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THE ISOLATION, CHARACTERIZATION AND INTERGENERIC TRANSFER OF TWO SOYBEAN (GLYCINE MAX L.) BETA-TUBULIN GENES

University of California, Irvine PH.D. 1986

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The Isolation, Characterization and Intergeneric Transfer of Two Soybean (*Glycine max* L.) Beta-Tubulin Genes

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Biological Sciences

by

Mark John Guiltinan

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Professor Franz Hoffmann

1986

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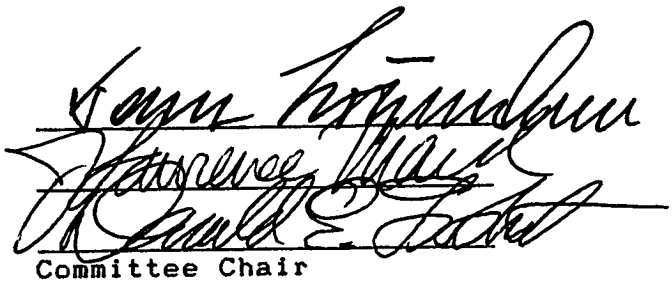

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1986

DEDICATION

I dedicate this thesis to my wife Shelley
and our soon to arrive child (Jenna or Gaven?)

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PUBLICATIONS

Fosket, D., D.N. Radin, M.Guiltinan. 1983. Chemical induction of carotenogenesis and its relationship to chromoplast differentiation in cultured plant tissue. In, W.W. Thomson; J.B. Mudd; M. Gibbs eds. Biosynthesis and Function of Plant Lipids. Publisher; American Society of Plant Physiologists.

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Guiltinan, M., Bustos, M., Cyr, R., and D. E. Fosket. The expression of a chimeric soybean beta-tubulin gene in transgenic tobacco plants. (submitted to EMBO)

ABSTRACTS

Guiltinan, M., Cyr, R., Bustos, M., and D. E. Fosket. (1984). The organization and expression of tubulin genes in higher plants J. Cell. Biochem. Supp 8b:61.

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Radin, D.N., Guiltinan, M.J., Fosket, D.E. Chemical induction of carotenogenesis in callus and cell suspension cultures. At A conference on genetic engineering of plants U.C. Davis Aug. 15-19 1982

ABSTRACT OF THE DISSERTATION

The Isolation, Characterization and Intergeneric Transfer of Two Soybean (*Glycine max* L.) Beta-Tubulin Genes

by

Mark John Guiltinan

Doctor of Philosophy in Biological Sciences
University of California, Irvine, 1986

Professor Donald E. Fosket, Chair

Thirty-five clones were isolated from a soybean genomic lambda library, using a beta-tubulin cDNA from *Chlamydomonas reinhardtii* as a probe. Analysis of these clones by restriction mapping, Southern hybridization and DNA sequencing indicated the presence of 2 classes of structurally different beta-tubulin genes in the soybean genome (designated SB-1 and SB-2). Partial nucleotide and deduced amino acid sequences of these genes showed 62% to 80% homology with each other and with beta-tubulin genes of four evolutionarily diverse organisms. Northern blot hybridization of soybean seedling poly A+ RNA to homologous soybean beta-tubulin probes indicated the presence of several classes of message in the expected size range (1.7 to 2.0 kb). A chimeric tubulin gene was constructed by the fusion of a genomic sequence containing a truncated soybean beta-tubulin gene and 2 kilobases of upstream DNA to the 3' untranslated region and a polyadenylation signal from transcription unit 7 of the octopine Ti plasmid pGV117. The chimeric gene was incorporated into the Ti plasmid transformation vector pGV3850::pAP2034, along with a plant active selectable marker gene. Strains of *Agrobacterium tumefaciens* harboring the plasmids were used to transform *Nicotiana tabacum* cells by the leaf disk method and plants were regenerated from the transformed cells. Transgenic plants were self crossed and the segregation of kanamycin resistance was assayed for several generations. DNA and RNA were extracted, and hybridized to a probe specific for the chimeric gene to assess its structure and expression in the transgenic plants. The chimeric gene was stably integrated into the tobacco genome without rearrangements and it was expressed as a polyadenylated RNA of 1.7 kb in the transformants. Genetic analysis revealed that the kanamycin resistance phenotype was inherited in a Mendelian fashion over two generations.

CHAPTER 1

ISOLATION AND INITIAL CHARACTERIZATION OF SOYBEAN BETA-TUBULIN GENES

INTRODUCTION

MICROTUBULES

Microtubules participate in important processes in all eukaryotic cells, such as cell division, motility, and intracellular transport of organelles. They also interact with other proteins to serve a structural role within the cytoskeleton [7].

Microtubules have several additional functions in plant cells [14]. In interphase cells, the principle microtubular array is located in the cortical cytoplasm adjacent to the plasmalemma and cell wall. These cortical cytoplasmic microtubules are thought to control the orientation in which cellulose microfibrils are deposited [14]. The orientation of cellulose microfibrils within the cell wall determines the vectors of cell expansion during growth [17,34]. Therefore, microtubules exert indirect but precise control over the vectors of subsequent cell enlargement and ultimately plant morphogenesis in general. During prophase in many plant species an array of microtubules known as the preprophase band appears. Its role in cell division is still

unknown but it has been suggested that it may in part determine the orientation of the mitotic spindle in cell division [13,15]. Additionally, during late telophase another unique array of microtubules, the phragmoplast, participates in the synthesis of the cell plate [14]. Golgi vesicles containing non-cellulosic components of the cell wall are transported via microtubules to the equatorial zone of the dividing cell.

TUBULIN

Microtubules are composed principally of the dimeric protein tubulin whose subunit polypeptides, designated alpha- and beta-tubulin, have molecular weights near 50 K daltons [21,31]. Tubulin is found in the cell principally as a soluble dimer or polymerized to form microtubules [7]. Frequently these two states are in a dynamic equilibrium which can be altered by a number of factors including Ca+, temperature, GTP, microtubule associated proteins (MAPS) as well as by several tubulin binding ligands such as colchicine and taxol [7].

Recent studies have indicated that higher plant tubulins exhibit significant differences from vertebrate tubulins in their ligand binding characteristics and immunological properties [29,30,36]. Peptide mapping studies suggest that the beta subunits of plant and vertebrate tubulins are more similar than their alpha-subunits. Plant alpha-tubulins differs markedly from those of animals [25].

It remains to be seen if these differences result in any important functional consequences.

TUBULIN GENES

The tubulins are encoded by multigene families in all 14 eukaryotes examined thus far, except Saccharomyces cerevisiae and Tetrahymena which each have only one beta-tubulin gene [7]. Although no sequence data have been published for higher plant tubulin, the two beta-tubulin genes have been sequenced from the alga Chlamydomonas reinhardi, and the amino acid sequences derived from them are identical [40]. A comparison of these sequences with those of vertebrate tubulins shows them to be 78-80% homologous. Comparison of sequence data from 15 different beta-tubulin sequences indicates that their amino acid sequences are highly conserved, and that the divergence between isotypes is clustered primarily in one highly variable domain (amino acid positions 430 to the C-terminus) [7]. A highly constant region also can be identified (amino acid positions 401-425) which shows nearly 100% conservation between divergent species. Interspecific conservation of the variable domains and 3' untranslated sequences of several tubulin genes indicates strong evolutionary pressure to maintain multiple isotypes within a species [7]. Differential and/or developmental regulation of tubulin gene

expression has been demonstrated in a number of organisms [7]. In addition, in several systems, tubulin mRNA levels seem to be regulated in response to changes in the intracellular concentration of soluble tubulin dimers [4, 5, 6].

In light of our knowledge of the multiple functions tubulin can assume in vivo, these results suggest that the individual isotypes may have some degree of functional specificity. The multitubulin hypothesis states that individual tubulin isoforms might be utilized for specific microtubular functions [10]. Although in a few cases individual tubulin isotypes have been shown to be stage or tissue specific [7, 18, 19, 20, 32, 38], in most species, the functional significance of the multiplicity of tubulin genes is not known. A complete understanding of these relationships is essential to our knowledge of the fundamental mechanisms of cellular growth and development of both plants and animals.

MICROHETEROGENEITY

Different tissues and cells in different developmental stages of a given organism can exhibit diverse patterns of tubulin protein isoforms [8,22,23,24,27]. In some cases this is the result of the regulated expression of several members of a tubulin gene family [24]. Alternatively tubulin heterogeneity also may arise as a result of post-

translational modification of the tubulin proteins [3,22,23], or a combination of both.

Analysis of tubulin function can be complicated by differential expression of multiple genes, post-translational modifications and by interactions of tubulin with other factors such as the MAPS (microtubule associated proteins). Thus, in order to understand how microtubules are formed and how they function in higher plants, it is necessary to first characterize the tubulin gene families and their products from a plant species and to learn how the expression of the individual members are regulated.

Chapter 1 of this thesis documents the isolation and characterization of the beta-tubulin gene family from soybean. Cloned DNA fragments containing putative soybean beta-1 and beta-2-tubulin genes were characterized by restriction mapping, hybridization analysis and DNA sequencing, and have been used as probes for the analysis of tubulin mRNA levels in various plant tissues (see Chapter 2 and 3 as well as Bustos, M., Ph.D. Thesis, 1986 and Cyr, R., Ph.D. Thesis, 1986).

In Chapter 2, high resolution restriction mapping, genomic blot hybridization and DNA sequencing results are presented which further characterize the soybean beta-tubulin gene family. This study shows that there are two divergent members in this family (SB1, SB2), but cannot rule out the possibility of multiple copies or alleles of these

genes. Chapter 2 also presents experiments which demonstrate that beta-tubulin transcripts can be detected in poly A+ RNA from soybean seedlings. Finally, Chapter 3 describes experiments which show that the beta-1-tubulin gene also is expressed in transformed tobacco plants as poly-adenylated mRNA. These experiments have pioneered the way for experiments which will address questions fundamental to plant molecular, cellular and morphogenic biology.