Title	Negative autoregulation of the Arabidopsis homeobox gene ATHB-2 [1] / Complexes of MADS-box protein are sufficient to convert leaves into floral organs [2] / An upstream region of the CDC2aAt gene directs transcription during trichome development [3] (MOLECULAR BIOLOGY AND INFORMATION - Molecular Biology)
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Citation	ICR annual report (2002), 8: 50-51
Issue Date	2002-03
URL	http://hdl.handle.net/2433/65307
Right	
Type	Article
Textversion	publisher

Molecular Biology and Information - Molecular Biology -

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Scope of Research

The major subjects are mechanisms involved in signal transduction and regulation of gene expression responsive to environmental stimuli, differentiation and development of plant organs, and plant-microbe interaction. As of December 2001, study is being concentrated on the roles of two-component response regulators and homeodomain proteins of higher plants in signal transduction and developmental processes.

Research Activities (Year 2001)

Presentations

Transcriptional networks regulated by *Arabidopsis* HD-Zip proteins, Aoyama T, Muramoto T, Ohgishi M, Morelli G, Ruberti I, Oka A; Target gene analysis of *Arabidopsis* ATHB-1, Muramoto T, Tukuda M, Oka A, Tabata S, Ruberti I, Morelli G, Aoyama T; Characterization of the homeobox gene, *ATHB-10/GL2*, Ohashi Y, Oka A, Ruberti I, Morelli G, Aoyama T, Ann Meeting of Jpn. Soc. Plant Physiol., 24-26 March (Fukuoka).

Function of GL2/ATHB-10 in epidermal cellular differentiation, Ohashi Y, Oka A, Ruberti I, Morelli G, Aoyama T, 29-30 November (Tsukuba), International Symposium "New era of transcription factor research in plants" organized by Takatsuji H and Aoyama T.

The *Arabidopsis* ARR1 response regulator is a transcription factor for genes immediately responsive to cytokinins, Sakai H, Honma T, Aoyama T, Oka A; Entopically additive expression of ATHB-10/GL2 alters the frequency and spacing of trichome initiation, Ohashi Y, Oka A, Ruberti I, Morelli G, Aoyama T, Ann Meeting of Mol. Biol. Soc. Jpn., 9-12 December (Yokohama).

Grants

Oka A, Research project for network mutually controlling plant responses to environmental stimuli with morphogenesis: Hierarchy of transcriptional controls in plant signal transduction, Special Coordination Fund of the Ministry of Education, Culture, Sports, Science, and Technology of Japan, 1 April 1997 - 31 March 2003

Aoyama T, Functional analysis of homeodomain proteins controlling the flexibility of plant morphogenesis, Grant from the Bio-oriented Technology Research Advancement Institution (BRAIN), 1 April 1998 - 31 March 2003

Aoyama T and Oka A, Molecular mechanism of adaptive responses controlled by *Arabidopsis* His-Asp phosphorelay signal transduction, Grant-in-Aid for Scientific Research on Priority Areas (B), 1 April 2000 - 31 March 2003

Award

Honma T, Complexes of MADS-box proteins are sufficient to convert leaves into floral organs, The ICR Special Award for Young Scientists.

Topics

Negative autoregulation of the *Arabidopsis* homeobox gene *ATHB-2* [1]

ATHB-2 is a transcription factor belonging to the *Arabidopsis* homeodomain-leucine zipper (HD-Zip) protein family. The *ATHB-2* gene is tightly regulated by light signals, and thought to direct morphological changes during shade avoidance responses. To understand how ATHB-2 mediates light signals in plant morphogenesis, we investigated its transcriptional network. We constructed a gene encoding a chimeric transcription factor (HD-Zip-2-V-G) that is expected to activate target genes of ATHB-2 in a glucocorticoid-dependent manner. In transgenic *Arabidopsis* plants expressing HD-Zip-2-V-G, glucocorticoid treatment activates the *ATHB-2* gene itself independently of *de novo* protein synthesis. An *in vitro* DNase I-footprinting experiment showed that the recombinant ATHB-2 protein specifically binds to an *ATHB-2* promoter region. These complementary results indicate that ATHB-2 recognizes its own promoter. Consistent with the fact that ATHB-2 itself has been shown to act as a repressor, expression of the endogenous *ATHB-2* gene was repressed in transgenic plants overexpressing an *ATHB-2* transgene. Moreover, target-gene analyses using the HD-Zip-2-V-G suggested that ATHB-2 recognizes other HD-Zip II subfamily genes. Thus, ATHB-2 has a negative autoregulatory loop and may be involved in a complicated transcriptional network including paralogous genes, like that of animal homeobox genes.

1. M. Ohgishi1, A. Oka, G. Morelli, I. Ruberti, and T. Aoyama1, *Plant J.*, **25**, 389-398 (2001) .

Complexes of MADS-box proteins are sufficient to convert leaves into floral organs [2]

Genetic studies, using floral homeotic mutants, have led to the ABC model of flower development. This model proposes that the combinatorial action of three sets of genes, the A, B and C function genes, specify the four floral organs (sepals, petals, stamens and carpels) in the concentric floral whorls. However, attempts to convert vegetative organs into floral organs by altering the expression of ABC genes have been unsuccessful. Here we show that the class B proteins of *Arabidopsis*, PISTILLATA (PI) and APETALA3 (AP3), interact with APETALA1 (AP1, a class A protein) and SEPALLATA3 (SEP3, previously AGL9), and with AGAMOUS (AG, a class C protein) through SEP3. We also show that vegetative leaves of triply transgenic plants, *35S::PI*; *35S::AP3*; *35S::AP1* or *35S::PI*; *35S::AP3*; *35S::SEP3*, are transformed into petaloid organs and that those of *35S::PI*; *35S::AP3*; *35S::SEP3*; *35S::AG* are transformed into staminoid organs. Our findings indicate that the formation of ternary and quaternary complexes of ABC proteins may be the molecular basis of the ABC model, and that the flower-specific expression of SEP3 restricts the action of the ABC genes to the flower.

2. T. Honma and K. Goto, *Nature*, **409**, 525-529 (2001).

An upstream region of the *CDC2aAt* gene directs transcription during trichome development [3]

Proliferation of eukaryotic cells proceeds according to a common cell cycle program. The cell cycle is regulated at two checkpoints at least (i.e., the G1-to-S phase transition and entry into mitosis) through a particular class of protein kinase activity. Since these kinases require an associating protein, cyclin, for their activity, they are called cyclin-dependent kinases (CDKs). The *Arabidopsis CDC2aAt* gene is thought to encode such a protein kinase, since it is actively transcribed in proliferating tissues and can complement defects in the *Schizosaccharomyces pombe cdc2* gene. We analyzed the functional structure of the *CDC2aAt* promoter, using fusion genes between various upstream regions of *CDC2aAt* and the *Escherichia coli* β-glucuronidase (GUS) gene. A 595-base pair (bp) DNA fragment upstream from the transcription start site conferred GUS activity on developing trichomes, but not on proliferating tissues. On the other hand, another upstream fragment extending to the 5' non-coding transcribed region gave GUS activity to both proliferating tissues and developing trichomes (Figure 1). Under the *gl2* mutant background, GUS activity directed by the 595-bp fragment was detected in single-stalk cells, but not in giant cells without obvious polar extension growth. These results revealed that the 595-bp fragment lacks *cis* element(s) essential for proliferating-cell-specific promoter activity, but can direct transcription in a specific period during trichome development, which doesn't include cell division. These results suggests that CDC2aAt functions during cell morphogenesis as well as cell proliferation.

3. Y. Imajuku, Y. Ohashi, T. Aoyama, K. Goto, and A. Oka, *Plant Mol. Biol.* **46**, 205-213 (2001).

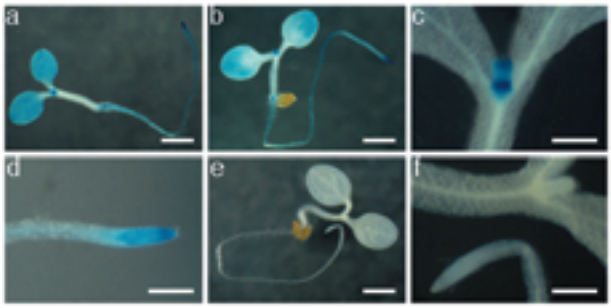

Figure 1. Histochemical analysis of *CDC2aAt* promoter activity in seedlings. Transgenic *Arabidopsis* 5 days after germination carrying *P(-1299/+677)::GUS* (a), *P(-591/+677)::GUS* (b-d), or *P(-591/+4)::GUS* (e and f) were examined histochemically. Close-up pictures of apical and root meristems are shown for *P(-591/+677)::GUS* (c and d, respectively) and *P(-591/+4)::GUS* (f). The bars in a, b, and e = 1 mm, and the bars in c, d and f = 0.2 mm. Quoted from ref 3.