

# Thiol-mediated redox regulation of DICER-LIKE RNaseIII and small RNA metabolism

Cyril Charbonnel<sup>1</sup>, Adnan Khan Niazi<sup>1</sup>, Emilie Elvira-Matelot<sup>2</sup>, Matthias Zytnicki<sup>3</sup>, Anne de Bures<sup>1</sup>, Edouard Jobet<sup>1</sup>, Pascale Comella<sup>1</sup>, Alisson Opsomer<sup>4</sup>, Marcin Nowotny<sup>5</sup>, Christine Carapito<sup>4</sup>, Nahid Shamandi<sup>2</sup>, Hervé Vaucheret<sup>2</sup>, Julio Sáez-Vásquez<sup>1</sup>, Jean-Philippe Reichheld<sup>1\*</sup>

<sup>1</sup>Laboratoire Génome et Développement des Plantes, UMR 5096, CNRS, Université Perpignan Via Domitia, Laboratoire Génome et Développement des Plantes, 66860, Perpignan, France.

<sup>2</sup>Institut Jean-Pierre Bourgin, UMR1318 INRA AgroParisTech CNRS, Université Paris-Saclay, 78000 Versailles, France

<sup>3</sup>UMIAT, INRA UR-875, F-31326 Castanet-Tolosan, France

<sup>4</sup>Institut Pluridisciplinaire Hubert Curien, UMR7178 CNRS/UdS, Strasbourg, France

<sup>5</sup>Laboratory of Protein Structure, International Institute of Molecular and Cell Biology, 02-109 Warsaw, Poland

Environmental constraints have major impacts on living organisms. Plants, as other species, develop various panels of mechanisms to cope with the effects of these stresses on growth and survival. Redox homeostasis is among the key players of cellular metabolism and cell responses to environmental constraints. It is sensitive to environmental changes and can signal these changes to response pathways, for example by modifying redox status of thiols residues in proteins. In eukaryotic cells, small RNAs (siRNA and miRNA) are major regulators for gene expression, involved in most developmental and stress response processes. The biogenesis of small RNAs is orchestrated by RNaseIII endonuclease enzymes called DICER-LIKE (DCL) and RNASE THREE-LIKE (RTL), which mature almost all classes of double-stranded RNA precursors. We have showed that the RNaseIII activity of DCL and RTL family members in *Arabidopsis thaliana* depends on the oxidation state of specific cysteine thiols. Interestingly, the four DCL and the three RTL that carry dsRBD share a conserved cysteine (C230 in *Arabidopsis* RTL1) in their dsRBD. C230 is essential for RTL1 and DCL1 activities and is subjected to posttranscriptional modification. Indeed, under oxidizing conditions, glutathionylation of C230 inhibits RTL1 cleavage activity in a reversible manner involving glutaredoxins. Moreover, through whole-genome analyses, we showed that the repertoire of small RNA changes with the redox environment of the cell, suggesting that redox regulation of RNaseIII endonuclease enzymes might signal environmental changes to regulate small RNAs metabolism. We conclude that the redox state of the dsRBD ensures a fine-tune regulation of dsRNA processing by plant RNase III that might relay environmental signals to gene expression regulation.