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Symbiotic Leghemoglobins Are Crucial for Nitrogen Fixation in Legume Root Nodules but Not for General Plant Growth and Development

Thomas Ott[1], Joost T. van Dongen[1], Catrin Günther[1], Lene Krusell[1], Guilhem Desbrosses[2], Helene Vigeolas[1], Vivien Bock[1], Tomasz Czechowski[1], Peter Geigenberger[1], and Michael K. Udvardi[1]
[1]Max Planck Institute of Molecular Plant Physiology, Golm, Germany
[2]Université Montpellier, Montpellier, France

Abstract:
Hemoglobins are ubiquitous in nature and among the best-characterized proteins. Genetics has revealed crucial roles for human hemoglobins, but similar data are lacking for plants. Plants contain symbiotic and nonsymbiotic hemoglobins; the former are thought to be important for symbiotic nitrogen fixation (SNF). In legumes, SNF occurs in specialized organs, called nodules, which contain millions of nitrogen-fixing rhizobia, called bacteroids. The induction of nodule-specific plant genes, including those encoding symbiotic leghemoglobins (Lb), accompanies nodule development. Leghemoglobins accumulate to millimolar concentrations in the cytoplasm of infected plant cells prior to nitrogen fixation and are thought to buffer free oxygen in the nanomolar range, avoiding inactivation of oxygen-labile nitrogenase while maintaining high oxygen flux for respiration. Although widely accepted, this hypothesis has never been tested in planta. Using RNAi, we abolished symbiotic leghemoglobin synthesis in nodules of the model legume *Lotus japonicus*. This caused an increase in nodule free oxygen, a decrease in the ATP/ADP ratio, loss of bacterial nitrogenase protein, and absence of SNF. However, LbRNAi plants grew normally when fertilized with mineral nitrogen. These data indicate roles for leghemoglobins in oxygen transport and buffering and prove for the first time that plant hemoglobins are crucial for symbiotic nitrogen fixation.

Keywords: symbiotic leghemoglobin, symbiotic nitrogen fixation, oxygen-labile nitrogenise, L. Japonicus, legume root nodules