IDENTIFICATION AND SEQUENCE OF A cDNA CLONE CORRESPONDING TO A GENE INVOLVED IN DEVELOPMENT OF *UNDARIA PINNATIFIDA*

HOU He-shen (侯和胜), LI Ning (李凝), WU Chao-yuan (吴超元)
(Department of Biology, Liaoning Normal University, Dalian 116029, China)
(Department of Biology, The Hong Kong University of Science and Technology, Hong Kong)
(Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China)

Abstract  During the induction of gamete-producing gametangia, induced gametophytes were collected at 4 days intervals (0, 4, 8, 12 d) and total RNAs were isolated by CsCl gradient ultracentrifugation. Some stage-specific expressed mRNAs were identified by differential display of mRNAs from different developing stages of the gametophytes. The cDNA of one specific mRNA was verified, cloned and sequenced. This gene was specifically expressed during 4 days of induction, and had partial homologous sequence with tobacco IAA-binding protein gene. It suggests that this cDNA may represent a gene which is related to the IAA regulating function during the development of the gametophytes.

Key words: Undaria pinnatifida, development, cDNA cloning, DNA sequence

INTRODUCTION

A critical developmental event in higher plant is the transition from vegetative to reproductive growth, which is marked by the onset of flowering. The analysis of mutations affecting flower structure has led to the identification of some of the genes that direct flower development. Cloning of these genes such as LFY, AP3, AG in Arabidopsis (Jack et al., 1992; Weigel et al., 1992) and FLO, DEF, PLE in Antirrhinum (Coen et al., 1991; Schwarz-Sommer et al., 1990) provided insight into the underlying molecular regulatory mechanisms of flowering, which is a key developmental step in plant. Although morphologically simple multicellular organisms, macrophytic algae undergo a number of intriguing developmental processes. Many brown algae, for examples, Laminaria and Undaria, which are important commercial seaweeds, have a life cycle that alternates between two dissimilar phases: the filamentous gametophyte and the foliose sporophyte. The gamete-producing gametangia, the organs of reproduction, can be induced by radiation, temperature or photoperiod from both male and female. The development of gametangia is generally assumed to be controlled at the level of gene expression. It offers an opportunity to study developmentally regulated genes that are expressed in a stage-specific manner.

* Contribution No. 3387 from the Institute of Oceanology, Chinese Academy of Sciences.
Projects 39670584 and 961165301 supported by NSFC and Science and Technology Commission of Shandong Province respectively.
Despite the importance of the transition from vegetative to reproductive growth, very little is known about the underlying molecular mechanisms in brown algae. Thus for mechanism understanding and eventual biotechnological reasons we isolated and sequenced a cDNA which may represent a stage-specific expressed gene involved in the transition of the different developmental stages in _Undaria pinnatifida_. The possible function of the gene is discussed here.

**MATERIALS AND METHODS**

**Algal material**

_Undaria pinnatifida_ was collected at Fujiazhuang, Dalian, in May 1995, and their gametophytes were maintained in culture at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao. The samples were cultured in PESI medium (Tatewaki, 1966) at 18°C in darkness and 20°C in 3000 lx light, under a 14:10 h LD cycle. Cultures were harvested at 4 days intervals (0, 4, 8, 12 d) after initiation of a light period, washed twice with 1% sarcosyl and were stored after quick freezing in liquid nitrogen at −70°C until use.

**Methods**

Standard methods for Northern blot analysis and hybridization, plasmid DNA isolation, DNA and RNA gel electrophoresis, were carried out according to Sambrook et al. (1989).

**RNA isolation**

Ten grams of frozen samples were ground in liquid nitrogen into a fine powder with precooled pestle and mortar and transferred to a 50 ml tube containing 10 ml of extraction buffer (6.0 mol/L guanidium thiocyanate, 25 mmol/L sodium citrate, pH 7.0, 100 mmol/L mercaptoethanol, 0.5% sarcosyl and 0.1% sigma anti-form). The frozen powder was dispersed by centrifugation to remove insoluble material in the vortex; the aqueous phase was collected using a Pasteur pipette and layered carefully over 11 ml of cesium chloride (CsCl) solution (5.7 mol/L CsCl, 10 mmol/L EDTA) in a centrifuge tube (Beckman, SW 28). This solution was centrifuged at 20°C for 22 h at 45000 r/min. The pellet was washed twice with 70% ethanol and then resuspended in 400 μl DEPC-treated ddH₂O. The resulting solution was extracted twice with phenol; chloroform (1:1), once with chloroform. RNA was precipitated by addition of 2.5 volumes of precooled absolute ethanol and 0.1 volume of 3 mol/L sodium acetate, followed by incubation at −20°C for 2 h. The precipitate was collected by centrifugation, rinsed twice with 80% ethanol, and resuspended in 200 μl of DEPC-treated ddH₂O. The concentration of the RNA was determined by diluting an aliquot of the resuspended material and reading its absorbance at 260 nm.

**The cDNAs differential display**

First strand cDNA synthesis, cDNA labeling, cDNA amplification were performed according to Liang et al. (1992). The putative specific cDNA bands displayed on the DNA sequence gel were cut off with 3 mm filter paper and were transferred together to a 1.5 ml eppendorf tube. The tube was then incubated in a boiling water bath for 15 min after 100 μl ddH₂O was added. The cDNA