GENE NOTE

CrMYC1, a Catharanthus roseus elicitor- and jasmonate-responsive bHLH transcription factor that binds the G-box element of the strictosidine synthase gene promoter*

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Abstract

A cDNA encoding a bHLH transcription factor was isolated by the yeast one-hybrid system from a Catharanthus roseus cDNA library using the G-box element of the Strictosidine synthase gene promoter as bait. The corresponding protein (named CrMYC1) was shown to bind specifically to the G-box in yeast. In C. roseus suspension cells CrMYC1 mRNA levels are induced by fungal elicitor and jasmonate suggesting that CrMYC1 may be involved in the regulation of gene expression in response to these signals.

Key words: bHLH, Catharanthus roseus, cMyc, elicitor, G-box, jasmonate, strictosidine synthase, transcription factor.

The genome of Arabidopsis thaliana contains 133 genes encoding transcription factors characterized by a basic helix–loop–helix (bHLH) domain. Although some have been shown to be involved in the regulation of development or secondary metabolism, for most of them functional data, including DNA-binding target sequence and specificity, are lacking (Heim et al., 2003). In this paper the isolation, DNA-binding and expression analysis of a Catharanthus roseus transcription factor are described. In C. roseus cell suspensions, the biosynthesis of pharmaceutically valuable terpenoid indole alkaloids (TIAs) is stimulated by methyl jasmonate and auxin (Menke et al., 1992) and up-regulated by fungal elicitor and MeJa (Menke et al., 1999). The JERE is recognized by elicitor- and MeJa-responsive AP2/ERF-domain transcription factors (ORCAs) that activate Str gene expression in response to these signals (Menke et al., 1999). Interestingly, a functional G-box (CACGTG) was also identified in the Str promoter, located at –108 to –103 close to the JERE element (Ouwerkerk and Memelink, 1999). G-boxes have been suggested to participate in MeJa responsiveness of plant genes (Menkens et al., 1995). In order to identify putative new regulators of Str gene expression, a tetramerized 18 bp fragment of the Str gene promoter containing the G-box was used as bait in a yeast one-hybrid screening. This resulted in the isolation of several cDNAs encoding C. roseus transcription factors (Pré et al., 2000). One of these cDNAs encoded a N-terminal truncated protein possessing a typical basic helix–loop–helix domain (bHLH) found among others in cMyc transcription factors.

Expression of the partial C. roseus protein fused to the yeast GAL4 activation domain in yeast reporter strains containing the His3 reporter gene under the control of a minimal yeast promoter containing a TATA box, or under the control of the same promoter plus tetramerized DNA fragments with mutated G-boxes (CACcaG or aACGTG), showed that the protein was only able to bind the native G-box (Fig. 1). These data are in accordance with the fact that the amino acid sequence of the basic domain possesses typical features of the B group of bHLH transcription factors, which are known preferentially to bind to G-boxes (Archley and Fitch, 1997).

A 5’-RACE PCR strategy resulted in a full-length (1433 bp) cDNA encoding a 271 amino acid protein, which was named CrMYC1. The bHLH domain is located between amino acids 151 and 200. The four arginine residues in positions 83–87 as well as residues 147–RR[XL]150ERARR–163 could constitute putative nuclear localization signals (NLS). Sequence comparison with other bHLH transcription factors show that CrMYC1 is highly homologous (78% identical at the amino acid level) to the A. thaliana bHLH031 transcription factor (accession number: AB028232), which belongs to sub-family XII of the A. thaliana bHLH transcription factor family (Heim et al., 2003). No functional data have been published for the AtbHLH031 transcription factor. Crmyc1 mRNA levels in response to fungal elicitor, MeJa and auxin, signals known to

* The sequence of the Crmyc1 cDNA is deposited in the GenBank Nucleotide Sequence Database under the accession number AF283506.

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regulate the Str gene expression, were determined in suspension cells.

Figure 2 shows that the Str and Crmyc1 mRNA levels were up-regulated by elicitation and MeJa. The up-regulation of the Crmyc1 mRNA level by these signals occurred after the increase in the Str mRNA level. In response to auxin, Str gene expression was transiently down-regulated while the Crmyc1 mRNA level remained constant. Given these expression kinetics, it is doubtful that CrMYC1 is involved in regulation of Str gene expression in response to MeJa, elicitors or auxin. However, further experiments are necessary to establish whether CrMYC1 has an effect on Str gene expression. Nevertheless, it is interesting that these data show that CrMYC1 is able to interact with the DNA G-box motif and a role is suggested for CrMYC1, and probably its orthologue in other species, in the regulation of plant gene expression in response to pathogen attack or other stresses involving (Me)Ja as secondary messenger.

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References


