

## Final Technical Report

**Grant No.** DE-FG02-99ER20324

**Project Title** Molecular bases and photobiological consequences of light intensity adaptation in photosynthetic organisms

**Project Duration** September 1, 1998 to March 1, 2002

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## Introduction

All photosynthetic organisms continuously adjust their rate of light harvesting to match, in so far as possible, the optimum rate of electron transport (Falkowski and LaRoche). This phenomenon, called photocclimation is formally defined as a set of developmentally independent, reversible responses that physiologically compensate for changes in light intensity. Photoacclimation involves a nested set of processes (Falkowski and Chen, in press). On short time scales, the responses primarily are associated with changes in the effective absorption cross section that are controlled by the xanthophyll cycle and state transitions. On longer time scales, photoacclimation involves in changes in gene expression that leads to alterations in the activity and concentration of photosynthetic complexes and cofactors, thylakoid density and plastid structure. In all eucaryotic photosynthetic organisms, the majority of chloroplast genes are located in the nucleus. Hence, a feedback between photosynthetic processes and nuclear encoded gene expression requires biochemical communication between chloroplast and nuclei. The immediate goal of this project is to identify those pathways, however the long term significance of the research is in identifying how environmental information is transduced by the photosynthetic apparatus to affect nuclear gene expression.

## Project Overview

Several years ago, with funding from DOE, we demonstrated that the redox status of the plastoquinone pool affects the expression of nuclear located photosynthetic genes in a eukaryotic green alga, *Dunaliella tertiolecta* (Escoubas, et al., Proc. Nat. Acad. Sci. 92:10237-41). That finding was the first to establish redox regulation of a nuclear gene in a photosynthetic organism. The results were rather straightforward. When the PQ pool was oxidized, nuclear genes encoding the light harvesting complexes were upregulated and the cell accumulated more light harvesting complexes (low light adapted). In contrast, when the PQ pool was reduced, the cell down regulated the expression of the light harvesting proteins with a concomitant loss of antenna pigment protein complexes (high light adapted). We set out to identify components involved in the signal transduction pathway that relays the perceived irradiance signals from the chloroplast to the nucleus.

Lacking a transformation system, our options were constrained to molecular biological and biochemical approaches. From gel mobility shift assays, we identified a DNA binding protein that corresponded to a "high light" condition, and appeared to be correlated with the down regulation of expression of a light harvesting protein associated with photosystem II. Hence, it seemed reasonable to set a short-term goal to characterize the key DNA binding factors and to assess how the activity of these factors is related to the redox poise of the photosynthetic

electron transport system. We also investigated the effect of redox modulation in the photosynthetic electron transport chain on the expression of various genes in various photosynthetic organisms that are physiologically and ecologically important, such as nitrate reductase in *Chlamydomonas reinhardtii*, and nitrogenase in a marine diazotrophic cyanobacterium *Trichodesmium* spp.

### Summary and Significance of the Major Results

We use the unicellular green eucaryotic alga, *Dunaliella tertiolecta*, as a model organism.

#### A. Regulation of nuclear-located *cab1* gene by photosynthetic electron transfer in chloroplast

By applying a combination of light transitions, uncouplers, and inhibitors of photosynthetic electron transport inhibitors we modulate the redox poise of many components in the plastid and examine the pattern of expression of *cab1* gene. This gene encodes the major light harvesting protein that services photosystem II. While our results have confirmed our own previous finding that light intensity regulation of *cab1* gene expression is signaled by the redox state of the PQ pool, we have also identified additional sensor(s) located in the PET chain.

#### B. Redox regulation of multiple DNA-binding complexes in the *cab1* promoter of *Dunaliella*

Using a gel mobility shift assay (EMSA), we investigated the *in vitro* DNA-binding activities in a 180 bp *cab1* promoter region (from -367 to -188 bp) in *D. tertiolecta* cells under a wide variety of conditions. Our results reveal a set of multiple DNA-binding complexes that correspond to transcriptional repressors and enhancers. The activities of the DNA-binding complexes appears to be regulated by both the redox status of the PQ pool and by the cytochrome *b<sub>6</sub>* complex. Sequence analysis of the promoter region also revealed three tentative binding sites that are presented with almost the same organization in two non-overlapping regions. The proposed binding sites may represent novel *cis*-acting elements whose motifs differ from those of the previous identified *cis*-elements involved in phytochrome-mediated light regulation of *cab* genes in higher plants. Thiol-groups, but not phosphorylated amino acids, seem to be involved in their *in vitro* binding to the targeted *cab1* promoter regions. EMSA results with thiol-modifying reagents suggest that the binding complexes share some common motifs. Moreover, the interactions among the binding factors, and their subsequent binding activity, requires thiol-group modification. Redox-regulation of DNA-binding activities via thiol-groups has been reported for numerous transcription factors in higher plants and animal cells. Our results suggest that the redox-regulated signal transduction information, relayed from the plastid to the nucleus, involves mechanisms other than, or in addition to, a

phosphorylation cascade that had been hypothesized previously (Escoubas et al. 1995). Our results suggest that chloroplast redox signals regulate *cab1* expression by modulating protein-protein interactions responsible for the formation of different binding complexes that act as either transcription repressors or enhancers. The results of this research will be submitted to a peer reviewed journal within the coming two months.

C.

### Education and Training

Contract DE-FG02-99ER20324 provided support for the training of three undergraduate students from Rutgers University as research assistants:

Ms. Mary Ann Tran (Jan. 2000-Sept. 2001)

Mr. Huy Nguyen (Jan. 2000-May 2000)

Mr. Carlos Gonzalez (Sept. 2001-May 2002)

The contract supported one Post-doctoral Research Fellow:

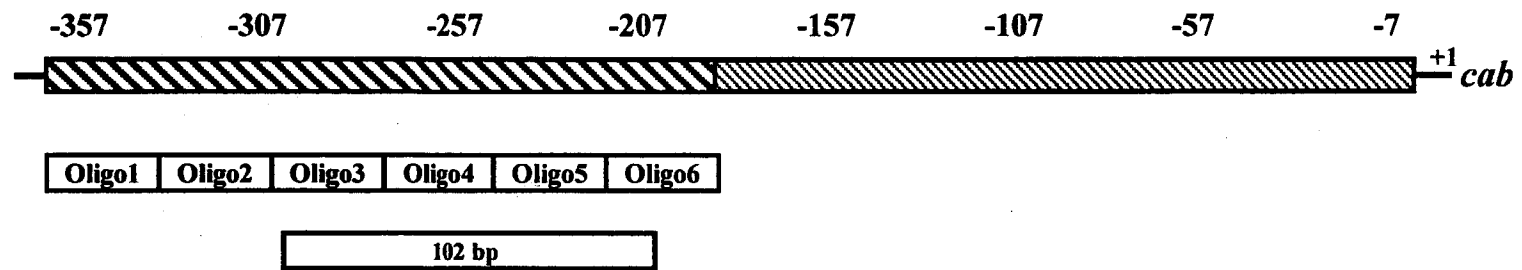
Dr. Yi-bu Chen

And one visiting scientist:

Dr. Mario Giordano

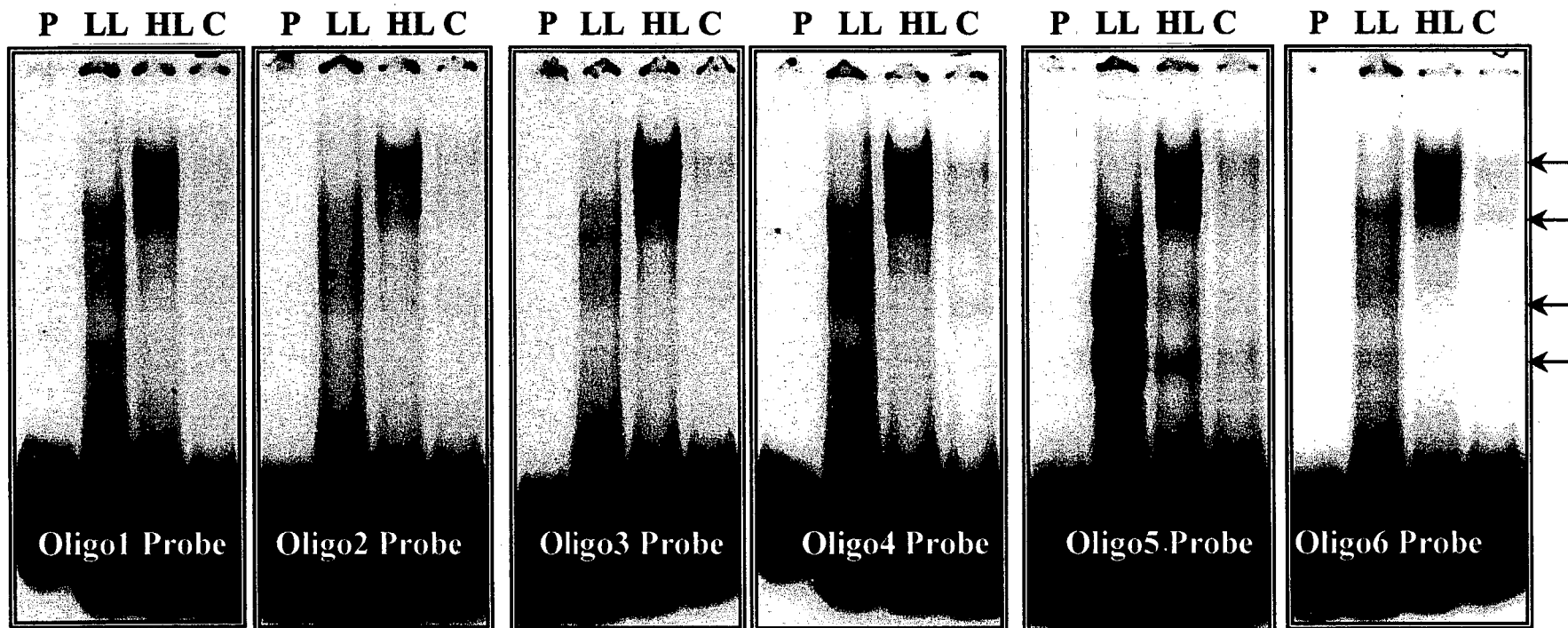
### Publications

1. **Berman-Frank, I., P. Lundgren, Y.-B. Chen, H. Küpper, Z. Kolber, B. Bergman, and P. G. Falkowski.** 2001. Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacteria *Trichodesmium*. *Science* **294:1534-1537**
2. **Falkowski, P. G. and Y.-B. Chen.** 2002. Photoacclimation of Light Harvesting Systems in Eucaryotic Algae. In B.R. Green and W.W. Parson (eds): "Light-harvesting antennas". Dordrecht; Boston, Kluwer Academic Publishers. In press
3. **Chen, Y.-B., D. Durford and P. G. Falkowski.** DNA-binding proteins regulated by chloroplast redox signals in *Dunaliella tertiolecta*. In preparation.



**Figure 1.** Schematic presentation of the six oligo DNA constructed within a 180 bp long *cab1* promoter region (-367 to -188 bp relative to the starting codon) and the original 102 bp promoter fragment of *Dunaliella tertiolecta*. These 30 bp long oligos were commercially synthesized and used as unlabelled competitors or labeled probes in EMSA.

**Figure 2.** EMSA of DNA-binding activities in the 180 bp *cab1* promoter region represented sequentially by Oligo1-6. Protein samples were extracted from high- or low-light acclimated culture and fractionated by a single step ammonium sulfate precipitation at 50% saturation level. P: probe only; LL: protein extracted from low-light culture; HL: protein sample from high-light culture; C: HL protein sample with addition of 50 ng of the same oligo DNA unlabeled as the competitor.





**Figure 3:** EMSA of DNA-binding activities at *cab1* promoter regions represented by Oligo 4-5 using protein samples extracted from high- and low-light acclimated culture and fractionated by a single or multiple-step of ammonium sulfate precipitations. P: oligo probe only; L: protein extracted from low-light culture and precipitated by a single step of 50% ammonium sulfate fractionation; H: protein sample from high-light culture with one-step of 50% ammonium sulfate fractionation; 1-6: protein samples from high-light culture and sequentially fractionated with ammonium sulfate at 20 % (F20), 30 % (F30), 40 % (F40), 50% (F50), 60% (F60) and 80% (F80) of the saturation level; 7-12: protein samples from low-light culture subjected to the same multiple-step ammonium sulfate fractionation.

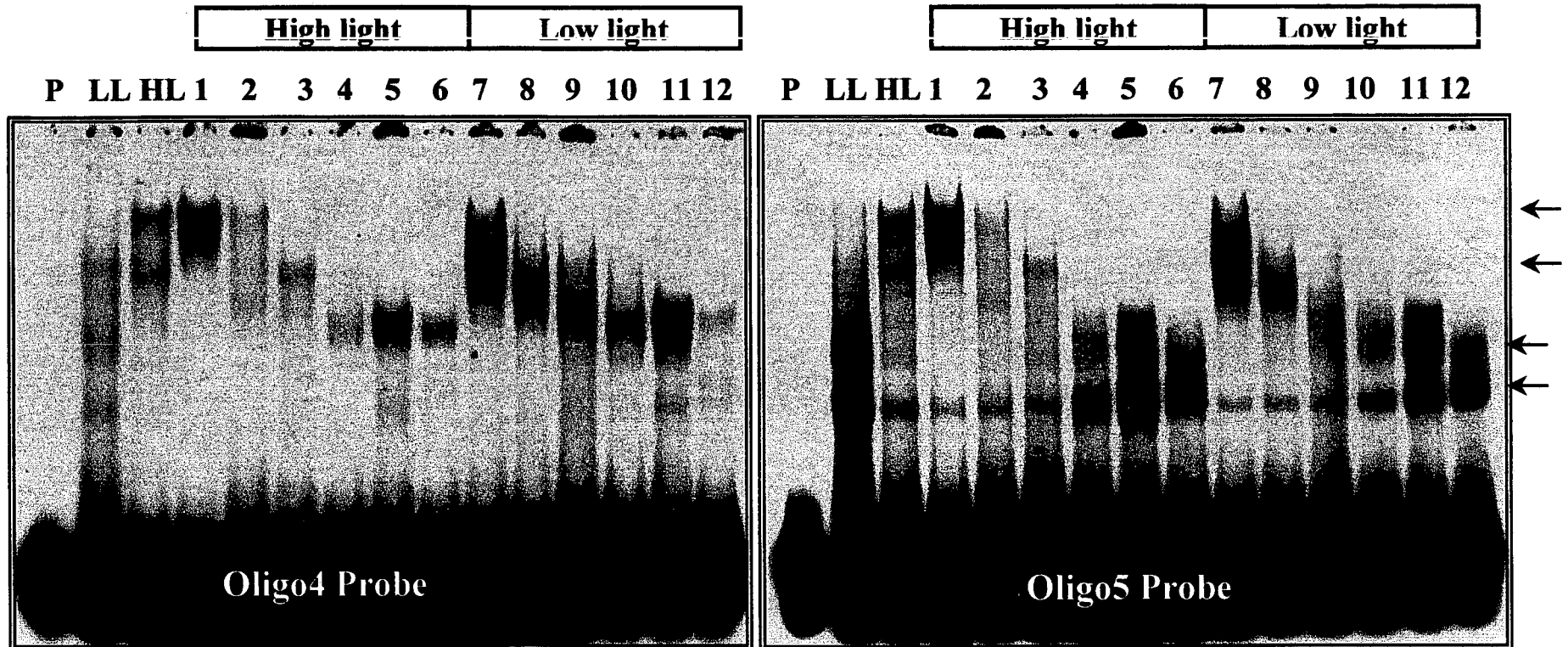
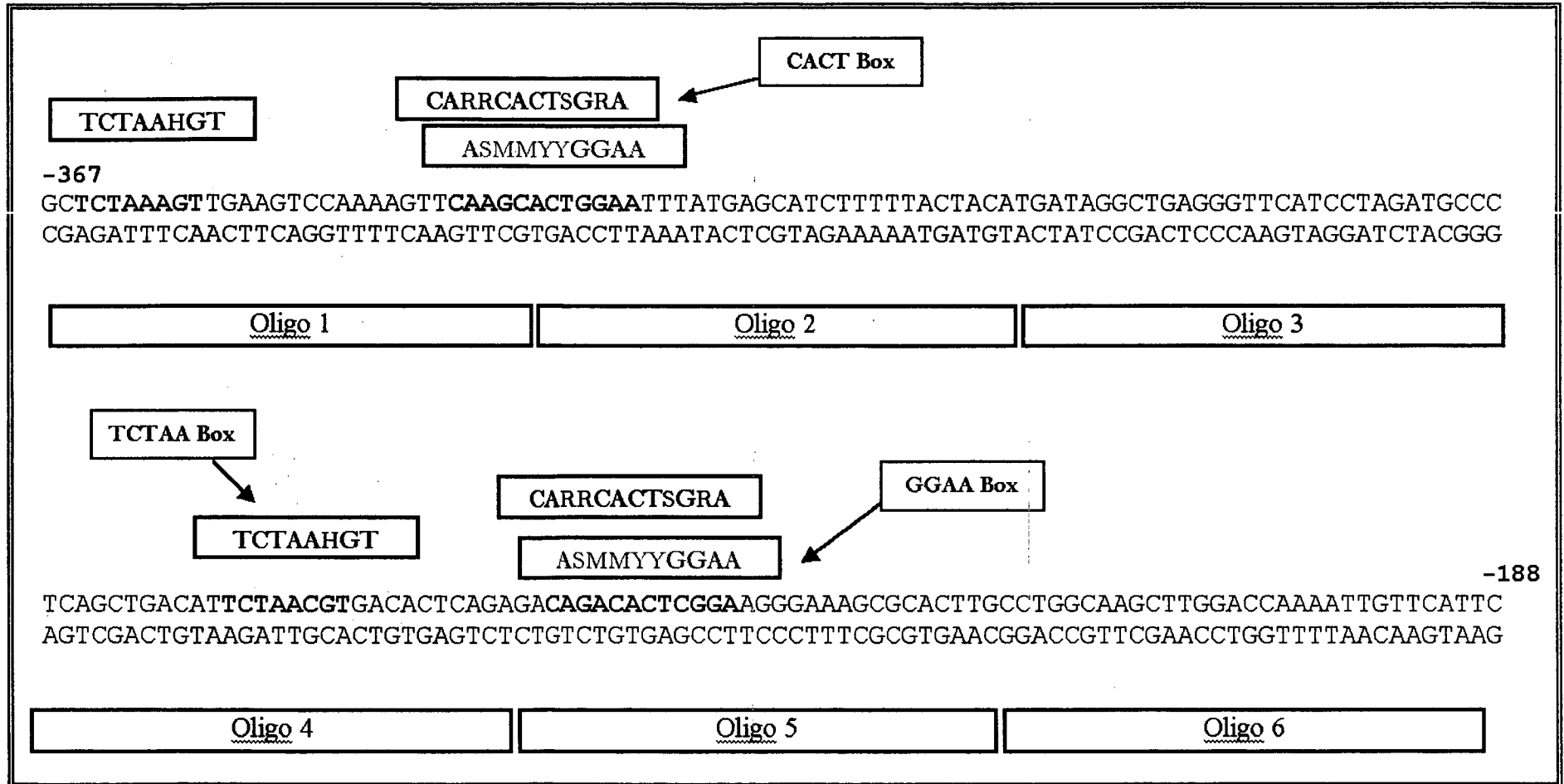


Figure 4. Tentative multiple and repetitive binding sites in *D. tertiolecta cabl* 180 bp promoter region (from -367 to -188 bp).



**Figure 5.** The tentative TCTAA motif in promoters of *cab* genes from different organisms.

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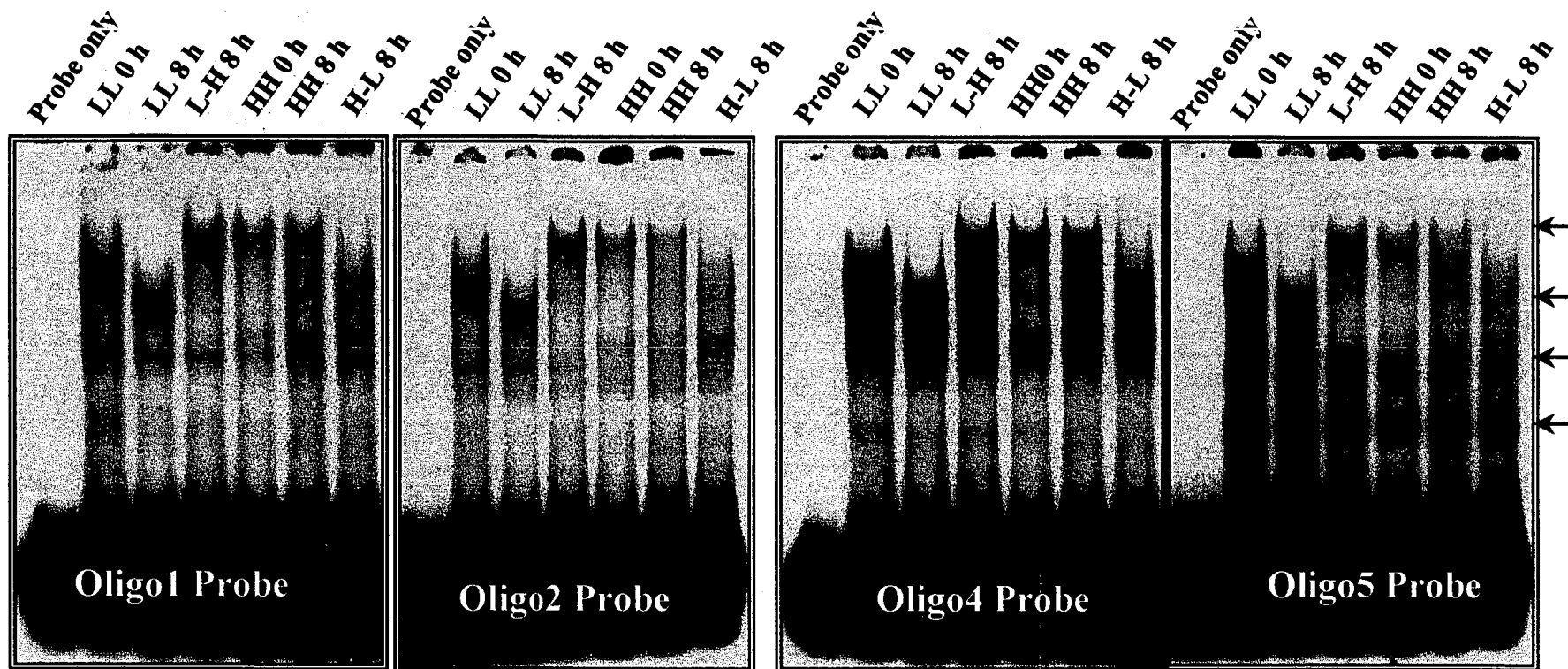
1. TACACCTCGACGTTGAACCCGCCAGCCTCCATAAATTCTCTAAATGTTGATATGTCAACGTTGTGCGCCTGGCCCCGAGGGCTCTAGATCTGGCTTCGGCCCCAA
2. TGAATCATGATAAGAAAAAAGTAGCTATATGTTCTCTAAAGTCTAAACCCATGATGATGAACATGCCATATGAATTAATATAGTTCTTTCATGTTCTTA
3. TCAATGTTATTGTAAATTGGTAGTGATGTTGGACTTCTAATGTTACTCTCTATGATGTTTCGGTCATCGGTATCACACTATCTTTACTTTTATTTAAAGG
4. GCTGACATCTAAACGTGACACTCAGAGACAGACACTCGGAAGGG
5. GCTCTAAAGTTGAAGTCCAAAAGTTCAAGCACTGGAATTTATGAGCATCTTTTTACTACATGATAGGCTGA

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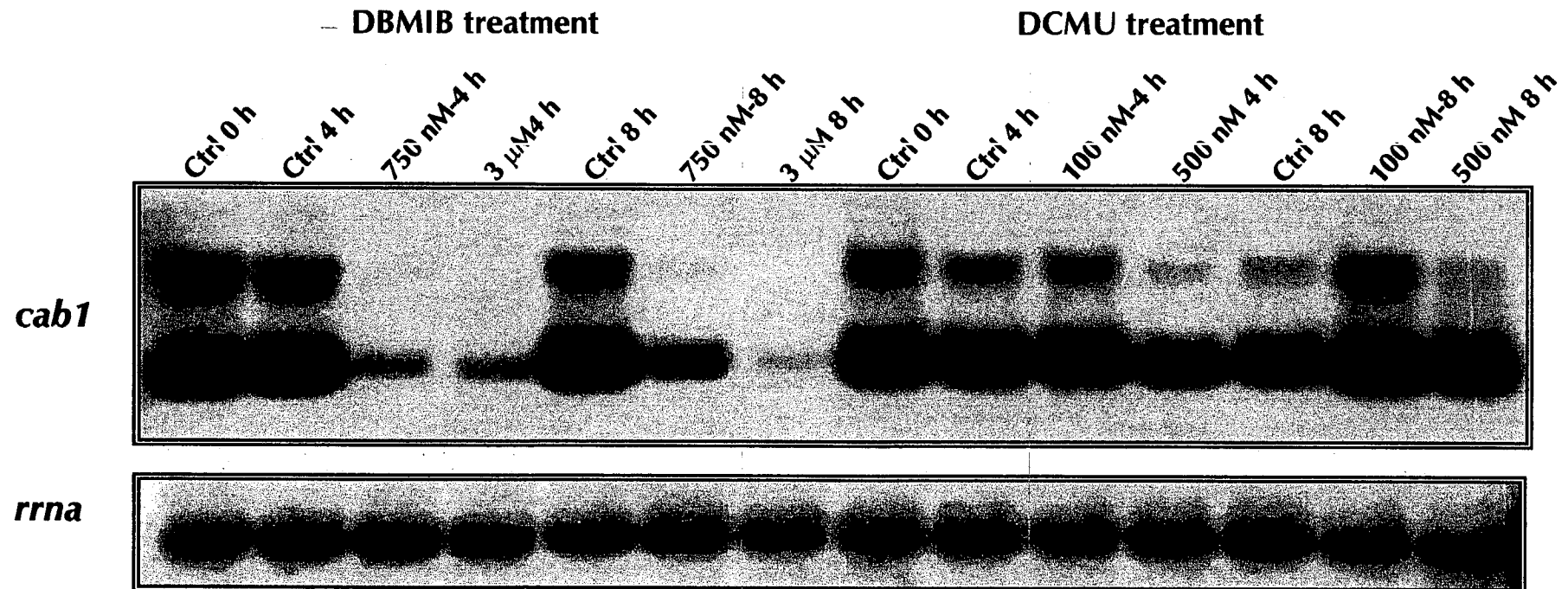
1. *Chlamydomonas reinhardtii cab1* promoter -500 to -400 bp
2. *Arabidopsis thaliana cab2* promoter -723 to -623 bp
3. *Arabidopsis thaliana cab3* promoter -552 to -442 bp
4. *Dunaliella tertiolecta cab1* promoter -274 to -231 bp
5. *Dunaliella tertiolecta cab1* promoter -367 to -297 bp



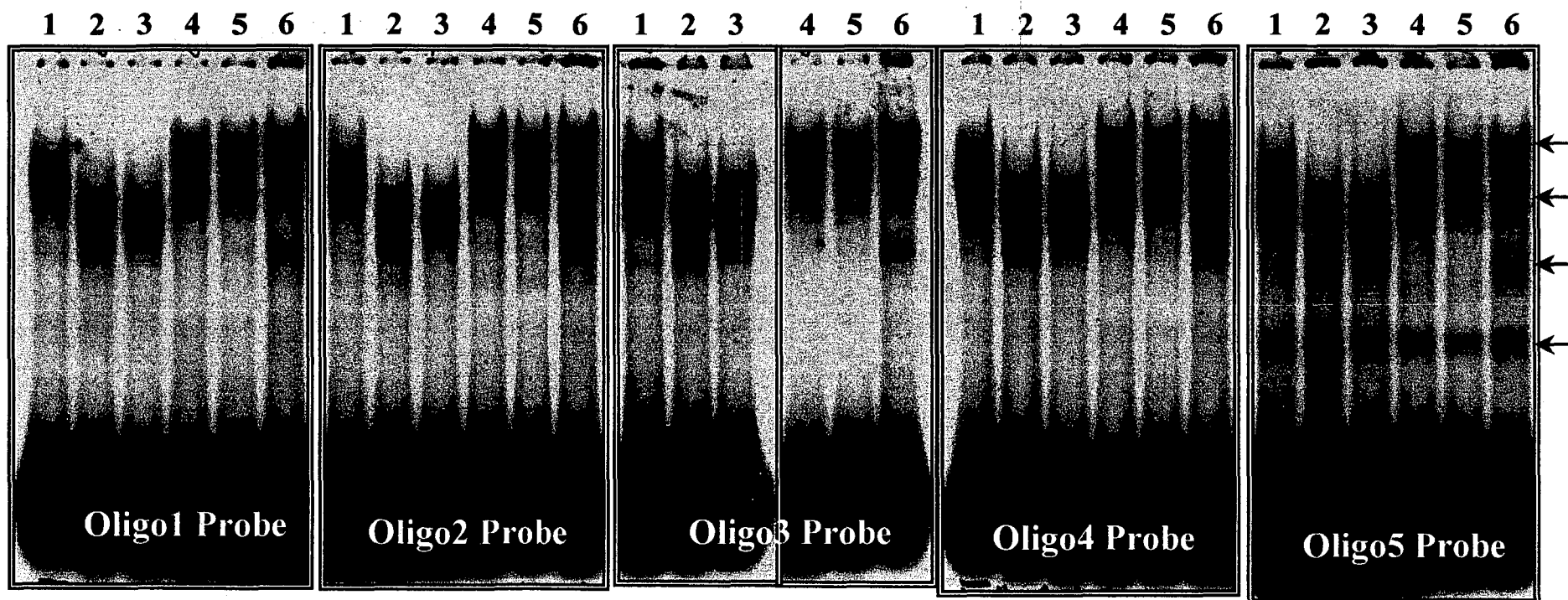
**Figure 7.** EMSA of changes of DNA-binding activities in different *cab1* promoter region following the light intensity transition. All protein samples were subjected to a single step ammonium sulfate precipitation at 50% saturation level. LL 0 h: low-light acclimated culture prior to the light transition; LL 8 h: control culture remained in the low-light condition at the end of 8 h of incubation; L-H 8 h: 8 h after the culture was transferred to high-light condition; HL 0 h: high-light acclimated culture prior to light transition; HL 8 h: culture remained in high-light conditions 8 h after the incubation; H-L 8 h: 8 h after the culture was transferred to low-light conditions.



**Figure 8.** Effects of photosynthetic electron transfer inhibitors on *cab1* transcript abundance. Northern blots of *cab1* and *rrna* 4 and 8 hours in *Dunaliella* after the addition of 100 nM DCMU or 750 nM DBMIB.



**Figure 9.** EMSA of effects of DCMU and DBMIB on DNA-binding activities in different *cab1* promoter region. All protein samples were subjected to a single step ammonium sulfate precipitation at 50% saturation level. DBMIB treatments—1: Control at zero time; 2: control at 8 h; 3: 750 nM DBMIB treatment for 8 h; DCMU experiment—4: control at zero time; 5: control at 8 h; 6: 100 nM DCMU treatment for 8 h.



**Figure 10.** Effects of thiol-group modifying reagents on DNA-binding activities of various DNA-binding factors in *Dunaliella cabl* promoter region represented by Oligo 4 and Oligo 5. Protein samples extracted from HL and LL acclimated cultures were subjected to a single step ammonium sulfate precipitation at 50% saturation level.

