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Distinct regulation of Arabidopsis ADP-ribose/NADH pyrophosphohydrolases, AtNUDX6 and 7, in biotic and abiotic stress responses

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Abbreviations: At, Arabidopsis; KO, knockout; Nudix, nucleoside diphosphates linked to some moiety X; NUDX, nudix hydrolases; PQ, paraquat; ROS, reactive oxygen species; SA, salicylic acid

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and
Ishikawa K, Ogawa T, Hirosue E, Nakayama Y, Harada K, Fukusaki E, et al. Modulation of the poly(ADP-ribosyl)ation reaction via the ArabidopsisADP-ribose/NADH pyrophosphohydrolase, AtNUDX7, is involved in the response to oxidative stress. Plant Physiol 2009; 151:741–54; PMID: 19656905; DOI: 10.1104/pp.109.140442.

Among Arabidopsis Nudix hydrolases (AtNUDX1~27), AtNUDX6 and AtNUDX7 having ADP-ribose/NADH pyrophosphohydrolase activities have been found to contribute to keeping the energy and redox homeostasis, and/or modulating defense responses against biotic and abiotic stress. Interestingly, AtNUDX6 had an opposite effect to AtNUDX7 on the regulation of immune responses. A comparison of the activities of ADP-ribose/NADH pyrophosphohydrolase among wild-type, knockout (*KO*)-*nudx6*, and *KO-nudx7* plants revealed AtNUDX7 to contribute more than AtNUDX6 to the total pyrophosphohydrolase activity toward both ADP-ribose and NADH under normal conditions and oxidative stress, while AtNUDX6 accounted for the majority of total NADH pyrophosphohydrolase activity under salicylic acid treatment. These results support the idea that the metabolism of ADP-ribose and/or NADH needs to be finely tuned for accurate regulation of cellular responses to biotic and abiotic stress.

Nudix (nucleoside diphosphates linked to some moiety X) hydrolases distributed among all classes of organisms from archaea to vertebrates have the potential to hydrolyze a wide range of substrates such as dinucleoside polyphosphates, various coenzymes, nucleotide sugars, ribo- and deoxynucleoside triphophates, and alcohols.[1-3] Recently, Nudix hydrolases having hydrolysis activity toward other compounds containing pyrophosphate bounds, such as nucleoside diphophates, the mRNA cap, 5'triphosphorylated RNA, and guanosine 3',5'-bispyrophosphate, and non-nucleoside substrates such as diphosphoinositol polyphosphates, 5-phosphoribosyl 1-diphosphate, thiamine pyrophosphate, and dihydroneopterin triphosphate, have been identified.[3] Several of these substrates are potentially toxic compounds, cell signaling molecules, metabolic intermediates, or coenzymes. Nudix hydrolases are thus considered to be associated with various cellular processes by hydrolytically removing these substrates.

Arabidopsis thaliana has 27 genes encoding Nudix hydrolase (*AtNUDX1-27*), which can be classified into three types by their predicted subcellular localization, the cytosol (*AtNUDX1*~11 and *25*), mitochondria (*AtNUDX12*~18), or chloroplasts (*AtNUDX19*~24, *26* and *27*).[4,5] It is remarkable that there are a large number of AtNUDXs having ADP-ribose or NADH pyrophosphohydrolase activity (AtNUDX2, 6, 7, 10, 14, 19 and 23); the number (7) of enzymes in the subfamily is greater than that in humans, which have 5 genes encoding the putative ADP-ribose or NADH pyrophosphohydrolase. Recombinant forms of AtNUDX2, 6 and 7 have showed the pyrophosphohydrolase activity toward both ADP-ribose and NADH with high affinity in vitro.[4] Recent studies have demonstrated that the modulation of ADP-ribose and/or NADH levels through the hydrolysis by AtNUDX2, 6 and 7 contributes to

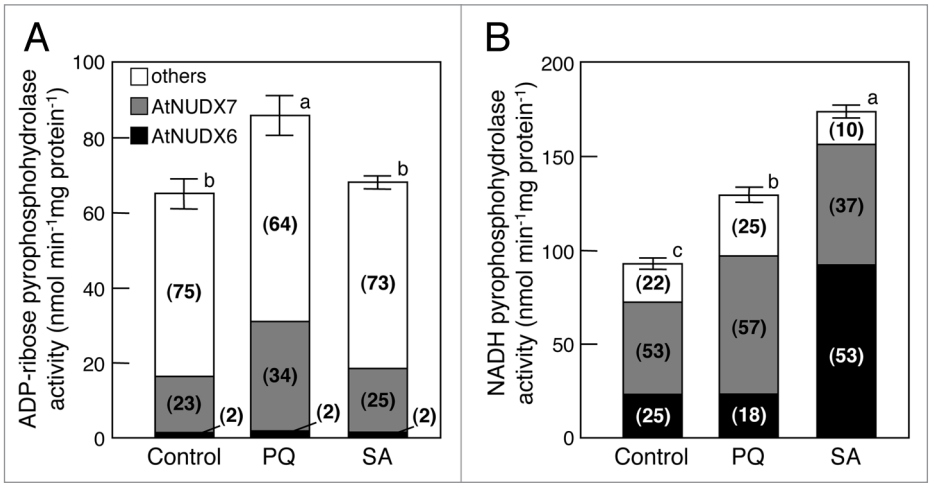

Figure 1. Changes in the ADP-ribose/NADH pyrophosphohydrolase activity of AtNUDX6 and 7 in Arabidopsis leaves under treatment with PQ or SA. The activities of pyrophosphohydrolase toward ADP-ribose (A) and NADH (B) in the leaves of wild-type plants grown on MS medium for 2 weeks under long-day conditions [16 h of light (100 μmol photons m^{-2} s^{-1}), 25°C/8 h of dark, 22°C] are shown as Control. PQ treatment was imposed by growing 2-week-old plants in MS medium containing the agent at 3 μM for 7 days under long-day conditions (PQ). SA treatment was imposed by growing 2-week-old plants in MS medium containing 0.5 mM SA for 24 h under long-day conditions (SA). The ADP-ribose and NADH pyrophosphohydrolase activities were measured as described previously.[8] The contributions (%) of AtNUDX6 and AtNUDX7 to total ADP-ribose/NADH pyrophosphohydrolase activity under treatment with PQ and SA were estimated from the decrease in activity in the respective knockout mutants (*KO-nudx6* and *KO-nudx7*)[7,8] and are indicated in parentheses. Data are the mean ± SD for three individual experiments (n = 3) using plants grown independently. Different letters indicate significant differences ($p < 0.05$).

keeping the energy and redox homeostasis, and/or modulating defense responses to both biotic and abiotic stress,[6-10] indicating the diverse roles of Nudix hydrolases in plants. AtNUDX2 might not function physiologically, because of its low levels even under stressful conditions.[6] It should be noted that the physiological role of AtNUDX6 differs considerably from that of AtNUDX7, although their enzymatic properties in vivo are partly the same: we previously demonstrated that AtNUDX7 acts in the hydrolysis of both ADP-ribose and NADH in cells, while AtNUDX6 acts only on NADH.[7,8]

It was demonstrated that AtNUDX7 acts as a negative regulator to prevent excessive stimulation of the defense response, which is dependent on and independent of Nonexpresser of Pathogenesis-Related genes 1, a master regulator of salicylic acid (SA)-induced defense genes, and SA accumulation,[10] while AtNUDX6 acts as a positive regulator through NPR1-dependent SA signaling pathways.[8] In addition, AtNUDX7, but not AtNUDX6, modulated the poly(ADP-ribosyl)ation reaction, which is one of the early responses to DNA damage caused by oxidative stress.[7] These observations raise the question of how AtNUDXs control such different processes.

To evaluate the physiological importance of each AtNUDX, here we compared AtNUDX6 and 7 in ADP-ribose and/or NADH pyrophosphohydrolase activity in Arabidopsis cells under various conditions. From the difference in activity of extracts prepared from the leaves of wild-type, knockout (*KO*)-*nudx6*, and *KO-nudx7* plants grown under normal conditions for 2 weeks, it was estimated that AtNUDX7 accounts for 23% of the total ADP-ribose pyrophosphohydrolase activity, but AtNUDX6 barely contributes to the activity (**Fig. 1**). Oxidative stress caused by 3 μM paraquat (PQ) for 7 days caused an increase in the total ADP-ribose pyrophosphohydrolase activity. Under oxidative stress, the contribution of AtNUDX7 to the activity increased to 34%. Treatment with 0.5 mM SA, a signaling molecule necessary for the onset of systemic acquired resistance, had no effect on the activity.

AtNUDX6 and 7 accounted for 25 and 53%, respectively, of the total pyrophosphohydrolase activity toward NADH under normal conditions (**Fig. 1**). The activity was increased by oxidative stress, with AtNUDX7 contributing 57%. On the other hand, under treatment with SA, the total NADH pyrophosphohydrolase activity was increased and AtNUDX6 accounted for 53% of the activity. These results indicated that AtNUDX7 contributed more than AtNUDX6 to the total pyrophosphohydrolase activity toward both ADP-ribose and NADH under normal conditions and oxidative stress.[7] On the other hand, AtNUDX6 accounted for the majority of the total NADH pyrophosphohydrolase activity under SA treatment.[8]

Plants are simultaneously exposed to abiotic and biotic hazards in nature. There is increasing evidence of crosstalk among the signaling pathways for biotic and abiotic stress.[12,13] It is worth noting that the expression of AtNUDX7, but not AtNUDX6, is regulated by intracellular levels of reactive oxygen species (ROS), since it is induced by not only pathogen infections but also oxidative stress including PQ treatment, all of which are known to cause the production of ROS in the cells.[8-11] On the other hand, the expression of *AtNUDX6* was induced only by the application of SA and its analogues, 2,6-dichloroisonicotinic acid or acibenzolar-S-methyl benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester, and not by oxidative stress.[8,14,15] Therefore, the expression of *AtNUDX6* was thought

to be regulated by intracellular SA levels, although it was also induced by pathogenic attacks causing local excessive production of both ROS and SA.[9] The differences in the regulation of AtNUDX6 and 7 and timing of production of ROS and SA in response to biotic stress raise the possibility that the total activity of ADP-ribose/NADH pyrophosphohydrolase and subsequent metabolism of ADP-ribose and/or NADH must be finely tuned for accurate regulation of such cellular responses. The activation of metabolism caused by the accumulation of either ROS or SA at specific phases in plant cells might have different effects on cellular responses through cooperation with other factors.

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