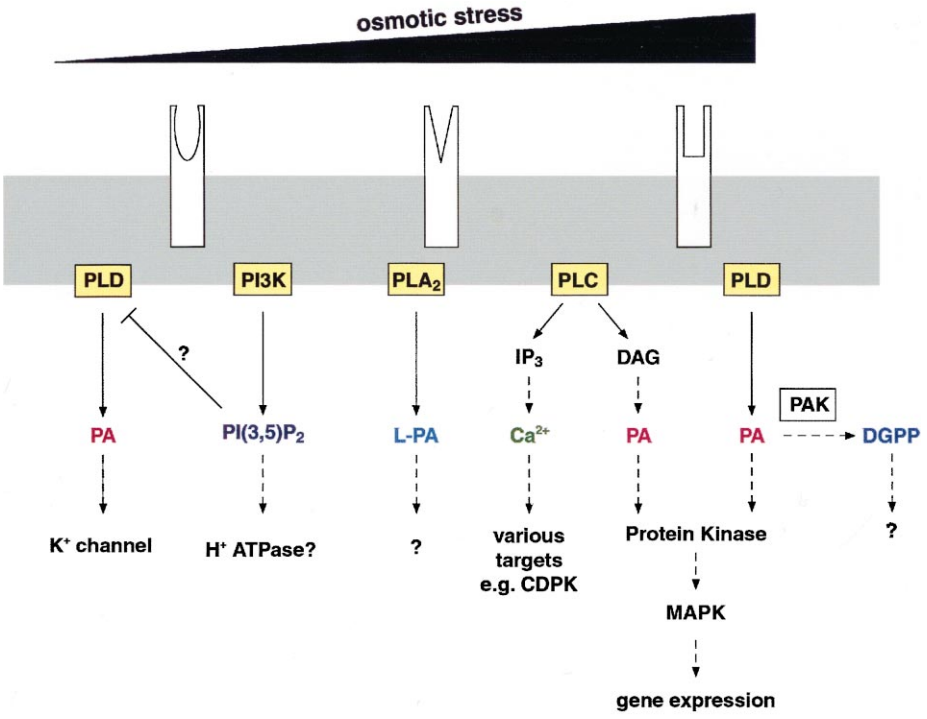

Fig. 3. Model showing osmotic stress activates distinct lipid and MAPK signalling pathways. The model should be viewed from top to bottom. It represents stress as a graded phenomenon that activates different receptors, dependent on the stress level. They in their turn activate different lipid signalling pathways. The second messengers produced are listed together with some potential targets. The targets are speculative but based on literature reports discussed in the text. Many details have been omitted for simplicity. Abbreviations: CDPK, calmodulin-like domain protein kinase; DAG, diacylglycerol; DGPP, diacylglycerol pyrophosphate; IP_3, inositol 1,4,5-trisphosphate; L-PA, lyso-PA; MAPK, mitogen-activated protein kinase; PA, phosphatidic acid; PI3K, phosphoinositide 3-kinase; $PI(3,5)P_2$, phosphatidylinositol 3,5-bisphosphate; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D.

These ideas are summarised in the model presented in Fig. 3. Each signalling pathway is presented as an independent route even though we have emphasised that each receptor can activate several effector enzymes. One must also appreciate that the cross-talk between pathways, absent from our scheme, will eventually prove to be extensive, although now hard to justify. The best example has recently been published and shows that PA, generated by PLD or PLC/DGK pathways, can activate a specific MAPK pathway [84]. While this is an exciting report that underlines the significance of PA as a plant signal, we do not know whether the response represents a downstream step in the same pathway or cross-talk between different pathways. Certainly a number of PA targets have recently been identified (reviewed in [12]). Other potential examples of cross-talk are seen in the optima exhibited by the signalling responses in Fig. 1. For example, PLD signalling is down-regulated at KCl concentrations above 50 mM KCl, therefore the PI3K pathway, that is activated above 50 mM, could be negatively regulating it. However, cross-talk is not yet our prime concern. The first challenge is to identify all the signalling pathways and assess their relevance to short- and long-term responses. Only then can we build up a picture of their interactions to produce an integrated model of osmo-stress detection and signalling.

A new technique that will help elucidate osmo-stress signalling is based on the expression (or injection) of GFP chimeras in plant cells. For example, GFP–PH and GFP–FYVE constructs have already been used to locate $PI(4,5)P_2$ and PI3P in plant cells [29,85]. They can also be used to monitor the changes in concentration associated with signalling, as originally illustrated by Stauffer et al. [86] for rat basophilic leukaemia cells. Thus prior to signalling, $PI(4,5)P_2$ in the plasma membrane was labelled by GFP–PH but when hydrolysed by PLC, the label dissociated into the cytosol, producing a much lower membrane: cytosol fluorescence ratio as a quantitative measure of the response. The technique has great potential because GFP chimeras can be produced for other lipid signals such as $PI(3,5)P_2$ and PA [30,31]. Since GFP exists in different spectral variants, different forms of lipid signalling can be measured in real time in the same cell. Such methods should not only help identify and locate the osmo-stress signalling pathways in plant cells but also accurately integrate them on a timescale.

We emphasise that the data in Fig. 1 represent the initial signals formed in response to osmo-stress. All the changes in lipid metabolism took place within 5 min of treatment and should be distinguished from the adaptation responses, that involve increased expression of signalling genes. Assuming increased expression results in higher enzyme activity, it could be involved in a second round of signalling events, for example to fragment the vacuole further ($PI(3,5)P_2$) or to further enhance membrane remodelling (PLD). Unfortunately, we do not yet know whether expression of the initial signalling enzymes is enhanced or whether different isozymes with different functions are being expressed. Nor do we know the general significance of different signalling isozymes in plants; what does it mean that a PLD belongs to class α, β, γ or δ [49,87,88]? Consequently, an immediate goal of present research is to assess enzyme function and location via gene knockouts and isozyme specific antibodies.

One of the consequences of adapting to osmo-stress is the modification of stress signalling. When *Chlamydomonas* was grown in 100 mM NaCl and then stressed by additional salt (same additions as in Fig. 1), the same signalling pathways were still activated in the same response pattern [89]. This is interesting because the osmo-sensors and their signalling pathways are now responding to much higher salt concentrations. Since this seems unlikely, if they detect salt concentrations, we can conclude that they detect a consequence of increased salt, such as loss of turgor. This suggests that the osmo-sensors are stretch receptors that respond to changes in membrane pressure and so remain operative irrespective of whether the cells are osmo-adapted or not. However, the change in turgor when cells are shifted from 100 to 200 mM salt should be less than when shifted from 1 to 100 mM salt, as in Fig. 1. Accordingly, although the pattern of signalling in adapted cells remained the same, all optima were shifted to higher concentrations and in general less signal was formed.

This review is the first devoted solely to osmo-stress-induced signalling. It therefore reviews 'what promises to be' rather than well established facts. Still, there is no denying that the most excitement in any research field is generated from getting the new plane into the air. In other words, now is the time to become involved.

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