Acta Biochimica Polonica

Vol. 55 No. 4/2008, 663–671

on-line at: www.actabp.pl

Review

The plant Nudix hydrolase family

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Received: 04 October, 2008; revised: 19 November, 2008; accepted: 04 December, 2008
available on-line: 16 December, 2008

Nudix hydrolases are a family of proteins defined by a conserved amino-acid sequence GX_5-EX_7REUXEEXGU, where U is a hydrophobic residue. These enzymes are widely distributed among all classes of organisms and catalyze, with varying degrees of substrate specificity, the hydrolysis of a variety of nucleoside diphosphate derivatives: nucleoside di- and triphosphates and their oxidized forms, dinucleoside polyphosphates, nucleotide sugars, NADH, coenzyme A and the mRNA cap. Nudix proteins are postulated to control the cellular concentration of these compounds. The genome of the model plant *Arabidopsis thaliana* contains 29 genes coding for putative Nudix hydrolases. Recently, several *Arabidopsis* Nudix genes have been cloned and their products characterized. This review summarizes current knowledge on these plant enzymes and discusses their possible cellular functions.

Keywords: MutT, Nudix, hydrolase, pyrophosphohydrolase, plant, *Arabidopsis thaliana*

INTRODUCTION

Nudix hydrolases are a family of pyrophosphatases containing a highly conserved amino-acid sequence, the Nudix box GX_5Ex_7REUXEEXGU, where U is a bulky hydrophobic amino acid such as Ile, Leu or Val. The first member of the Nudix family to be characterized was the MutT protein of *Escherichia coli*, hence the original name of this group of enzymes, the MutT family. The MutT hydrolase preferentially hydrolyzes 8-oxo-(d)GTP, a modified nucleotide that occurs in the cellular nucleotide pool as a result of oxidative stress, with the potential to cause both replicational and transcriptional errors (for review see Arczewska & Kuśmierek, 2007).

It was subsequently established that enzymes sharing the characteristic amino-acid motif catalyze the hydrolysis of a variety of **nu**cleoside **di**phosphate compounds linked to a moiety, **x**, hence the acronym, Nudix. The hydrolysis reaction can be described by the equation NDP-X + $H_2O \rightarrow$NMP + P-X, where NDP-X represents a nucleoside diphosphate linked to the moiety X, NMP is a nucleoside monophosphate, and P-X is phosphate linked to the moiety X. The range of substrates includes (d)NTPs (both canonical and modified), dinucleoside polyphosphates, various coenzymes, nucleotide sugars, and alcohols (Bessman *et al*., 1996). In addition, other compounds containing pyrophosphate bonds may also be hydrolysed. Hydrolysis of NDPs (Fisher *et al*., 2004; Xu *et al*., 2004; Hori *et al*., 2005; Ito *et al*., 2005), the mRNA cap and 5′ triphosphorylated RNA (Wang *et al*., 2002; Gunawardana *et al*., 2008 & Deana *et al*., 2008), non-nucleoside substrates such as diphosphoinositol polyphosphates DIPs (Safrany *et al*., 1998; 1999a; 1999b), 5-phosphoribosyl 1-diphosphate PRPP (Fisher *et al*., 2002), thiamine pyrophosphate TPP (Lawhorn *et al*., 2004), and dihydroneopterin triphosphate DHNTP (Klaus *et al*., 2005; Gabelli *at al*., 2007) has also been observed in the presence of Nudix enzymes. All these substrates are either potentially toxic compounds, cell signaling molecules,

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Abbreviations: Ap_3A, diadenosine-5′,5‴-P^1,P^3-triphosphate; Ap_4A, diadenosine-5′,5‴-P^1,P^4-tetraphosphate; Ap_5A, diadenosine-5′,5‴-P^1,P^5-pentaphosphate; Ap_6A, diadenosine-5′,5‴-P^1,P^6-hexaphosphate; DHNTP, 7,8-dihydroneopterin triphosphate, 8-oxo(d)GTP, 8-oxo-7,8-dihydro-2′-(deoxy)guanosine 5′-triphosphate; PRPP, 5-phospho-α-D-ribosyl diphosphate; PP-$InsP_5$, diphosphoinositol pentakisphosphate.

or metabolic intermediates whose concentrations require modulation during the cell cycle. Thus, it has been postulated that the role of Nudix hydrolases is to sanitize or regulate the accumulation of these metabolites (Bessman *et al*., 1996).

Usually, Nudix hydrolases are small proteins (16–35 kDa) consisting of two domains: an N-terminal domain and a C-terminal catalytic domain. The Nudix box, usually located in the C-terminal domain, forms a β strand-loop-α helix-loop structure which functions as a cation-binding and catalytic site. This motif, in association with additional regions which differ depending on the specificity of the enzyme, form the Nudix fold, a structural α/β/α sandwich that is commonly present in Nudix hydrolases (for review see Mildvan *et al*., 2005). This structural feature is shared by the C-terminal domain of isopentenyl diphosphate isomerases (Bonanno *et al*., 2001) and MutY-type DNA glycosylases (Volk *et al*., 2000). It has been suggested that the MutY-type DNA glycosylases and isopentenyl diphosphate isomerases, together with hydrolases, form a large Nudix suprafamily with a common evolutionary origin (McLennan, 2006).

In the majority of cases studied, substrate hydrolysis occurs by nucleophilic substitution at phosphorus, with variation in the number and role of divalent cations. However, in members of the GDP-mannose-mannosyl hydrolase (GDPMH) family which have a modified Nudix sequence, hydrolysis is carried out by nucleophilic substitution at carbon (Gabelli *et al*., 2004; Legler *et al*., 2000). This variation illustrates the mechanistic diversity of Nudix enzymes. Crystallographic and mutational studies of several Nudix enzymes have confirmed the involvement of the conserved residues of the Nudix box in the coordination of divalent cations and in catalysis (for review see Mildvan *et al*., 2005).

Most Nudix hydrolases require an alkaline pH and the presence of divalent cations (usually Mg^{2+} or Mn^{2+}) for full activity. Many members of this family are also strongly inhibited by low levels of fluoride (McLennan, 2000). It has been suggested that this inhibition may result from the blocking of the active site by an MgF_3 complex (Fletcher *et al*., 2002).

The defining characteristic of Nudix enzymes is their ability to catalyze the hydrolysis of a variety of nucleoside diphosphate derivatives with varying degrees of specificity. The diversity of substrates implies a mechanism for the discrimination and recognition of specific molecules. Indeed, the presence of particular amino-acid residues, located up or downstream of the Nudix box, is often predictive of specificity for a particular substrate. It has been suggested that Nudix enzymes may be divided into subfamilies based on their preferred substrate (Xu *et al*., 2000). Table 1 summarizes present knowledge of these additional conserved regions identified in some Nudix proteins.

A recent BLAST search (Altschul *et al*., 1997) of sequence databases has revealed over 2500 open reading frames (ORFs) potentially encoding Nudix proteins, from more than 300 species. The number of Nudix representatives in each species varies from one in *Mycoplasma* sp. to over 50 in eukaryotes (Kraszewska, unpublished).

In the recently sequenced genomes of *Arabidopsis thaliana* (130 Mb), *Oryza sativa* (430 Mb), *Populus trichocarpa* (480 Mb) and *Vitis vinifera* (500 Mb), the numbers of genes coding for putative Nudix proteins are 32, 33, 53 and 30, respectively. In relation to genome size, the highest number of putative Nudix proteins is present in *Arabidopsis* (listed in Table 2). This could be due to the environmental adaptability of this plant species, as has previously been suggested for *Deinococcus radiodurans*, a bacterium extremely resistant to radiation (Xu *et al*., 2001).

To date, several *Arabidopsis* genes encoding Nudix proteins have been cloned and characterized. This review summarizes these results with emphasis on the possible cellular functions of the proteins.

MULTI-SUBSTRATE AtNUDT1 HYDROLASE

The first characterized Nudix hydrolase from *A. thaliana* was a protein encoded by the At1g68760 gene. As this enzyme displays none of the conserved amino acids predictive for a particular substrate (Table 1), its activity was tested with several compounds. In the presence of 5 mM Mn^{2+} the preferred

Table 1. Additional conserved regions identified in some Nudix proteins

Additional motif	Major substrate	References
Proline (P) 15 or 16 aa downstream of the Nudix box	ADP-ribose	Dunn *et al.*, 1999
Tyrosine (Y) 16–18 aa downstream of the Nudix box	Ap_4A	Dunn *et al.*, 1999
LLTXR[SA]X_3RX_3GX_3FPGG	Coenzyme A	Gasmi *et al.*, 2001
SQX_2WPXPXS (e.g. SQPWPFPQS)	NADH	Frick *et al.*, 1995
GGGX_5EX_7REUXEEXGUX_2GX_6G	Ap_nA (n>4), PP-InsP	Yang *et al.*, 1999
NGD or GE	UDP-sugars	Xu *et al*., 2004
L[VL]VRK and AANE	m^5UTP and UTP	Xu *et al*., 2003

Table 2. The Nudix hydrolases of *Arabidopsis thaliana*

Locus	Protein name	Protein length (aa)	Predicted cellular localization*	Predicted substrate	Recognized major substrates	Reference
Chromosome 1						
At1g68760	AtNUDT 1	148	C	unknown	NADH, DHNTP, dNTP, 8-oxo-(d)GTP	Dobrzanska *et al*., 2002; Klaus *et al*., 2005; Ogawa *et al*., 2005; Yoshimura *et al*., 2007
At1g12880	AtNUDT12	204	M	Ap_6A, PP-InsP		
At1g14860	AtNUDT18	177	M	Ap_6A, PP-InsP		
At1g18300	AtNUDT4	208	C	Ap_6A, PP-InsP		
At1g28960	AtNUDT15	294	M	Coenzyme A	CoenzymeA	Ogawa *et al*., 2008
At1g30110	AtNUDT25	176	C	Ap_4A	Ap_4A	Yoshimura *et al*., 2007; Szurmak *et al*., 2008
At1g73540	AtNUDT21	199	CH	Ap_6A, PP-InsP		
At1g79690	AtNUDT3	733	C	unknown		
Chromosome 2						
At2g01670	AtNUDT17	183	M	Ap_6A, PP-InsP		
At2g04430	AtNUDT5	284	C	ADP-ribose		
At2g04440	AtNUDT26	216	C	unknown		
At2g04450	AtNUDT6		C	unknown	ADP-ribose, NADH	Ogawa *et al*., 2005
At2g33980	AtNUDT22	303	CH	Coenzyme A	Coenzyme A	Szurmak & Kraszewska, unpublished
At2g42070	AtNUDT23		CH	unknown	FAD	Ogawa *et al*., 2008
Chromosome 3						
At3g10620	AtNUDT28	217	CH	Ap_4A	Ap_4A, Ap_5A	Ogawa *et al*., 2008
At3g12600	AtNUDT16	181	M	Ap_6A, PP-InsP		
At3g26690	AtNUDT13	203	M	Ap_6A, PP-InsP	Ap_6A, Ap_5A, p_4A	Olejnik *et al*., 2007
At3g46200	AtNUDT9	312	C	unknown		
Chromosome 4						
At4g11980	AtNUDT14	310	M	ADP-ribose	ADP-ribose, ADP-glucose	Munoz *et al*., 2006; Ogawa *et al*., 2008
At4g12720	AtNUDT7	283	C	unknown	ADP-ribose, NADH	Olejnik & Kraszewska, 2005; Ogawa *et al*., 2005; Jambunathan & Mahalingam, 2006
At4g25434	AtNUDT10	305	C	unknown	ADP-ribose	Ogawa *et al*., 2005
Chromosome 5						
At5g06340	AtNUDT29	228	CH	Ap_4A	Ap_4A, Ap_5A	Ogawa *et al*., 2008
At5g13570	AtNUDT27	374	C	mRNA cap (m^7GTP-RNA)	mRNA cap (m^7GTP-RNA)	Gunawardana *et al*., 2008
At5g20070	AtNUDT19	439	CH	NADH	NADH, NADPH	Ogawa *et al*., 2008
At5g45940	AtNUDT11	223	C	Coenzyme A	Coenzyme A	Ogawa *et al*., 2005
At5g47240	AtNUDT8	370	C	unknown		
At5g47650	AtNUDT2	279	C	unknown	ADP-ribose, NADH	Ogawa *et al*., 2005; Ogawa *et al*., 2008
At5g19460	AtNUDT20	375	CH	unknown		
At5g19470	AtNUDT24	366	CH	unknown		

*C, cytoplasm; M, mitochondria; CH, chloroplasts

substrate of this hydrolase was NADH. It was also shown that the AtNUDT1 protein exists as a dimer in solution (Dobrzańska *et al*., 2002). Further studies, conducted by other researchers, have revealed that the AtNUDT1 hydrolase is the closest plant homologue of the *Lactococcus lactis* YlgG protein that removes pyrophosphate from dihydroneopterin triphosphate (DHNTP). This reaction is the second step in the pterin branch of the folate synthesis pathway. In the presence of a different divalent cation (1 mM Mg^{2+}) AtNUDT1 was found to efficiently hydrolyze DHNTP. However, under the same reaction conditions, the most favored substrates were (d)NTPs (Klaus *et al*., 2005). A similar switch in substrate

preference in the presence of different cations has also been observed for a murine Nudix hydrolase (Hua *et al*., 2003). This might indicate that the *in vivo* substrate specificity of some Nudix hydrolases depends on the cellular fluxes and local concentrations of cofactors.

The preference of AtNUDT1 for (d)NTPs was further corroborated by Ogawa *et al*. (2005) and Yoshimura *et al*. (2007). Those authors also found that AtNUDT1 efficiently catalyzes the hydrolysis of 8-oxo-(d)GTP. In a transcriptional mutational analysis using a *mutT*-deficient strain of *E. coli* expressing the AtNUDT1 protein, Yoshimura and coworkers established that this plant hydrolase can partially compensate for the lack of the MutT protein in the bacterial cells. In addition, they observed an increase in 8-oxo-(d)G levels in the genomic DNA of mutant *Arabidopsis* plants lacking AtNUDT1 activity. These results led those authors to conclude that the physiological role of AtNUDT1 is the elimination of potentially mutagenic oxidized nucleotides from the cellular pool. However, the *ATNUDT1* mutant plants did not exhibit any noticeable changes in their phenotype under normal or stressful conditions. Therefore, the physiological significance of this AtNUDT1 multi-substrate hydrolase remains to be determined.

DIADENOSINE POLYPHOSPHATE HYDROLASES AtNUDT25 AND AtNUDT13

Diadenosine polyphosphates, $Ap_{2-6}A$, found in a number of prokaryotic and eukaryotic organisms, have been implicated in a range of physiological processes such as DNA replication and repair, stress responses, neurotransmission and apoptosis (for review see McLennan, 2000). In addition, diadenosine heptaphosphate (Ap_7A) has also been observed in human cells (Jankowski *et al*., 1999) The cellular level of diadenosione polyphosphates is precisely controlled by various enzymes, including some with the Nudix signature (for review see Guranowski 2000; McLennan *et al*., 2001). The Nudix hydrolases that are active on diadenosine polyphosphates may be divided into two subfamilies, preferentially hydrolysing Ap_4A or Ap_5A, and Ap_6A, respectively. Interestingly, members of the latter subfamily also act as phosphohydrolases utilizing non-nucleotide diphosphoinositol polyphosphates, PP-InsP (DIPs), the most highly phosphorylated compounds of the inositol-based cell signaling family (Safrany *et al*., 1999). In addition, members of both subfamilies of diadenosine polyphosphate Nudix hydrolases are able to hydrolyse 5-phospho-α-D-ribosyl diphosphate (PRPP), which is both a substrate and a regulator of purine and pyrimidine synthesis (Fischer *et al*., 2002).

The first plant diadenosine polyphosphate Nudix hydrolase was cloned from polyadenylated RNA isolated from the cotyledons of *Lupinus angustifolius*. In the presence of Mg^{2+} ions the recombinant protein catalyzed the asymmetric cleavage of Ap_4A to ATP and AMP. Typically for Nudix hydrolases, the enzyme was inhibited by low levels of NaF (Maksel *et al*., 1998). Further NMR and mutagenesis studies demonstrated that the C-terminal domain of the protein displays a typical Nudix fold and that the conserved glutamate residues in the Nudix motif contribute significantly to catalysis (Swarbrick *et al*., 2000; Maksel *et al*., 2001).

Recently, an *Arabidopsis* homologue of the *L. angustifolius* Ap_4A hydrolase has been described independently by two groups (Yoshimura *et al*., 2007; Szurmak *et al*., 2008). The former study showed that in the presence of 5 mM Mg^{2+} ions the AtNUDT25 hydrolase, encoded by the At1g30110 gene, catalyzes the hydrolysis of Ap_4A, Ap_5A, NADH and CoA. In contrast, Szurmak and coworkers (2008) found that AtNUDT25 exhibited almost no enzymatic activity with any of these substrates in the presence of magnesium cations. However, when Mg^{2+} was replaced by Mn^{2+}, Ap_4A hydrolysis was observed. Thus, it appears that in the presence of the appropriate cofactor the hydrolase is far more specific. By the use of isothermal titration calorimetry, Szurmak and coworkers showed that AtNUDT25 preferentially hydrolyses Ap_4A in the form of an Mn^{2+} complex.

A subfamily of Nudix hydrolases that hydrolyse long-chain dinucleotide polyphosphates, including diadenosine hexaphosphate (Ap_6A) and diadenosine pentaphosphate (Ap_5A), with low or zero activity towards diadenosine tetraphosphate (Ap_4A) or diadenosine triphosphate (Ap_3A), has been described in yeast and mammals. Interestingly, these enzymes also hydrolyse non-nucleoside substrates, diphosphoinositol polyphosphates (Safrany *et al*., 1998; Yang *et al*., 1999; Caffrey *et al*., 2000).

The AtNUDT13 protein encoded by the At3g26690 gene was the first characterized plant Nudix hydrolase active on long-chain diadenosine polyphosphates (Olejnik *et al*., 2007). It was established that in the presence of Mg^{2+} the most favored substrate of AtNUDT13, Ap_6A, was cleaved asymmetrically yielding ADP and p_4A. This hydrolase also degraded Ap_5A and p_4A, but far less efficiently. No activity was observed using Ap_4A, Ap_3A, dNTPs, NTPs, PP-InsP_5 or PRPP as substrates. It was also found that magnesium ions are absolutely essential for catalysis and cannot be replaced by manganese ions. Chemical crosslinking and size exclusion chromatography were used to demonstrate that the protein exists as a monomer in solution. Subcellular localization studies indicated that the AtNUDT13 protein is mitochondrial. Taken together, these findings

suggest that diadenosine polyphosphates, which are much more stable than ATP, could serve as a store of the latter compound in mitochondria. Thus, the mitochondrial Nudix hexaphosphate hydrolase AtNUDT13 might be involved in the turnover of ATP and ADP in these organelles (e.g., during stress) (Olejnik *et al*., 2007). Interestingly, of the seven putative *Arabidopsis* Ap_6A hydrolases, five are thought to be targeted to mitochondria, one to chloroplasts and only one resides in the cytoplasm (Table 2). This observation supports the hypothesis that *Arabidopsis* Ap_6A hydrolases are specifically involved in mitochondrial metabolism (e.g., ATP turnover). In comparison, of the three *Arabidopsis* Ap_4A hydrolases two are putatively targeted to chloroplasts and one resides in the cytoplasm. The physiological functions of plant diadenosine polyphosphate hydrolases are largely unknown and require further investigation. Analysis of mutant *Arabidopsis* lines deprived of a particular enzyme may provide some insight, although other hydrolases from the same subfamily might complement the absence of the mutated one. In addition, the physiological functions of diadenosine polyphosphates in plant cells remain unclear although several plant enzymes, besides Nudix hydrolases, that are involved in the cellular metabolism of these compounds have been identified (for review see Guranowski, 2004).

RNA DECAPPING HYDROLASE AtNUDT27 (ATDCP2)

Messenger RNA turnover is critical for the regulation of gene expression. In particular, removal of the m^7GTP cap from the 5′ end of mRNA is an essential step in the 5′-3′ decay pathway. The decapping of mRNA that occurs inside cytoplasmic processing bodies (P bodies) is a highly regulated process which involves several proteins, including the DCP2 protein (for review see Coller & Parker, 2004). DCP2 is a Nudix enzyme that acts on m^7GTP-RNAs, generating m^7GDP and 5′-phosphorylated RNAs which are substrates of 5′-3′ exonuclease. Thus, the catalytic activity of DCP2 is absolutely required for mRNA decapping and further RNA degradation. The DCP2 Nudix hydrolases were initially identified in mammals and yeast (Wang *et al*., 2002; She *et al*., 2006).

Recently, an *Arabidopsis* homologue of the DCP2 protein was identified and characterized (Xu *et al*., 2006; Iwasaki *et al*., 2007). The product of the At5g13570 gene, the AtDCP2 (AtNUDT27) protein, like its yeast and mammalian counterparts, catalyzes the hydrolysis of m^7GTP-RNAs to m^7GDP and 5′-phosphorylated RNAs. This protein is part of a decapping complex localized in cytoplasmic foci, which are putative *Arabidopsis* P bodies. Null mutants of *AtDCP2* accumulate capped mRNA and display a lethal phenotype at the seedling cotyledon stage, indicating that it is essential for mRNA turnover in postembryonic development. Mutational analysis of conserved glutamate residues in the Nudix motif of AtDCP2 has shown that this enzyme employs the catalytic mechanism common to Nudix hydrolases. However, unlike other Nudix enzymes, AtDCP2 is refractory to inhibition by fluoride (Gunawardana *et al*., 2008), and it appears to be unique in *Arabidopsis* cells rather than a member of a subfamily like, for example, Ap_nA hydrolases.

NUCLEOTIDE SUGAR HYDROLASES ATNUDT7, ATNUDT2 (AtNUDX2) AND ATNUDT14 (ATASPP)

The first characterized *Arabidopsis* Nudix hydrolase active on nucleotide sugars was the AtNUDT7 (formerly AtGFG) protein encoded by At4g12720 gene. In the presence of Mg^{2+} ions this enzyme was shown to hydrolyse a variety of substrates with a preference for ADP-ribose. Thus, it was postulated that this hydrolase might help to utilize any excess of free ADP-ribose present in the cell (Olejnik & Kraszewska, 2005). Free ADP-ribose, a highly reactive molecule, is a major product of NAD^+, poly(ADP)ribose, and cyclic-ADP-ribose catabolism. It can also be released from mono-ADP-ribosylated proteins (Kim *et al*., 1993, Han *et al*., 2002). Due to its ability to modify proteins by non-enzymatic mono-ADP-ribosylation and to bind to ATP-activated K^+ channels, ADP-ribose is potentially cytotoxic (Jacobson *et al*., 1994; Cervantes-Laurean *et al*., 1996; Kwak *et al*., 1996). Detailed characterization of the AtNUDT7 protein showed that its catalytic activity was not affected by low concentrations of NaF. Chemical crosslinking studies demonstrated that AtNUDT7 exists in solution as a dimer (Olejnik & Kraszewska, 2005). Further studies have confirmed that ADP-ribose is the favored substrate of AtNUDT7, although substantial hydrolysis of NADH was also observed (Ogawa *et al*., 2005).

Loss of function *AtNUDT7* mutant plants display a pleiotropic phenotype including small size, curled leaves, microscopic cell death, increased resistance to bacterial pathogens, and increased levels of reactive oxygen species (ROS) and NADH. It was concluded that AtNUDT7 helps to maintain cellular homeostasis during stress by hydrolysing excess NADH, which is a potential source of superoxide (Jambunathan & Mahalingam, 2006). Independent studies on *AtNUDT7* mutant plants by Bartsch and coworkers confirmed the pleiotropic phenotypic effects caused by this mutation. Using genetic analyses, they also established that growth inhibition, en-

hanced basal resistance to bacterial pathogens and cell death were dependent on a functional enhanced disease susceptibility 1 (EDS1) protein, which together with phytoalexin-deficient 4 (PAD4) controls defense activation and programmed cell death (Bartsch *et al*., 2006). Further studies on *AtNUDT7* mutants have revealed that this mutation leads to the perturbation of cellular redox homeostasis and increased levels of NADH in pathogen-challenged plants. However, no significant changes in the cellular level of ADP-ribose were observed in the mutant plants. In addition, it was shown that the hydrolytic activity of AtNUDT7 protein is essential for its function. It was concluded that the alteration in cellular redox homeostasis caused by the *AtNUDT7* mutation primes the cells for an enhanced defense response. Thus, the proposed function of the AtNUDT7 protein is to modulate the defense response to prevent excessive stimulation (Ge *et al*., 2007). However, the precise mechanism by which the AtNUDT7 hydrolase regulates this process remains unknown.

Similarly to AtNUDT7, when tested *in vitro*, the AtNUDT2 hydrolase shows preference for ADP-ribose and NADH (Ogawa *et al*., 2005). It was observed that overexpression of AtNUDT2 increases tolerance of *Arabidopsis* plants to oxidative stress. It was suggested that this phenomenon was due to the maintenance of NAD^+ and ATP levels by nucleotide recycling from free ADP-ribose molecules under stress (Ogawa *et al*., 2008).

Another recently described *Arabidopsis* Nudix hydrolase active on nucleotide sugars is an ortholog of the *E. coli* ADP-sugar pyrophosphatase ASPP, an enzyme that hydrolyses a variety of ADP-sugars including ADP-ribose and ADP-glucose. The latter is the bacterial glycogen and starch precursor. It was shown that in bacteria the ASPP hydrolase controls glycogen biosynthesis through regulating the intracellular level of ADP-glucose (Moreno-Bruna *et al*., 2001). Similarly to its bacterial counterpart, the *Arabidopsis* AtNUDT14 (AtASPP) protein, encoded by the At4g11980 gene, was found to catalyze the hydrolytic breakdown of ADP-ribose and ADP-glucose *in vitro*, with a preference for the former. However, leaves of plants overexpressing AtNUDT14 showed a significant decrease in ADP-glucose level, followed by a reduction in starch content. In spite of high K_m for ADP-glucose estimated *in vitro*, this result suggests that ADP-glucose rather than ADP-ribose is the preferred *in vivo* substrate of this hydrolase. Despite the prediction concerning its localization based on the protein sequence, it was shown that AtNUDT14 is not mitochondrial (Munoz *et al*., 2006). Subcellular localization studies with AtNUDT14 fused to green fluorescent protein (GFP) have shown that the protein is targeted to the chloroplasts (Munoz *et al*., 2008).

CONCLUSIONS

The 29 genes encoding putative Nudix proteins present in the *Arabidopsis* genome are spread across all chromosomes, with the highest number present on chromosomes 1, 2 and 5 (8, 6 and 8 genes, respectively). The remaining seven genes are located on chromosomes 3 and 4 (4 and 3, respectively). Based on their predicted localizations, the encoded proteins are evenly distributed between the cytoplasm and organelles, i.e. mitochondria and chloroplasts (14 and 15, respectively). Interestingly, none of the proteins display a nuclear targeting signal suggesting that these hydrolases are predominantly involved in processes that occur outside the nucleus. Recently, the proteins were numbered according to their predicted cellular localization: presumably cytosolic AtNUDT1-11; predicted to be transported to mitochondria AtNUDT12-18; and targeted to chloroplasts AtNUDT19-24 (Ogawa *et al*., 2005). Three additional Nudix proteins (Ap_4A hydrolases) identified in sequence databases, encoded by genes At1g30110, At3g10620 and At5g06340, were subsequently added to this list (Yoshimura *et al*., 2007). However, two presumably cytosolic Nudix proteins, one with an unpredicted substrate and the other active on mRNA cap, encoded by the At2g04440 and At5g13570 genes, respectively, were missed. I propose to name these proteins AtNUDT26 and AtNUDT27 for the At2g04440 and At5g13570 gene products, respectively. The putative chloroplastic Ap_4A hydrolases encoded by the genes At3g10620 and At5g06340 would then become AtNUDT28 and AtNUDT29, respectively.

Through a search of the InterPro database, two presumably chloroplastic proteins encoded by the At3g02780 and At5g16440 genes were found to contain domains with structural similarity to the Nudix fold and therefore they were included in the *Arabidopsis* Nudix hydrolase family by Munoz *et al*. (2006). It has previously been shown that these proteins exhibit isopentenyl diphosphate isomerase (IPP isomerase) activity, which is required for interconversion of isopentenyl diphosphate (IPP) in the isoprenoid pathway (Campbell *et al*., 1997). Despite the structural similarities between the C-terminal domains of IPP isomerases and the Nudix proteins, the hydrolysis of the IPP pyrophosphate bond by a Nudix-like domain would be unlikely and also insufficient for the isomerisation. IPP isomerisation also requires functions of the unique N-terminal part of the protein. Therefore, it has been suggested that the Nudix-like motifs of the IPP-processing enzymes are catalytically inefficient and act only to bind the pyrophosphate moiety of the IPP substrate (Smit & Mushegian, 2008).

Similarly, a direct involvement of the Nudix-like domain of the MutY glycosylase in the removal of adenine mispaired with guanine or 8-oxo-guanine from DNA would be unlikely. The MutY glycosylase employs a different catalytic mechanism and this activity resides in the N-terminal part of the protein. It has been suggested that the Nudix-like domain of MutY may play some role in the recognition of 8-oxoG in DNA (Volk *et al*., 2000).

Neither the IPP isomerases nor MutY-like glycosylases contain a canonical Nudix motif and most probably they do not exhibit a pyrophosphohydrolase activity, two features that are diagnostic for member of the Nudix hydrolase family. Therefore, I propose to exclude both IPP isomerases and the predicted *Arabidopsis* MutY-like glycosylase (encoded by the At4g12740 gene) from the *Arabidopsis* Nudix hydrolase family.

The variety of substrates, many of which remain unrecognized, and the different enzymatic specificities of the Nudix hydrolases impede the determination of the biological functions of individual family members. In future, the ease of transformation of the model plant *A. thaliana* and the existence of mutant lines are likely to be vital in determining the *in vivo* roles of the Nudix hydrolases. This information will in turn help to establish the functions of homologous proteins in other species.

Acknowledgements

I would like to express my gratitude to Professor Jerzy Buchowicz for his many years of support. I would also like to thank Dr. Kamil Olejnik for fruitful collaboration on the AtNUDT7 and AtNUDT13 proteins and Dr. Marta Dobrzańska and Dr. Blanka Szurmak for their contributions to ongoing studies on plant Nudix hydrolases.

This work was supported by the Ministry of Scientific Research and Information Technology grant no. 2P06A03427 and no. NN303351635.

REFERENCES

Arczewska KD, Kuśmierek JT (2007) Bacterial DNA repair genes and their eukaryotic homologues: 2. Role of bacterial mutator gene homologues in human disease. Overview of nucleotide pool sanitization and mismatch repair systems. *Acta Biochim Polon* **54**: 435–457.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.

Bessman MJ, Frick DN, O'Handley SF (1996) The MutT proteins or "Nudix" hydrolases, a family of versatile widely distributed, "housecleaning" enzymes. *J Biol Chem* **271**: 25059–25062.

Bonanno JB, Edo C, Eswar N, Pieper U, Romanowski MJ, Ilyin V, Gerchman SE, Kycia H, Studier FW, Sali A, Burley SK (2001) Structural genomics of enzymes involved in sterol/isoprenoid biosynthesis. *Proc Natl Acad Sci USA* **98**: 12896–12901.

Caffrey JJ, Safrany ST, Yang XN, Shears SB (2000) Discovery of molecular and catalytic diversity among human diphosphoinositol-polyphosphate phosphohydrolases-an expanding NUDT family. *J Biol Chem* **275**: 12730–12736.

Campbell M, Hahn FM, Poulter CD, Leustek T (1997) Analysis of the isopentenyl diphosphate isomerase gene family from *Arabidopsis thaliana*. *Plant Mol Biol* **32**: 323–328.

Cervantes-Laurean D, Jacobson EL, Jacobson MK (1996) Glycation and glycoxidation of histones by ADP-ribose. *J Biol Chem* **271**: 10461–10469.

Coller J, Parker R (2004) Eukaryotic mRNA decapping. *Annu Rev Biochem* **73**: 861–890.

Deana A, Celesnik H, Belasco JG (2008) The bacterial enzyme RppH triggers messenger RNA degradation by 5′ pyrophosphate removal. *Nature* **451**: 355–358.

Dobrzańska M, Szurmak B, Wyslouch-Cieszynska A, Kraszewska E (2002) Cloning and characterization of the first member of the Nudix family from *Arabidopsis thaliana*. *J Biol Chem* **277**: 50482–50486.

Fisher DI, Safrany ST, Strike P, McLennan AG, Cartwright JL (2002) Nudix hydrolases that degrade dinucleoside and diphosphoinositol polyphosphates also have 5-phosphoribosyl 1-pyrophosphate (PRPP) pyrophosphatase activity that generates the glycolytic activator ribose 1,5-bisphosphate. *J Biol Chem* **277**: 43555–43559.

Fisher DI, Cartwright JL, Harashima H, Kamiya H, McLennan AG (2004) Characterization of a Nudix hydrolase from *Deinococcus radiodurans* with a marked specificity for (deoxy) ribonucleoside 5′-diphosphates. *BMC Biochem* **5**: 7.

Fletcher JI, Swarbrick JD, Maksel D, Gayler KR, Gooley PR (2002) The structure of Ap(4)A hydrolase complexed with ATP-MgF(x) reveals the basis of substrate binding. *Structure* **10**: 205–213.

Gabelli SB, Bianchet MA, Azurmendi HF, Xia Z, Sarawat V, Mildvan AS, Amzel LM (2004) Structure and mechanism of GDP-mannose glycosyl hydrolase, a Nudix enzyme that cleaves at carbon instead of phosphorus. *Structure* **12**: 927–935.

Gabelli SB, Bianchet MA, Xu W, Dunn CA, Niu ZD, Amzel LM, Bessman MJ (2007) Structure and function of the *E. coli* dihydroneopterin triphosphate pyrophosphatase: a Nudix enzyme involved in folate biosynthesis. *Structure* **15**: 1014–1022.

Gunawardana D, Cheng HC, Gayler KR (2008) Identification of functional domains in *Arabidopsis thaliana* mRNA decapping enzyme (AtDCP2). *Nucleic Acids Res* **36**: 203–216.

Guranowski A (1990) Fluoride is a strong and specific inhibitor of (asymmetrical) Ap_4A hydrolases. *FEBS Lett* **262**: 205–208.

Guranowski A (2000) Specific and nonspecific enzymes involved in the catabolism of mononucleoside and dinucleoside polyphosphates. *Pharmacol Therapeut* **87**: 117–139.

Guranowski A (2004) Metabolism of diadenosine tetraphosphate (Ap_4A) and related nucleotides in plants; review with historical and general perspective. *Front Biosci* **9**: 1398–1411.

Han MK, Kim JS, Park YR, Shin YM, Park HJ, Park KJ, Park KH, Kim HK, Jang SI, An NH, Kim UH (2002) Antidiabetic effect of a prodrug of cysteine, L-2-oxothiazolidine-4-carboxylic acid, through CD38 dimerization and internalization. *J Biol Chem* **277**: 5315–5321.

Hori M, Fujikawa K, Kasai H, Harashima H, Kamiya H (2005) Dual hydrolysis of diphosphate and triphosphate derivatives of oxidized deoxyadenosine by Orf17 (NtpA), a MutT-type enzyme. *DNA Repair* **4**: 33–39.

Hua LV, Hidaka K, Pesesse X, Barnes LD, Shears SB (2003) Paralogous murine Nudt10 and Nudt11 genes have differential expression patterns but encode identical proteins that are physiologically competent diphosphoinositol polyphosphate phosphohydrolases. *Biochem. J* **373**: 81–89.

Ito R, Hayakawa H, Sekiguchi M, Ishibash T (2005) Multiple enzyme activities of *Escherichia coli* MutT protein for sanitization of DNA and RNA precursor pools. *Biochemistry* **44**: 6670–6674.

Iwasaki S, Takeda A, Motose H, Watanabe Y (2007) Characterization of Arabidopsis decapping AtDCP1 and AtDCP2, which are essential for postembryonic development. *FEBS Lett* **581**: 2455–2459.

Jacobson EL, Cervantes-Laurean D, Jacobson MK (1994) Glycation of proteins by ADP-ribose. *Mol Cell Biochem* **138**: 207–212.

Jambunathan N, Mahalingam R (2006) Analysis of *Arabidopsis* growth factor gene 1 (AtGFG1) encoding a nudix hydrolase during oxidative signaling. *Planta* **224**: 1–11.

Jankowski J, Tepel M, van der Giet M, Tente IM, Henning L, Junker R, Zidek W, Schulter H (1999) Identification and characterization of P(1), P(7)-di(adenosine-5′)-heptaphosphate from human platelets. *J Biol Chem* **274**: 23926–23931.

Kim UH, Kim MK, Kim JS, Han MK, Park BH, Kim HR (1993) Purification and characterization of NAD glycohydrolase from rabbit erythrocytes. *Arch Biochem Biophys* **305**: 147–152.

Klaus SMJ, Wegkamp A, Sybesma W, Hugenholtz J, Gregory JF, Hanson AD (2005) A nudix enzyme removes pyrophosphate from dihydroneopterin triphosphate in the folate synthesis pathway of bacteria and plants. *J Biol Chem* **280**: 5274–5280.

Kwak YG, Park SK, Kim UH, Han MK, Eun JS, Cho KP, Chae SW (1996) Intracellular ADP-ribose inhibits ATP-sensitive K^+ channels in rat ventricular myocytes. *Am J Physiol* **271**: C464–C468.

Lawhorn BG, Gerdes SY, Begley TP (2004) A genetic screen for the identification of thiamin metabolic genes. *J Biol Chem* **279**: 43555–43559.

Legler PM, Massiah MA, Bessman MJ, Mildvan AS (2000) GDP-mannose mannosyl hydrolase catalyzes nucleophilic substitution at carbon, unlike all other Nudix hydrolases. *Biochemistry* **39**: 8603–8608.

Maksel D, Guranowski A, Ilgoutz SC, Moir A, Blackburn MG, Gayler KR (1998) Cloning and expression of diadenosine 5′,5‴-P^1,P^4–tetraphosphate hydrolase from *Lupinus angustifolius* L. *Biochem J* **329**: 313–319.

Maksel D, Gooley PD, Swarbrick JD, Guranowski A, Gange C, Blackburn MG, Gayler KR (2001) Characterization of active-site residues in diadenosine tetraphosphate hydrolase from *Lupinus angustifolius*. *Biochem J* **357**: 399–405.

McLennan AG (2000) Dinucleoside polyphosphates — friend or foe? *Pharmacol Ther* **87**: 73–89.

McLennan AG (2006) The nudix hydrolase superfamily. *Cell Mol Life Sci* **63**: 123–143.

McLennan AG, Barnes LD, Blackburn GM, Brenner C, Guranowski A, Miller AD, Rovira JM, Rotllan P, Soria B, Tanner JA, Sillero A (2001) Recent progress in the study of the intracellular functions of diadenosine polyphosphates. *Drug Development Res* **52**: 249–259.

Mildvan AS, Xia Z, Azurmendi HF, Saraswat V, Legler PM, Massiah MA, Gabelli SB, Bianchet MA, Kang LW, Amzel LM (2005) Structures and mechanisms of nudix hydrolases. *Arch Biochem Biophys* **433**: 129–143.

Moreno-Bruna B, Baroja-Fernandez E, Munoz FJ, Bastarrica-Berasategui A, Zandueta-Criado A, Rodrigez-Lopez M, Lasa I, Akazawa T, Pozueta-Romero J (2001) Adenosine diphosphate sugar pyrophosphatase prevents glycogen biosynthesis in *Escherichia coli*. *Proc Natl Acad Sci USA* **98**: 8128–8132.

Munoz FJ, Baroja-Fernandez E, Moran-Zorzano MT, Alonso-Casajus N, Pozueta-Romero J (2006) Cloning, expression and characterization of Nudix hydrolase that catalyzes the hydrolytic breakdown of ADP-glucose linked to starch biosynthesis in *Arabidopsis thaliana*. *Plant Cell Physiol* **47**: 926–934.

Munoz FJ, Baroja-Fernandez E, Ovecka M, Li J, Mitsui T, Sesma MT, Montero M, Bahaji A, Ezquer I, Pozueta-Romero J (2008) Plastidial localization of potato Nudix hydrolase of ADP-glucose linked to starch biosynthesis. *Plant Cell Physiol*, in press.

Ogawa T, Ueda Y, Yoshimura K, Shigeoka S (2005) Comprehensive analysis of cytosolic Nudix hydrolases in *Arabidopsis thaliana*. *J Biol Chem* **280**: 25277–25283.

Ogawa T, Ishikawa K, Harada K, Fukusaki E, Yoshimura K, Shigeoka S (2008a) Overexpression of an ADP-ribose pyrophosphatase AtNUDX2, confers enhanced tolerance to oxidative stress on *Arabidopsis* plants. *Plant J*, PMID: 18798872 (Epub ahead of print).

Ogawa T, Yoshimura K, Miyake H, Ishikawa K, Ito D, Tanabe N, Shigeoka S (2008b) Molecular characterization of organelle-type Nudix hydrolases in *Arabidopsis thaliana*. *Plant Physiol* **148**: 1412–1421.

Olejnik K, Kraszewska E (2005) Cloning and characterization of an *Arabidopsis thaliana* Nudix hydrolase homologous to the mammalian GFG protein. *Biochim Biophys Acta* **1752**: 133–141.

Olejnik K, Murcha MW, Whelan J, Kraszewska E (2007) Cloning and characterization of AtNUDT13, a novel mitochondrial *Arabidopsis thaliana* Nudix hydrolase specific for long-chain diadenosine polyphosphates. *FEBS J* **274**: 4877–4885.

Safrany ST, Caffrey JJ, Yang XY, Bembenek ME, Moyer MB, Burkhart WA, Shears SB (1998) A novel context for the MutT module, a guardian of cell integrity, in a diphosphoinositol polyphosphate phosphohydrolase. *EMBO J* **17**: 6599–6607.

Safrany ST, Cartwright JL, Yang X, Shears SB (1999a) Diphosphoinositol polyphosphates: the final frontier for inositide research. *Biol Chem* **380**: 945–951.

Safrany ST, Ingram SW, Cartwright JL, Falck JR, McLennan AG, Barnes LD, Shears SB (1999b) The diadenosine hexaphosphate hydrolases from *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* are homologues of the human diphosphoinositol polyphosphate phosphohydrolase. Overlapping substrate specificities in a MutT-type protein. *J Biol Chem* **274**: 21735–21740.

She M, Decker CJ, Chen N, Tumat S, Parker R, Song H (2006) Crystal structure and functional analysis of Dcp2p from *Schizosaccharomyces pombe*. *Nat Struct Mol Biol* **13**: 63–70.

Smit A, Mushegian A (2008) Biosynthesis of isoprenoids via mevalonate in Archea: The lost pathway. *Genome Res* **10**: 1468–1484.

Swarbrick JD, Bashannyk T, Maksel D, Zhang XR, Blackburn GM, Gayler KR, Gooley PR (2000) The three-dimensional structure of the nudix enzyme diadenosine tetraphosphate hydrolase from *Lupinus angustifolius* L. *J Mol Biol* **302**: 1165–1177.

Szurmak B, Wysłouch-Cieszyńska A, Wszelaka-Rylik M, Bal W, Dobrzańska M (2008) A Adenosine 5′,5′′-P^1P^4

tetraphosphate (Ap_4A) hydrolase from *Arabidopsis thaliana* that is activated preferentially by Mn^{2+} ions. *Acta Biochim Polon* **55**: 151–160.

Volk DE, House PG, Thiviyanathan V, Luxon BA, Zhanf S, Lloyd RS, Gorenstein DG (2000) Structural similarities between MutT and the C-terminal domain of MutY. *Biochemistry* **39**: 7331–7336.

Wang Z, Jiao X, Carr-Schmid A, Kiledjian M (2002) The hDcp2 protein is a mammalian mRNA decapping enzyme. *Proc Natl Acad Sci USA* **99**: 12663–12668.

Xu WL, Shen JY, Dunn CA, Desai S, Bessman MJ (2001) The nudix hydrolases of *Deinococcus radiodurans*. *Mol Microbiol* **39**: 286–290.

Xu WL, Shen JY, Dunn CA, Bessman MJ (2003) A new subfamily of the nudix hydrolase superfamily active on 5-methyl-UTP (Ribo-TTP) and UTP. *J Biol Chem* **278**: 37492–37497.

Xu WL, Dunn CA, Jones CR, D'Souza G, Bessman MJ (2004) The 26 nudix hydrolases of *Bacillus cereus*, a close relative of *Bacillus anthracis*. *J Biol Chem* **279**: 24861–24865.

Xu J, Yang J-Y, Niu Q-W, Chua N-H (2006) *Arabidopsis* DCP2, DCP1 and Varicose form a decapping complex required for postembryonic development. *Plant Cell* **18**: 3386–3398.

Yang XN, Safrany ST, Shears SB (1999) Site-directed mutagenesis of diphosphoinositol polyphosphate phosphohydrolase, a dual specificity NUDT enzyme that attacks diadenosine polyphosphate and diphosphoinositol polyphosphates. *J Biol Chem* **274**: 35434–35440.

Yoshimura K, Ogawa T, Ueda Y, Shigeoka S (2007) AtNUDX1, an 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate pyrophosphohydrolase, is responsible for eliminating oxidized nucleotides in *Arabidopsis. Plant Cell Physiol* **48**: 1438–1449.