

# CAM7 and HY5 genetically interact to regulate root growth and abscisic acid responses

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CAM7, a member of CaM family in *Arabidopsis*, acts as a transcriptional regulator and enhances photomorphogenic growth and light regulated gene expression under various light conditions. HY5, a bZIP transcription factor, promotes photomorphogenesis at multiple wavelengths of light including far red, red, and blue light. Very recently, it has been shown that CAM7 and HY5 directly interact with the *HY5* promoter to regulate the transcriptional activity of *HY5* during *Arabidopsis* seedling development. In this study, we have investigated the root phenotype of *cam7 hy5* double mutants and shown that *CAM7* and *HY5* genetically interact to control the root growth. We have further shown an interdependent function of HY5 and CAM7 in abscisic acid (ABA) responsiveness.

Light plays a major role for proper growth and development of plants from seed germination till flowering.<sup>1</sup> During the transition from dark to light, the seedlings follow photomorphogenesis, which is characterized at the cellular level by suppression of cell elongation and expansion in hypocotyl and cotyledon, and onset of growth by cell division in both shoot and root meristem. On the other hand, phytohormones also play important roles for the regulation of cell expansion and division. Thus, light and hormone signaling pathways are likely to intersect with each other to evoke the desired responses. It has already been shown that various plant hormones are involved in light signaling and influence cell expansion, division, and light-regulated gene expression.<sup>2-4</sup>

In *Arabidopsis thaliana*, several transcription factors involved in switching the skotomorphogenic to photomorphogenic growth have been identified. These transcription factors are either positive or negative regulators of light signal transduction pathways and are specific to single or multiple wavelengths of light. Among them, LONG HYPOCOTYL 5 (HY5), the basic leucine zipper (bZIP) transcription factor, and CALMODULIN7 (CAM7/ZBF3) are the only known transcription factors acting downstream of multiple wavelengths of light including far red light, red light, and blue light, and positively regulate light signaling pathways.<sup>5-8</sup> It has been shown that unlike other calmodulins, CAM7 acts as a unique transcriptional factor that directly binds to promoters of several light-inducible genes.<sup>8</sup> The ectopic expression of CAM7 causes hyperphotomorphogenic growth under various light conditions. Whereas *cam7* mutants do not exhibit any altered photomorphogenic phenotype, *cam7 hy5* double mutant seedlings exhibit a super-tall phenotype.<sup>8</sup> Recently it has been revealed that CAM7 and HY5 directly interact with the E- and T/G box of HY5 promoter and positively regulate the expression of *HY5* in

a concerted manner at various stages of development.<sup>9</sup> HY5 is a major and versatile molecule, which plays an important role in light-regulated gene expression. Loss-of-function *hy5* mutants exhibit dark-grown characteristics in the light<sup>10</sup> with the elongated hypocotyl and severely affected root morphogenesis. The most obvious phenotype of *hy5* seedlings is the increased number of lateral roots. These lateral roots along with primary roots grow faster and are negatively gravitropic.<sup>10</sup>

HY5, the key molecule of light signal transduction pathway, has also been involved in multiple hormone signaling pathways like GA, cytokinin, auxin, and abscisic acid (ABA),<sup>11-14</sup> and thus acts as a convergence point of these pathways. Recently, the gene ontology analysis by Zhang et al., 2011, has shown that HY5-dependent developmental programs are mostly controlled by HY5-regulated transcription factors. Chip-on-chip analyses have also shown that genes involved in auxin, ethylene, cytokinin, and jasmonic acid pathways are extremely enriched in HY5-regulated genes. Thus, HY5 acts as a master molecule that coordinates the hormonal responses with the light signaling pathways.<sup>15</sup> Although the molecular and genetic interactions between HY5 and CAM7 have been studied,<sup>8,9</sup> the cross talk of these two molecules with respect to phytohormones is still to be elucidated. In this study, we have reported that *CAM7* and *HY5* genetically interact to control the root phenotype. We have also shown the genetic relationship between *CAM7* and *HY5* in ABA responsiveness.

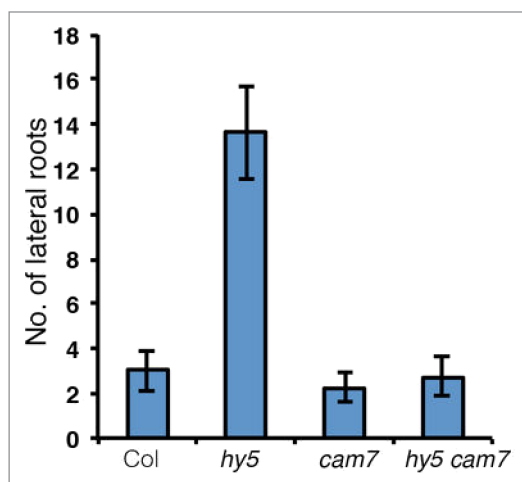
## Additional mutation in *CAM7* partly suppresses the root phenotype of *hy5* mutants

It has been found that mutation in *HY5* causes an increase in the number of lateral roots.<sup>10</sup> To determine the genetic relationship between *cam7* and *hy5* in controlling the lateral root formation, we examined the lateral roots formed in *cam7 hy5*

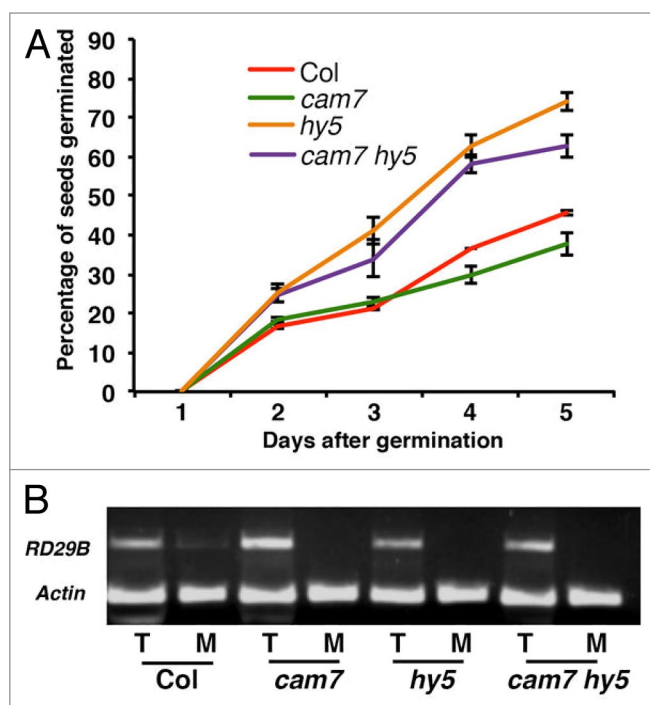
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**Figure 1.** Formation of lateral roots in *cam7 hy5* double mutants. Quantification of lateral roots formed in wild type and various mutants of 10-d-old seedlings.



**Figure 2.** Response of *cam7 hy5* double mutant to ABA. (A) Quantification of percentage of seeds germinated in presence of ABA at various days in constant WL ( $30 \mu\text{mol}/\text{m}^2/\text{s}$ ). (B) Expression of ABA pathway marker gene (*RD29B*) in the wild type and various mutants from 6-d-old seedlings grown in constant WL ( $30 \mu\text{mol}/\text{m}^2/\text{s}$ ) mock treated (MS solution) or treated with ABA ( $5 \mu\text{M}$ ). M stands for mock control, and T stands for treated with ABA.

double mutant seedlings grown for 10 d in white light ( $100 \mu\text{mol}/\text{m}^2/\text{s}$ ). While counting the number of lateral roots, the number was found to be significantly higher in *hy5* mutants, consistent with the observation made by Oyama et al., 1997. Although *cam7* mutants did not show any significant alteration in the number of lateral roots formed, the number of lateral roots were significantly reduced in *cam7 hy5* double mutants. These results suggest that additional mutation in *CAM7* is able to suppress the defect of lateral root formation in *hy5* (Fig. 1).

#### CAM7 and HY5 work in an antagonistic manner in ABA-mediated inhibition of seed germination

Recently, it has been shown that *hy5* mutant is tolerant to the inhibitory effect of ABA in seed germination.<sup>14</sup> We investigated whether *CAM7* mutation alters ABA responsiveness of *hy5* mutant. For this study, freshly harvested seeds of wild type, single, and double mutant plants were inoculated on MS media without or with ABA. The rate of germination in wild type and various mutant seeds was found to be similar in the absence of ABA. On the other hand, ABA reduced the germination rate of wild type seeds ( $\sim 45\%$ ). The rate of seed germination was found to be further diminished in *cam7* mutants as compared with wild type ( $\sim 35\%$ ), suggesting that *cam7* mutants are more susceptible to ABA-mediated inhibition of seed germination. The *hy5* mutants showed increased rate of seed germination ( $\sim 72\%$ ) in the presence of ABA (Fig. 2A). However, the rate of germination of *hy5* seeds was reduced with the additional mutation in *CAM7*. The *cam7 hy5* double mutant seeds showed  $\sim 60\%$  germination in the presence of ABA (Fig. 2A). These results suggest that *CAM7* and *HY5* work in an antagonistic manner in ABA-mediated inhibition of seed germination, and the additional mutation in *CAM7* is able to partly suppress the rate of seed germination in *hy5* mutants in the presence of ABA.

*RD29B* is one of the marker genes involved in ABA signaling pathways, and its expression is induced by ABA treatment.<sup>14</sup> We monitored the expression of *RD29B* in *cam7 hy5* double mutants. RT-PCR analyses without or with ABA revealed that *RD29B* expression was slightly reduced in *hy5* mutants, whereas it was strongly increased in *cam7* mutant background. The level of expression of *RD29B* was found to be similar to wild type in *cam7 hy5* double mutants. These results suggest that additional mutation in *HY5* is able to suppress the higher level of induction of *RD29B* in *cam7* mutant background (Fig. 2B).

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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## References

- Deng XW, Quail PH. Signalling in light-controlled development. *Semin Cell Dev Biol* 1999; 10:121-9; <http://dx.doi.org/10.1006/scdb.1999.0287>; PMID:10441064
- Chory J, Reinecke D, Sim S, Washburn T, Brenner M. A role for cytokinins in de-etiolation in *Arabidopsis* (*det* mutants have an altered response to cytokinins). *Plant Physiol* 1994; 104:339-47; <http://dx.doi.org/10.1104/pp.104.2.339>; PMID:12232085
- Neff MM, Fankhauser C, Chory J. Light: an indicator of time and place. *Genes Dev* 2000; 14:257-71; <http://dx.doi.org/10.1101/gad.14.3.257>; PMID:10673498
- Vandenbussche F, Vriezen WH, Smalle J, Laarhoven LJ, Harren FJ, Van Der Straeten D. Ethylene and auxin control the *Arabidopsis* response to decreased light intensity. *Plant Physiol* 2003; 133:517-27; <http://dx.doi.org/10.1104/pp.103.022665>; PMID:12972669
- Ang LH, Chattopadhyay S, Wei N, Oyama T, Okada K, Batschauer A, Deng XW. Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of *Arabidopsis* development. *Mol Cell* 1998; 1:213-22; [http://dx.doi.org/10.1016/S1097-2765\(00\)80022-2](http://dx.doi.org/10.1016/S1097-2765(00)80022-2); PMID:9659918
- Osterlund MT, Hardtke CS, Wei N, Deng XW. Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* 2000; 405:462-6; <http://dx.doi.org/10.1038/35013076>; PMID:10839542
- Jiao Y, Lau OS, Deng XW. Light-regulated transcriptional networks in higher plants. *Nat Rev Genet* 2007; 8:217-30; <http://dx.doi.org/10.1038/nrg2049>; PMID:17304247
- Kushwaha R, Singh A, Chattopadhyay S. Calmodulin7 plays an important role as transcriptional regulator in *Arabidopsis* seedling development. *Plant Cell* 2008; 20:1747-59; <http://dx.doi.org/10.1105/tpc.107.057612>; PMID:18621945
- Abbas N, Maurya JP, Senapati D, Gangappa SN, Chattopadhyay S. *Arabidopsis* CAM7 and HY5 physically interact and directly bind to the HY5 promoter to regulate its expression and thereby promote photomorphogenesis. *Plant Cell* 2014; 26:1036-52; <http://dx.doi.org/10.1105/tpc.113.122515>; PMID:24610722
- Oyama T, Shimura Y, Okada K. The *Arabidopsis* HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. *Genes Dev* 1997; 11:2983-95; <http://dx.doi.org/10.1101/gad.11.22.2983>; PMID:9367981
- Cluis CP, Mouchel CF, Hardtke CS. The *Arabidopsis* transcription factor HY5 integrates light and hormone signaling pathways. *Plant J* 2004; 38:332-47; <http://dx.doi.org/10.1111/j.1365-313X.2004.02052.x>; PMID:15078335
- Alabadi D, Gallego-Bartolomé J, Orlando L, García-Cárcel L, Rubio V, Martínez C, Frigerio M, Iglesias-Pedraz JM, Espinosa A, Deng XW, et al. Gibberellins modulate light signaling pathways to prevent *Arabidopsis* seedling de-etiolation in darkness. *Plant J* 2008; 53:324-35; <http://dx.doi.org/10.1111/j.1365-313X.2007.03346.x>; PMID:18053005
- Vandenbussche F, Habricot Y, Condiff AS, Maldiney R, Van der Straeten D, Ahmad M. HY5 is a point of convergence between cryptochrome and cytokinin signalling pathways in *Arabidopsis thaliana*. *Plant J* 2007; 49:428-41; <http://dx.doi.org/10.1111/j.1365-313X.2006.02973.x>; PMID:17217468
- Chen H, Zhang J, Neff MM, Hong SW, Zhang H, Deng XW, Xiong L. Integration of light and abscisic acid signaling during seed germination and early seedling development. *Proc Natl Acad Sci U S A* 2008; 105:4495-500; PMID:18332440; <http://dx.doi.org/10.1073/pnas.0710778105>; <http://dx.doi.org/10.1073/pnas.0710778105>
- Zhang H, He H, Wang X, Wang X, Yang X, Li L, Deng XW. Genome-wide mapping of the HY5-mediated gene networks in *Arabidopsis* that involve both transcriptional and post-transcriptional regulation. *Plant J* 2011; 65:346-58; <http://dx.doi.org/10.1111/j.1365-313X.2010.04426.x>; PMID:21265889
- Boter M, Ruíz-Rivero O, Abdeen A, Prat S. Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and *Arabidopsis*. *Genes Dev* 2004; 18:1577-91; <http://dx.doi.org/10.1101/gad.297704>; PMID:15231736
- Zhai Q, Li CB, Zheng W, Wu X, Zhao J, Zhou G, Jiang H, Sun J, Lou Y, Li C. Phytochrome chromophore deficiency leads to overproduction of jasmonic acid and elevated expression of jasmonate-responsive genes in *Arabidopsis*. *Plant Cell Physiol* 2007; 48:1061-71; <http://dx.doi.org/10.1093/pcp/pcm076>; PMID:17567636