Plant natriuretic peptides - emerging roles in fluid and salt balance

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Abstract

Immunological and physiological evidence has suggested the presence of a biologically active natriuretic peptide hormone like system in plants. Vertebrate atrial natriuretic peptides (ANPs) and immunoaffinity purified plant natriuretic peptides (PNPs) can elicit a number of plant responses that are essential in homeostasis and growth. These responses include tissue specific ion movements and the regulation of stomatal guard cell movements and thus plant gas exchange. Access to the complete genome of
the model plant Arabidopsis thaliana has since allowed us to identify Arabidopsis PNPs and elucidate the evolutionary relationships with animal natriuretic peptides. PNPs are small proteins related to the wall loosening expansins and are thus heterologues of animal natriuretic peptides and similarities are the result of convergent evolution. Plant and animal NPs share some sequence and structural similarity, both are systemically mobile, signal via the second messenger cGMP and play a key role in ion and fluid homeostasis. Currently experiments are underway to test biotechnological applications of PNPs with a view to confer increased stress tolerance to plants. Finally, it is conceivable that PNPs can also exert effects on humans and thus be of future therapeutic use.

Introduction

The first direct evidence of an endocrine link between the heart and the kidney was provided when it was observed that intravenous infusion of heart atrial homogenates into rats induced a marked reduction in blood pressure that was accompanied by a potent renal natriuresis and diuresis [1]. Subsequently, peptides of varying sizes were purified from atrial tissue that possessed natriuretic, diuretic and vasorelaxant activity [2]. Structurally related natriuretic peptides (NPs) have since been isolated and identified in various organs in the body and these peptides are collectively known as the NP family. In mammals the three main NPs are: atrial NP (ANP), synthesised mainly in atrial myocytes [3]; the B-type NPs, originally identified in the brain [4]; and C-type NP, which was first identified in the central nervous system of mammals [5]. While ANP and BNP are synthesized as prepropeptides and CNP as a propeptide, significant, but by no means all, biological activity of the three peptides resides in mature C-terminal peptide fragments with a conserved 17 amino acid disulfide ring structure [6, 7]. Two main classes of NP receptors (NPR) have been identified and the main physiological effects of NPs are mediated through guanylate cyclase-linked NPRs (NPR-GC). The NPR-GCs are transmembrane proteins that consist of an extracellular ligand binding domain, a single transmembrane domain, and a guanylate (or guanylyl) cyclase intracellular domain. When activated by NPs, GC-linked NPRs generate the intracellular second messenger, cGMP, from hydrolysis of guanosine triphosphate. Increases in cGMP levels in cells have been shown to cause many of the observed physiological effects of NPs [8].

There are three known classes of cGMP binding proteins; cGMP-dependent protein kinases (PKG), cGMP-binding phosphodiesterases (PDEs) and cyclic nucleotide-gated ion channels in plants (CNGC). In animals the most established cGMP signaling effects occur through activation of PKGs [9]. The
PDEs metabolise cGMP to an inactive monophosphate form, thereby acting to reduce intracellular cGMP concentrations.

The vasorelaxant effects of ANP have been shown to be dependent on cGMP-induced activation of PKGI. PKGI induces relaxation of smooth muscle cells by modulating the activity of several calcium channels resulting in reduced intracellular calcium levels and by decreasing the calcium sensitivity of the contractile system [10].

In the kidney, the NP-induced natriuresis and diuresis is mediated by a cGMP-induced inhibition of sodium and water reabsorption throughout the nephron. In the proximal tubules, cGMP inhibits angiotensin II stimulated sodium and water transport, apical Na\(^+\)/H\(^+\) exchange and K\(^+\) channels which depolarises the proximal tubule cells and lowers the electrical driving force for electrogenic Na\(^+\) transport [11]. In the inner-medullary collecting ducts, cGMP has been shown to reduce apical epithelial sodium reabsorption by inhibiting an amiloride-sensitive cation channel via a dual mechanism, a direct phosphorylation-independent mechanism and by activating PKG [12].

In addition to mammals, NP systems have been identified in each vertebrate class. In fish, amphibians, birds and reptiles NP families have been identified and NPR-GC have commonly been found to be localized in organs involved in cardiac and osmoregulatory and salt homeostasis. In amphibians, birds and fish NPs have been shown to be involved in the regulation of blood fluid volume and composition thus documenting that the NP system is integral to the regulation of fluid volume and composition in diverse forms of life [13]. In the following sections, we shall examine if such a system also operates in plants, and if so what its mode of action and evolutionary history are.

### 2. Early physiological indications of an analogous system in plants

The first indication of the existence of NPs in plants came from immunoassays on tissue extracts from Florida beauty (Dracena godseffiana) [14]. In this study, antibodies against the N-terminus (ANP, 1-98), midportion (ANP, 31-67) and the C-terminus (ANP, 99-126) of ANP prohormone detected molecules in leaves and stems. A follow-up study presented further immunological evidence for NP-like proteins in several other plant classes including the Euglenophyceae, and data from high performance gel permeation chromatography also projected that pro-NPs and NPs from plants might share similar molecular masses with vertebrate ANPs [15]. It was also revealed that the rate of transpiration, solute flow and solute uptake in carnation and chrysanthemum was rapidly and significantly increased after exogenous application of synthetic human ANP (1-30), ANP (31-67) or ANP (79-98) at
concentrations of < 5-9 pg ml\(^{-1}\) (approx. 3 pM) [15]. Remarkably, ANP (99-126) prohormone did not seem to affect these processes at equivalent peptide concentrations. Subsequently it was demonstrated that synthetic rat ANP can induce stomatal opening in *Tradescantia* sp. in a concentration dependent manner albeit at concentrations of ≥ 10\(^{-6}\) M [16]. While such concentrations are several orders of magnitude higher than expected for a response to a peptide hormone, this may be a consequence of the large taxonomic divergence between hormone source and test organism. It is also noteworthy that, unlike animal systems, Na\(^+\) was not required in the medium for activity [16], indicating effects other than on Na\(^+\) transport. This was not to say that rat ANP cannot affect Na\(^+\) transport in plants but implied that K\(^+\) transport is modulated by NPs in preference. The first evidence for a NP effect on Na\(^+\) transport in plants came from further experiments on stomata where high extracellular Na\(^+\) was shown to inhibit auxin-induced opening. This inhibition was, in part, overcome in the presence of the Na\(^+\) channel inhibitor amiloride as well as rat ANP [16] thus establishing a putative link between rat ANP and Na\(^+\) transport in plants. The rat ANP molecule contains six charged amino acids (five arginines and one aspartic acid) and forms a loop due to a disulphide bond between cysteines in positions 7 and 23. To prove that the observed effects of rat ANP in plants are not solely due to its charges but reside in the native conformation, the molecule was reduced and irreversibly linearized by an S-carboxymethylation reaction [17]. In contrast to the native circular molecule, the linearized rat ANP showed no biological activity in stomatal opening assays thereby excluding a non-specific charge effect residing in the primary structure. Such a loss of biological activity after irreversible reduction of the molecule has previously been demonstrated in animal systems [7] and was taken as evidence of a receptor-mediated effect which requires interaction with a ligand of specific three dimensional conformation. Additional evidence for specific receptor-ligand interactions came from competitive *in vitro* binding assays where isolated leaf membranes were incubated with radiolabelled rat (3-[\(^{125}\)I]iodotyrosol\(^{28}\)) ANP and increasing concentrations of unlabelled rat ANP [16, 18]. This showed that 50% of the labelled ligand could be displaced by 0.1 µM rat ANP, indicating that plant membranes contain a specific low-affinity NP binding site for the heterologous rat ANP since half maximal specific binding for peptide hormones in homologous vertebrate systems is typically substantially lower (< 0.1 nM).

The involvement of cGMP in rat ANP-dependent signalling in plants was also investigated since cGMP acts as the second messenger for ANP in vertebrate systems and evidence indicates that cGMP may have a similar role in plant signal transduction [19, 20]. While rat ANP-induced increases in stomatal aperture are reversibly inhibited by the guanylate cyclase inhibitors, methylene blue and LY 83583, the cell permeant cGMP analogue 8-Br-cGMP
can mimic the effect of rat ANP when applied alone [17, 21]. It is also noteworthy that the 8-Br-cGMP effect is not observed in the presence of the (drought-) stress response hormone abscisic acid (ABA) [17]. ABA does not signal via cGMP-dependent signal pathways [22]. Data are therefore consistent with guanylate cyclase activation in stomatal opening in response to rat ANP and indicate the presence of putative NP receptors with guanylate cyclase domains. This was further supported by observed increases in cGMP concentrations in guard cell protoplasts in response to rat ANP [21].

In addition, measurements of dye movements, and determination of tissue water exchange ratios by $^2$H NMR, suggest that rat ANP significantly increases radial water movements out of the xylem of shoots of *Tradescantia multiflora* [23]. This enhancement is also observed in response to 8-Br-cGMP while the water channel inhibitor mercuric chloride and the guanylate cyclase inhibitor LY 83583 both significantly inhibit radial water movement. Thus, evidence suggests that NPs are involved in controlling radial water movement out of the xylem and this effect may be mediated via regulation of guanylate cyclases and water channels. In support of the latter notion, cGMP-dependent phosphorylation has been reported to regulate water channel activity of a seed-specific aquaporin [24].

In addition, we have demonstrated that a synthetic peptide identical to the C-terminus (amino acids 99–126) of the rat ANP modulates osmotically-induced swelling of mesophyll cell protoplasts (MCPs) in a concentration and time-dependent manner [25]. Osmotically-induced volume changes in MCPs are enhanced by plant extracts with NP immunoreactivity and this effect is concentration-dependent.

Finally, we isolated and purified by immuno-affinity chromatography biologically active PNP immuno-analogues (irPNPs) from a number of different species including ivy and potato [17, 21, 25, 46]. Exogenous irPNPs stimulate stomatal opening and activate the H+-ATPase [25]. Moreover, irPNPs rapidly and specifically induce transient elevation of cGMP levels in the conductive stele tissue of maize roots [26] and stomatal guard cell protoplasts [21]. IrPNPs also enhance osmoticum-dependent volume changes in leaf mesophyll protoplasts[25]. The immuno-reactants modulate ion fluxes across plant membranes leading to a rapid (<60 sec) net influx of H$^+$ and a delayed (>20 min) net influx of K$^+$ and Na$^+$ in maize stele tissue [27]. We obtained N- and C-terminal sequence data from potato PNPs (StPNPs) [25] and in silico identified two closely related homologous *Arabidopsis thaliana* genes, AtPNP-A (AAD08935) and AtPNP-B (CAB79756) [28].

In summary, these findings are consistent with a NP-dependent effect on plant cell volume regulation and an important role in cell and whole plant homeostasis for peptides that are recognized by antibodies directed against the C-terminus of vertebrate ANPs [29, 30].
3. Molecular identification of PNPs in *Arabidopsis thaliana*

We have since isolated and characterised the genes and proteins of an immunoreactive PNP from *Arabidopsis thaliana* (AtPNP-A) and several closely related sequences in different plant species [28]. AtPNP-A is a small protein of 126 amino acids in length (MW, 14016 kD; pI, 9.22) that is encoded by a gene with a single intron of 100 bp (accession no., AAD08935). The protein contains a 25 amino acid signal peptide (MW, 2249) that directs the protein into the extracellular space (Fig. 1). The part most conserved between PNP-As from different plant species (between amino acids 33 and 66) has also been shown to be the key to its biological function [31] and is the one that shares some similarity to animal NPs and in particular hANP(99-126) (Fig. 1).

![Figure 1](image_url)

**Figure 1.** (A) *Arabidopsis thaliana* PNP-A. The N-terminal first 25 amino acids are the signal peptide (SP) that direct the protein into the extracellular space. Amino acids 33 to 66 convey the homeostasis regulating biological activity (in red). The C-terminus also contains a rare lipoprotein A (RlpA) domain (DPBB 1; e value = 0.002) marked in yellow. The amino acid sequence of AtPNP-A (33-66) is compared to human ANP (hANP (99-126) delineated by purple triangles) and asterisks (*) signify identical amino acids, colons (:) are conservative substitutions, full stops (.) are semi-conservative replacements and the lozenges (♦) indicate cysteine residues in AtPNP-A that can form disulfur bridges. (B) Alignment of amino acid sequences of NP -like molecules. Identical amino acids are in red, conservative substitutions are in blue and the lozenges (♦) indicate cysteines in AtPNP-A that can form disulfur bridges. Note that in the literature the numbering of AtPNP-A residues includes the 25 residues of the signal peptide. For consistency, if numbering was based on the prohormone as in animals, residues 33 - 66 of AtPNP-A would become residues 7 - 40.
However, AtPNP-A, its related sequence AtPNP-B and orthologues in other higher plant species, share domains with the cell wall loosening expansins (Fig. 2) and can be classified as expansin-related on the basis of this homology [32]. Interestingly, the C-terminal domain of expansins [33] is encoded by an entire exon that is absent in PNP-like molecules [28]. Expansins are distantly related to glucanases and cellulases; in the latter, the C-termini have been proven to be cell wall binding [34] and the same function has been proposed for the expansin C-terminus [33].

Since expansins, which are the closest relatives of PNPs, and the more distantly related glucanases and cellulases, all contain the C-terminus; it is reasonable to argue that PNP-like molecules have lost this domain. This is in keeping with the fact that the domain is delineated by an intron–exon border. Loss of the wall-binding domain could result in increased mobility of PNP-like molecules. It also follows from the phylogenetic data, that similarities between AtPNP-A and ANP (Fig. 2) may be the result of convergent evolution.

Interestingly, the bacterial citrus pathogen *Xanthomonas axonopodis* pv. *citri* str. 306 also contains a gene encoding a PNP-like protein and we found that the *Xanthomonas axonopodis* PNP-like protein shares significant sequence

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**Figure 2.** Model of the molecular evolution of PNPs. The ancestral molecule is a hydrolase (glucan hydrolase 45; GH45 domain) with a signal peptide (SP) and a cell wall binding or carbohydrate binding domain (CB domain). Both, expansins and PNPs lose hydrolytic activity but retain the SP and GH45 like domain. PNPs, contrary to expansins also lost the CB domain, and thus gaining systemic mobility.
similarity and an identical domain organisation with PNP [35]. We also observed a significant excess of conserved residues within the domain previously identified as being sufficient to induce biological activity [31]. Structural modeling predicts identical six stranded double-$\psi$ $\beta$ barrel fold for both proteins thus supporting the hypothesis of similar modes of action (Fig. 3).

No significant similarity between the *Xanthomonas axonopodis* PNP-like protein and other bacterial proteins from GenBank was found. The similarity between the *Xanthomonas axonopodis* PNP-like molecule and the *Arabidopsis thaliana* PNP (AtPNP-A), the shared domain organisation and the incongruent phylogeny suggest that the encoding gene may have been acquired by the bacteria in an ancient lateral gene transfer event [35]. Biological activity of a recombinant *Xanthomonas axonopodis* protein in plants and changes in symptoms induced by a *Xanthomonas axonopodis* mutant with a knocked-out PNP-like gene is underway to experimentally test the hypothesis of molecular mimicry. If this hypothesis proves true, it could at least in part explain why the citrus pathogen, *Xanthomonas campestris*, that does not contain a PNP-like gene, produces dry corky lesions while the closely related *Xanthomonas axonopodis*

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**Figure 3.** A fold model of AtPNP-A (amino acid 26 to 126). The model shows the six stranded double-$\psi$ $\beta$ barrel structure that assumes a pseudo-two fold axes in which parallel strands form structures. The $\alpha$-helices are in red, the 6 $\beta$-strands are in blue. The signal peptide was not included in the model and the domain conferring activity is between solid triangles. The model was generated using the software MOLSCRIPT [54].
forms wet lesions. It also suggests that genes typically found in the host, horizontally transferred or heterologous, can help to explain aspects of the physiology of the host-pathogen interactions.

4. Synthesis and degradation in planta

Loci of synthesis and degradation as well as induction patterns of immunoreactant PNPs are currently under investigation. RT-PCR has revealed *AtPNP-A* transcripts in leaf tissue from unstressed Arabidopsis plants [28], indicating that the protein is not only found, but synthesised, in leaves. It is also possible that stress induced up-regulation of *AtPNP-A* transcription occurs either in leaves or elsewhere. Microarray data (accessed through Genevestigator) documents transcriptional up-regulation of *AtPNP-A* (*At2g18660*) in response to ozone, nitrogen and potassium deficiency, salicylic acid, osmotic and salt stress, and syringolin and the plant pathogens *Erysiphe orontii*, *Erysiphe cichoracearum*, *Agrobacterium tumefaciens*, and *Botrytis cinerea*.

Experimental data on *AtPNP-B* (*At4g30380*) comes from the citrus homologue (CjBAp12; accession no., AAD03398). While the biological role of the molecule is not as yet determined, published data suggest that CjBAp12 transcription occurs in the root only and is up-regulated in response to blight infection [36]. The protein is also found in shoots [36], thus strongly suggesting that the molecules are transported, possibly in the xylem, and have a systemic mode of action. Microarray experiments accessed through "Genevestigator" confirm transcriptional up-regulation of *AtPNP-B* in response to abiotic stresses such as anoxia, hypoxia, cyclohexamide, salicylic acid and osmotic stress and the pathogen *Pseudomonas syringae*. However, it appears that *AtPNP-A* is much more responsive at the transcript levels compared to *AtPNP-B* and *AtPNP-A* is also more readily detected at a protein level.

Our *in situ* localisation data indicate that immunoreactant PNPs are systemically mobile proteins since they are found in conductive tissue; a highly unlikely place for synthesis, but not transport. We have also detected biologically active immunoreactants in xylem sap of *Erucastrum strigosum* and this is indicative of synthesis in the root [37]. It was also noted from western blots that abiotic stress induction with NaCl or sorbitol increased PNP levels. Interestingly, recombinant *AtPNP-A* effects such as induction of protoplast swelling required active protein synthesis [37]. Further information on temporal and spatial induction patterns will be forthcoming when results from transgenic plants containing GUS or GFP promoter fusions become available.
5. Elucidating the biological role and mechanisms of action of PNPs

Since PNPs are systemically mobile, as indicated from the structure and processing of the molecules [28] as well as the in situ localisation studies [38], we have tested the effect of added recombinant AtPNP-A on ion flux in intact roots [39].

It was demonstrated that AtPNP-A causes rapid H⁺ influx in the elongation zone of Arabidopsis thaliana roots but not in the mature zone. AtPNP-A also induces significant K⁺ and Na⁺ efflux and this effect is seen in the mature root zone only. Whether these responses are caused directly by the recombinant molecule (e.g. by binding to ion channels) or indirectly via second messengers such as cGMP that is known to modulate cGMP-gated ion channels remains to be elucidated (Fig. 4). An interplay between PNP, calcium and cGMP has been observed [21, 40]. In any case these observations suggest that responses to AtPNP-A are developmental stage and tissue specific and point to a complex role in plant physiology.

Figure 4. Model of PNP action at the cellular level. The model proposes that PNPs can dock to receptor like molecules with guanylyl cyclase function (pGCs) and that cGMP acts as second messenger affecting cytosolic Ca²⁺ levels, modulating ion channels, activating phosphorylation through kinases and affecting the transcriptome. Phosphodiesterases (PDEs) in turn metabolise cGMP to GMP.
Microarray data reveals two additional pointers to the importance and complexity of the role of AtPNP-A. Firstly, it appears that the strongest transcriptional up-regulation is seen after K⁺ starvation. K⁺ is the most important inorganic solute in the plant and contrary to Na⁺, it is not toxic. The K⁺ uptake from the soil into the root and transport to the shoot and into the growing tissue is essential since K⁺ uptake into cells will contribute essentially the osmotic pressure required to draw H₂O and thus allow normal plant expansion growth. It is conceivable that AtPNP-A plays a major role in compensation for the lack of the osmoticum by regulating K⁺ channels (activate input channels and/or block efflux carriers) or by stimulating the synthesis of compatible osmotica. The latter mechanism is supported by the extraordinary capacity of immunoaffinity purified PNP and recombinant AtPNP-A to induce protoplast swelling caused by H₂O up-take [25, 31]. Notably, AtPNP-A-induced volume increases occur in osmotically highly unfavourable conditions not unlike those one might expect during K⁺ starvation [31].

A second indication from microarray data comes from a expression coefficient analysis (Table 1). Here we identified genes that are transcriptionally co-regulated with AtPNP-A.

Such an analysis can shed light on the biological context of a target gene, in this case AtPNP-A. In this case we note that the most strongly expression correlated gene is At3g57260, which is annotated hydrolase hydrolyzing O-glycosyl compounds. Most importantly, this gene is also annotated as encoding a protein that is part of the systemic acquired resistance (SAR), a vital and complex plant mechanism that protects plants from succumbing to pathogens. This association may suggest that AtPNP-A has a part in host defense responses, e.g. in counteracting the host-induced distortion of the homeostatic balance.

Experimental data suggested that immunoreactant PNP increases in shoots in response to NaCl stress [37]. A PNP response to environmental disturbance is not unexpected and in agreement with the blight-induced protein [36]. The pathogen causes severe disruption and plugging of xylem vessels leading to wilting and limb death. Accordingly, CjBAp12, found predominantly in the xylem, may have a role in counteracting the severe homeostatic disturbance caused by the pathogen.

The link of AtPNP-A with a role in host-pathogen interactions is further supported by the unusual presence of a PNP-like protein in the citrus pathogen Xanthomonas axonopodis [35]. Moreover, observations suggest that reduced AtPNP-A expression in transgenic Arabidopsis plants alters lesion sizes in Botrytis infected plants (Lara Donaldson, unpublished observation). Other up-regulated genes (Table 1), notably the aspartic endopeptidase, the triphosphatase and the ubiquitin ligase also point to a response to biotic and/or
Table 1. Genes that are expression correlated with AtPNP-A (At2g18660)

<table>
<thead>
<tr>
<th>Locus:</th>
<th>r-value</th>
<th>Annotation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>At3g57260</td>
<td>0.731</td>
<td>Hydrolyzing O-glycosyl compounds, SAR(^2)</td>
</tr>
<tr>
<td>At5g10760</td>
<td>0.681</td>
<td>Aspartic endopeptidase</td>
</tr>
<tr>
<td>At2g04450</td>
<td>0.676</td>
<td>Triphosphatase activity, stress response</td>
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<tr>
<td>At5g52760</td>
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<td>Metal ion transport</td>
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<td>At2g17040</td>
<td>0.659</td>
<td>Transcription factor activity, developmental process</td>
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<tr>
<td>At5g55450</td>
<td>0.647</td>
<td>Lipid binding and lipid transport</td>
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<tr>
<td>At1g21250</td>
<td>0.645</td>
<td>Kinase activity and cell wall (WAK1), signal transducer</td>
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<tr>
<td>At4g23610</td>
<td>0.641</td>
<td>Hin1 - role in hypersensitive response</td>
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<td>At1g13470</td>
<td>0.634</td>
<td>Mitochondrial protein of unknown function</td>
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<tr>
<td>At4g14365</td>
<td>0.634</td>
<td>Ubiquitin ligase and zinc ion binding</td>
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<td>At1g14120</td>
<td>-0.425</td>
<td>Oxidoreductase acting on paired donors</td>
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<td>Protein binding and signal transduction</td>
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<td>Trehalose phosphatase activity, trehalose biosynthesis</td>
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<td>Leaf morphogenesis, monopolar growth</td>
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<tr>
<td>At2g45400</td>
<td>-0.454</td>
<td>UDP-glucose 4-epimerase activity, flavonoid biosynthesis</td>
</tr>
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</table>

1 r-value; Correlation coefficient of genes transcriptionally co-regulated with AtPNP-A (At2g18660). 2 Systemic acquired resistance

abiotic stress. In the group of the ten most negatively correlated genes, one (At5g04770) is involved ATP synthesis and a second (At4g36380) in leaf morphogenesis and monopolar growth, both processes we would expect to be reduced in a stress response situation. Finally, an additional experimental clue
to AtPNP-A’s function and importance comes from the fact that T-DNA insertion mutants with reduced expression show distinct stress response and growth phenotypes (Donaldson, Denby and Gehring, unpublished observation). There are no reports of homozygous mutants with a complete absence of expression since they are most likely lethal.

6. Towards a general model of PNP action

In the following discussion an attempt is made to formulate a general theory of the role and action of this essential plant homeostasis regulating system involving PNPs. The model which is essentially based on data from *Arabidopsis thaliana* (Fig. 5) represents the two major spatial components, the root and the shoot and proposes that AtPNP-A plays a role in the signaling between the two.

**Figure 5.** Model of PNP effects at the whole plant level. The root shoot boundary is indicated by a horizontal bar. The xylem (blue) functions mainly as conductive tissue for H$_2$O and minerals and the phloem (light green) transports nutrients down from the leaves to the stem and root system. The PNP-A transcription inducing stresses are NaCl in the root and low K$^+$ and high osmotic pressure in the shoot. The proposed specific PNP-binding molecules (receptors) light grey boxes and we propose that binding increases cGMP levels which in turn affects cellular processes notably the regulation of cyclic nucleotide gated channels (CNGCs), K$^+$ release and activation of aquaporins.
Notably the fact that AtPNP-A transcription is increased by different stimuli in roots and shoots supports a role in integration. In the root high Na\(^{+}\) concentration, a cation that needs to be excluded, triggers transcription (see "Genevestigator" data; www.genevestigator.ethz.ch) of AtPNP-A and most likely processing and transport into the intracellular (apoplastic) space and/or into the xylem for transport to the shoot. In the root AtPNP-A is likely to have a differential tissue specific effect [39] and given that PNP triggers rapid cGMP increases [21, 26], it is conceivable that these increases directly modulate cation channels and thus influence ion homeostasis in general and Na\(^{+}\) efflux and salinity tolerance in particular [41, 42]. The root tissue synthesised AtPNP-A that is transported to the shoot in the xylem can presumably also modulate ion flux via cGMP and in addition may play a major role in drawing water into cells even under osmotically unfavorable conditions [25, 31]. In the xylem, immunoreactant PNP has also been shown to increase lateral water movements out of the conductive tissue and thus contribute to the water homeostasis and solute both in shoot and root.

In the shoot, two abiotic stresses are known that trigger transcription of the AtPNP-A encoding gene. The first is K\(^{+}\) starvation leading to low K\(^{+}\) levels in the leaves and thus a shortage of the key inorganic ion and solute required for elongation growth, the second is osmotic stress. In the case of K\(^{+}\) shortage, lacking osmotic pressure required for elongation growth may be compensated by the ability of AtPNP-A to enable H\(_2\)O up-take under osmotically adverse conditions by mechanisms that require de novo protein synthesis [31, 37] and may at least in part involve the synthesis and/or re-compartmentalisation of compatible organic solutes. Under opposite conditions, osmotic stress in the shoot, AtPNP-A synthesis is also induced and can again counteract it drawing H\(_2\)O through the mechanisms described above. Since immunoreactant PNPs have also been identified in the phloem [38] we may postulate a role in shoot root signalling in addition to a direct or indirect enhancement of H\(_2\)O up-take into the root.

7. Future applications in agricultural biotechnology, medicine and nutrition

Since climate change will continue to occur and stresses from climatic extremes are likely to increase we can expect increasing difficulties in growing crops in many parts of the world [43, 44]. Food security is therefore heavily dependent on the development of crop plants with increased resistance to abiotic stresses such as drought and salinity. The urgent need to use rational approaches to develop crop plants with increased abiotic stress tolerance has led to an impressive body of work in the area of plant genetics, plant physiology, plant biochemistry and plant molecular biology and a realization...
that only an integrated and systems based approach can possibly deliver biotechnological solutions [45]. Since proteins that systemically affect homeostasis are a target candidate group for biotechnology, we have tested Arabidopsis mutants with different levels of AtPNP-A expression for changes in abiotic stress responses. Early results have confirmed that AtPNP-A expression mutants do affect NaCl tolerance (Lara Donaldson, unpublished observation). Perhaps surprisingly, T-DNA insertion mutants with reduced expression significantly cope better with increased NaCl loads in the soil than the wild-type, at least during germination and early growth phases, which is often a difficult period in plant development. This suggests that it may be important to dynamically regulate PNP expression so that it is reduced during germination and enhanced when required at more mature stages by the plant.

Since the active region of PNP-A has structural similarities to ANP (Fig. 1), as also attested by the cross recognition of PNP and ANP by anti-hANP antibodies [e.g. 25, 38, 46] it is interesting to speculate if PNP could have any potential therapeutic uses in humans. As described above, the effects of PNP can be mimicked by ANP in plants indicating that the plant receptor recognises both peptides; possibly the reverse is true. ANP mediates its effects through NPR1 which generates cGMP and activates PKG1. ANP has marked antihypertrophic effects on cardiomyocytes both in culture and in vivo [47] and has both antimitogenic and apoptotic effects [48] and can inhibit growth of cancer cells in vitro and in vivo [49]. Preliminary studies have shown that recombinant AtPNP-A can induce the apoptotic cascade in CHO cells (Pironcheva and Gehring, unpublished results) and this exciting finding is being pursued. Furthermore, ANP is present in the intestine where it has paracrine functions modulating water and nutrient intake [50] and we are currently investigating if recombinant AtPNP-A can induce such effects. Interestingly, ANP induces lipolysis of stored triacylglycerols in human fat cells via a cGMP-mediated mechanism [51] raising the possibility that ANP and functional homologues such as PNP could be investigated as potential anti-obesity treatments. Clinically, intravenous administration of ANP and BNP reduce arterial blood pressure in patients with chronic heart failure [52]. Nesiritide (a recombinant BNP) was approved for treatment of acute decompensation in heart failure in the US in 2001 but concerns have been raised about its safety [53].

In summary, PNPs are an essential and novel systemically mobile plant signaling molecule with a role in homeostasis and potential applications in agricultural biotechnology and health.

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