Requirement for the plastidial oxidative pentose phosphate pathway for nitrate assimilation in Arabidopsis

John D. Bussell1, Olivier Keech1,†, Ricarda Fenske2 and Steven M. Smith1,*

1Australian Research Council Centre of Excellence in Plant Energy Biology (M316), University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia, and
2Metabolomics Australia, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

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*For correspondence (e-mail steven.smith@uwa.edu.au).
†Present address: UPSC, Department of Plant Physiology, Umeå University, 90187 Umeå, Sweden.

SUMMARY
Sugar metabolism and the oxidative pentose phosphate pathway (OPPP) are strongly implicated in N assimilation, although the relationship between them and the roles of the plastidial and cytosolic OPPP have not been established genetically. We studied a knock-down mutant of the plastid-localized OPPP enzyme 6-phosphogluconolactonase 3 (PGL3). pgl3-1 plants exhibited relatively greater resource allocation to roots but were smaller than the wild type. They had a lower content of amino acids and free NO in leaves than the wild type, despite exhibiting comparable photosynthetic rates and efficiency, and normal levels of many other primary metabolites. When N-deprived plants were fed via the roots with 15NO exhibited normal induction of OPPP and nitrate assimilation genes in roots, and amino acids in roots and shoots were labeled with 15N at least as rapidly as in the wild type. However, when N-replete plants were fed via the roots with sucrose, expression of specific OPPP and N assimilation genes in roots increased in the wild type but not in pgl3-1. Thus, sugar-dependent expression of N assimilation genes requires OPPP activity and the specificity of the effect of the pgl3-1 mutation on N assimilation genes establishes that it is not the result of general energy deficiency or accumulation of toxic intermediates. We conclude that expression of specific nitrate assimilation genes in the nucleus of root cells is positively regulated by a signal emanating from OPPP activity in the plastid.

Keywords: Arabidopsis thaliana, nitrate, nitrogen assimilation, oxidative pentose phosphate pathway, plastid, 6-phosphogluconolactonase.

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Regulating the redox gatekeeper: vacuolar sequestration puts glutathione disulfide in its place
Graham Noctor*, Amna Mhamdi, Guillaume Queval, Christine H Foyer*
Institut de Biologie des Plantes, UMR CNRS 8618, Université de Paris sud, 91405 Orsay cedex, France
G.N., A.M.) and Centre for Plant Sciences, University of Leeds, Leeds, UK (G.Q., C.H.F.)

ABSTRACT
Oxidative stress signaling is an important player in plant responses to the environment, and is the subject of ever-intensifying interest in relation to processes such as phytohormone regulation. One well documented biochemical response to intracellular oxidation is accumulation of the disulfide form of glutathione, GSSG. In addition to its potential usefulness as a stress marker, this response may be of functional importance, given emerging evidence that glutathione status is important in oxidative stress signaling. Within this context, we present a novel view of the role of compartmentalization of glutathione in the signaling processes, highlighting and discussing the implications of recent studies revealing that oxidative stress can drive changes in the subcellular
distribution of GSSG, with a focus on vacuolar transport and the proteins that could be involved.

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**Altered Ribostasis: RNA-Protein Granules in Degenerative Disorders**

Mani Ramaswami,1,* J. Paul Taylor,2,* and Roy Parker3,*

1School of Genetics and Microbiology and School of Natural Sciences, Smurfit Institute of Genetics and Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland
2Department of Developmental Neurobiology, St. Jude Children’s Research Hospital, Memphis, TN 38120, USA
3Department of Biochemistry and Chemistry and the Howard Hughes Medical Institute, University of Colorado, Boulder CO 80309, USA

*Correspondence: mani.ramaswami@tcd.ie (M.R.), jpaultaylor@stjude.org (J.P.T.), roy.parker@colorado.edu (R.P.)

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**SUMMARY**

The molecular processes that contribute to degenerative diseases are not well understood. Recent observations suggest that some degenerative diseases are promoted by the accumulation of nuclear or cytoplasmic RNA-protein (RNP) aggregates, which can be related to endogenous RNP granules. RNP aggregates arise commonly in degenerative diseases because RNA-binding proteins commonly self-assemble, in part through prion-like domains, which can form self-propagating amyloids. RNP aggregates may be toxic due to multiple perturbations of posttranscriptional control, thereby disrupting the normal ‘‘ribostasis’’ of the cell. This suggests that understanding and modulating RNP assembly or clearance may be effective approaches to developing therapies for these diseases.


Keith

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**Structure and function of Hip, an attenuator of the Hsp70 chaperone cycle**


Zhuo Li, F Ulrich Hartl & Andreas Bracher

Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany.

The Hsp70-interacting protein, Hip, cooperates with the chaperone Hsp70 in protein folding and prevention of aggregation. Hsp70 interacts with non-native protein substrates in an ATP-dependent reaction cycle regulated by J-domain proteins and nucleotide exchange factors (NEFs). Hip is thought to delay substrate release by slowing ADP dissociation from Hsp70. Here we present crystal structures of the dimerization domain and the tetratricopeptide repeat (TPR) domain of rat Hip. As shown in a cocrystal structure, the TPR core of Hip interacts with the Hsp70 ATPase domain through an extensive interface, to form a bracket that locks ADP in the binding cleft. Hip and NEF binding to Hsp70 are mutually exclusive, and thus Hip attenuates active cycling of Hsp70–substrate complexes. This
mechanism explains how Hip enhances aggregation prevention by Hsp70 and facilitates transfer of specific proteins to downstream chaperones or the proteasome.

**Biodirectional processing of pri-miRNAs with branched terminal loops by Arabidopsis Dicer-like1**


Hongliang Zhu, Yuyi Zhou, Claudia Castillo-González, Amber Lu, Chunxiao Ge, Ying-Tao Zhao, Liusheng Duan, Zhaohu Li, Michael J Axtell, Xiu-Jie Wang & Xiuren Zhang

Texas A&M University, College Station, Texas, USA. China Agricultural University, Beijing, China. Penn State University, University Park, Pennslyvania, USA.

MicroRNAs (miRNAs) originate from primary transcripts (pri-miRNAs) with characteristic stem-loop structures, and their accurate processing is required for the production of functional miRNAs. Here, using the pri-miR-166 family in *Arabidopsis thaliana* as a paradigm, we report the crucial role of pri-miRNA terminal loops in miRNA biogenesis. We found that multibranched terminal loops in pri-miR-166s substantially suppress miR-166 expression *in vivo*. Unlike canonical processing of pri-miRNAs, terminal loop–branched pri-miRNAs can be processed by Dicer-like 1 (DCL1) complexes bidirectionally from base to loop and from loop to base, resulting in productive and abortive processing of miRNAs, respectively. In both cases, DCL1 complexes canonically cut pri-miRNAs at a distance of 16–17 bp from a reference single-stranded loop region. DCL1 also adjusts processing sites toward an internal loop through its helicase domain. These results provide new insight into the poorly understood processing mechanism of pri-miRNAs with complex secondary structures.