Characterization of a Unique GATA Family Gene That Responds to Both Light and Cytokinin in Arabidopsis thaliana

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For higher plants, light is one of the most prominent environmental signals. It regulates almost all aspects of development as well as physiological processes. The model higher plant Arabidopsis thaliana has at least three types of common photoreceptors: phytochromes (phyA–phyE) for red and far-red light, and cryptochromes (cry1 and cry2) and phototropins (phot1 and phot2) for blue light.1) These photoreceptors act coordinately at every developmental stage from the germination of seeds to flowering. Such light-dependent signal transduction pathways must be properly integrated into the downstream global regulatory network at the level of transcription.2) Thus, characterization of light-responsive transcription factors is one of the major paradigms of current plant biology.3) It has been suggested that a subfamily of type-IV zinc-finger transcription factors in plants is implicated in a light-responsive transcriptional network.5) The members of this family of DNA-binding proteins are collectively designated GATA factors, because many light-responsive promoters contain the cis-acting GATA motif, and some of these Arabidopsis GATA factors were suggested to function as nuclear GATA-binding proteins.5)

For higher plants, light is an important external signal, whereas cytokinin acts as an internal hormonal signal, and both are crucial for almost all aspects of development and physiological states. Here we identified and characterized a unique gene, CGA1, encoding a GATA factor, whose expression was rapidly induced by both the light and cytokinin signals in Arabidopsis thaliana.

Key words: cytokinin; GATA family transcriptional factor; light

We have been characterizing the molecular mechanisms of phosphorelay-mediated cytokinin signal transduction, which is primarily propagated by the cytokinin-receptor histidine (His) protein kinases, named AHK2, AHK3, and AHK4/CRE1 (see Fig. 3C).9–11) During the course of our previous transcriptome studies on cytokinin-dependent signal transduction, a gene encoding a GATA factor was suggested to be one of the early cytokinin-responsive genes.12) Here, we characterized this particular gene (At4g26150), named CGA1 (CYTOKININ-RESPONSIVE GATA FACTOR 1), with special reference to a link between the cytokinin and light signal transduction.8)

A. thaliana has 29 members of the GATA family, each of which commonly has a highly conserved zinc-finger type DNA-binding motif (Fig. 1A). It has also been long been suspected that in A. thaliana there is a link between light-signal transduction and the action of cytokinin, a class of plant hormones that regulates almost all aspects of development of plants.6) For instance, when dark-grown seedlings are treated externally with cytokinin in medium, the resulting seedlings display many of the characteristics of light-grown plants (e.g., short hypocotyls and open cotyledons).7) Again, it was reported a cytokinin signaling component, ARR4, which is a member of the cytokinin-inducible response regulator (type-A ARR) family, interacts physically with the phyB photoreceptor, implying a link between the cytokinin and light signal transduction.8)

Abbreviations: CGA1, CYTOKININ-RESPONSIVE GATA FACTOR 1; Col, Columbia-0; GNC, GATA nitrate-inducible carbon metabolism-involved; t-Zeatin, trans-zeatin
Arabidopsis seedlings were grown in the dark, and subsequently the etiolated seedlings were exposed to white light (65 μmol/m² s⁻¹) (Fig. 1B). We found that transcripts of CGA1 rapidly accumulated in the seedlings upon the onset of white light treatment. This white light-inducible expression of CGA1 was largely, if not absolutely, dependent on the phyA/phyB red light receptors, because induction of CGA1 was severely attenuated in mutant seedlings lacking both phyA and phyB (Fig. 1B). The transcription of GNC was also up-regulated by light, albeit to a far smaller extent. We then carried out a similar experiment with a certain fluence rate of monochromatic red light (660 nm, 40 μmol/m² s⁻¹) (Fig. 1C). The expression of CGA1 was induced by red light, and this induction was dependent on the phyA/phyB red light receptors.

We then asked the second critical question, as to whether CGA1 is indeed a cytokinin-responsive gene. We also wondered whether the closest homolog (GNC) is also a cytokinin-induced gene. To answer these questions, we conducted the experiments as follows: When the wild-type young plants (14-d-old) were treated with cytokinin, transcripts of CGA1 rapidly accumulated in leaves within 20 min (Fig. 2A). This observation is consistent with that in our preliminary report. In addition, it was found that induction of GNC by cytokinin was less evident, if there was any. These results are presented in a quantitative manner (Fig. 2B). Taken together, the results of this study indicate that the expression properties of CGA1 are unique in that its transcription in plants is regulated by both red light and cytokinin. In this respect, CGA1 appears to be distinct from its closest homolog, GNC.

We also examined whether such plant hormone-dependent induction of CGA1 was specific to cytokinin. We carried out experiments to address this issue in a previous study, and found that both cytokinin derivatives (t-Zeatin and 6-benzylaminopurine) specifically induced expression of CGA1, but that the other hormones tested (such as 2,4-dichlorophenoxy acetic acid, 1-aminocyclopropane-1-carboxylic acid, gibberellin, and abscisic acid) did not do so at all. We further examined the cytokinin-responsive expression of CGA1 in terms of organ-specificity and/or developmental stage-specificity. Transcripts of CGA1 were specifically detected in light-grown seedlings, but not in dark-grown seedlings (Fig. 3A). The basal-level expression of CGA1 in the light-grown seedlings was further enhanced by cytokinin, while its expression was not induced by cytokinin in the dark-grown seedlings, at least under the conditions tested. In adult plants, the transcripts of CGA1 were predominantly detected in leaves, and not at all in roots. The expression of CGA1 in reproductive organs (e.g., flowers and siliques) was much less evident. These results are consistently interpreted by assuming that the occurrence of CGA1 is not ubiquitous, and this DNA-binding protein plays a specialized role in leaves in a manner dependent on both light and cytokinin.

The next question was whether cytokinin-inducible expression of CGA1 is primarily mediated by AHK His-kinase-dependent signal transduction. This appears to be...
Cytokinin was significantly if not completely attenuated in the case (Fig. 3B). Cytokinin-dependent induction of CGA1 was induced predominantly in leaves, specifically by cytokinin, in a manner dependent on the cytokinin receptors. Expression of CGA1 was examined for various stages and/or organs prepared for the wild-type Col plants. RNA samples were prepared from light-grown and etiolated seedlings (3 d old), roots and rosettes of 14-d-old plants, and cauline leaves, stems, flowers, and siliques of 40-d-old plants grown under continuous fluorescent light, and subjected to semi-quantitative RT-PCR analysis. B, RNA samples were prepared from wild-type Col and ahk2 ahk3 double mutant plants grown under continuous white fluorescent light for 15 d, after being treated with or without t-Zeatin (20 μM), as indicated, and subjected to semi-quantitative RT-PCR analysis. The transcript of ARR15 was analyzed as a positive reference with specific primers (5’-CCGTGACCTATGGCTCTCAGAGATTTATC-3’ and 5’-CCGTGACCTAACCCCTAGACTGCTAATTTCG-3’). C, A schematic representation of the novel findings in this study. It should be noted that the cytokinin-dependent pathway was observed in light-grown adult plants, whereas the light-induced pathway was seen during early de-etiolation of seedlings. The results of this study are discussed in the context of His-Asp phosphorelay-mediated signal transduction, and details are given in the text. D, RNA samples were prepared from wild-type Col and phyB double mutant plants grown under continuous white fluorescent light for 15 d, after being treated with or without t-Zeatin (20 μM), as indicated, and subjected to semi-quantitative RT-PCR analysis. Semi-quantitative RT-PCR experiments were performed, as described in the legend to Fig. 1. It should be noted that the cycles of PCR were varied for the various RNA samples (from 20 to 27 cycles) in order to amplify dsDNA in a semi-quantitative manner. After optimizing the cycles, the most quantitative results are presented here. The numbers in parentheses indicate the cycles of PCR performed in each experiment.

Fig. 2. Expression of CGA1 Was Induced in Response to Cytokinin. A, Wild-type Col plants were grown on vertically oriented MS-agar plates under continuous white fluorescent light for 20 d. After the resulting young plants being treated with t-Zeatin (20 μM), RNA samples were prepared from whole plants at intervals, as indicated, and the transcripts of CGA1 and GCN in response to cytokinin were examined, according to the procedures given in the legend to Fig. 1. The numbers in parentheses indicate the cycles of PCR performed in each experiment. B, The intensity of each stained band detected in panel (A) was quantified by Multi Gauge Ver. 3.0 Software (FUJIFILM, Tokyo), and the values were normalized using the values of ACT8 as loading references. The results were expressed by taking the maximum value of CGA1 as 100. Hence the values are relative amounts of transcripts.
the phyA phyB mutant (Fig. 3D). This is consistent with the scheme in Fig. 3C, in which the cytokinin-signaling and light-signaling pathways are assumed to act on the CGA1 gene in a parallel manner. We observed earlier that cytokinin-induction of CGA1 is severely attenuated in dark-grown etiolated seedlings (Fig. 3A). This interesting phenomenon remains to be explained in some other way. A possible explanation is that there is a dark-specific, phyA/phyB-independent inhibitory mechanism that interferes with the cytokinin-induced expression of CGA1 in the dark.

In summary, we uncovered an interesting Arabidopsis GATA transcription factor the expression of which is regulated by both cytokinin and light. During the last half-decade, intensive studies on the model higher plant Arabidopsis have begun to shed light on the molecular mechanism underlying the phosphorelay-mediated cytokinin signal transduction pathway, as schematically summarized. In this context, one can envisage a-priori that there must be many target genes other than type-A ARRs, which are regulated directly by type-B ARR transcription factors.14,15) The results of this study suggest that CGA1 might be such a new target. This gene is even more intriguing in that its expression is also induced by light in a manner dependent on phyA/phyB photoreceptors. It is tempting to speculate that CGA1 play a unique role in the integration of both the cyto-

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