ABSTRACT

TOR, target of rapamycin, is an atypical kinase, which controls the regulatory network in yeast and animal cells to regulate their growth in response to environmental cues. In response to nutrients, starvation, and hormones, TOR protein regulates ribosome biogenesis, and translation and transcription of a subset of genes involved in translational machinery (Cutler et al., 2001). It has been shown that TOR modulates translation in eukaryotes by phosphorylation of the downstream effectors and thus regulates the abundance of ribosomes and the translation factors (Dennis et al.1999). Integration of energy levels, nutrient sufficiency and stress information to regulate the translational machinery allows TOR to act as a "master regulator" that strikes a balance between nutrient availability, metabolic processes, protein synthesis, and cell growth (Abraham, 2002). Such a balance is crucial for the cell to ensure its survival. TOR is also at the heart of growth signaling pathways with its substarte AtS6K mediating translational regulation by phosphorylation of ribosomal protein S6 (RPS6;Turck et al., 2004). There is another regulatory role of TOR at the transcriptional level; TOR through its interaction with a phosphatase affects the phosphorylation status of some transcriptional factors and thereby changing their localization from cytoplasm to the nucleus (Cutler et al. 2001 and Beck et al. 1999). Under osmotic

stress or nutrient limitation, these transcription factors may shuttle into the nucleus to drive the expression of a set of stress related genes. However, the identification of such transcription factors is yet to be determined in plant cells. It is likely that based on the source of the signal AtS6K in a TOR dependent and TOR independent manner. For example, PDK1 may activate AtS6K under a certain set of signals that are different from that of TOR.

Despite the importance of this pathway in eukaryotes, little is known of the regulation of this central signaling mechanism in plants. This pathway may link various stress signals to the growth signaling pathway optimizing plant growth under a variety of environmental conditions. In this study, we cloned all plant TOR kinase genes from Arabidopsis thaliana. In order to study the regulation of TOR kinase pathway in plants, the interaction of FKBP12 and the FRB domain of pTOR in the presence of rapamaycin was tested. pFKBP12 does not interact with the pTOR-FRB in a yeast two hybrid and *in vitro* translation experiments. However, the human FKBP12 (hFKBP12) was found to interact with pTOR-FRB domain in a rapamycin-dependent manner. The immunoprecipitated AtS6K was found to be active and phosphorylated the RPS6 in the kinase reactions. Among various treatments tested, osmotic stress was found to inhibit the kinase activity of AtS6K. AtS6K was found to interact with the regulatory TOR associated protein (RapTOR) *in vivo* when both constructs were expressed in tobacco leaves.

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ABBREVIATIONS

aa	amino acids
AMP	Adenosine Mono Phosphate
ATP	Adenosine Tri Phosphate
bp	base pairs
cDNA	complementary DNA
CNBR	Cyanogen bromide
C	degree centigrade
DNA	Deoxyribo Nucleic Acid
DTT	Di Thio Threitol
EDTA	Ethylene Diamine Tetra Acetic Acid
FKBP12	FK506-Binding Protein 12
GST	Glutathione-S-Transferase
IPTG	Isopropyl Thio Galactoside
kb	kilo base
kDa	kilo Dalton
MALDI	Matrix assisted Laser Desorption Ionization
mM	milliMolar
mRNA	messenger RNA
m RNP	messenger Ribo Nucleo Protein complex
MS	Mass Spectrometry
μM	microMolar

NLS	Nuclear Localization Signal
nM	nanoMolar
ORF	Open Reading Frame
PAGE	Poly Acrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PDK1	Phosphatidylinositol Dependent Kinase 1
PI3K	Phosphatidyl Inositol 3 phosphate Kinase
PKB	Protein Kinase B
PKC	Protein Kinase C
PP2A	Protein Phosphatase
PP4	Protein Phosphatase type 4
PP6	Protein Phosphatase type 6
PTEN	Phosphatase TENSIN homologue
PVP	Poly Vinyl Pyrrolidone
RNA	Ribo Nucleic Acid
rDNA	ribosomal DNA
rRNA	ribosomal RNA
SDS	Sodium Dodecyl Sulfate
Ser	Serine
Tap42	TOR associated Protein 42
TCA	Tri Carboxylic Acid
Thr	Threonine
TOF	Time Of Flight
TOR	Target of Rapamycin

TOS	TOR Signaling
tRNA	transfer RNA