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REGULAR PAPER

Identification of *Arabidopsis thaliana* NRT1/PTR FAMILY (NPF) proteins capable of transporting plant hormones

Yasutaka Chiba · Takafumi Shimizu · Shinya Miyakawa · Yuri Kanno · Tomokazu Koshiba · Yuji Kamiya · Mitsunori Seo

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Abstract NRT1/PTR FAMILY (NPF) proteins were originally identified as nitrate or di/tri-peptide transporters. Recent studies revealed that this transporter family also transports the plant hormones auxin (indole-3-acetic acid), abscisic acid (ABA), and gibberellin (GA), as well as secondary metabolites (glucosinolates). We developed modified yeast two-hybrid systems with receptor complexes for GA and jasmonoyl-isoleucine (JA-Ile), to detect GA and JA-Ile transport activities of proteins expressed in the yeast cells. Using these GA and JA-Ile systems as well as the ABA system that we had introduced previously, we determined the capacities of Arabidopsis NPFs to transport these hormones. Several NPFs induced the formation of receptor complexes under relatively low hormone concentrations. Hormone transport activities were confirmed for some NPFs by direct analysis of hormone uptake of yeast cells by liquid chromatography–tandem mass spectrometry. Our results suggest that at least some NPFs could function as hormone transporters.

Keywords Abscisic acid (ABA) · Arabidopsis (*Arabidopsis thaliana*) · Gibberellin (GA) · Jasmonoyl-isoleucine (JA-Ile) · NRT1/PTR FAMILY (NPF) · Transport

Introduction

Plant hormones are a group of endogenous, bioactive small molecules that induce various physiological responses throughout plant life cycles (Kende and Zeevaart 1997; Santner et al. 2009; Santner and Estelle 2009). Hormone-dependent physiological responses are regulated on several levels including hormone biosynthesis, catabolism (inactivation), transport, perception and signal transduction. The balance between biosynthesis and catabolism, in association with transport, determines local hormone concentrations in a particular cell. On the other hand, the recognition of hormones by receptors and the transduction of the signals regulate the sensitivities and responsiveness of a cell to hormones. Plant hormones exist in plant tissues at low concentrations; thus, these processes must be regulated very precisely.

In animals, hormones are synthesized in specific cell types and are transported to target cells to induce physiological processes. In the case of plant hormones, the transport of auxin (indole-3-acetic acid; IAA) has been thoroughly characterized (Blakeslee et al. 2005; Petrasek and Friml 2009). The plant-specific PIN transporter family and some members in subgroup B of the ATP-binding cassette (ABC)-type transporter family (ABCB) function as IAA exporters whereas the amino acid permease-like AUX/LUX family mediates IAA uptake (Habets and Offringa 2014; Swarup and Peret 2012; Yang and Murphy 2009). Another example in which transport has been

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Y. Chiba · S. Miyakawa · T. Koshiba · M. Seo
Department of Biological Sciences, Tokyo Metropolitan University, Hachioji-shi, Tokyo 192-0387, Japan

Y. Chiba · T. Shimizu · Y. Kanno · Y. Kamiya · M. Seo (✉)
RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa 230-0045, Japan
e-mail: mitsunori.seo@riken.jp

extensively studied is abscisic acid (ABA) (Boursiac et al. 2013; Seo and Koshiba 2011). ABA acts as a signal that is produced in roots under water deficit and is transmitted to leaves to induce stomatal closure (Jiang and Hartung 2008). In Arabidopsis, immunohistochemical analysis with antibodies raised against the ABA biosynthesis enzymes AtNCED3, AtABA2, and AAO3 detected these enzymes in vascular tissues, suggesting that ABA is synthesized in these tissues (Endo et al. 2008; Koiwai et al. 2004). This implies that ABA synthesized in vascular tissues has to be transported to guard cells to induce stomatal closure. So far, four ABA transporters have been identified in Arabidopsis, and two of them are subgroup G ABC transporters, namely AtABCG25 and AtABCG40 (Kang et al. 2010; Kuromori et al. 2010). AtABCG25 is likely expressed in vascular tissues and involved in the export of ABA from ABA synthesizing cells, whereas AtABCG40 mediates ABA uptake into guard cells leading to stomatal closure. It is interesting to note that an ABCG protein is required for the exudation of storigolactone from roots in Petunia (Kretzschmar et al. 2012), and that the Arabidopsis AtABCG14 is involved in the transport of different types of cytokinins between shoots and roots through the xylem and/or phloem (Ko et al. 2014; Zhang et al. 2014b). The third ABA transporter in Arabidopsis is a member of the NRT1/PTR FAMILY (NPF) proteins. There are 53 genes encoding NPF proteins in Arabidopsis, and some have been identified as nitrate or peptide (di/tri-peptide) transporters (Leran et al. 2014; Tsay et al. 2007). NPF4.6 initially has been characterized as a nitrate transporter, NRT1.2 (Huang et al. 1999), but we demonstrated it functions also as an ABA transporter (Kanno et al. 2012). NPF4.6 is expressed in the vasculature and possibly regulates the amount of ABA transported toward the guard cells in association with AtABCG25. As the fourth Arabidopsis ABA transporter, a DTX/MATE proteins called AtDTX50 was recently identified as an ABA efflux transporter expressed in vascular tissues and guard cells (Zhang et al. 2014a). One may conclude from these facts that the ABA transport system is highly complex and redundant.

NPF4.6 is not the only NPF that transports substrates other than nitrate or peptides. At least three additional Arabidopsis NPFs, NPF4.1, NPF4.2, and NPF4.5, transported ABA in yeast (Kanno et al. 2012). Interestingly, NPF4.1 transported gibberellin (GA; GA_3) as well (Kanno et al. 2012). NPF6.3/NRT1.1/CHL1, which had been identified as a nitrate transporter, also functions as an IAA transporter (Krouk et al. 2010). NPF2.10/GTR1 and NPF2.11/NRT1.10/GTR2 are involved in the transport of glucosinolates (Nour-Eldin et al. 2012). Considering that dipeptides are substrates of certain NPFs, we speculated that the amino acid-conjugated, bioactive hormone jasmonoyl-isoleucine (JA-Ile) might also be transported by NPFs. Transport of GA and JA-Ile within plants has been suggested (Matsuura et al. 2012; Pimenta Lange et al. 2012). Thus, in the present study, we screened additional NPFs for hormone transport activities. To perform a comparative investigation, we developed modified yeast two-hybrid (Y2H) systems to detect GA and JA-Ile transport activities of proteins expressed in the system. To this end, we used receptor complexes for the different hormones as we did previously for ABA. The capacities of 45 out of the 53 Arabidopsis NPFs to transport ABA, GA and JA-Ile were examined.

Materials and methods

Construction of Y2H systems

For expression of DBD-GID1a and DBD-COI1 in yeast, *GID1a* (At3g05120) and *COI1* (At2g39940) cDNAs were amplified with primer combinations presented in Table S1 and cloned into pT7-Blue (Novagen). After sequencing, the *GID1a* and *COI1* cDNAs were excised by digestions with *Bam*HI and *Pst*I, or with *Sma*I *and Sal*I, respectively, and cloned into the pGBT9 vector.

For expression of AD-GAI and AD-JAZ3 in yeast, the *GAI* (At1g14920) and *JAZ3* (At3g17860) cDNAs were amplified with primer combinations presented in Table S1. After sequencing, the *GAI* and *JAZ3* cDNAs were excised using *Bam*H I and *Pst* I, and *Eco*R I and *Sal* I, respectively, and cloned into the pGAD424 vector.

DBD-PYR1 and AD-ABI1 constructs were generated as described previously (Kanno et al. 2012).

Cloning of NPF cDNAs

NPF cDNAs were amplified with primer combinations listed in Table S2, and cloned into pENTR/D-TOPO (Invitrogen) or pDONR207 (Invitrogen). For cloning into pDONR207, cDNAs amplified with primers that contained a parts of the *att*B1 (forward) and *att*B2 (reverse) sequences in their 5′ regions were first cloned into pT7-Blue, and then re-amplified with primers 5′-GGGGACAAGTTTGTAC Aaaaaagcaggct-3′ (forward) and 5′-GGGGACCACTTTG TACAagaaagctgggt-3′ (reverse) to add complete *att*B1 and *att*B2 recombination sequences, respectively. Nucleotides shown in lowercase overlap with parts of the *att*B1 and *att*B2 recombination sequences added to the first primers. The amplified cDNAs with recombination sequences were introduced into pDONR207 by BP reactions. After sequencing, NPF cDNAs cloned into pENTR/D-TOPO or pDONR207 were cloned into pYES-DEST52 in which the *GAL1* promoter had been replaced by the *ADH1* promoter by LR reactions.

Eleven NPF cDNAs of subgroup 4 and 6 were prepared as described previously (Kanno et al. 2012).

Y2H transport assays

The DBD, AD, and NPF constructs were transformed into the yeast strain PJ69-4A according to standard protocols. Yeast cells derived from 10 independent original transformants cultured overnight in liquid media [synthetic dextrose (SD); -Trp, -Leu, -Ura] were collected by centrifugation and re-suspended in buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) so that the OD_{600} became 1 (approximately 10^7 cells/ml), 0.1, or 0.01. Ten µl cell suspensions (10^5, 10^4, or 10^3 cells) were inoculated on plates (SD, -Trp, -Leu, -Ura, -His) containing the desired concentrations of hormones, and were incubated at 30 °C.

Direct transport assays by LC–MS/MS

Direct hormone transport assays were performed essentially as described previously (Takeuchi et al. 2014), with some modifications. Hormones were added to reaction mixtures at 10 µM. ABA and GA_3 were extracted and purified from yeast cells as described previously (Kanno et al. 2012). GA_1, GA_4 and JA-Ile were extracted and purified as GA_3 was. LC–MS/MS analysis was performed with a Nexcera (Shimazu)/Triple TOF 5600 (AB SCIEX) system with an ZORBAX Eclipse XDB-C18 column (Agilent). LC conditions and MS/MS parameters are shown in Tables S3 and S4.

Chemicals

ABA and GA_3 were purchased from Sigma-Aldrich and Wako, respectively. D_6-ABA, D_2-GA_1, D_2-GA_3, D_2-GA_4, GA_1 and GA_4 were purchased from Olchemim. JA-Ile and $^{13}C_6$-JA-Ile were synthesized as described previously (Jikumaru et al. 2004).

Results

Construction of Y2H systems to detect hormone-dependent formation of receptor complexes

We previously had developed a Y2H system to characterize ABA transport activities of proteins expressed in yeast cells by detecting ABA-dependent interactions between the ABA receptor PYR/PYL/RCAR and PP2C protein phosphatases (Kanno et al. 2012). Arabidopsis PYR1 fused to the GAL4 DNA binding domain (DBD; DBD-PYR1) and ABI1 fused to the GAL4 activation domain (AD; AD-ABI1) were expressed in yeast strain PJ69-4A, in which marker gene (*HIS3*) expression was activated by the ABA-dependent formation of DBD-PYR1/AD-ABI1 complexes (Fig. S1). In the present study, we spot-inoculated selective media with a certain amount of cells to compare relative growth between yeast lines (Fig. 1a). As we reported previously, yeast cells expressing NPF4.6/NRT1.2/AIT1 grew on selection media in the presence of 0.1 µM ABA whereas cells without the transporter did not. Yeast cells without NPF4.6/NRT1.2/AIT1 grew at relatively high concentrations (1 µM) of ABA, but not as rapidly as lines expressing NPF4.6/NRT1.2/AIT1.

In the presence of their hormone ligand, the receptors of GA, JA-Ile and IAA form specific protein complexes: the GA receptor GID1 interacts with DELLA proteins, the JA-Ile receptor COI1 interacts with JAZ proteins, and the IAA receptor TIR1/AFB interacts with Aux/IAA proteins (Lumba et al. 2010). Therefore, we expected that Y2H systems with the GA-, JA-Ile-, and IAA-specific receptor complexes could be used to detect hormone transport activities of NPFs.

We have shown by LC–MS/MS that NPF4.1/AIT3, originally identified based on its ABA transport activity, also transported GA (GA_3) (Kanno et al. 2012). Therefore, we constructed a Y2H system with the Arabidopsis GID1a and GAI (Fig. S1), and tested whether GA transport activity of NPF4.1/AIT3 could be detected (Fig. 1b). GA_1, GA_3 and GA_4 were used as substrates because they are recognized by the GA receptor (Nakajima et al. 2006). GA_1 and GA_4 are bioactive GAs synthesized in higher plants, whereas GA_3 is a major GA produced by the fungus *Gibberella fujikuroi*. However, GA_3 induces similar physiological responses in plants as GA_1 and GA_4 when exogenously applied. Because the three GAs have different receptor affinities (Nakajima et al. 2006), we had to determine the appropriate GA concentrations to be used in the assay. Significant growth of yeast without NPF4.1/AIT3 was observed on media containing 1 µM GA_1 or GA_3, although cells expressing NPF4.1/AIT3 grew better than the control (Fig. 1b). In contrast, only cells expressing NPF4.1/AIT3 grew at 0.1 µM GA_1 or GA_3. As expected from the particularly high affinity of the GA receptor GID1a to GA_4, yeast cells grew similarly well on selection media regardless of the presence of NPF4.1/AIT3 when GA_4 was available at 0.1 µM. A marked difference between cells with and without NPF4.1/AIT3 was observed when 20 nM GA_4 was present in the selection media. At 2 nM GA_4, only cells expressing NPF4.1/AIT3 could grow. These results showed that GA transport activities of NPFs could be detected by the Y2H transport assay.

The Arabidopsis JA-Ile receptor COI1 and the JAZ3 protein were used to construct a Y2H system. Significant growth of yeast containing DBD-COI1 and AD-JAZ3 was observed with 100 µM but not with 50 µM JA-Ile in the selection medium (Fig. 1c). We expected that this system could be used to determine JA-Ile transport activities of NPFs although we had no positive controls to confirm the possibility.

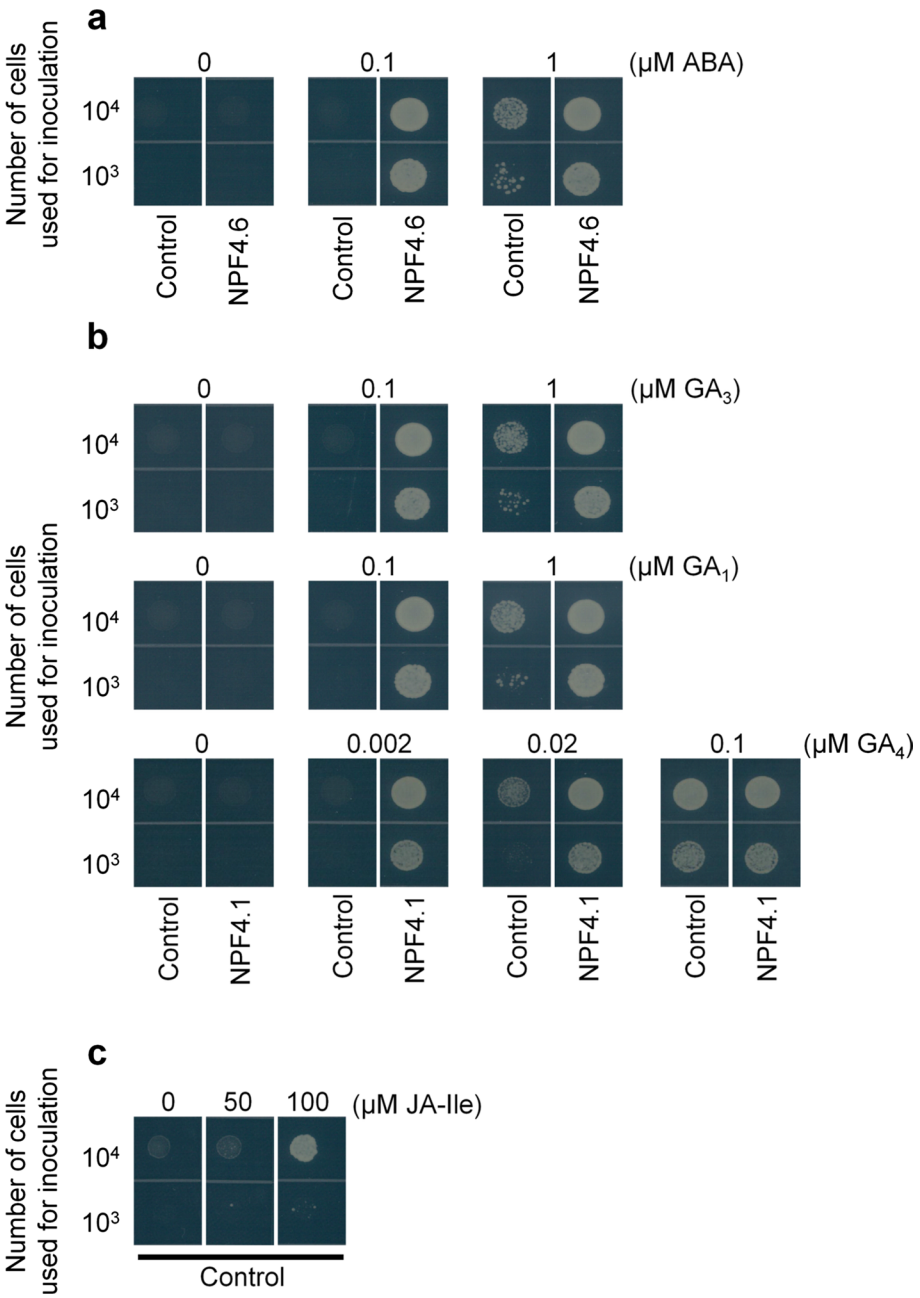

Fig. 1 Detection of hormone transport activities of NPF proteins using Y2H systems. **a** *NPF4.6* (*NRT1.2/AIT1*) cDNA cloned into the yeast expression vector pYES-DEST52 in which the *GAL1* promoter had been replaced with the *ADH1* promoter, or the empty vector (*control*) was introduced into the yeast strain PJ69-4A containing DBD-PYR1 and AD-ABI1 constructs. Selection media (SD, -Trp, -Leu, -Ura, -His) containing 0, 0.1 or 1 μM ABA were inoculated with 10^3 or 10^4 cells. Photos were taken 3 days after inoculation. **b** *NPF4.1* (*AIT3*) cDNA cloned into the yeast expression vector pYES-DEST52 in which the *GAL1* promoter had been replaced with the *ADH1* promoter, or the empty vector (*control*) was introduced into the yeast strain PJ69-4A containing DBD-GID1a and AD-GAI constructs. Selection media (SD, -Trp, -Leu, -Ura, -His) containing GA_1 (0, 0.1 or 1 μM), GA_3 (0, 0.1 or 1 μM) or GA_4 (0, 0.002, 0.02 or 0.1 μM) were inoculated with 10^3 or 10^4 cells. Photos were taken 2 days (GA_4) or 3 days (GA_1 and GA_3) after inoculation. **c** The yeast strain PJ69-4A containing DBD-COI1 and AD-JAZ3 constructs was transformed with the yeast expression vector pYES-DEST52 of in which the *GAL1* promoter had been replaced with the *ADH1* promoter (*control*). Selection media (SD, -Trp, -Leu, -Ura, -His) containing 0, 50 or 100 μM JA-Ile were inoculated with 10^3 or 10^4 cells. Photos were taken 5 days after inoculation

We also attempted to construct a Y2H system to detect IAA-dependent interactions between the TIR1/AFB receptor and Aux/IAA proteins. We used AD-IAA1, AD-IAA3 and AD-IAA5 as interactors of DBD-TIR1. However, none of the combinations allowed yeast growth on selection media even with 100 μM IAA. Therefore we were unable to

characterize IAA transport activities of NPFs using the Y2H approach.

Detection of hormone transport activities of NPFs by the Y2H systems

To determine whether there are unidentified Arabidopsis NPFs capable of transporting hormones, we cloned as many NPF cDNAs as possible (45 out of 53 Arabidopsis NPF cDNAs) into yeast expression vectors and introduced them into Y2H systems together with hormone receptor complexes.

ABA transport activities of NPFs were detected by determining the growth of yeast containing DBD-PYR1 and AD-ABI1 on selection media in the presence of 0.1 μM ABA (Fig. 2). Although we had already examined the ABA transport activities of 11 NPFs (NPF4.6/NRT1.2/AIT1, NPF4.5/AIT2, NPF4.1/AIT3, NPF4.2/AIT4, NPF6.3/NRT1.1, NPF4.4/NRT1.13, NPF4.3/NRT1.14, NPF6.2/NRT1.4, NPF6.4/NRT1.3, NPF6.1 and NPF4.7) that were formerly categorized to group I according to their phylogenetic relationships (Tsay et al. 2007), these NPFs were included in the present assay again to compare the activities of all NPFs under identical conditions. Colonies of yeast cells expressing NPF2.5, NPF4.1/AIT3, NPF4.5/AIT2, NPF4.6/AIT1 and NPF5.2/PTR3 grew well even when they were started with a relatively small number of cells (10^3), suggesting that these NPFs transported ABA very efficiently. Significant growth of colonies consisting of cells expressing NPF1.1, NPF8.2/PTR5, NPF5.1, NPF5.3 and NPF5.7 was observed when a larger amount of starter cells (10^4) was used, indicating that these NPFs also transported ABA. Although we previously found weak ABA transport activity of NPF4.2/AIT4 (Kanno et al. 2012), the activity was under the detection level in the present assay conditions. Actually, yeast expressing NPF4.2/AIT4 grew slightly better than cells without the transporter when large amounts of starter cells (10^5) were incubated for a long period (Fig. S2).

Next, we determined the capacities of NPFs to transport GA_1, GA_3 and GA_4 using the Y2H system with DBD-GID1a and AD-GAI (Fig. 2). The growth of yeast on selection media containing 0.1 μM GA_1 suggested that NPF2.3, NPF2.5, NPF2.12/NRT1.6, NPF2.13/NRT1.7, NPF1.1/NRT1.12, NPF1.2/NRT1.11, NPF3.1/Nitr, NPF4.1/AIT3, NPF4.2/AIT4, NPF5.1, NPF5.2/PTR3 and NPF5.7 efficiently transported GA_1 into the yeast cells. Cells expressing NPF2.4, NPF2.6, NPF2.7/NAXT1, NPF2.10/GTR1, NPF8.2/PTR5, and NPF5.6 grew better than cells without the transporters under the same conditions, suggesting that they also transported GA_1. Similar trends were observed when 0.1 μM GA_3 was used as a substrate except that the cells expressing NPF2.6, NPF8.2/PTR5 and NPF5.6 failed to grow. Most of the NPFs that induced

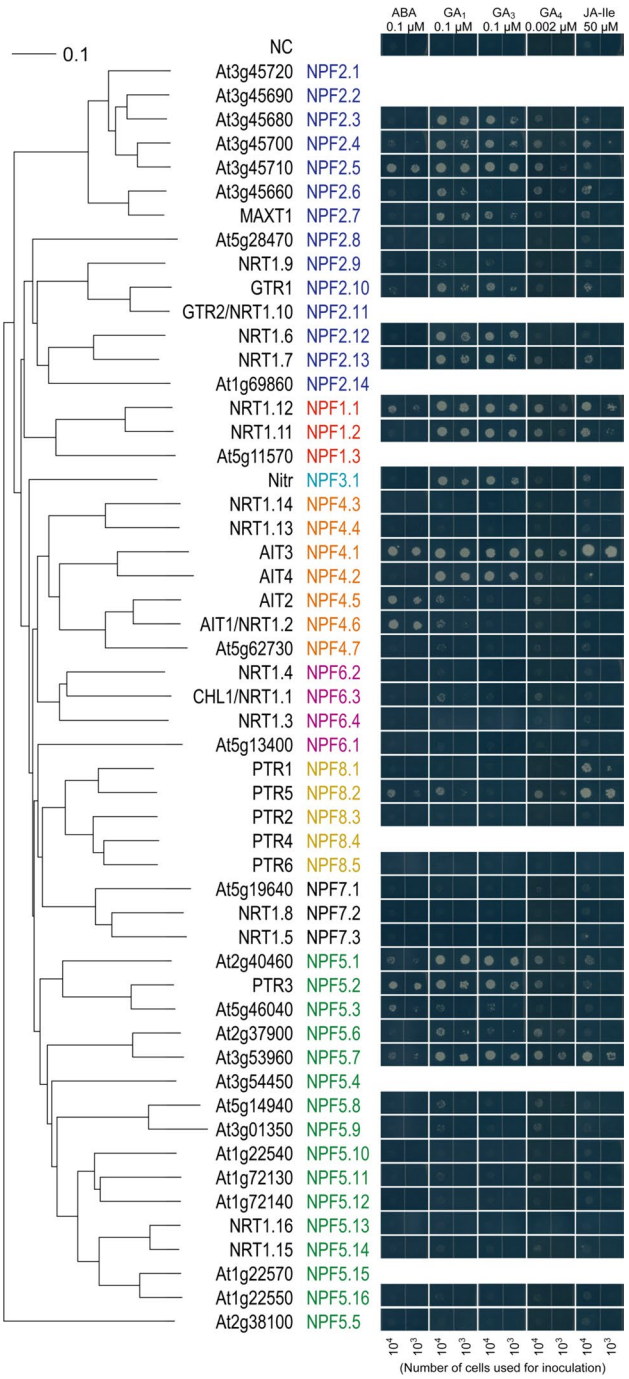

Fig. 2 Detection of hormone transport activities of NPFs. The yeast strain PJ69-4A containing the Y2H receptor complex systems for ABA, GA or JA-Ile was transformed with *NPF* cDNAs cloned into the yeast expression vector pYES-DEST52 in which the *GAL1* promoter had been replaced with the *ADH1* promoter. Selection media (SD, -Trp, -Leu, -Ura, -His) containing 0.1 μM ABA, 0.1 μM GA_1, 0.1 μM GA_3, 0.002 μM GA_4 or 50 μM JA-Ile were inoculated with 10^3 or 10^4 cells. Photos were taken 2 days (GA_4), 3 days (ABA, GA_1 and GA_3), or 5 days (JA-Ile) after inoculation. The *scale bar* indicates a branch length corresponding to 0.1 substitution per site

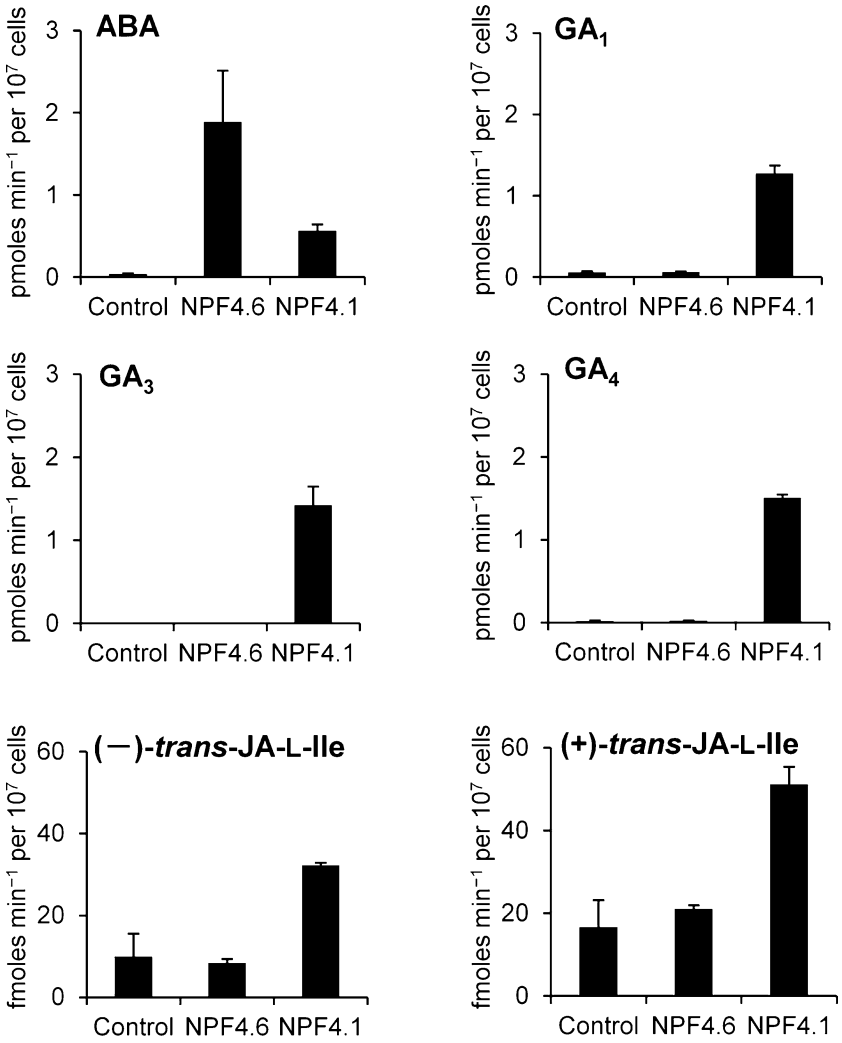

Fig. 3 Direct measurements of hormone transport activities by LC–MS/MS. ABA, GA (GA_1, GA_3 and GA_4) and JA-Ile transport activities (p moles/10^7 cells/min for ABA and GA, f moles/10^7 cells/min for JA-Ile) of NPF4.6/NRT1.2/AIT1 and NPF4.1/AIT3 were determined in the presence of 10 μM of the potential substrates. JA-Ile was applied as a mixture (1:1) of (−)-*trans*-JA-L-Ile and (+)-*trans*-JA-L-Ile, and activities for each compound were calculated independently. Amounts of substrates taken up by yeast cells transformed with the empty vector are shown as control. Mean values of three biological replicates are given with standard deviations

yeast growth in the presence of 0.1 μM GA_3 or GA_1 also induced growth in response to 2 nM GA_4, although NPF2.12/NRT1.6, NPF3.1/Nitr and NPF4.2/AIT4 were less effective.

The JA-Ile transport activities of NPFs were determined by examining their growth effects on yeast cells containing DBD-COI1 and AD-JAZ3 on selection media supplemented with 50 μM JA-Ile (Fig. 2). Colonies of cells expressing NPF1.1/NRT1.12, NPF1.2/NRT1.11, NPF4.1/AIT3, NPF8.1/PTR1, NPF8.2/PTR5 and NPF5.7 exhibited significant growth regardless of the number of cells used for inoculation. Colonies of cells expressing NPF2.4, NPF2.6, NPF2.7/NAXT1, NPF2.10/GTR1, NPF2.13/NRT1.7, NPF3.1/Nitr and NPF5.1 showed slow growth.

Direct examination of hormone transport activities of NPFs by LC–MS/MS

The results presented in Fig. 2 suggest that several NPF proteins transported ABA, GA and/or JA-Ile. However, as the hormone transport activities were detected indirectly based on yeast growth, we could not exclude the possibility that the NPFs induced growth independently of their hormone transport activities. We had demonstrated that NPF4.6/NRT1.2/AIT1 transported ABA whereas NPF4.1/AIT3 transported ABA and GA_3, by directly measuring the hormones taken up by yeast cells using LC–MS/MS (Kanno et al. 2012). These results were confirmed in the present study (Fig. 3). Also, as expected from the Y2H-based transport assays described above, it was shown that NPF4.1/AIT3 transported GA_1 and GA_4 whereas NPF4.6/NRT1.2/AIT1 did not (Fig. 3). In addition, we confirmed that NPF4.1/AIT3 but not AIT4.6/NRT1.2/AIT3 transported JA-Ile (Fig. 3). NPF4.1/AIT3 did not discriminate between (−)-*trans*-JA-L-Ile and (+)-*trans*-JA-L-Ile, as the substrate used was a mixture of both compounds which accumulated to similar levels in yeast cells expressing NPF4.1/AIT3.

Discussion

In the present study, we demonstrated that Y2H systems with receptor complexes for GA, JA-Ile, and ABA enabled the detection of hormone transport activities. As expected, several NPFs enhanced the hormone-dependent formation of receptor complexes in yeast cells in response to exogenous hormones (Fig. 2). LC–MS/MS analysis confirmed that yeast cells expressing the NPFs accumulated the expected hormones at significantly higher levels compared to control cells (Fig. 3).

We previously showed that four NPFs, namely NPF4.1/AIT3, NPF4.2/AIT4, NPF4.5/AIT2 and NPF4.6/NRT1.2/AIT1, transported ABA as indicated by the Y2H assay and/or LC–MS/MS, and that one of them, NPF4.1/AIT3, also transported GA_3 according to LC–MS/MS analysis (Kanno et al. 2012). The present study revealed that a large number of additional NPFs were capable of translocating ABA, GA and/or JA-Ile. We have to caution that there is some uncertainty regarding the detected hormone transport activities of NPFs, for several reasons. First, the growth rates of yeast were determined at times for each substrate: numerous NPFs seemed to promote yeast growth if growth was determined over prolonged incubation periods whereas few NPFs promoted growth significantly over shorter periods. Second, we could not determine the levels of active NPF proteins in the yeast cells. Because we previously found that the addition of a tag to NPF proteins for immuno-detection inhibited their activity, we expressed NPFs without tags in the present assay (Kanno et al. 2012). Also, it has to be noted that the chemical nature of the substrates used in the present assays differed in terms of receptor affinity, membrane permeability, stability, etc. Therefore different substrate concentrations were used and yeast growth was determined over different periods, depending on which hormone was tested. Nevertheless, it appears possible to estimate relative substrate preferences or substrate specificities of the different NPFs. For example, NPF4.6/NRT.12/AIT1 transported ABA but not GA (GA_1, GA_3, GA_4), JA-Ile, jasmonic acid and IAA, suggesting that this NPF protein had a comparatively high specificity for ABA (Fig. 2; Kanno et al. 2012). Consistent with this interpretation, NPF4.6/NRT1.2/AIT1 has been shown to function as an ABA transporter in vivo (Kanno et al. 2012). Similarly, NPF8.1/PTR1 was relatively specific to JA-Ile (Fig. 2). On the other hand, NPF4.1/AIT3 showed a relatively broad substrate specificity for ABA, GA and JA-Ile (Fig. 2) while it did not transport JA and IAA (Kanno et al. 2012). NPF2.12/NRT1.6 and NPF5.6 were relatively specific to GAs, but the two proteins preferred different GA species (Fig. 2). In many cases, closely related NPFs showed similar substrate specificities (Fig. 2). However, NPFs capable of transporting a particular substrate are widely distributed across the protein family, and phylogenetic relationships are not sufficient to predict the substrate specificity of a particular NPF protein. Recently the 3D structure of NPF6.3/NRT1.1/CHL1 has been resolved and possible mechanisms of nitrate recognition have been discussed (Parker and Newstead 2014; Sun et al. 2014). In the near future, it should become possible to correlate NPF substrates specificities with 3D structures.

Several NPFs have been characterized as nitrate or peptide transporters, leading to the assumption that nitrate transporters (NRTs) do not transport peptides whereas peptide transporters (PTRs) do not transport nitrate (Tsay et al. 2007). More recently it became clear that NPFs accepted multiple substrates (Kanno et al. 2012; Krouk et al. 2010; Nour-Eldin et al. 2012). Here we identified additional NPFs capable of transporting ABA, GA and/or JA-Ile, and it is conceivable that at least some of them function as hormone transporters in vivo. Since NPFs transport the same substrates, their physiological functions might be highly redundant. In apparent contrast to this interpretation, NPF mutants often s exhibit characteristic phenotypes (Almagro et al. 2008; Fan et al. 2009; Guo et al. 2003; Hsu and Tsay 2013; Huang et al. 1999; Karim et al. 2007; Komarova et al. 2008; Li et al. 2010; Lin et al. 2008; Wang and Tsay 2011). Although these phenotypes have been discussed mainly in the context of nitrate and peptide transport, the phenotypic effects may be due to defects in hormone transport. In the case of NRT1.1, the competition between two potential substrates, nitrate and IAA, determines the physiological responses (Krouk et al. 2010). Similar competitive interactions between nitrate, peptides, and various hormones may form the basis for the integration of environmental and physiological information that is linked to the relative availability of the different substrates.

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