Artificial Control in Transgenic Plants of the Activity of Transcription Factors

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For artificial control in transgenic plants of the activity of transcription factors, constructed was a DNA cassette encoding the transcriptional activation domain of Herpes Simplex viral protein VP16 and the hormone binding domain of rat glucocorticoid receptor. Combining with DNA segments coding for the DNA binding domain of transcription factors, the VG cassette produced fusion proteins whose activity can be induced in transgenic plants by the exogenous steroid hormone. Thus plant phenotypes specifically induced by the hormone give information on *in vivo* roles of the transcription factors. In addition, this system provides a tool by which we identifies direct target genes of the transcription factors. The *Arabidopsis* ATHB-1 and ATHB-2 transcription factors containing the homeodomain and leucine zipper motif have been studied with this system.

Keywords: Transcription/ Steroid hormone/ Homeodomain/ Transgenic plant

It is generally accepted that transcriptional initiation is the most critical step at which gene expression is regulated. In eukaryotic cells, transcription factors are largely involved in control of gene expression, and frequently play key roles in the regulation of cell differentiation, organ development, ontogenesis, and so on. However, their actual roles remain unclear in many cases, especially in higher eukaryotes because of the difficulty in the *in vivo* analysis of transcription factors. The purpose of this study is to construct a generally applicable tool for artificial control in transgenic plants of transcription factors, and then to apply it to functional analysis of the *Arabidopsis* transcription factors, ATHB-1 and ATHB-2. We developed a DNA cassette encoding a protein whose function of transcriptional activation can artificially be modulated, and fused it to another DNA segment that codes for a transcription factor or its portion containing the DNA-binding domain. To construct an artificially-regulable activator domain, we took advantage of well-characterized functional domains, the acidic domain of the Herpes Simplex viral protein VP16 [1] and the hormone binding domain of the rat glucocorticoid receptor (GRHBD) [2]. The VP16 domain is thought to act as a powerful transactivating domain in various organisms including plants by interacting with basal transcription factors [3-5]. The GRHBD domain suppresses the function of its neighboring domain in the absence of glucocorticoid, and the hormone binding to GRHBD results in release from the suppression [6-8]. As shown in Figure 1a, the DNA fragment encoding GRHBD was fused to the 3' end of the fragment encoding the VP16 domain in an in-frame manner. The restriction sites existing be-

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Scope of research

Attempts have been made to elucidate structure-function relationships of genetic materials and various gene products. 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 1999, study is being concentrated on the roles of homeo domain proteins, MADS box proteins, and DDK response regulators of higher plants in developmental and signal transduction processes.

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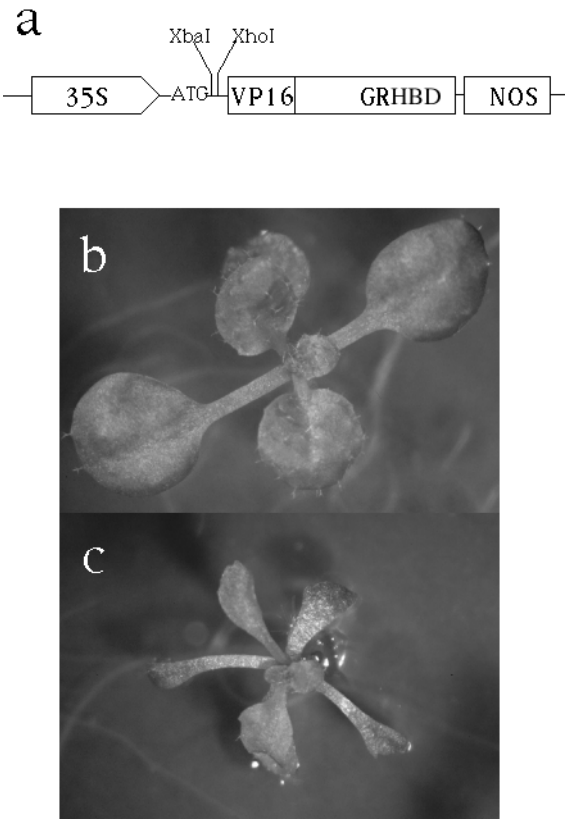

Figure 1. Architecture of the VG cassette vector (a), and phenotypes of *Arabidopsis* plants transgenic for 35S-HDZip1-VG grown under the absence (b) and presence (c) of hormone.

tween the translation initiation codon and the VP16 domain can be used for making a fusion protein with transcription factors or their DNA-binding domains. In the vector plasmid DNA, the VP16-GRHBD segment (VG cassette) is preceded by the cauliflower mosaic virus 35S promoter [9] and followed by the polyA-addition signal sequence of the nopaline synthase gene of the *Agrobacterium* Ti plasmid. This architecture of the cassette provides the expectancy that the resulting chimeric protein acts as a strong transcriptional activator only when glucocorticoid is exogenously supplied.

The *Arabidopsis* ATHB-1 and ATHB-2 [10-12] are transcription factors containing the homeodomain and leucin zipper motif. To analyze their function, the DNA segment encoding their DNA-binding domain (termed HDZip1 and HDZip2, respectively) was cloned into the VG cassette vector. The resulting chimeric genes (35S-HDZip1-VG and 35S-HDZip2-VG) were introduced into *Arabidopsis* for transgenic plants. In the absence of glucocorticoid, transgenic plants carrying either of 35S-HDZip1-VG and 35S-HDZip2-VG were indistinguishable from the wild-type plants (Figure 1b). On the other hand, treatment with dexamethasone, an agonist of glucocorticoid, led to transgene-specific phenotypes. On agar plates containing 10 μM of dexamethasone, seedlings carrying 35S-HDZip1-VG had very thin cotyledons. True leaves also showed similar thin phenotypes and lobes of leaves were extremely exaggerated (Figure 1c). From these phenotypes induced, ATHB-1 itself or genes recognized by its DNA-binding domain is thought to be involved in leaf development. 35S-HDZip2-VG seedlings under similar conditions had each a short and radially swollen hypocotyl and petioles (data not shown). Another transgenic plant simply overexpressing the native ATHB-2 gene, on the contrary, gave an opposite phenotype (data not shown). These results suggest that ATHB-2 itself acts originally as a repressor but not an activator, and that the opposite phenotype with 35S-HDZip2-VG is brought by inducible activation of transcription with the foreign VG domain. The specific inducible phenotypes described above indicate that the chimeric transcription factors were activated in transgenic plant cells inducibly by exogenously added dexamethasone.

This hormone-induction system is useful for analysis of the regulatory network of gene expression downstream from a transcription factor of interest. The most advantageous point of this system is no requirement of *de novo* protein synthesis for induction of transcriptional activation. Combining with an inhibitor of protein synthesis (e.g. cycloheximide), this system makes it possible to isolate mRNAs (or cDNAs) derived from genes that are directly activated by the transcription factors without secondary effects. In other words, the VG cassette system allows us to identify direct target genes of the transcription factor. We have actually found several candidates for the target of ATHB-1 and ATHB-2.

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