Molecular genetic analysis of the maize *terminal ear1* gene and *in silico* analysis of related genes

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This thesis is dedicated to Ben, Charlie and Alex.

THESIS ABSTRACT

Mutants of the maize *terminal ear1* (*te1*) gene have shortened internodes, abnormal phyllotaxy, leaf pattern defects and partial feminisation of tassels. The *te1* gene encodes an RNA recognition motif (RRM) protein, and is expressed in the vegetative shoot apex in semicircular rings that laterally oppose the positions of leaf primordia (Veit 1998). This project aimed to further characterise the molecular biology and function of the *te1* gene.

Molecular genetic studies aimed to further characterise the genes structure and expression. Genomic clones were sequenced revealing the intron exon structure. 5' RACE was used to predict a 5' transcription start site. Competitive RT-PCR showed that *te1* transcripts were highest in vegetative shoot meristems and embryos, lower in ears, roots and tassels, and undetectable in leaves. Two *te1* mutant alleles were cloned and the junctions sequenced, a further five alleles were characterised incompletely.

The TE1 peptide belongs to a subclass of RRM proteins which includes the *Schizosaccharomyces pombe* protein MEI2. More than 30 putative plant *Mei2*-like genes were identified in Genbank, no examples have been found in metazoans. Seven *Mei2*-like genes were predicted from the completed *Arabidopsis* genome. Exon structure and amino acid sequence supported three groupings of *Mei2*-like genes. Structural predictions of *Mei2*-like proteins indicate that the third RRM contained some novel structural features not present in canonical RRM proteins.

Attempts to study the function of the TE1 protein *in vitro* were limited by the inability of both *E. coli* and *Pichia pastoris* expression systems to express the full length protein, probably due to codon bias. Antibodies produced to a C-terminal portion of the protein did not specifically detect the TE1 protein in plant extracts without incurring non-specific activity.

The *te1* cDNA was ectopically expressed in *Arabidopsis* from a copper-inducible promoter both with and without the SV40 nuclear localisation signal (NLS). Although both *te1 and NLS:te1* transgenes were detected in transformants no phenotypes consistently correlated with transgene expression.

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ABBREVIATIONS

2-ME	2-mercaptoethanol (β-mercaptoethanol)
Amp	ampicillin (superscript, concentration in μg/ml)
BCIP	x-phosphate/5-bromo-chloro-indolyl-phosphate
Cefo	Cefotaxime (superscript, concentration in μg/ml)
DEPC	diethylpyrocarbonate
DIG	Digoxigenin
DMPC	dimethylpyrocarbonate
DMSO	dimethylsulphoxide
dNTPs	deoxyribonucleotide triphosphates
DTT	dithiothreitol
EDTA	disodium ethylenediaminetetra-acetate
EMS	ethylmethane sulfonate
GA_3	Giberellic acid 3
Gent	gentamycin (superscript, concentration in μg/ml)
IPTG	Isopropylthio-β-D-galactaside
Kan	kanamycin (superscript, concentration in μg/ml)
LB	Luria Bertanni medium
NaOAc	sodium acetate
NBT	4 Nitroblue tetrazolium chloride
PBS	phosphate buffered saline
PCR	Polymerase chain reaction
PCR	polymerase chain reaction
pNPP	p-Nitrophenyl phosphate disodium
polyA+	Polyadenylated RNA
rpm	revolutions per minute
RRM	RNA recognition motif
RT-PCR	Reverse transcription-polymerase chain reaction
SAM	shoot apical meristems
SDS	sodium dodecyl sulfate
SDS-PAGE	SDS polyacrylaminde gel electrophoresis
Spec	spectinomycin (superscript, concentration in μg/ml)
SSC	sodium chloride, sodium citrate 20 x SSC is 3 M NaCl, 0.3M sodium citrate pH 7.0
Tris	Tris(hydroxymethyl)aminomethane
xg	multiples of gravitational force

TABLE OF GENES

A consistent nomenclature is used throughout this thesis to describe genes and proteins. Genes names are always italicised, proteins always listed in block capitals with suffixes describing the amino acids included for partial peptides. e.g. the portion of the *te1* gene was expressed to produce the peptide TE1$^{1-286}$.

Gene symbol	Gene	Species
ACE1	metallothionein regulatory protein	*Saccharomyces cerevisiae*
AML1, AML2, AML3, AML4, AML5	Arabidopsis Mei2-like	*Arabidopsis thaliana*
ANT	*AINTEGUMENTA*	*Arabidopsis thaliana*
CDC2a	cell division cycle 2a	*Arabidopsis thaliana*
CLV1, CLV2, CLV3	*CLAVATA*	*Arabidopsis thaliana*
CUC1, CUC2	*CUPSHAPED COTYLEDONS1, 2*	*Arabidopsis thaliana*
FIL	*FILAMENTOUS FLOWER*	*Arabidopsis thaliana*
gn1	*Gnarly*	*Zea mays*
GR	glucocorticoid receptor	*mammalian*
kn1	*knotted1*	*Zea mays*
MBP	maltose binding protein	*E. coli*
Mei2	Meiosis deficient 2	*Schizosaccharomyces pombe*
MGO1, MGO2	*MGOUN*	*Arabidopsis thaliana*
nptII	neomycin phoshotransferase	synthetic
OSH1	*Oryza sativa* homeobox1	*Oryza sativa*
STM	*SHOOTMERISTEMLESS*	*Arabidopsis thaliana*
T710	phage T7 protein 10	phage T7
te1	*terminal ear1*	*Zea mays*
TEL1, TEL2	*terminal ear1-like*	*Arabidopsis thaliana*
UFO	*UNUSUAL FLORAL ORGANS*	*Arabidopsis thaliana*
WUS	*WUSCHEL*	*Arabidopsis thaliana*
YAB3	*YABBY3*	*Arabidopsis thaliana*