Volume 48(1-4):91, 2004
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARY

Characterisation of the promoter of *Medicago sativa* cyclin dependent kinase - Cdc2MsF

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The eukaryotic cell cycle is regulated at multiple points and most of these involve the activation of a special class of serine-threonine protein kinases, which functionally require binding for activity to a regulatory protein known as a cyclin and are therefore named cyclin-dependent kinases (CDKs). Alfalfa (*Medicago sativa* L.) is one of the most important forage crops and it is used as a model plant in molecular biology. It was demonstrated that at least six Cdc2 homologous genes, members of the CDK family, are expressed in tetraploid alfalfa (Magyar et al., 1997). The mRNA level of one of them – cdc2MsF – showed a peak during G2/M phases. Our aim is to characterize the cdc2MsF promoter (*fpr*), which would develop further the study of the cell cycle.

Cloning of *fpr*, plant transformation and histochemical assays

We cloned a genomic DNA fragment from tetraploid alfalfa, approximately 400bp upstream from the transcription start of cdc2MsF gene.

Since the G2/M specific expression of cdc2MsF mRNA and protein was described in synchronized cell cultures (Magyar et al. 1997), our main question was if *fpr* directed reporter genes have the same pattern in a living plant organism as the native cdc2MsF protein has.

In order to follow the promoter activity, we made pCambia3301-derived constructs where the *fpr* regulates reporter genes coding for β-glucuronidase (GUS) or luciferase. The constructs were introduced in alfalfa plants by *Agrobacterium*-mediated transformation. Throughout present work the spatial and developmental expression of *fpr* driven reporter genes was investigated by analysing callus tissue, somatic embryos and 2-3 week old plantlets. In addition, for first time, histochemical detection of the native cdc2MsF protein was performed and correlated to the expression of the recombinant GUS and luciferase proteins.

Different parts from GUS stained alfalfa plants were observed under light microscope. Ultrathin sections were made from GUS stained root tips and analyzed. Immunolocalization of cdc2MsF protein was done on sections from GUS stained emerging lateral roots. Single-labelling immunohistochemistry was performed on alfalfa root tip with cdc2MsF and luciferase antibodies. For visualizing the immunoreactions secondary antibodies conjugated with horseradish peroxidase and DAB (diaminobenzidine) substrate were used. Also, we examined the activity of the luciferase enzyme after addition of luciferin substrate.

The GUS staining experiments showed that in alfalfa the reporter product accumulates preferentially in meristematic regions in which the cell division is frequent. This observation was confirmed by the cdc2MsF and luciferase immunopositivity of alfalfa root tip in the apical root meristem. In addition, there is a correlation between the GUS staining and the cdc2MsF protein expression in emerging lateral roots. Light excitation was detected during the stages of active cell division.

Synchronization of cell cultures from transformed alfalfa plants

For detailed analysis of the cell phase expression of *fpr* regulated reporter genes, we cultivated *in vitro* cell cultures from transformed alfalfa plants. Samples were taken in order to check the cell cycle progression of synchronized cells. The luciferase and cdc2MsF (as a control) transcript levels were investigated by RT-PCR and by Northern blot assay.

Reference

Magyar Z, Mészaros T, Miskolczi P, Deák M, Fehér A, Brown S, Kondorosi E, Athanasiadis A, Pongor S, Bilgin M, Bakó L, Koncz C, Dudits D (1997) Cell cycle phase specificity of putative cyclin dependent kinase variants in synchronized alfalfa cells. Plant Cell 9:223-235.

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