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Commentary

Plant nitrilase: a new job for an old enzyme

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Nitrilases are versatile enzymes that hydrolyze nitriles to carboxylic acids and ammonia, but many members of this family lack defined biological functions. In plants, nitrilases have been associated with detoxification of cyanide-containing compounds and auxin biosynthesis; however, recent work suggests that the chemical versatility of these proteins contributes to metabolite repair. In this issue of the *Biochemical Journal*, Niehaus et al. demonstrate that the Nit1 nitrilase from *Arabidopsis thaliana* functions as a metabolite repair enzyme that removes deaminated glutathione from the cytoplasm and plastids.

In the era of genomes and high-throughput approaches, the classic challenge of determining enzyme function remains as daunting as ever. Traditionally, biochemists relied on assaying for an enzymatic activity to find the right protein. Yet, now even with abundant (albeit not always well annotated) sequence data to guide experiments, it is still the right assay that reveals function. Recent work by Niehaus et al. [1] provides new insight into the role of a plant nitrilase (Nit1), which has a history of possible biochemical roles. This study reveals a new job for Nit1 from the model plant *Arabidopsis thaliana* as a metabolite repair enzyme that eliminates deaminated glutathione formed from non-specific transamination reactions. Importantly, this is one of a growing number of studies that highlight how biochemically versatile enzymes are exploited for the repair of damaged metabolites [2–4].

Nitrilases (or nitrile aminohydrolases) catalyze the hydrolysis of nitriles to carboxylic acids and ammonia [5]. Metabolically, these enzymes accept a variety of substrates, which makes assigning biochemical function problematic; however, that substrate promiscuity provides a readily available toolkit for chemists seeking biocatalysts to use in synthetic schemes and industrial-scale processes [6]. Depending on the organism of interest, nitrilases with varying substrate preferences for diverse aromatic, aliphatic, and arylacetonitriles have been described [6,7].

In plants, nitrilases were originally associated with the detoxification of β-cyanoalanine, a side product of ethylene biosynthesis [8], but later work suggested a potential role in the biosynthesis of the major plant hormone indole acetic acid (IAA or auxin) [9–11]. As a biosynthetic route to IAA, the nitrilases could hydrolyze indole-3-acetonitrile to IAA and displayed elevated expression of certain isoforms around wounds resulting from bacterial pathogen infection [9–11]. Subsequently, the major route to auxin was discovered and the contribution of indole-3-acetonitrile and the nitrilases to IAA synthesis downgraded to a minor pathway [12]. Later biochemical studies of the four nitrilases in *A. thaliana* showed that each could form the auxin, but with much lower catalytic efficiency compared with other substrates [13,14]. One isoform (Nit4) in Arabidopsis appears to primarily detoxify β-cyanoalanine through its breakdown to asparagine, aspartic acid, and ammonia, whereas the other three isoforms (Nit1–3) catalyzed the conversion of molecules associated with the breakdown of cyanogenic glycosides and glucosinolates found in Brassicaceae, such as Arabidopsis [13,14]. The cyanogenic glycosides and glucosinolates are natural plant products with anti-herbivory activity. Thus, the textbook answer to 'what do nitrilases do?' would be linked to nitrogen cycling in a set of plant-specific processes — detoxification of cyanide-containing molecules generated from ethylene biosynthesis and catabolism of specialized metabolites in one family of plants.

In comparison, the role of nitrilases in mammals was less well defined. Changes in expression of two (Nit1 and Nit2) of the eight nitrilase isoforms in humans affected cell growth and both were implicated as possible tumor suppressors, but substrates for the enzymes were unknown [15].

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Given the lack of ethylene, auxin, and glucosinolates in humans, the insights from the plant enzymes were of little use to understand the biochemistry of the nitrilases in mammals. In 2017, Peracchi et al. [16] discovered an unexpected role for nitrilases in mammals and microbes. Using a combination of *in vitro* and *in vivo* approaches, the mammalian and yeast Nit1 proteins were shown to function as amidases using deaminated glutathione, which contains a carbonyl group in place of the free amino group, as a substrate [16]. Metabolically, deamination of glutathione results from the non-specific activity of various enzymes that perform transamination between amino acid and α-keto acid substrates; such a 'mistake' needs to be repaired. Additional examination of Nit1 homologs in *Escherichia coli* and other bacteria that synthesize glutathione showed that the activity and need for repair of the damaged glutathione were not limited to mammals and yeast [16]; however, the potential role of the enzyme in plants was not examined.

In the current work [1], Niehaus and co-workers return to test the possible role of the Nit1 nitrilase from Arabidopsis and demonstrate that the plant enzyme also serves as a repair system that removes deaminated glutathione. As reported with nitrilases from non-plant organisms, the plant homolog Nit1 displayed significant activity with deaminated glutathione and deaminated ophthalmic acid (a chemical analog of deaminated glutathione). Homology modeling of the Arabidopsis Nit1 active site suggests critical amino acid changes that would allow the enzyme to easily accommodate the deaminated glutathione molecule. *In vitro* transcription/translation experiments showed that two translation products resulted from alternative start sites that allow for dual targeting of Nit1 to either the cytoplasm or plastid in plants. Interestingly, this localization pattern mimics that observed for glutathione synthetase in plants, which also has alternate start sites to target cellular localization [17,18]. Thus, the cellular localization the highly active glutathione biosynthesis enzyme and a repair enzyme overlap.

Yet, analysis of the role of Nit1 in planta suggests a more nuanced role in metabolism. As expected based on the biochemical study, Arabidopsis *nit1* knockout lines displayed a loss in the production of deaminated glutathione with accumulation of higher levels of the glutathione breakdown products cysteinylglycine and cystathionine; however, there were no obvious growth phenotypes under normal conditions or under a variety of conditions associated with oxidative stress [1]. Lipid metabolite analysis did identify 22 different triglycerides with at least a 10-fold decrease in the *nit1* mutants compared with wild-type plants, which suggests a possible link to lipid metabolism through the inhibition of glutathione-dependent peroxidases; however, additional work is needed to confirm this metabolic hypothesis.

Given the prevalence of glutathione in eukaryotes and many prokaryotes, the metabolite repair function of the nitrilases may represent their major metabolic function and that the other activities ascribed to it in plants (i.e. detoxification of cyanide-containing molecules) are the result of adaptation to plant-specific metabolic rocesses. It would be interesting to compare the biochemical and *in vivo* functions of nitrilase isoforms from various organisms more broadly to assess potential metabolic functions. Nonetheless, the nitrilases remain a highly versatile family of enzymes that still have a few tricks up their sleeve. In summary, nitrilases join the growing ranks of enzyme superfamilies that have been modified through evolution to serve as damage control officers to either repair damaged metabolites or inactivate potentially toxic molecules [2–4].

Abbreviations

IAA, indole acetic acid.

Competing Interests

The Author declares that there are no competing interests associated with this manuscript.

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