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Functional Analysis of Plant Mei2-like Proteins

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Thesis Abstract

Molecular techniques were used to analyse the function of a novel class of RNA-binding proteins in plants, termed Mei2-like. The biochemical function of this class of proteins is unclear. Although the conserved presence of three RNA recognition motifs (RRMs) in all members of the family suggests the importance of an RNA binding activity, the precise biochemical mechanism by which these proteins act is unknown. Genetic and molecular analyses of the founding member of the family, *Schizosaccharomyces pombe* Mei2p, provide of a conceptual framework for the studies of the plant Mei2-like proteins presented here. Therefore, the aims of this thesis were to 1) study the cellular localisation of Mei2p in plant cells, and 2) deduce the functions of plant *Mei2-like* genes by identifying the protein(s) that physically interact(s) with Mei2-like proteins.

Transient expression of GFP-fused Mei2p in onion epidermal cells was performed to show that Mei2p localised into the nucleus in the presence of meiRNA, a non-coding mRNA. Thus plants seem to share the capacity with *S. pombe* for meiRNA-dependent nuclear localisation of Mei2p. Moreover, intracellular localisation of one of the plant Mei2-like proteins, TERMINAL EAR-Like 2 (TEL2), was studied in onion epidermal cells. The GFP-fused TEL2 localised into the nucleus without co-expression of any special RNA, suggesting that either some RNA species that assist nuclear localisation of TEL2 are already present in onion epidermal cells, or the mechanism of intracellular localisation of TEL2 is different from Mei2p.

The yeast two-hybrid system was utilised to identify protein interactors with TEL2. Six proteins were identified, including the well-studied KORRIGAN

(KOR) protein. Based on the proteins identified, speculation is offered on how these proteins interact with TEL2. Since TEL genes are expressed in the central zone (CZ) of meristems, and mitotic activity of cells in the CZ is low, TEL2 may be involved in controlling cell division in the CZ *via* interactions with these proteins.

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Gene nomenclature

Arabidopsis thaliana

GENES are capitalised and italicised

PROTEINS are capitalised

gene mutants are in lower case and italicised

Maize

genes are in lower case and italicised

PROTEINS are capitalised

gene mutants are named after the genes, in lower case and italicised

Fission yeast, *Schizosaccharomyces pombe*

genes are in lower case and italicised

Proteins are written with the first letter capitalised, and "p" is added after the name of gene, e.g Protein encoded by *mei2* is called

Mei2p

Budding yeast, *Saccharomyces cerevisiae*

GENES are capitalised and italicised

gene mutants are in lower case and italicised

Abbreviations

3-AT	3-amino triazol
BCIP	x-phosphate/5-bromo-chloro-indoyl-phosphate
CZ	central zone
EDTA	disodium ethylene diamine tetra acetate
mg	milligram
μ g	microgram
ng	nanogram
NBT	4 Nitroblue tetrazolium chloride
NaOAc	sodium acetate
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PZ	peripheral zone
RAM	root apical meristem
RRM	RNA recognition motif
RT-PCR	reverse transcription polymerase chain reaction
RZ	rib zone
SAM	shoot apical meristem
SDS	sodium dedecyl sulphate
TEL	terminal ear 1-like
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside