Embryogeny/Seed formation/Senescence/Germination

376(2pG01)

Isolation and characterization of *LEC1* homolog in somatic and zygotic embryos in carrot.

Katsumi Yazawa, Kiminori Takahata, Hiroshi Kamada (Univ. of Tsukuba, Inst. Biol. Sci.)

Embryogenesis in higher plants has been studied using Arabidopsis mutants. In Arabidosis, three genes, *ABSCISIC ACID-INSENSITIVE3* (*ABI3*), *FUSCA3* (*FUS3*) and *LEAFY COTYLEDON1* (*LEC1*), encoding transcription factors, are thought to play a important roles in embryogenesis. Particularly, *lec1* mutant shows abnormal embryogenesis in both morphology and seed maturation. Ectopic expression of *LEC1* gene induced embryo-like structures in vegetative cells. These reports suggest that *LEC1* gene is an important transcription factor controlling embryogenesis.

In our laboratory, carrot *ABI3* homolog (*C-ABI3*) and embryogenic cell proteins (ECPs) which are specifically expressed in late embryogenesis in carrot had been isolated and characterized.

To isolate a carrot homolog of *Arabidopsis LEC1* gene, a cDNA library made from carrot somatic embryos were screened using the *LEC1* gene as a probe. Ten positive clones were analyzed and all of them contained a partial fragment of the same gene (*C-LEC1*). Genomic Southern blot analysis shows that there are two copies of *C-LEC1* gene in carrot genome. *C-LEC1* gene shows 55.5% sequence similarity to *Arabidopsis LEC1* gene. Comparison of the overall amino acid sequence of the open reading frame revealed that C-LEC1 shows 52.9% similarity to LEC1. Particularly, B domain that located in the central part of C-LEC1 has high similarity to that of LEC1. This domain has similarity to the HAP3 subunit of the CCAAT box-binding factor (CBF) in mammals and yeast and contains conserved DNA binding site and subunit interaction site. According to the expression analysis during the development of somatic embryos, no signal was detected just after the induction of somatic embryos. The expression of *C-LEC1* was gradually increased with embryo development and decreased after globular stage. In developing carrot seeds, the same result was obtained.

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EXPRESSION ANALYSIS OF EMBRYOGENESIS RELATED GENES USING *IN SITU* HYBRIDIZATION.

Kiminori TAKAHATA, Tsutomu SAITOU, Katsumi YAZAWA, Hajime SHIOTA[1], Hiroshi KAMADA, Institute of Biological Sciences, Univ. of Tsukuba, Ibaraki, 305-8572, [1]Fac. Sci., Yokohama City Univ., Yokohama 236-0027

Somatic embryogenesis can be induced from somatic cells which differentiated having a specific function and has been extensively used as a model system of zygotic embryogenesis, because somatic embryos develop into seedlings through morphological changes similar to those of zygotic embryogenesis.

In our laboratory several embryogenic cell proteins (ECP) had been identified as proteins which are specifically found in embryogenic cells (EC) of carrot. We had also cloned the corresponding genes and the expression of the genes was found to be induced by abscisic acid (ABA) only in embryonic tissues.

C-ABI3, a carrot homologue of *Arabidopsis ABI3*, was also cloned and it was found that *C-ABI3* regulates expression of ECP genes through ABA signal transduction in embryos and plays an important role in acquisition of desication tolerance. More recently, *C-LEC1*, a carrot homologue of *Arabidopsis LEC1* which is known as embryo specific transcription factor, was also cloned.

In this study we investigated expression pattern of *ECP31,40,45,63,C-ABI3,C-LEC1* genes in EC and each developmental stage of somatic embryos using *in situ* hybridization. We also investigated the effect of ABA on the expression pattern of the genes in somatic embryos. It was found that the expression of these genes was found in all parts of EC and somatic embryos at each developmental stage. It is noteworthy that expression of *ECP31* gene was strongly enhanced by ABA treatment at each developmental stage of somatic embryos.

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Isolation and characterization of outermost cell specific homeobox genes in rice.

Momoyo ITO, Naoki SENTOKU, Asuka NISHIMURA, Makoto MATSUOKA, Bioscience Center, Nagoya Univ., Nagoya 464-8601, Japan.

We have isolated five HDGL2-type homeobox genes, *Roc1-5* (rice outermost cell specific gene), which is specifically expressed in the epidermal cell layer of shoot and/or root. In situ expression analyses have revealed that all genes are specifically expressed in the epidermal cell layer, while they can be divided into two subclasses by whether each gene is expressed in the root epidermal cell or not. Therefore, one subclass expressed in the shoot and root epidermal cells may be involved in the development of both organs, while the other class expressed only in shoot epidermal cells may be involved in the shoot development.

The precise analyses of the *Roc1* expression pattern have revealed that the outermost cell specific expression is established at the moment when the embryo cells can be physically recognized as the inner or outer cells at the very early stage of embryogenesis. *Roc1* gene is also expressed in the outermost cell of callus without any tissue formations. These findings suggest that the *Roc1* expression in the outermost cells may be based on the physical-positional information of the embryo or callus before the cell fate determination of the L1 layer. In other words, the *Roc1* expression caused by the physical information may be prerequisite for the differentiation of the epidermal cell during the rice embryogenesis. Based on these analyses, we will discuss the function of *Roc1* gene in rice embryogenesis.

379(2pG04)

Analysis of the *globular arrest 1* mutant of *Arabidopsis thaliana* which has a defect in the embryogenesis

Takaaki ISHIKAWA[1], Yasushi YOSHIOKA[1], Chiyoko MACHIDA[1] and Yasunori MACHIDA[1] ([1] Div. Biol. Sci., Grad. Sch. Sci., Nagoya Univ.)

To study the embryogenesis of higher plants by the genetic and molecular analysis, we isolated embryo defective mutants from a screening of T-DNA transformed lines. The *globular arrest 1* (*gla1*) mutant is one of the their mutants. Embryos of the *gla1* mutant normally developed up to the globular stage, but these embryos failed to undergo the transition to the heart stage in which the cotyledonary primordias emerge. The *gla1* mutation was tightly linked to the T-DNA. Then we researched the genomic sequence surrounding the insertion site of T-DNA. The T-DNA was inserted in the coding region of the gene which was predicted to code folylpolyglutamate synthetase (FPGS). FPGS catalyzes tetrahydrofolate polyglutamates synthesis. Tetrahydrofolate polyglutamate is a precursor for one-carbon substituted folates which have importance in biosynthesis of purines, thymidylate, glycine and methionine. These results suggest that the luck of these metabolisms cause the phenotype of the *gla1* mutants.