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ALCOHOL DEHYDROGENASE GENE EXPRESSION IN CALLUS AND SOMATIC EMBRYO OF CARROT

S. RAMAGOPAL* and Z. R. SUNG

Departments of Genetics and Plant Pathology, University of California, Berkeley, California 94720, U.S.A.

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Key Word Index—*Daucus carota*; Umbelliferae; alcohol dehydrogenase; mRNA; anaerobiosis; embryogenesis.

Abstract—A carrot mRNA of about 1700 nucleotides hybridized specifically to a cloned maize alcohol dehydrogenase (Adh) gene. Hybridization of mRNAs from callus and somatic embryo to the maize probe suggests that Adh expression in carrot is similar in these two cell types.

INTRODUCTION

Many plant cells in tissue culture undergo somatic embryogenesis and regeneration. Factors that trigger embryogenesis and molecular events that regulate this process are not fully understood. Withdrawal of hormone from the culture medium [1] or anaerobic conditions [2, 3] are among the factors which appear to contribute for the development of embryos. Alcohol dehydrogenase is a developmentally regulated enzyme whose distribution varies with tissue-type [4] and also is induced by anaerobic stress [5]. Whether expression of alcohol dehydrogenase is regulated during somatic embryogenesis is not known. Molecularly cloned Adh probes from maize were used in this study to investigate Adh expression in a carrot culture.

RESULTS AND DISCUSSION

We used two recombinant DNA clones containing maize Adh sequences in our study. The plasmid pB428 is a genomic clone of a 11.5 Kb Bam HI fragment of maize DNA that included the Adh sequences [6]. pZML84 is a cDNA clone representing 900 bases of the 1650-base-long maize Adh 1 mRNA [7]. Both probes hybridized to carrot genomic DNA in Southern blots (data not shown).

Poly A(+) RNA was prepared from callus cultures and somatic embryos of carrot, denatured, resolved by electrophoresis and transferred to nitrocellulose filters. The plasmid DNA from pB428 was digested with Hind III, separated on an agarose gel and the desired fragments purified after electroelution. A 2.3 Kb fragment containing majority of the exons and introns of the Adh gene and an adjacent 5 Kb fragment with a small portion of the 3′ end of Adh gene [6] were isolated. Pst I digestion yielded the Adh insert from the cDNA clone. The purified fragments were nick translated and used as hybridization probes.

The 2.3 Kb fragment hybridized to a discrete mRNA species of about 1700 nucleotides from carrot (Fig. 1B). Therefore, carrot Adh mRNA appears to be in the same size range as that of maize [7]. The 5 Kb maize DNA did not hybridize to any of the carrot mRNAs (Fig. 1A). This suggests that the portion of the 3′ end of Adh gene in the 5 Kb fragment has insufficient homology to carrot mRNA. In addition, the data indicate that the other sequences in the maize 5 Kb DNA fragment also lack homology to carrot mRNA. Hybridization results with pZML 84 were similar to those observed with the 2.3 Kb genomic clone (not shown). This indicates that exon sequences in the 2.3 Kb fragment were responsible for hybridization with carrot mRNA. Adh expression was qualitatively and quantitatively identical in callus and somatic embryo (Fig. 1B).

Zygotic embryos of angiosperms appear to develop in an anaerobic environment [8, 9]. In bean pods, a high Adh activity was observed in embryos compared to seed coats. In addition, Adh showed differences in isoenzyme pattern [9]. Anaerobic conditions also prevail during somatic embryo development and a lowering of oxygen in suspension cultures of carrot was found to promote embryogenesis [2, 3]. Our data obtained above with sensitive gene probes suggest that Adh expression is unaltered during somatic embryogenesis in the carrot culture. Also, no differences in Adh isoenzyme pattern were discernible between callus cultures and somatic embryos (data not shown). Why alcohol dehydrogenease is regulated differently in somatic and zygotic embryos is not clear. The organizational environment in which a zygotic embryo grows in a developing seed is not entirely equivalent to somatic embryo development in a tissue culture system. Moreover, anaerobic conditions cause a pleiotropic response in plants and Adh is one of the many induced polypeptides [5]. Whether the other anaerobic proteins are important for the development of embryo in seeds and in cell cultures remain to be investigated.

EXPERIMENTAL

Cell line and culture. A diploid cell line of *Daucus carota*, L. (W001C) was grown in a Murashige and Skoog medium and callus (suspension cultured cells) and somatic embryos were

*To whom correspondence should be addressed at: USDA/ARS, Experiment Station, HSPA, P.O. Box 1057, Aiea, Hawaii 96701, U.S.A.

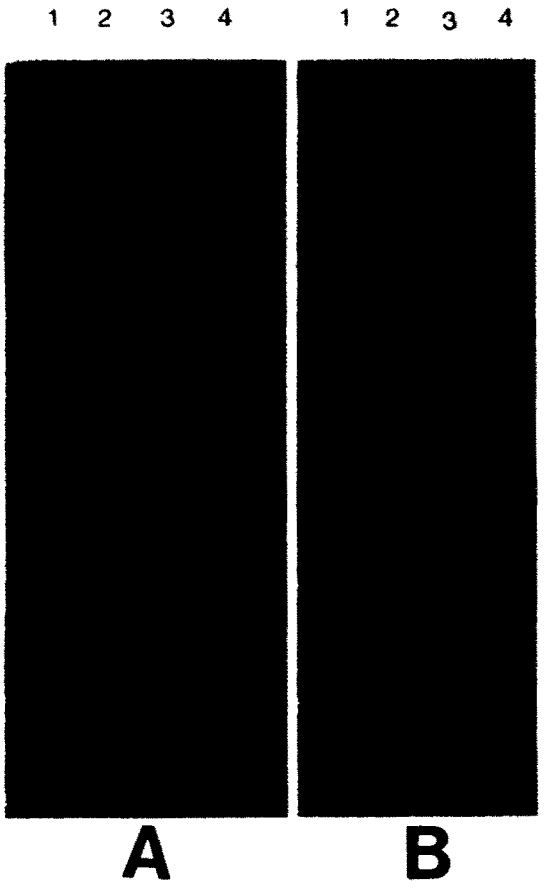

Fig. 1. Northern blot analysis of carrot poly A(+) RNA from callus and somatic embryo. Lanes 1 and 2 contained 1.75 and 3.5 μg of RNA, respectively, from callus culture and lanes 3 and 4 contained 1.85 and 3.7 μg of RNA, respectively, from somatic embryo. Electrophoresis and blot hybridization were performed as described in Experimental. The following probes were used: (A) 5 Kb fragment, (B) 2.3 Kb fragment. Using rRNA as markers, the size of the mRNA (arrow) is estimated as 1700 nucleotides.

obtained as described [10]. The callus cultures were harvested at the exponential phase of growth. The embryogenic cultures which consisted mainly of heart and torpedo but also some globular stages were harvested after 12–14 days of induction.

Carrot mRNA preparation and northern transfer. Poly A(+) mRNA was isolated from growing, suspension cultures and somatic embryos. Total RNA was extracted by phenol and precipitated with ethanol. The ethanol precipitates were washed extensively with 2 LiCl [11] and applied to an oligo dT-cellulose column. Poly A(+) RNA was purified by two cycles of chromatography. mRNA samples were denatured by heating at 65° (10 min), separated on 1% agarose, 2.2 M formaldehyde gels [12] and transferred to nitrocellulose filter. Hybridization and washing conditions were modified from Maniatis *et al.* [12] and Fyrberg *et al.* [13]. Prehybridization was in 50% formamide, 5 × SSC, 5 × Denhardt's solution, 0.1% SDS, 0.05% NaPPi, 10 μg/ml poly A and 250 μg/ml denatured salmon sperm DNA at 37°, overnight. Hybridization buffer was similar to prehybridization buffer except that salmon sperm DNA was 100 μg/ml and contained 10% sodium dextran sulphate and ^{32}P-probe DNA. After 48 hr of hybridization at 37°, the filter was washed once in 2 × SSC, 0.5% sarkosyl, 0.02% NaPPi at room temperature followed by three times in 0.2 × SSC, 0.05% sarkosyl and 0.01% NaPPi at 50°. Nitrocellulose filters, after exposing to X-ray film, were stained with methylene blue to visualize RNA markers.

Plasmids and hybridization probes. The plasmid pB428 was a generous gift of M. Freeling. A sample of pZML84 DNA was kindly provided by E. Dennis and was used to transform *Escherichia coli* HB 101. The Adh clone was identified by colony hybridization using the 2.3 Kb DNA fragment from pB428 [6, 14]. Plasmids were propagated in HB 101, DNA extracted by the alkaline method [12] and purified by CsCl gradient. Probe DNA fragments were prepared after digestion of total plasmid DNA with restriction enzyme, gel electrophoresis, electroelution and DEAE-Sephacel chromatography. Hybridization probes were labeled by nick translation [15] with [α-^{32}P] dCTP (3000 Ci/mmole, Amersham). Carrier tRNA was added to the reaction mixture and extracted once each with phenol–$CHCl_3$ and Et_2O and passed through a Sephadex G50 column [14].

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