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**A unified nomenclature of NITRATE TRANSPORTER 1/PEPTIDE
TRANSPORTER family members in plants**

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12

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

25

1

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

3 In plants, a group of membrane proteins belonging to the NITRATE TRANSPORTER
4 1/PEPTIDE TRANSPORTER (NRT1/PTR) family display sequence homology with
5 proteins ubiquitously present across all major kingdoms of life [1] such as bacteria
6 [2], fungi [3], and animals [4, 5]. In these organisms, several family names are used
7 to describe these proteins: PROTON-COUPLED OLIGOPEPTIDE TRANSPORTER
8 (POT), PEPTIDE TRANSPORTER (PepT/PTR) or SOLUTE CARRIER 15 (SLC15).
9 These membrane proteins display a predicted conserved structural arrangement of
10 12 transmembrane domains connected by short peptide loops. The three-
11 dimensional arrangement of these domains was recently characterized for two
12 bacterial homologs (PepTSo and PepTSt) in both open and closed conformations [2,
13 6]. Most non-plant members of this family are involved in nitrogen acquisition in the
14 form of di- and tripeptides [7]. For example, the human PepT1 and PepT2 proteins
15 are involved in dietary nitrogen uptake. These proteins are also involved in the
16 transport of β -lactam antibiotics [7]. The rather high substrate selectivity of the above
17 mentioned transporters is not shared by their plant counterparts.

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

1 that an even wider range of molecules are transported by some family members [11,
2 12]. Auxin, ABA and glucosinolates are three new NRT1/PTR substrates [13-16].
3 Furthermore, some members are able to transport two different substrates:
4 nitrate/IAA, nitrate/ABA, nitrate/glucosinolates or peptides/amino acids. The role of
5 specific members is described below.

6

7 **Phylogenetic relationship is the basis of the unified nomenclature**

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

16 The next step was to establish the phylogenetic relationships between the
17 different family members. To that end, we first identified the amino acid sequence of
18 all members of this family within the 31 fully sequenced genomes and two outgroups
19 (*Physcomitrella patens* and *Selaginella moellendorffii*, two primitive non-seed plants),
20 using the BigPlant phylogenomic pipeline [17]. Briefly, proteins from all species were
21 queried against each other, using BLAST, to determine the level of homology, if any,
22 between them. The BLAST e-value was used as a proxy for distance to cluster the
23 proteins, using OrthoMCL [18], and identify gene families across 33 genomes (Figure
24 1 and Figure S1 in the supplementary material online). This fully autonomous

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

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

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

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

7

8 **Subfamilies NPF1 to NPF8: from structure to function**

9

10 *NPF1 (192 members)*

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

16

17 *NPF2 (365 members)*

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

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

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

10

11

12 *NPF3 (109 members)*

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

1

2 *NPF4 (350 members)*

3 AtNPF4.6 (NRT1.2/ AIT1) was the second nitrate transporter to be characterized in
4 plants [31]. Members of this subfamily have recently been demonstrated to be ABA
5 importers [14]. When expressed in yeast, AtNPF4.1 (AIT3) transported GA (GA₃) as
6 well as ABA [14].

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

13

14

15 *NPF5 (663 members)*

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

20

21

22 *NPF6 (227 members)*

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

1 it is a dual affinity nitrate influx transporter and a nitrate sensor. The nitrate-
2 dependent lateral root phenotype of a *chl1* mutant is due to its ability to transport
3 auxin [37]. Nitrate efflux activity by AtNPF6.3 (and AtNPF6.2) was also demonstrated
4 [39].

5 Three other members have been demonstrated to be nitrate transporters:
6 AtNPF6.2 (AtNRT1.4, [40]) AtNPF6.4 (AtNRT1.3, [41]) and MtNPF6.8 (MtNRT1.3,
7 [42]). Expression of *MtNPF6.8* in *Xenopus* oocytes showed that the encoded protein
8 is a dual affinity nitrate transporter [42]. The same study provided evidence for the
9 involvement of MtNPF6.8 in the mediation of the effect of nitrate on primary root
10 elongation in *Medicago truncatula* seedlings. Future studies will have to show
11 whether or not these members operate as transceptors too.

12

13

14 *NPF7 (206 members)*

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

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

21

22

23 *NPF8 (273 members)*

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

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

9

10 **Concluding remarks**

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

18

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4

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Table 1: Revised nomenclature for the characterized NPF members^a

Species	Previous Name	New Name	Locus identification number	Substrates	Refs.	
<i>Arabidopsis thaliana</i>	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]	
	<i>Medicago truncatula</i>	NIP/LATD	MtNPF1.7	Medtr1g009200.1	Nitrate/unknown	[23]
		MtNRT1.3	MtNPF6.8	Medtr5g085850.1	Nitrate	[42]
	<i>Oryza sativa</i>	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]	
<i>Cucumis sativus</i>	CsNitr1	CsNPF3.2	Cuca.337560.1	Nitrite	[30]	

^aThe NPF members with an affected previous name are listed with their new name, locus identification number and, if known, substrate(s).

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1 **Legends**

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

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