

February 8, 1996
DOE/ER/20032--T1**Final Technical Report**

DOE DE-FG02-91ER20032 "Iron regulation of Gene Expression in the *Bradyrhizobium japonicum*/Soybean Symbiosis" 6/1/91 to 5/31/95. \$173,464 direct costs.

Iron, heme and oxygen regulation of *hemA*. *B. japonicum* produces ALA, the universal precursor of tetrapyrroles, in a reaction catalyzed by the product of the *hemA* gene. Expression of the *B. japonicum hemA* gene is affected by iron availability (Page et al., 1994). Activity of a *hemA-lacZ* fusion is increased approximately 3-fold by iron and RNA analysis indicates that iron regulation is at the level of mRNA accumulation. Deletions of regions upstream of the *hemA* promoter, including a putative "iron box", did not eliminate this differential expression (Page, 1994). However, deletion of most of the 5' untranslated region, excluding the Shine-Dalgarno sequence, eliminated the increased expression observed in the presence of iron (Page, 1994). This deletion also resulted in overall expression which is less than 20% of that seen for cells grown in the absence of iron; however, this same fusion is still subject to oxygen regulation. Because regulation by oxygen is known to occur at the transcriptional level, via the *fixLJ* system (see below), this result demonstrates that, although expression is lower, transcriptional regulation of *hemA* expression still occurs and suggests that regulation by iron may occur via changes in mRNA stability.

To address the question of how the 5' untranslated region of the *hemA* transcript is involved in iron regulation, we constructed evenly spaced 10 bp deletions within the *hemA* leader region and determined their effect on *hemA-lacZ* expression. Deletions were constructed such that 16 bp of wild type sequence were replaced with the 6 bp recognition sequence for the restriction enzyme *Bgl*III resulting in an overall change of 10 bp. The deletions were designed to eliminate potential "stem loop" structures within the *hemA* transcript as well as disrupt two potential peptides upstream of the *hemA* structural gene. The *Bgl*III restriction site provides a screen for identification of plasmids containing the deletion. Cells containing *hemA-lacZ* fusions with each of the four 10 bp deletions were grown in minimal xylose medium with and without added iron. Assays of β -galactosidase activity showed that fusions containing deletions 3 and 4 are expressed at wild type levels under all conditions. Expression of the fusion containing deletion 2 is ~30% of wild type when grown under both conditions but the fold induction by iron is the same as the wild-type fusion. The fusion containing deletion 1 is also expressed at 30% of wild-type under iron deficient conditions but is no longer induced by the presence of iron.

The *hemA* gene of *B. japonicum* is also regulated by oxygen (Page and Guerinot, 1995). Up to 9 fold induction of β -galactosidase activity is seen when cultures of *B. japonicum* containing either a plasmid encoded or chromosomally integrated *hemA-lacZ* fusion are shifted to restricted aeration. The oxygen effect is mediated via the FixLJ two-component regulatory system that regulates the expression of a number of genes involved in the nitrogen fixation process in response to low oxygen conditions (Anthamatten and Hennecke, 1991); oxygen induction is lost when the *hemA-lacZ* fusion is expressed in strains of *B. japonicum* carrying mutations in *fixL* or *fixJ*. The *B. japonicum hemA* promoter region contains a sequence identical to the *E. coli* Fnr binding site (-46 to -33 relative to the *hemA* transcription start). Fnr is a regulatory protein necessary for the oxygen-regulated expression of anaerobic respiratory genes (Spiro, 1994). Activity of a *hemA-lacZ* fusion construct in which the Fnr box-like sequence was replaced with a *Bgl*III site is not induced in *B. japonicum* cultures grown under restricted aeration. The *fnr* homolog *fixK* has been cloned from several rhizobial species, including *B. japonicum*, and transcription of *fixK* is FixLJ dependent. Collectively, these data suggest a role for the rhizobial Fnr-like protein, FixK, in the regulation of *hemA*. Furthermore, the coregulation

MASTER

W

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

of *hemA* with symbiotically important genes via FixLJ is consistent with the idea that *hemA* is required in the nodule as well as under free-living conditions.

The *hemA* deletion strain MLG1 of *B. japonicum* is an ALA auxotroph (Guerinot and Chelm, 1986) and therefore cannot synthesize heme without the addition of ALA. However, MLG1 is capable of growth without ALA supplementation in a glucose/salts/yeast extract medium (Frustaci et al., 1991). Remarkably, and most importantly for our studies, such cells appear to have no measurable heme. By introducing a *hemA-lacZ* fusion into strain MLG1, we have been able to examine whether heme itself regulates *hemA* expression. Heme is a strong negative regulator of *hemA* (Page, 1994); β -galactosidase levels are 8 fold higher in the *hemA* mutant than in wild type cells carrying the same construct. Addition of ALA or hemin to the culture restores the level of *hemA-lacZ* expression to that of wild type. Therefore, we tentatively conclude that heme is normally repressing expression of *hemA* in wild type cells. MLG1 is totally deficient in ALAS activity, lacks heme, and is relieved of this negative control of *hemA* expression. Levels of *hemA-lacZ* fusion mRNA do not change with differences in heme availability, suggesting that heme regulates *hemA* posttranscriptionally.

Publications resulting from previous support:

- Guerinot, M.L. 1993. Iron and the nodule. pp 197-217. *In* Iron Chelation in Plants and Soil Microorganisms, L.L. Barton and B. Hemming, (eds.), Academic Press, New York.
- Page, K.P., E. Connolly and M.L. Guerinot. 1994. The effect of iron availability on expression of the *Bradyrhizobium japonicum hemA* gene. *J. Bacteriol.* 176: 1535-1538.
- Page, K. 1994. The effect of iron, oxygen and heme on the expression of the *Bradyrhizobium japonicum hemA* gene. Ph.D. Dartmouth College.
- Guerinot, M.L. 1994. Microbial Iron Transport. *Annu. Rev. Microbiol.* 48: 743-772.
- Page, K.M., and M.L. Guerinot. 1995. Oxygen control of the *Bradyrhizobium japonicum hemA* gene. *J. Bacteriol.* 177: 3979-3984.
- Page, K.P., D.J. Westenberg and M.L. Guerinot. 1996. Iron and heme regulation of the *Bradyrhizobium japonicum hemA* gene. *J. Bacteriol.* To be submitted by 3/1/96.

Presentations at meetings:

- Page, K.M. and M.L. Guerinot. 1992. Iron regulation in *Bradyrhizobium japonicum*. Sixth International Symposium on Molecular Plant-Microbe Interactions. Seattle, WA. 7/11-16/92 CONF-9207238
- Kurtz, H. and M.L. Guerinot. 1992. Identification of an iron-regulated gene of *Bradyrhizobium japonicum* using *TnphoA* mutagenesis. Sixth International Symposium on Molecular Plant-Microbe Interactions. Seattle, WA.
- Guerinot, M.L. Iron uptake, storage and regulation in *Bradyrhizobium japonicum*. 14th North American Conference on Symbiotic Nitrogen Fixation. Minneapolis, MN. July, 1993. CONF-9307235
- Page, K. and M.L. Guerinot. Effect on iron, oxygen and heme on the *Bradyrhizobium japonicum hemA* gene. 7th International Symposium on Molecular Plant-Microbe Interactions. Edinburgh, Scotland. June, 1994. CONF-9406431

References

- Anthamatten, D. and H. Hennecke. 1991. The regulatory status of the *fixL*- and *fixJ*-like genes may be different from that found in *Rhizobium meliloti*. *Mol. Gen. Genet.* 225:38-48.
- Frustaci, J. M., I. Sangwan and M. R. O'Brian. 1991. Aerobic growth and respiration of a Δ -aminolevulinic acid synthase (*hemA*) mutant of *Bradyrhizobium japonicum*. *J. Bacteriol.* 173:1145-1150.

- Guerinot, M. L. and B. K. Chelm. 1986. Bacterial aminolevulinic acid synthase activity is not essential for leghemoglobin formation in the soybean/*Bradyrhizobium japonicum* symbiosis. *Proc. Natl. Acad. Sci. USA.* **83**:1837-1841.
- Page, K. 1994. The effect of iron, oxygen and heme on the expression of the *Bradyrhizobium japonicum hemA* gene. Ph.D. Dartmouth College.
- Page, K.P., E. Connolly and M.L. Guerinot. 1994. The effect of iron availability on expression of the *Bradyrhizobium japonicum hemA* gene. *J. Bacteriol.* **176**: 1535-1538.
- Page, K. and M. L. Guerinot. 1995. Oxygen Control of the *Bradyrhizobium japonicum hemA* gene. *J. Bacteriol.* **177**:3979-3984.
- Spiro, S. 1994. The Fnr family of transcriptional regulators. *Antonie van Leeuwenhoek.* **66**:23-36.

3. Product/Report Description

b. Conference/Meeting/Presentation

Conference Title: 14th North American Conference on Symbiotic Nitrogen Fixation.

Location: Minneapolis, MN

Dates: 7/25/93 to 7/29/93

Sponsor: University of Minnesota

9307235-

Conference Title: 6th International Symposium on Molecular Plant Microbe Interactions.

Location: Seattle, WA

Dates: 7/11/92 to 7/16/92

Sponsor: International Society for Molecular Plant Microbe Interactions

9207238

*Preprints + reprints
removed and cycled
separately.*