PROGRESS REPORT
1993-1994

**ANALYSIS OF STRUCTURAL DOMAINS REQUIRED FOR PHYTOCHROME
FUNCTION BY *IN VITRO* MUTAGENESIS.**

Grant Number: DE-FG02-88ER13968

Principal Investigator: RICHARD D. VIERSTRA

Phytochrome is a photoreceptor that has a central role in light-regulated development in plants. It is synthesized as a red light-absorbing form (Pr) which upon irradiation with red light is converted to a biologically active, far-red light-absorbing form (Pfr). Phytochrome-controlled responses span the life cycle of plants, from seed germination and de-etiolation, to flowering and senescence. During the 40 years since its initial discovery, a great body of information has accumulated concerning the properties of the chromoprotein *in vitro* and the various responses controlled by Pfr. However, little is known about how phytochrome functions at the molecular level as an activator of photomorphogenesis. Such knowledge would be useful in designing chemical and molecular genetic tools to more effectively control light-regulated plant development.

Several years ago, we succeeded in expressing a chimeric oat phytochrome A gene in transgenic tobacco that results in increased levels of a functional photoreceptor. By all criteria, the recombinant oat protein synthesized in tobacco is biochemically indistinguishable from that synthesized naturally including its rapid Pfr-specific conjugation with ubiquitin and degradation. Importantly, plants that overexpress oat phytochrome exhibit a striking "light exaggerated" phenotype. This phenotype represents the first assay of the receptor's function *in vivo*. The goal of this project is to combine the transgenic system with *in vitro* mutagenesis to identify phytochrome domains potentially important to synthesis, dimerization, chromophore attachment, Pr/Pfr phototransformation, Pfr-enhanced degradation, and biological activity.

In the last year, we successfully expressed in tobacco a series of 8 nested deletions of phytochrome A that involved the removal of both amino- and carboxy-terminal sequences. From examination of the encoded proteins synthesized *in planta*, we have identified several domains important for phytochrome structure and function. Sequences necessary for chromophore attachment *in vivo* were localized to the amino-terminal 398 residues because mutant proteins this small had covalently bound chromophore. Deletion mutants from the carboxy terminus to residue 653 were spectrally indistinguishable from the full-length chromoprotein. In contrast, further truncation to residue 399 resulted in a chromoprotein with a bleached Pfr absorbance spectrum; Pr and Pfr absorbance maxima shifted toward shorter wavelengths; and reduced Pfr to Pr phototransformation efficiency. These results identified residues between 399 and 652 as essential for spectral integrity, but are not essential for chromophore attachment. Sequence(s) between residues 919 and 1093 appear to be necessary for dimerization. Carboxy-terminal mutants containing this region behaved as dimers under nondenaturing conditions *in vitro*, whereas truncations without this region behaved as monomers.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

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

With respect to biological activity, two domains required for full biological activity were identified. One is located near the amino terminus between residues 7-69 and the other is located at the carboxy terminus between residues 1094 and 1129. Removal of either rendered the chromoprotein biologically inactive. The amino-terminal domain is of interest because it undergoes a conformation change during Pr/Pfr transformations and is necessary for many of the physical properties of the chromoprotein including its Pr and Pfr absorbance spectra and the stability of Pfr. This suggests that removal of these amino acids affects the native structure of the protein especially in its active form. More refined mapping of this region with 5 smaller deletion mutants potentially identified a short stretch of amino acids (residues 12-47) that is biologically essential. Whereas, other deletions in this regions still render the chromoprotein biologically active, deletion of this region results in inactive proteins. Biochemical analysis of these deletions revealed that removal of residues 12-47 destabilizes Pfr such that it reverts more rapidly to Pr and shifts the absorbance spectra of both Pr and Pfr to shorter wavelengths. Thus residues 12-47 may be necessary for maintaining the correct conformation of Pfr.

The carboxy-terminal 35-amino acid region is of interest because by all criteria measured, its removal does not affect the biochemical properties of the chromoprotein. It behaves as a dimer, has unaltered Pr and Pfr absorbance spectra, and shows equivalent efficiency in the phototransformation between Pr and Pfr. Therefore, removal of these carboxy-terminal residues would seem to render the chromoprotein inactive not because of general physicochemical perturbations, but because it is missing an essential component of the active site. The potential biological function of the carboxy-terminal domain is unclear. Interestingly, despite its importance, its amino acid sequence is not well conserved when compared to various phytochromes (A, B, and C) from other species. Even though the domain is predicted to be hydrophobic and not to reside on the surface, it is readily accessible to proteolytic attack, especially in the Pr form. The biological activity of this region does strengthen the recent hypothesis that the carboxy terminus has a catalytic activity based on its amino acid sequence homology with a group of bacterial sensor proteins that act as protein kinases. While the 35-amino acid stretch identified here is within the proposed catalytic domain, it is distal to the specific residues proposed to be directly involved in protein phosphorylation. More refined mapping of this region is underway to test its proposed kinase function. We have also discovered that removal of this C-terminal domain does stabilize Pfr to rapid degradation. Instead of being rapidly degraded following red light photoconversions, chromoproteins missing this C-terminal domain appear very stable. The possible relationship between function and degradation of Pfr is intriguing.

Further refined mapping of structurally and functionally important domains is in progress to identify specific residues. Of special interest is the location of residues within the amino and carboxy terminus required for biological activity. Toward this goal a series of nested deletions and alanine-scanning mutations within the amino-terminal 69 residues has been made. Their expression in transgenic tobacco and subsequent biochemical analysis of the proteins are in progress. Completion of this work will represent an important step in the identification of domains essential to the proper assembly and function of this essential photoreceptor.

PUBLICATIONS DERIVED FROM CURRENT DOE GRANT DURING 1993.

1. Cherry, JR, D Hondred, JM Walker, JM Keller, HP Hershey, and RD Vierstra (1993) Carboxy-Terminal Deletion Analysis of Oat Phytochrome A Reveals the Presence of Separate Domains Required for Structure and Biological Activity. *Plant Cell*. 5: 565-575.
2. Vierstra, RD (1993) Protein Degradation in Plants. *Annu Rev. Plant Physiol. Molec. Biol.* 44: 385-410.
3. Vierstra, RD (1993) Illuminating Phytochrome Function(s): There is Light at the End of the Tunnel. *Plant Physiol.* 103: 679-684.
4. Vierstra, RD (1993) Phytochrome Degradation. In: *Photomorphogenesis in Plants* (RE Kendrick and GHM Kronenberg, eds.) Martinus Nijhoff Publishers, Dordrecht, Netherlands. pp 141-162.
5. Cherry, JR, and RD Vierstra (1993) The Use of Transgenic Plants to Examine Phytochrome Structure/Function. In: *Photomorphogenesis in Plants* (RE Kendrick and GHM Kronenberg, eds.) Martinus Nijhoff Publishers, Dordrecht, Netherlands. pp 271-297.
6. Casal, JJ, RA Sanchez, and RD Vierstra (1994) *Avena* Phytochrome A Overexpressed in Transgenic Tobacco Seedlings Differentially Affects Red/Far-Red Reversible and Very-Low-Fluence Responses (Cotyledon Unfolding) during De-etiolation. *Planta* 192: 306-309.
7. Ballaré, CL, Scopel AL, ET Jordan, and RD Vierstra (1994) Signaling Among Neighboring Plants and the Development of Size Inequalities in Plant Populations. *Proc. Natl. Acad. Sci. USA*. (in press).
8. Jordan, E, PM Hatfield, D Hondred, M Talon, JAD Zeevaart, and RD Vierstra (1994) Phytochrome A Overexpression in Tobacco: Dwarf Phenotype Potentially Mediated by High Concentrations of the Photoreceptor in Vascular Tissue Attenuating Gibberellin Levels. *Plant Physiol.* (in preparation).

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, makes 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.