

## IN THIS ISSUE

# Light Regulation of Plant Development: HY5 Genomic Binding Sites

Photomorphogenesis is a critical developmental process in plants involving numerous signaling pathways that coordinately regulate the inhibition of stem elongation, differentiation of chloroplasts, accumulation of chlorophyll, and leaf expansion that accompany the transition from dark to light as a seedling emerges from the soil. *Arabidopsis* HY5 encodes a bZIP transcription factor that is a positive regulator of photomorphogenesis, and previous studies suggest that it is a key regulator of numerous genes during photomorphogenesis (Oyama et al., 1997; Ang et al., 1998).

In the dark, HY5 activity is repressed by the key negative regulator of photomorphogenesis, COP1, an E3 ubiquitin ligase that interacts directly with HY5 in the nucleus and targets it for degradation via the ubiquitin-proteasome pathway (Osterlund et al., 2000; Saijo et al., 2003). In hypocotyls of light-grown seedlings, COP1 is initially inactivated and subsequently excluded from the nucleus, allowing derepression of HY5 (reviewed in Quail, 2002). This suggests that HY5 acts at or near the top of the transcriptional cascade that follows the dark-to-light transition during photomorphogenesis.

In this issue of *The Plant Cell*, Lee et al. (pages 731–749) use in vivo chromatin immunoprecipitation combined with microarray analysis (ChIP-chip) to map genome-wide in vivo HY5 binding sites in *Arabidopsis*. HY5 binding sites were mapped to 3894 genes, including numerous genes encoding early light-responsive genes and other transcription factors, and binding activity was observed preferentially in promoter regions of transcribed genes. The work thus supports a model wherein HY5 functions as a high hierarchical regulator of numerous transcriptional cascades operating during photomorphogenesis.

## ChIP-Chip METHOD

ChIP-chip involves immunoprecipitating chromatin associated with a transcription

factor or other chromatin-associated protein and using the immunoprecipitated DNA as a probe for hybridization to a genomic microarray to identify the sites bound by the factor (reviewed in Buck and Lieb, 2004). Lee et al. generated transgenic *Arabidopsis* expressing hemagglutinin (HA)-tagged HY5 driven by the cauliflower mosaic virus 35S promoter to use a commercially available and highly specific HA antibody for immunoprecipitation. The authors demonstrated that the 35S:HA-HY5 transgene completely rescued the phenotype of the *hy5* mutant, indicating that the expressed HA-HY5 protein was fully functional. Transgenic lines were selected that exhibited HA:HY5 protein levels similar to endogenous HY5 protein levels for use in ChIP-chip experiments.

ChIP was performed on 4-d-old seedlings grown on agar plates under a variety of light conditions. The seedlings were fixed with formaldehyde to cross-link genomic DNA with associated factors. Chromatin was then extracted, sonicated, and immunoprecipitated with HA antibody, the immunocomplexes were isolated and reverse cross-linked, and the resulting DNA purified for use in microarray analysis.

The authors used high-density oligonucleotide microarrays representing the entire *Arabidopsis* genome, constructed using maskless array synthesizer technology (Singh-Gasson et al., 1999). The arrays contain 193,751 60-nucleotide targets positioned every 500 nucleotides along one strand of the genome. To determine the in vivo genomic HY5 binding sites, the arrays were hybridized with Cy5-labeled probes constructed from immunoprecipitated DNA and Cy3-labeled probes from purified untreated chromatin DNA as a reference sample. HY5 binding sites would therefore be identified as microarray targets showing a Cy5: Cy3 ratio significantly greater than background.

## AN ABUNDANCE OF BINDING SITES

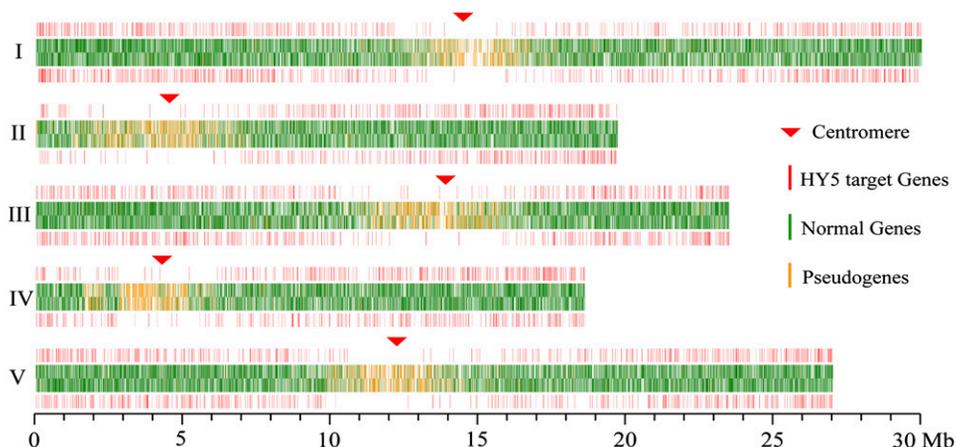
Statistical analysis of the ChIP-chip data led to the identification of 3894 genes as

putative HY5 binding targets in the *Arabidopsis* genome. The targets were distributed on all of the five chromosomes but were noticeably depleted in pericentromeric regions rich in pseudogenes (see figure). The ChIP-chip results were confirmed using a conventional ChIP-PCR assay for a number of randomly selected genes and specific genes from selected functional categories. The results were further validated in a number of ChIP-PCR experiments for specific genes using HY5 antibody and untransformed wild-type and *hy5* mutant plants. These results support the conclusion that most of the putative HY5 binding targets identified using the ChIP-chip procedure are likely to be bona fide HY5 target genes.

The large number of HY5 target genes was surprising but supports conclusions from previous studies suggesting that HY5 is a high hierarchical regulator of transcriptional cascades in numerous pathways associated with photomorphogenesis. By contrast, Thibaud-Nissen et al. (2006) used a similar ChIP-chip procedure to identify 51 putative binding sites for the transcription factor TGA2 in *Arabidopsis* leaves. TGA2 is known to be a transcription factor involved in triggering systemic acquired resistance by binding the promoter region and activating transcription of the *PR-1* gene.

Work in other systems has shown that it is not unreasonable to expect that certain transcription factors will target and influence transcription of a very large number of genes. Cawley et al. (2004) identified binding sites for three important transcription factors in human cells, Sp1, c-Myc, and p53, on tiling arrays covering chromosomes 21 and 22 and produced minimal estimates of 12,000 targets for Sp1, 25,000 for c-Myc, and 1600 for p53 when extrapolated to the entire genome. Wei et al. (2006) used a somewhat different method of ChIP coupled with paired-end ditag sequencing (ChIP-PET) to identify 1766 p53 targets (542 with high confidence) in a colorectal cancer cell line in which p53 is activated.

## IN THIS ISSUE



Genome-Wide HY5 Binding Targets.

ChIP-chip analysis identified 3894 genes as putative HY5 binding targets. Targets are distributed among all five chromosomes, except for a significant depletion of sites in pericentromeric regions rich in pseudogenes.

ChIP-PET involves extracting two 18-bp sequence tags, one from each end of a DNA fragment isolated by ChIP, joining the two together, and sequencing the resulting concatenated tag to identify the putative binding sites. Holstege and Clevers (2006) found that it was somewhat disconcerting to note that there was little overlap between the sets of putative p53 target sites in these two studies. Wei et al. (2006) noted that the p53 binding sites on chromosomes 21 and 22 identified by the ChIP-PET approach were significantly enriched for known p53 binding motifs (85% versus 10%) compared with sites identified by the ChIP-chip approach of Cawley et al. (2004) and therefore concluded that the ChIP-PET approach was likely more specific than the ChIP-chip procedure. However, they also noted that the two experiments employed different induction treatments and time points, and previous work (Crosby et al., 2004) showed that binding of p53 to target sites is highly dynamic and changes significantly in the first 6 to 12 h after exposure to conditions that induce DNA damage. Therefore, the two experiments “may merely represent two snapshots of potentially very large and fluid bodies of transcriptional networks in response to different p53-activating signals,” and a comprehensive picture of p53 activity might be obtained “only through

sampling many combinations of biological settings and p53-activating signals” (Wei et al., 2006).

This discussion is germane to the work of Lee et al. in several respects. First, the authors found that >70% of the putative HY5 target genes have HY5 binding consensus sequences in the promoters (G box, C box, and CG and CA hybrid sequences). The authors concluded that this suggests, as other studies have shown, that chromatin structure affects the binding of transcription factors (in other words, chromatin structure might promote binding to particular sites that otherwise lack consensus binding sequences). Of course, it is also possible that at least some of the putative targets lacking any known HY5 binding consensus sequence are not true HY5 targets. Future work might focus on this subset of targets, first to validate these results by other means (including, for example, ChIP-PCR and use of ChIP with HY5 antibody) and, second, to investigate possible HY5 recognition elements or parameters associated with these target genes (e.g., those present in chromatin structure if not DNA sequence).

Another point of consideration raised by the work of Wei et al. (2006) is the possible dynamic nature of HY5 binding. Lee et al. examined whether different light conditions affect the *in vivo* binding of HY5 using the

ChIP-PCR assay against the promoters of the HY5 target genes *RbcS1A* and *CHS* (genes that were also identified as HY5 targets in the ChIP-chip experiments). These experiments showed no difference in HY5 binding to these promoters under different light conditions or, indeed, following a light-to-dark transition. Other experiments with *hy5* mutant plants showed that HY5 binding is nonetheless required for rapid transcription of these and other photosynthesis genes during photomorphogenesis. These results indicate that HY5 binding is not sufficient for transcriptional regulation of these light-inducible genes, and other factors are responsible for dynamic regulation. Therefore, it might further be concluded that using other conditions and time points would not be expected to identify a substantially different set of putative targets.

Further independent experiments certainly are necessary to determine the absolute validity of the ~3900 HY5 target genes identified by Lee et al. Nonetheless, the validation of a number of targets by ChIP-PCR, relatively high percentage of targets containing known HY5 binding consensus sites, and range of conditions tested support the likelihood that this set represents true *in vivo* HY5 targets. Large-scale microarray analysis of gene expression has suggested a model wherein

## IN THIS ISSUE

phytochrome signaling activates a master set of diverse transcriptional regulators, which in turn influence the expression of multiple downstream target genes (Tepperman et al., 2001, 2004). Lee et al. found that >60% of the early phyA- and phyB-induced genes identified by Tepperman et al. (2001, 2004) are putative HY5 binding targets, offering further support for the model wherein HY5 functions high in the hierarchy of regulators of the transcriptional cascades induced during photomorphogenesis.

**Nancy A. Eckardt**  
News and Reviews Editor  
neckardt@aspb.org

## REFERENCES

- Ang, L.-H., Chattopadhyay, S., Wei, N., Oyama, T., Okada, K., Batschauer, A., and Deng, X.W.** (1998). Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of *Arabidopsis* development. *Mol. Cell* **1**: 213–222.
- Buck, M.J., and Lieb, J.D.** (2004). ChIP-chip: Considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments. *Genomics* **83**: 349–360.
- Cawley, S., et al.** (2004). Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs. *Cell* **116**: 499–509.
- Crosby, M.E., Oancea, M., and Almasan, A.** (2004). p53 binding to target sites is dynamically regulated before and after ionizing radiation-mediated DNA damage. *J. Environ. Pathol. Toxicol. Oncol.* **23**: 67–79.
- Holstege, F.C.P., and Clevers, H.** (2006). Transcription factor target practice. *Cell* **124**: 21–23.
- Lee, J., He, K., Stolc, V., Lee, H., Figueroa, P., Gao, Y., Tongprasit, W., Zhao, H., Lee, I., and Deng, X.W.** (2007). Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. *Plant Cell* **19**: 731–749.
- Osterlund, M.T., Hardtke, C.S., Wei, N., and Deng, X.W.** (2000). Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* **405**: 462–466.
- Oyama, T., Shumura, Y., and Okada, K.** (1997). The *Arabidopsis* *HY5* gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyls. *Genes Dev.* **11**: 2983–2994.
- Quail, P.H.** (2002). Photosensory perception and signalling in plant cells: New paradigms? *Curr. Opin. Cell Biol.* **14**: 180–188.
- Saijo, Y., Sullivan, J.A., Wang, H., Yang, J., Shen, Y., Rubio, V., Ma, L., Hoecker, U., and Deng, X.W.** (2003). The COP1–SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes Dev.* **17**: 2642–2647.
- Singh-Gasson, S., Green, R.D., Yue, Y., Nelson, C., Blattner, F., Sussman, M.R., and Cerrina, F.** (1999). Maskless fabrication of light-directed oligonucleotide microrarrays using a digital micromirror array. *Nat. Biotechnol.* **17**: 974–978.
- Tepperman, J.M., Hudson, M.E., Khanna, R., Zhu, T., Chang, S.H., Wang, X., and Quail, P.H.** (2004). Expression profiling of phyB mutant demonstrates substantial contribution of other phytochromes to red-light-regulated gene expression during seedling de-etiolation. *Plant J.* **38**: 725–739.
- Tepperman, J.M., Zhu, T., Chang, H.-S., Wang, X., and Quail, P.H.** (2001). Multiple transcription-factor genes are early targets of phytochrome A signaling. *Proc. Natl. Acad. Sci. USA* **98**: 9437–9442.
- Thibaud-Nissen, F., Wu, H., Richmond, T., Redman, J.C., Johnson, C., Green, R., Arias, J., and Town, C.D.** (2006). Development of *Arabidopsis* whole-genome microarrays and their application to the discovery of binding sites for the TGA2 transcription factor in salicylic acid-treated plants. *Plant J.* **47**: 152–162.
- Wei, C.-L., et al.** (2006). A global map of p53 transcription-factor binding sites in the human genome. *Cell* **124**: 207–219.

## Light Regulation of Plant Development: HY5 Genomic Binding Sites

Nancy A. Eckardt

*Plant Cell* 2007;19;727-729

DOI 10.1105/tpc.107.052233

This information is current as of August 21, 2019

**References**

This article cites 15 articles, 4 of which can be accessed free at:

</content/19/3/727.full.html#ref-list-1>

**Permissions**

[https://www.copyright.com/ccc/openurl.do?sid=pd\\_hw1532298X&issn=1532298X&WT.mc\\_id=pd\\_hw1532298X](https://www.copyright.com/ccc/openurl.do?sid=pd_hw1532298X&issn=1532298X&WT.mc_id=pd_hw1532298X)

**eTOCs**

Sign up for eTOCs at:

<http://www.plantcell.org/cgi/alerts/ctmain>

**CiteTrack Alerts**

Sign up for CiteTrack Alerts at:

<http://www.plantcell.org/cgi/alerts/ctmain>

**Subscription Information**

Subscription Information for *The Plant Cell* and *Plant Physiology* is available at:

<http://www.aspb.org/publications/subscriptions.cfm>