Overview:

This is a final report for Department of Energy Grant No. DE-FG02-08ER15927 entitled "Molecular Genetic Analysis of Activation-Tagged Transcription Factors Thought to be Involved in Photomorphogenesis". Based on our preliminary photobiological and genetic analysis of the *sob1-D* mutant, we hypothesized that OBP3 is a transcription factor involved in both phytochrome and cryptochrome-mediated signal transduction. In addition, we hypothesized that OBP3 is involved in auxin signaling and root development. Based on our preliminary photobiological and genetic analysis of the *sob2-D* mutant, we also hypothesized that a related gene, *LEP,* is involved in hormone signaling and seedling development.

Recent Progress:

Jason Ward, a former graduate student in my lab at Washington University, completed the bulk of the first specific aim from this grant resulting in a publication in The Plant Cell (Ward et al. 2005). Additional studies related to the first specific aim will be continued by a post doc, Dr. Jiwen Qiu, who will be joining my lab in December 2008. These additional studies include: intact vs. excised cotyledon experiments; epistasis analysis between *OBP3* and *PP7*; molecular analysis of interactions between CYR1 and OBP3; and analysis of previously collected Affymetrix chip data comparing global gene expression as related to the genetic state of *OBP3*. In addition, Jason Ward tested the hypothesis that OBP3 is involved in auxin signaling. After completing all of the proposed experiments in the second specific aim of this grant, it appears that there is no direct connection between auxin signaling and OBP3. With this in mind, we are no longer pursuing this line of experiments. Jason Ward also completed the third specific aim of this grant resulting in an additional publication in The Plant Cell (Ward et al. 2006). Since these studies did not show a direct link between LEP and photomorphogenesis, we have focused our efforts on OBP3 and two recently identified DNA-binding proteins involved in photomorphogenesis, SOB3/AHL29 and its closest family member ESC/AHL27 (see below).

Problems Encountered:

Soon after Jason Ward left my lab for a postdoctoral position in the lab of Dr. Daphne Preuss, followed by employment at the Monsanto Corporation, I decided to move my research program from Washington University to Washington State University in Pullman WA. In the interim, Ian Street, another former graduate student in my lab, expanded the scope of this research based on his findings from studies of an AT-hook domain containing DNA-binding protein SOB3/AHL29 and its closest family member ESC/AHL27. These studies resulted in a publication in The Plant Journal (Street et al. 2008).

Expanding the Scope of this Research:

Ian Street's studies focused on *SOB3*, which encodes a plant-specific AT-hook motif containing protein, identified from an activation-tagging screen for suppressors of the long-hypocotyl phenotype of a weak *phyB* allele, *phyB-4*. *sob3-D* over-expressing seedlings have shorter hypocotyls and as adults, develop larger flowers and leaves, and are delayed in senescence compared to wild-type plants. At the nucleotide level, *SOB3* is closely related to *ESCAROLA* (*ESC*), which was identified in an independent activation-tagging screen. *ESC* over-expression also suppresses the *phyB-4* long-hypocotyl phenotype and confers an adult morphology similar to *sob3-D*, suggesting similar functions. Analysis of transgenic plants harboring SOB3:SOB3-β-glucuronidase (GUS) or ESC:ESC-GUS translational fusions driven

by their endogenous promoter regions showed GUS activity in the hypocotyl and vasculature tissue in light- and dark-grown seedlings. A loss-of-function *SOB3* allele (*sob3-4*) was generated through an EMS intragenic suppressor screen of *sob3-D phyB-4* plants, and this allele was combined with a predicted null allele, disrupting *ESC* (*esc-8*), to examine potential genetic interactions. The *sob3-4 esc-8* double mutant had a long hypocotyl in multiple fluence rates of continuous white, far-red, red and blue, light. *sob3-4 esc-8 phyB-9* and *sob3-4 esc-8 cry-103* triple mutants also had longer hypocotyls than photoreceptor single mutants. In contrast, the *sob3-4 esc-8 phyA-211* triple mutant was the same length as *phyA-211* single mutants. Taken together, these data suggest that *SOB3* and *ESC* act redundantly to modulate hypocotyl growth inhibition in response to light acting specifically down stream of the photoreceptor phyA.

List of Publications Resulting from this Grant:

Below is a list of publications resulting from this grant. Each is also attached to the end of this document in the order listed.

Directly Supported by this Grant:

Ward JM, Cufr CA, Denzel MA and **Neff MM (2005)** The Dof transcription factor, OBP3, modulates phytochrome and cryptochrome signaling in Arabidopsis. **Plant Cell** 17 475-485

Ward JM, Smith AM, Shah PK, Gallanti SE, Yi H, Demianski AJ, van der Graaff E, Keller B and **Neff MM (2006)** A New Role for the AP2 Transcription Factor, LEP, in Gibberellin-Induced Germination is Revealed by the Mis-Expression of a Homologous Gene, *SOB2/DRN-like.* **Plant Cell** 18 29-39

Street IH, Shah PK, Smith AM, Avery N, and **Neff MM (2008)** The AT-Hook Containing Proteins SOB3/AHL29 and ESC/AHL27 are Negative Modulators of Hypocotyl Growth in *Arabidopsis*. **Plant Journal** 54 1-14

Acknowledging Indirect Support from the Grant:

Turk EM, Fujioka S, Seto H, Shimada Y, Takatsuto S, Yoshida S, Wang H, Torres QI, Ward JM, Murthy G, Zhang J, Walker JC and **Neff MM (2005)** *BAS1* and *SOB7* Act Redundantly to Modulate *Arabidopsis* Photomorphogenesis via Unique Brassinosteroid Inactivation Mechanisms. **Plant Journal** 42 23-34

Zhang J, Wrage EL, Vankova R, Malbeck J, and **Neff MM (2006)** Overexpression of *SOB5* suggests the involvement of a novel plant protein in cytokinin-mediated development. **Plant Journal** 46 834-848

Nemri A, **Neff MM**, Burrell M, Jones JDG and Studholme DJ **(2007)** Marker development for the genetic study of natural variation in *Arabidopsis thaliana.* **Bioinformatics** 23 3108-3109

Chen H, Zhang J, **Neff MM**, Hong S-W, Deng XW and Xiong L **(2008)** Integration of light and abscisic acid signaling during seed germination and early seedling development. **Proceedings of the National Academy of Sciences USA** 105 4495-4500

Neff MM, Sanderson L and Tedor D **(2009)** Light-mediated germination in lettuce seeds: Resurrection of a classic plant physiology lab exercise. **The American Biology Teacher** 71 367-370