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To: Stanley Kyeyune, DOE, San Francisco Field Office,
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From: Renee Sung, Dept. of Plant Biology, UCB

Re: Final report on DOE grant no. DE-FG03-87ER13698
Characterization of embryo-specific genes, 4/1/87 to 3/31/92

The objective of the proposed research is to characterize the function and regulation of a set of embryonic genes which are expressed in the embryos, not in the plants. 22 cDNA clones were isolated from a cDNA library we constructed using mRNAs of carrot somatic embryos. These cDNA clones identified mRNA species that are present in the somatic and zygotic embryos, but not in adult plants (Choi et. al. 1987, Borkird et. al. 1988). The sequence of all 22 cDNA clones were determined; genomic clones for three cDNA clones, DC8, DC59, and DC49 were isolated and gene sequences determined (Franz et. al. 1989, Hatzopoulos et al. 1990a). DC8, DC49, and several other genes identified by the cDNA sequences belong to the category of late embryogenesis abundant protein genes, Lea. The function of these gens have not yet been determined, but they share common structural features, are regulated by ABA (Hatzopoulos et al. 1990b), and are speculated to play a role in seed desiccation (Dure et. al. 1989).

DC59 is a gene encoding a protein in the membrane of lipid body; its expression in the embryos is also dependent on the presence of ABA (Hatzopoulos et. al. 1991a).

To study the mechanism of DC8 and DC59 regulation, we performed gel retardation experiments, and found that embryo nuclei contain factors that can interact with specific regions of the promoters, but not leaf and root nuclei (Hatzopoulos et al. 1990a, Goupil et. al. 1992). The promoter of these 2 genes can interact with some of the same nuclei factors, suggesting that their coordinate regulation is controlled transcriptionally. To demonstrate transcriptional regulation, promoter deletion analysis using transgenic carrots containing promoter:gus constructs were carried out. We found that DC8 promoter contains an ABA-responsive region with 3 ABA elements, ACGTGG(T), but this region alone is insufficient to confer DC8 transcription. In addition to the ABA-responsive sequence, one of the two reduncant upstream sequence is required to cause gus expression (Goupil et. al. 1992).

Gus expression in transgenic plants provides a sensitive assay to investigate temporal and spatial expression of DC8. We found that DC8 expression can be induced as early as 14 days after callus induction from carrot leaves, i.e., before the initiation of somatic embryos. During zygotic embryogenesis, DC8:gus activity can be easily detected in the globular-stage embryo and the endosperm surrounding the globular embryo. This result shows that although DC8 expresses strongly in late embryogenesis, the promoter is active in early embryogenesis. When is the promoter inactivated? It is not inactivated until after germination. Germinating seedlings are gus-positive, but become gus-negative after substantial cell elongation. New cells produced from the meristems are gus-negative (Goupil et al. 1992). DC8 RNA synthesis in young seedlings is still responsive to ABA induction (unpublished results). We interprete this result to mean that DC8 promoter is active in all cells formed during seed development,but not in cells formed after germination. The loss of gus activity in the elongating cells of carrot seedling may be interpreted as follows. The promoter, once activated in an embryonic cell, is not easily inactivated, until the transcriptional factors are turned over during germination. Presumably, there is no new synthesis of the transcription factors after germination. Leaf primordia and root cells derived from the meristems cannot produce the factors needed to maintain an active transcription unit. Future experiments are directed at the identification of genes encoding the transcriptional factors and genes involved in the signal transduction pathway of ABA response.

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