Early Onset of Green Color in Nodules of *Rhizobium etli* Mutant Differs from Development of Green Color Due to Normal Senescence

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The root nodules of *Phaseolis vulgaris*, the common bean, infected by a cytochrome $bc1^-$ mutant *Rhizobium etli* display an early onset of intense green color. Healthy nodules display a red color due to the heme cofactor of leghemoglobin, which delivers oxygen from the plant cytosol to the bacteria. Oxygen cannot be reduced by the cytochrome $bc1^-$ mutant bacteria; therefore oxygen builds up in the nodule as leghemoglobin continues to bring oxygen to the bacteria. The high oxygen concentration in the nodule is dangerous to its persistence because oxygen can form reactive oxidative species (ROS) and damage the nodule. One way the plant may resist the accumulation of oxygen in a compromised nodule is by breaking down the heme cofactor of leghemoglobin so that no more oxygen will be delivered to the nodule. Heme is broken down by an enzyme called heme oxygenase. The breakdown product of this reaction is an organic molecule called biliverdin which has a green color. Biliverdin is also known to have antioxidant properties, which may be important to defend the nodule against the development of ROS. Spectral data supported the hypothesis that biliverdin was the source of the green color for the cytochrome $bc1^-$ mutant. Immunochemical detection on SDS-PAGE indicated heme oxygenase was upregulated in the nodules of the cytochrome $bc1^-$ mutant between 11 and 14 days after inoculation. As normal nodules senesce, they gradually take on a dark green hue and lose nitrogen fixing activity. This normal senescence occurs in nodules with wild-type bacteria two weeks later than the onset of green color in the cytochrome $bc1^-$ mutant. One possibility is that the plant's response to the mutant bacteria might be a premature, but otherwise normal, senescence. However, that does not appear to be the case. A common indicator of senescence is the complete degradation of protein in the plant cytosol. In nodules with wild-type bacteria, protein content decreased significantly with the onset of green color, whereas in nodules with the cytochrome $bc1^-$ mutant bacteria there was not a significant decrease in protein content with the onset of green color. Additionally, during the normal wild-type senescence heme oxygenase was not upregulated, which emphasizes the uniqueness of the upregulation of heme oxygenase in the cytochrome $bc1^-$ mutant. In conclusion, the data suggest that the plant upregulates production of heme oxygenase in response to high oxygen concentration in the nodule. It is possible that this is the plant's mechanism to defend against a build up of oxygen in an otherwise healthy nodule. This defense mechanism of breaking down heme to biliverdin would protect the nodule from damage by ROS. Induction of this mechanism by $bc1^-$ mutant deficiencies appears to be independent of the normal senescence program and, indeed, may delay the onset of senescence.