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Sophie Léran*[1], Kranthi Varala*[2], Jean-Christophe Boyer[1], Maurizio Chiurazzi[3], Nigel Crawford[4], Françoise Daniel-Vedèle[5], Laure David[5], Rebecca Dickstein[6], Emilio Fernandez[7], Brian Forde[8], Walter Gassmann[9], Dietmar Geiger[10], Alain Gojon[1], Ji-Ming Gong[11], Barbara A Halkier[12], Jeanne M Harris[13], Rainer Hedrich[10], Anis M Limami[14], Doris Rentsch[15], Mitsunori Seo[16], Yi-Fang Tsay[17], Mingyong Zhang[18], Gloria Coruzzi[2], Benoît Lacombe[1]

[1]Biochimie et Physiologie Moléculaire des Plantes, UMR CNRS/INRA/UM2/SupAgro, Institut de Biologie Intégrative des Plantes “Claude Grignon”, Place Viala, 34060 Montpellier, France

[2]New York University, Department of Biology, Center for Genomics & Systems Biology, 12 Waverly Place, New York, N.Y. 10003, USA

[3]Institute of Genetics e Biophysics "Adriano Buzzati-Traverso", CNR, Via Pietro Castellino 111, 80131 Naples, Italy

[4]Section of Cell and Developmental Biology, UC San Diego 9500 Gilman Dr., La Jolla, CA 92093, USA

[5]INRA AgroParisTech, UMR1318 Institut Jean-Pierre Bourgin, RD10, 78026 Versailles Cedex, France

[6]Department of Biological Sciences, University of North Texas, 1155 Union Circle #305220, Denton, TX 76203, USA

[7]Depto. de Bioquímica y Biología Molecular, Edificio Severo Ochoa Baja E, Campus de Rabanales, E-14071-Córdoba, Spain

[8]Centre for Sustainable Agriculture, Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

[9]Division of Plant Sciences, CS Bond Life Sciences Center and Interdisciplinary Plant Group, University of Missouri, Columbia, USA

[10]Universität Würzburg, Julius-von-Sachs-Institut für Biowissenschaften, Lehrstuhl für Molekulare Pflanzenphysiologie und Biophysik, Julius-von-Sachs-Platz 2, 97082 Würzburg, Germany

[11]National Key Laboratory of Plant Molecular Genetics and National Center for Plant Gene Research (Shanghai), Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

[12]DynaMo Centre of Excellence, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

[13]Department of Plant Biology, 315 Jeffords Hall, 63 Carrigan Drive, University of Vermont, Burlington, VT05405, USA

[14]UMR 1345 Research Institute of Horticulture and Seeds (INRA, Agrocampus-Ouest, University of Angers), SFR 4207 Quasav, 2 Bd Lavoisier, 49045 Angers cedex, France

[15]University of Bern, Institute of Plant Sciences, Altenbergrain 21, 3013 Bern, Switzerland

[16]RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa 230-0045, Japan

[17]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

[18]Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

* Contributed equally to this article

Corresponding author: Lacombe, B. (benoit.lacombe@supagro.inra.fr)

Members of the plant NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER (NRT1/PTR) family display protein sequence homology with the SLC15/PepT/PTR/POT family of peptide transporters in animals. Compared to their animal and bacterial counterparts, these plant proteins transport a wide variety of substrates: nitrate, peptides, amino acids, dicarboxylates, glucosinolates, IAA, and ABA. The phylogenetic relationship of the members of the NRT1/PTR family in 31 fully sequenced plant genomes allowed the identification of unambiguous clades, defining 8 sub-families. The phylogenetic tree was used to determine a unified nomenclature of this family named NPF, for NRT1/PTR FAMILY. We propose that the members should be named accordingly: NPFX.Y, where X denotes the subfamily and Y the individual member within the species.

Plant NRT1/PTR proteins display homology with proteins from other kingdoms

In plants, a group of membrane proteins belonging to the NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER (NRT1/PTR) family display sequence homology with proteins ubiquitously present across all major kingdoms of life [1] such as bacteria [2], fungi [3], and animals [4, 5]. In these organisms, several family names are used to describe these proteins: PROTON-COUPLED OLIGOPEPTIDE TRANSPORTER (POT), PEPTIDE TRANSPORTER (PepT/PTR) or SOLUTE CARRIER 15 (SLC15). These membrane proteins display a predicted conserved structural arrangement of 12 transmembrane domains connected by short peptide loops. The three-dimensional arrangement of these domains was recently characterized for two bacterial homologs (PepTSo and PepTSt) in both open and closed conformations [2, 6]. Most non-plant members of this family are involved in nitrogen acquisition in the form of di- and tripeptides [7]. For example, the human PepT1 and PepT2 proteins are involved in dietary nitrogen uptake. These proteins are also involved in the transport of β-lactam antibiotics [7]. The rather high substrate selectivity of the above mentioned transporters is not shared by their plant counterparts.

The first plant member discovered, known as AtNRT1.1 or CHL1, was identified in *Arabidopsis* (*Arabidopsis thaliana*) as a nitrate transporter [8]. The fifteen years following this identification were punctuated by the cloning of other nitrate transporters in different plants [9], but also by the demonstration that some plant NRT1/PTR family members could behave as dipeptide transporters [10]. As a consequence, the presence of nitrate transporters was believed to be a specific feature of this family in plants. But several studies in the last few years demonstrate

that an even wider range of molecules are transported by some family members [11, 12]. Auxin, ABA and glucosinolates are three new NRT1/PTR substrates [13-16]. Furthermore, some members are able to transport two different substrates: nitrate/IAA, nitrate/ABA, nitrate/glucosinolates or peptides/amino acids. The role of specific members is described below.

Phylogenetic relationship is the basis of the unified nomenclature

To date, the different family members were mainly named according to their first identified substrate: NRT1/nitrate, PTR/peptide, AIT/ABA, GTR/glucosinolate. But, as discussed above several members transport more than one substrate. Furthermore, sequence homologies do not correlate with substrate selectivity. We have therefore decided to develop a unified nomenclature for this protein family (Table 1). The first step was to identify a unique name for these proteins. An open and fruitful discussion was conducted within the research community and NPF (for NRT1 PTR FAMILY) was the consensus name agreed upon for this family.

The next step was to establish the phylogenetic relationships between the different family members. To that end, we first identified the amino acid sequence of all members of this family within the 31 fully sequenced genomes and two outgroups (*Physcomitrella patens* and *Selaginella moellendorffii,* two primitive non-seed plants), using the BigPlant phylogenomic pipeline [17]. Briefly, proteins from all species were queried against each other, using BLAST, to determine the level of homology, if any, between them. The BLAST e-value was used as a proxy for distance to cluster the proteins, using OrthoMCL [18], and identify gene families across 33 genomes (Figure 1 and Figure S1 in the supplementary material online). This fully autonomous

clustering algorithm grouped all known NPF members from *Arabidopsis thaliana* into a single protein family containing 2,398 sequences from 33 genomes. In each of these sequenced genomes, there are between 51 (in *Capsella rubella*) and 139 (in *Malus domestica*) unique members of this family (Figure S1 in the supplementary material online). All sequences were aligned using MAFFT [19] and the alignment was used to perform a parsimony analysis in PAUP* [20] according to this automated phylogenomic pipeline [17].

This analysis allows the identification of eight unambiguous clades in the family. We assigned a number (from 1 to 8) for each clade and designated them as subfamilies. Within the clades, a second number identifies each member, according to their position in the phylogenetic tree (Figure 1). This number does not imply relationship, chromosomal location or order of characterization. Of the 2,398 protein sequences, 14 were left out of all clades due to poor alignments to other family members and are hence placed outside the major clades in the phylogenetic tree. These sequences show a significant similarity to the NPF members, but align poorly with the other members. These sequences could represent proteins that have recently lost function and are in the process of degradation or alternately represent chimeric gene models. Each of the remaining 2,384 sequences in the analysis belongs to one of the clades and is assigned a specific two-number code (Table S1 in the supplementary material online).

We recommend that future numbering of newly identified members should take into account this convention, with two or three letters to identify the species, followed by NPF, followed by the clade number, and finally the specific number according to the order in which they are identified. Consequently, this second number

is used to differentiate genes within the species and does not reflect orthologous relationships, meaning that GmNPF6.1 is not necessarily closer to OsNPF6.1 than to OsNPF6.4. A similar rule was adopted in other nomenclatures such as for ATP-BINDING CASSETTE (ABC) proteins [21] and HIGH AFFINITY POTASSIUM TRANSPORTER (HKT) proteins [22] to avoid constant renumbering of members within a species.

Subfamilies NPF1 to NPF8: from structure to function

NPF1 (192 members)

In this subfamily, one member, MtNPF1.7 previously named NIP/LATD (NUMEROUS INFECTIONS AND POLYPHENOLICS/LATERAL ROOT-ORGAN DEFECTIVE), from *Medicago truncatula* has been characterized [23] and behaves as a high-affinity nitrate transporter with a second unknown function [24, 25]. MtNPF1.7 is involved in nodulation and root architecture [23].

NPF2 (365 members)

This subfamily contains well-established nitrate and glucosinolates transporters. Members of this family show a wide range of tissue and developmental specificity in *Arabidopsis*. Nitrate influx and efflux transporters belong to the NPF2 subfamily. In *Arabidopsis*, AtNPF2.1 to AtNPF2.7 were assigned to the same subgroup [26]. The efflux transporter AtNPF2.7 (NAXT1, [26]) is expressed in the cortex of mature roots, whereas AtNPF2.9 (NRT1.9, [27]) is expressed in root companion cells where it is involved in phloem loading. Nitrate-dependent early embryo development requires

the expression of the low affinity nitrate transporter AtNPF2.12 (NRT1.6, [28]). Source-to-sink nitrogen remobilization is an important physiological process, mediated by different forms of organic nitrogen forms and also by nitrate itself; the AtNPF2.13 protein (NRT1.7, [29]) is responsible for phloem loading in the source leaves.

Five *Arabidopsis* NPF members are capable of transporting glucosinolates: AtNPF2.9 (NRT1.9), 2.10 (GTR1), 2.11 (GTR2/NRT1.10), 2.13 (NRT1.7) and 2.14. Two of these NPF members, AtNPF2.10 (GTR1) and AtNPF2.11 (GTR2/NRT1.10), are nitrate transporters [15].

NPF3 (109 members)

This is the smallest subfamily. The first characterized member was *Cucumis sativus* CsNPF3.2 (CsNitr1). The *CsNPF3.2* gene gives rise to two splice isoforms, the longer of which (*CsNitr1-L*) encodes a protein with a chloroplast targeting signal and inner chloroplastic membrane localization. The shorter isoform of CsNPF3.2 (CsNitr1-S) expressed in yeast (*Saccharomyces cerevisiae*) causes reduced nitrite accumulation [30]. This led to the suggestion that CsNitr1-L functions as a chloroplast nitrite uptake transporter to remove toxic nitrite from the cytosol [30]. The *Arabidopsis* AtNPF3.1 and *Vitis vinifera* VvNPF3.2 orthologs do not possess predicted chloroplast targeting signals and AtNPF3.1 is a plasma membrane localized protein (L. David and F. Daniele-Vedèle, unpublished results). AtNPF3.1 and VvNPF3.2 mediate nitrate and nitrite uptake into *Xenopus* oocytes with similar low affinities (S. Pike and W. Gassmann, unpublished).

NPF4 (350 members)

AtNPF4.6 (NRT1.2/ AIT1) was the second nitrate transporter to be characterized in plants [31]. Members of this subfamily have recently been demonstrated to be ABA importers [14]. When expressed in yeast, AtNPF4.1 (AIT3) transported GA (GA_3) as well as ABA [14].

In rice (*Oryza sativa*), OsNPF4.1 (SP1, [32]) regulates the development of inflorescences. However, expression and analysis in *Xenopus* oocytes and yeast did not support a function of this protein in the transport of nitrate, dipeptide, or histidine. Sequence homology of OsNPF4.1 to *Arabidopsis* ABA transporters (Figure 1 and Table S1 in the supplementary material online) and the developmental role it plays suggests that it might be involved in ABA transport.

NPF5 (663 members)

This is the largest subfamily accounting for 25% of the sequences. Surprisingly, only one member has been functionally characterized, AtNPF5.2 (PTR3, [33]). Another member, OsNPF5.5, did not show di- or tri-peptide uptake activity when expressed in yeast (OsPTR3, [34]).

NPF6 (227 members)

This subfamily includes the first identified member of NRT1/PTR family: NRT1.1 [8], now named AtNPF6.3. This protein is the first transceptor identified in plants [35-38]:

it is a dual affinity nitrate influx transporter and a nitrate sensor. The nitrate-dependent lateral root phenotype of a *chl1* mutant is due to its ability to transport auxin [37]. Nitrate efflux activity by AtNPF6.3 (and AtNPF6.2) was also demonstrated [39].

Three other members have been demonstrated to be nitrate transporters: AtNPF6.2 (AtNRT1.4, [40]) AtNPF6.4 (AtNRT1.3, [41]) and MtNPF6.8 (MtNRT1.3, [42]). Expression of *MtNPF6.8* in Xenopus oocytes showed that the encoded protein is a dual affinity nitrate transporter [42]. The same study provided evidence for the involvement of MtNPF6.8 in the mediation of the effect of nitrate on primary root elongation in *Medicago truncatula* seedlings. Future studies will have to show whether or not these members operate as transceptors too.

NPF7 (206 members)

Three proteins in this subfamily have been functionally characterized. Two different substrates have been identified: nitrate for AtNPF7.2 (NRT1.8 [43]) and AtNPF7.3 (NRT1.5, [44, 45]); as well as dipeptides for OsNPF7.3 (OsPTR6, [34]). Furthermore, AtNPF7.3 is a bidirectional transporter involved in nitrate influx and efflux.

Expression of two other members in yeast, OsNPF7.1 (OsPTR4) and OsNPF7.4 (OsPTR5), did not confer di- or tri-peptide uptake activity [34]).

NPF8 (273 members)

In *Arabidopsis*, subfamily 8 contains dipeptide transporters: AtNPF8.1 (PTR1, [46]), AtNPF8.2 (PTR5, [46]) and AtNPF8.3 (PTR2). AtNPF8.3 is also able to transport histidine in yeast [47-49]. AtNPF8.4 (PTR4) and AtNPF8.5 (PTR6) are tonoplast-localized transporters, which have been resistant to functional characterization in yeast and Xenopus oocytes [50].

In rice OsNPF8.9 (OsNRT1/OsNRT1.1 [51]) is a low-affinity nitrate transporter. Expression of another member in yeast and Xenopus oocytes, OsNPF8.20 (OsPTR9), did not confer nitrate, di- or tri-peptide uptake activity [52].

Concluding remarks

It was the increasing recognition of the importance of the NPF proteins in *Arabidopsis*, as well as in other plants, that prompted us to propose our unified nomenclature. Besides the need to understand the specific role of each transporter, it is important to understand the molecular basis for their different selectivities. This will provide the foundation for future investigations into the relationship between function and phylogenetic position. Moreover, the phylogenetic analysis now provides a framework to inspire functional experimentation.

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Table 1: Revised nomenclature for the characterized NPF members[a]

Species	Previous Name	New Name	Locus identification number	Substrates	Refs.
Arabidopsis thaliana	NRT1.11	AtNPF1.2	AT1G52190.1	Unknown	[27]
	NAXT1	AtNPF2.7	AT3G45650.1	Nitrate	[26]
	NRT1.9	AtNPF2.9	AT1G18880.1	Nitrate/glucosinolates	[15, 27]
	GTR1	AtNPF2.10	AT3G47960.1	Glucosinolates/nitrate	[15]
	GTR2/NRT1.10	AtNPF2.11	AT5G62680.1	Glucosinolates/nitrate	[15]
	NRT1.6	AtNPF2.12	AT1G27080.1	Nitrate	[28]
	NRT1.7	AtNPF2.13	AT1G69870.1	Nitrate/glucosinolates	[15, 29]
	Nitr	AtNPF3.1	AT1G68570.1	Nitrate/nitrite	[30]
	AIT3	AtNPF4.1	AT3G25260.1	Aba/ga3	[14]
	AIT4	AtNPF4.2	AT3G25280.1	Unknown	[14]
	NRT1.14	AtNPF4.3	AT1G59740.1	Unknown	Unpublished
	NRT1.13	AtNPF4.4	AT1G33440.1	Unknown	Unpublished
	AIT2	AtNPF4.5	AT1G27040.1	Unknown	[14]
	NRT1.2/AIT1	AtNPF4.6	AT1G69850.1	Nitrate/ABA	[14, 31]
	PTR3	AtNPF5.2	AT5G46050.1	Dipeptides	[33]
	NRT1.16	AtNPF5.13	AT1G72125.1	Nitrate	Unpublished
	NRT1.15	AtNPF5.14	AT1G72120.1	Nitrate	Unpublished
	NRT1.4	AtNPF6.2	AT2G26690.1	Nitrate	[40]
	NRT1.1	AtNPF6.3	AT1G12110.1	Nitrate/IAA	[8, 37]
	NRT1.3	AtNPF6.4	AT3G21670.1	Nitrate	[41]
	NRT1.8	AtNPF7.2	AT4G21680.1	Nitrate	[43]
	NRT1.5	AtNPF7.3	AT1G32450.1	Nitrate	[44]
	PTR1	AtNPF8.1	AT3G54140.1	Dipeptides	[46]
	PTR5	AtNPF8.2	AT5G01180.1	Dipeptides	[46]
	PTR2/NTR1	AtNPF8.3	AT2G02040.1	Dipeptides	[47]
	PTR4	AtNPF8.4	AT2G02020.1	Unknown	[50]
	PTR6	AtNPF8.5	AT1G62200.1	Unknown	[50]
Medicago truncatula	NIP/LATD	MtNPF1.7	Medtr1g009200.1	Nitrate/unknown	[23]
	MtNRT1.3	MtNPF6.8	Medtr5g085850.1	Nitrate	[42]
Oryza sativa	OsPTR2	OsNPF2.2	Os12g44100.1	Unknown	[34]
	SP1	OsNPF4.1	Os11g12740.1	Unknown	[32]
	OsPTR3	OsNPF5.5	Os10g33210.1	Unknown	[34]
	OsPTR5	OsNPF7.4	Os04g50940.1	Unknown	[34]
	OsPTR6	OsNPF7.3	Os04g50950.1	Unknown	[34]
	OsPTR4	OsNPF7.1	Os07g41250.1	Unknown	[34]
	OsPTR9	OsNPF8.20	Os06g49250.1	Unknown	[52]
	OsNTR1.1	OsNPF8.9	Os03g13274.2	Nitrate	[51]
	OsPTR8	OsNPF8.5	Os03g51050.1	Unknown	[34]
	OsPTR1	OsNPF8.2	Os07g01070.1	Unknown	[34]
	OsPTR7	OsNPF8.1	Os01g04950.1	Unknown	[34]
Cucumis sativus	CsNitr1	CsNPF3.2	Cucsa.337560.1	Nitrite	[30]

[a]The NPF members with an affected previous name are listed with their new name, locus identification number and, if known, substrate(s).

Legends

Figure 1. Phylogenetic tree of the NPF gene family in plants. Parsimony analysis of the 2,398 sequences from 33 fully sequenced genomes (Listed in Table S1 in the supplementary material online) identifies 8 clades in the NRT1/PTR family. A graphical view of the tree has been generated using FigTree (the software can be freely downloaded, http://tree.bio.ed.ac.uk/software/figtree/). The NPF tree file is available in the supplementary material online.