AMP and GMP Catabolism in Arabidopsis Converge on Xanthosine which Is Degraded by a Nucleoside Hydrolase Heterocomplex

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Review timeline:

TPC2018-00889-RA	Submission received:	November 23, 2018
	1st Decision:	January 22, 2019 *revision requested*
TPC2018-00889-RAR1	1st Revision received:	January 25, 2019
	2nd Decision:	January 30, 2019 *acceptance pending, sent to sci editor*
	Final acceptance:	February 14, 2019
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REPORT:(The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2018-00889-RA 1st Editorial decision– *revision requested* January 22, 2019

We have received reviews of your manuscript entitled "AMP and GMP Catabolism in Arabidopsis Converge on Xanthosine which Is Degraded by a Nucleoside Hydrolase Heterocomplex." On the basis of the advice received, the board of reviewing editors would like to accept your manuscript for publication in The Plant Cell. This acceptance is contingent on revision based on the comments of our reviewers. In particular, please consider the following:
Reviewer #1 notes that earlier work had indicated NSH1/NSH2 heterocomplex formation (in vitro, by mixing of mutant extracts). This earlier work needs to be considered in the Discussion. Reviewers #2 and #3 provide further constructive comments for improvement of the Discussion and several Figures that we ask you to address during revision.
Please match bar thickness in Figure 12.
Please highlight all changes and include a detailed annotation to changes to the text, with line numbers, and noting your responses to the comments.

---------- Reviewer comments:

TPC2018-00889-RAR1 1st Revision received January 25, 2019

Reviewer comments and **author responses**:

Reviewer #1:

This manuscript provides insight into the biochemical mechanism of the purine catabolic pathway. On the one hand this study is a comprehensive mutant analysis in the purine degradation pathway, on the other hand it is an elegant in vitro and in vivo study demonstrating the formation of a NSH1-NSH2 heterocomplex and thus revealing NSH2 function.

Individual components of the mutant analyses have been shown and published previously to be involved, however, some combinations with triple mutants now confirm their importance for the purine degradation pathway.

Reviewer #2:

The work by Baccolini and Witte aims to unravel the initial reactions in purine nucleotide catabolism. This story follows previous work by the same lab where processes in purine catabolism further downstream were clarified. Plant-specific features of nucleotide metabolism including the biochemistry of the reactions involved and the corresponding regulation are a neglected topic in plant science; this also holds true for purine nucleotide catabolism.

The authors have hands on a wide set of single mutants of genes involved in purine catabolism and combine these to higher order mutants in clever combinations. These mutants are then analyzed for the composition of metabolic intermediates by highly sensitive mass spectrometry. By this, subtle changes in metabolite composition were detected and interpreted in favor of clarifying the impact of individual enzymes for the analyzed pathway. By this, the authors were able to present a revised model of purine nucleotide catabolism. An impressive amount of experimental data is presented in this work contributing to an improved detailed understanding of the pathway studied.

Point 1. In addition the authors discovered that NSH1 and NSH2 function in a complex where NSH1 is required for NSH2 activity. This explains why recombinant NSH2 could not be produced as functional enzyme in previous studies. However, I am uncertain about the benefit of this unusual regulation in contrast to a regulation of the individual enzymes. Obviously, plants with an exclusive NSH2 activity (by inducing a catalytically inactive NSH1) can grow without restrictions. Do the authors have an idea about this?

RESPONSE: As the reviewer, we wonder whether NSH2 activation by NSH1 has a deeper regulatory purpose or not. NSH2 amounts might be dynamically altered upon environmental stimuli to increase the catalytic capacity for purine nucleosides (more NSH1-NSH2 complex) while probably decreasing it for pyrimidine nucleosides (less NSH1 homomeric complex) or vice versa. In the last sentences of the discussion we mention this possibility (lines 573-578), but we have not (yet) investigated if such stimuli-dependent dynamic changes actually occur.

Point 2. The effects of dark stress (carbon starvation) have been studied with the help of a set of mutants. An attenuation of dark stress symptoms e.g. in *NSH1* knockout mutants by ribose release from nucleosides could be disproved (Schroeder et al., 2018). With these new data, can the authors build a hypothesis towards the origin of such dark stress phenotype?

RESPONSE: In our study, dark stress was only used as a means of enhancing purine catabolism whereas the origin of enhanced dark stress phenotypes was not in the focus of our investigation.

Schroeder et al. (2018) established conclusively that it is the high guanosine accumulation in the GSDA mutant that leads to increased dark stress symptoms detectable when plants are exposed for more than four days to darkness. Enhanced dark stress symptoms were also reported for *XDH* mutants (Brychkova et al., 2008) and the *NSH1* mutant (Jung et al., 2011), but compared to the phenotype enhancement observed in the *GSDA* knockout, these are rather weak (see Schroeder et al.). Indeed in our laboratory, an enhanced phenotype of the *NSH1* mutant in dark stress was never observed, and also the *XDH* mutant did not display an aberrant phenotype in the short-term experiment (only up to three days) of the study presented here. Therefore, we cannot build any new hypotheses regarding the origin of increased dark stress phenotypes in purine nucleotide catabolic mutants.

Point 3. The absence of IMPP puts inosine catabolism as a side activity in purine catabolism. It was shown that inosine feeding to rice, tomato, onion and sunflower leads to increased root growth and root nitrogen contents (Tokuhisa et al., 2009; doi.org/10.1111/j.1747-0765.2010.00452.x). This indicates a high capacity of inosine catabolism, probably via NSH (could be checked in future studies). Such uptake of purine nucleosides and bases via the rhizosphere (in soils rich in organic material) might contribute to the flux through purine catabolism and require/explain the presence of different input routes. The authors might add this thought to the discussion.

RESPONSE: A sentence referring to inosine uptake from soil as a potential nucleoside source for purine catabolism has been added to the Discussion in lines 532-534. The corresponding reference (Tokuisa et al., 2010) has been added in the References in lines 930-932.

Reviewer #3:

In this well conducted study, the authors provide an updated model of purine salvage and catabolism in *Arabidopsis thaliana* through mutant analysis, metabolite measurements, recombinant gene expression, and biochemical experiments. Their main findings are that inosine and hypoxanthine, are not major intermediate of purine nucleotide catabolism in vivo, and that NSH2 is activated to xanthosine hydrolase through formation of a heterocomplex with NSH1. The latter finding is very interesting an well supported by data. Mechanistic details on the formation of the complex and NSH2 activation are lacking, making it difficult to predict if this observation can be extended to other plants.

Point 1. In the metabolic schemes (Fig 1 and Fig 13), it is not clear which enzymatic activities are known or presumed (e.g. there is an evidence for IMPP and GMPP activities?). The two different cases could be distinguished by different graphical symbols.

RESPONSE: The schemes have been changed, highlighting those enzymes in blue, which are presumably involved in purine metabolism, but have not been genetically identified. The legends of the figures have been changed accordingly in lines 978-979 and lines 1122-1123.

TPC2018-00899-RAR1 2nd Editorial decision – *acceptance pending* **January 30, 2019**

We are pleased to inform you that your paper entitled "AMP and GMP Catabolism in Arabidopsis Converge on Xanthosine which Is Degraded by a Nucleoside Hydrolase Heterocomplex" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff.

Final acceptance from Science Editor **February 14, 2019**