Article

In silico prediction of three-dimensional structure and interactome analysis of Tubulin α subfamily of *Arabidopsis thaliana*

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Abstract
Microtubules are essential components of cytoskeleton, rigid hollow rods approximately 25 nm in diameter. Microtubules are dynamic structures being continuously assembled and disassembled within the cell. The basic building blocks of microtubules are heterodimers of globular α- and β-tubulin subunits In *Arabidopsis thaliana* tubulin subunits are encoded by small gene families, six for α-tubulin and nine for β-tubulin.Both α- and β-tubulin bind GTP, which functions analogously to the ATP bound to actin to regulate polymerization. It is shown that tubulin alpha forms hydrogen bonds with the GTPase domain of b-tubulin. Multiple sequence alignment revealed highsimilarity between the family subunits. Due to the missing of three dimensional structuresin *A. thaliana, structural models were predicted and validated. Additionally, protein domains search revealed that all tubulin* α family subunits contain GTPase domain as the tubulin C terminal domain, confirming previous research. Finally the interactome analysis revealed several interactomes.AtTUA6 shows strong interaction with embryosac development arrest 10 protein (EDA10), involved in stimulating the exchange of guanyl nucleotides, enabling the replacement of GDP by GTP in association with a GTPases.

Keywords 3D structure; interactome; microtubules; tubulin α; functional annotation.

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1 Introduction

Microtubules are dynamic cytoskeletal polymers essential for various cell functions such as intracellular organization, ordered vesicle transport, cell division and establishment of cell polarity. The basic building blocks of microtubules are heterodimers of globular α- and β-tubulin subunits. They are arranged in a head-to-tail fashion to form 13 protofilaments that constitute cylindrical microtubules with outer diameter around 25 nm. In *Arabidopsis thaliana* tubulin subunits are encoded by small gene families, six for α-tubulin (Schröder et al., 2001) and nine for β-tubulin (Snustad et al., 1992). It has been proposed that the function of microtubules is modulated by highly diverse posttranslational modifications of tubulin dimers (Blume et al., 2010). The alpha- and beta-tubulins are the major components of microtubules, while gamma-tubulin plays a major role in

the nucleation of microtubule assembly. Microtubules are capable of performing various tasks during the life cycle of eukaryotic cells in spite of ahighly conserved basic structure. The different functions appear to be, at least in part, mediated by differentialaction of diverse associated proteins, including motor proteins (Schröder et al., 2001). However, the in vivo threedimensional structure of Tubulin alpha proteins is not resolved. In this study the in silicon three dimensional structure of all Tubulin α proteins in Arabidopsis *thaliana* will be predicted, phylogenetic relationships and Interactome for new functional annotations as well.

Microtubules

Microtubules play central roles in several of the most basic processes of eukaryotic cells: cell division, cell motility, intracellular transport, and the control of cell shape. In plant cells, rigid cell walls obviate the need for direct cytoskeletal maintenance of cell shape (Steven et al., 1987). Microtubules of the eukaryotic cytoskeleton perform essential and diverse functions and are composed of a heterodimer of alpha and beta tubulins. The genes encoding these microtubule constituents belong to the tubulin superfamily, which is composed of six distinct families. Genes from the alpha, beta and gamma tubulin families are found in all eukaryotes. The alpha and beta tubulins represent the major components of microtubules, while gamma tubulin plays a critical role in the nucleation of microtubule assembly. There are multiple alpha genes, which are highly conserved among species. This gene encodes alpha tubulin and is highly similar to mouse and rat TubA1 gene (Levilliers et al., 1998; Jolly et al., 2007).

About 13 of these protofilaments associate in parallel making the microtubule wall, and giving rise to a polymer with a well-defined polarity. Essential to the function of microtubules is their ability to switch stochastically between growing and shrinking phases (dynamic instability), a non-equilibrium behaviour of tubulin that is based on nucleotide binding and hydrolysis. Each tubulin monomer binds one molecule of GTP. The nucleotide bound to αtubulin, at the so called N-site, is non-exchangeable. The resulting metastable microtubule structure is thought to be stabilized by a cap of remaining GTP-tubulin subunitsat the ends, the loss of which results in rapid depolymerization (Lowe et al., 2001).As mentioned above,the alpha tubulin heterodimer is the structural subunit of microtubules. Tubulin alpha shares minimum 40% amino-acid sequence identity with tubulin beta , exist in several isotype forms, and undergo a variety of posttranslational modifications. The structures of alpha and beta tubulins are basically identical: each monomer is formed by a core of two beta-sheets surrounded by alpha-helices. The monomer structure is very compact, but can be divided into three regions (domains) based on function: the amino-terminal nucleotide-binding region, binds GTP , the carboxy-terminal region which probably constitutes the binding surface for motor proteins and an intermediate taxol-binding region (Nogales et al., 1998).

It is important to note that various isotypes of the two major microtubule subunits, Alpha and beta tubulin, can occur even within a single organism and that these may interact differentially with the proteins. Tubulin isotypes comprise post-translational modifications of a common progenitor and/or members of multigene families (MacRae and Carrie, 1989).In higher plants, as in higher animals, it is now accepted that tubulins are generally encoded by multigene families. Even in Arabidopsis, with its 'minimal' genome, families with at least six different α and nine β tubulin members have been found (Kopczak et al., 1992; Chu et al., 1993).

Tubulin α subfamily in Arabidopsis thalina

Tubulin α subfamily is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain.This subfamilyincludessixtubulin α proteins, as show in Table 1 (Steven et al., 1987).

Alpha-tubulin 1 (TUA1) is encoded by the tua1 gene in *A. thaliana*.This protein is primarilyexpressed in stamens and mature pollen. TUA1 is one of the main constituent of cytoskeleton,involved in microtubule-

based process. The location of TUA1 was measure in microtubules cytoskeleton, cytosol and plasma membrane (Ludwig et al., 1987, 1988).

Alpha-tubulin 2 (TUA2) proteinis also located in cytoskeleton and represent the main structural constituent of it.This protein is involved in cadmium transport (salt stress), microtubule-based processes and in the response to salt stress.TUA2 is located in cytoskeleton, cytosol and cell wall and cell membrane and in chloroplast. TUA2 is expressed 26 plant structure during 15 growth stage (Ludwig et al., 1987).

Alpha-tubulin 3 (TUA3)is involved is the function of cellular response to gravity.Like all other tubulin alpha proteins it is an important structural part of cytoskeleton.TUA3 is mainly expressed in mature pollen stage and during the germination stage of pollen. TUA3 shares the location with other tubulin alpha members (Ludwig et al., 1987; Saito et al., 2003).

Alpha-tubulin 4 (TUA4) functions in structural constituent of cytoskeleton.TUA4 is involved in response to cadmium ions, microtubule-based process, cellular response to gravity. This protein is located in cytosol, cell wall, plasma membrane and chloroplast. TUA4 is expressed in 29 plant structures during 15 growth stages (Ludwig et al., 1987, 1988; Saito et al., 2003).

Alpha-tubulin 5(TUA5), together with all tubulin alpha members, represent an important structural constituent of cytoskeleton.TUA5 regulates cadmium ion transport and microtubule-based processes.As atubulin complex it is located in cytosol, cell wall and cell membrane.It is expressed ion 6 plant structures (Mattingly et al., 2001; Saito et al., 2003; Abe and Hashimoto, 2005).

Alpha-tubulin 6 (TUA6) protein, like other tubulin alpha members it is a structural constituent of cytoskeleton, involved in response to salt stress, microtubule cytoskeleton organization and cellular response to gravity. Furthermore, it is involved protein polymerisation (Ludwig et al., 1987; Mattingly et al., 2001; Saito et al., 2003; Abe and Hashimoto, 2005; Ban et al., 2013).

2 Methodology

The fasta sequences of Tubulin α subfamily proteins (TUA1, TUA2, TUA3, TUA4, TUA5 and TUA6) were obtained from NCBI databases. The Gene accession numbers from NCBI and TAIR database are shown in Table 1.

Table 1 Tubulin α proteins and their NCBI accession IDs.

Tubulin α members	NCBI accession IDs	TAIR IDs
TUA1	NP_176654.1	AT1G64740
TUA2	NP_175423.1	AT1G50010
TUA3	NP_197478.1	AT5G19770
TUA4	NP_171974.1	AT1G04820
TUA5	NP_197479.1	AT5G19780
TUA6	NP_849388.1	AT4G14960

The multiple sequence alignment was performed with the ClustalW2 program (Larkin et al., 2007). Default parameters were applied and aligned sequences were executed using this software. Phylogenetic tree was constructed using Maximum likelihood method in Robust Phylogenetic Analysis software, where phylogenetic relationships between amino acids of TUA subunits were reconstructed and analysed (Dereeper et al., 2008).

In order to predict the three dimensional structure of Tubulin alpha subfamily in *Arabidopsis thaliana*,protein data bank (Pdb) files were obtained based on homology modelling using CPH models 3.2

servers (Nielsen et al., 2010). Additionally to validate the 3-D structures for all TUAs, by testing stereochemistry quality using Ramachandran plots obtained from RAMPAGE program (Lovell et al., 2003).

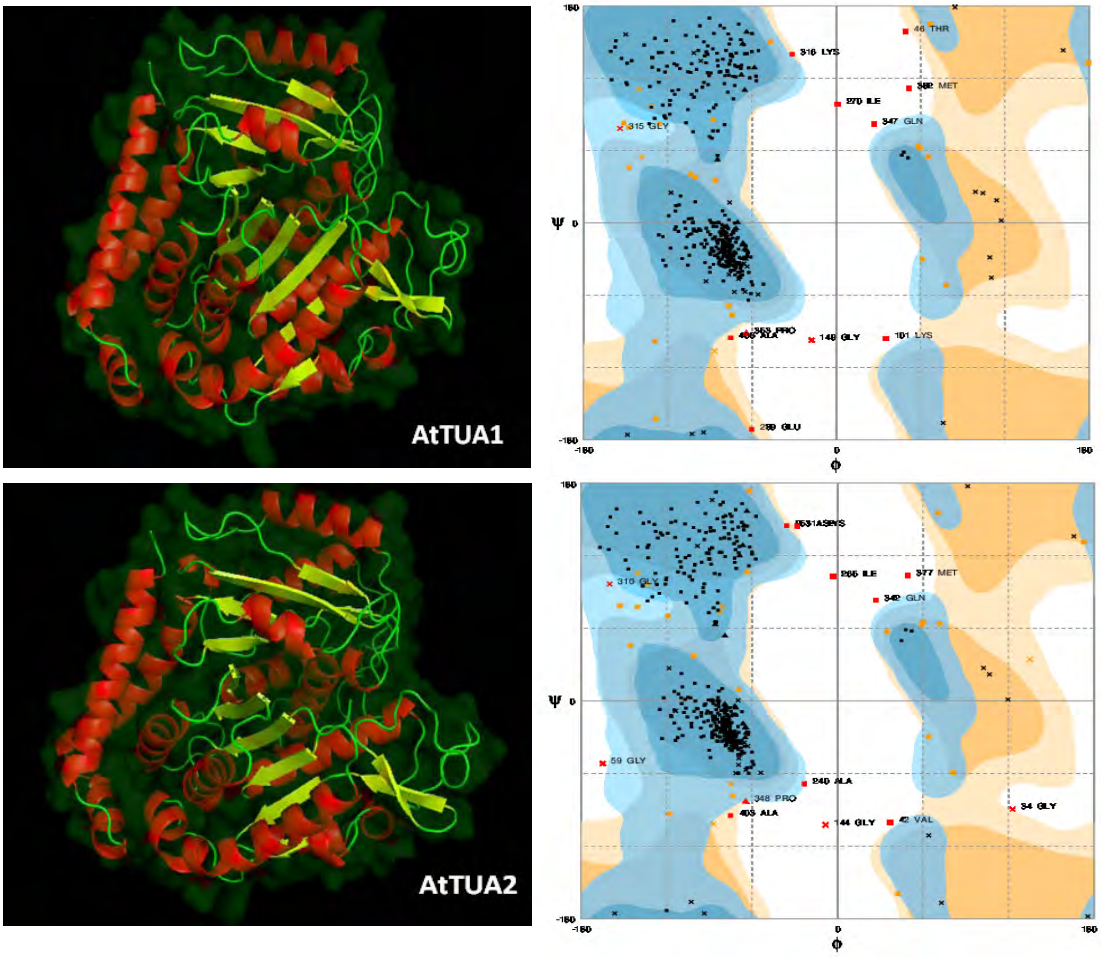
The protein's genetically mobile domain annotations were obtained using *Pfam* database analysis program (Finn et al., 2010). For the protein-protein interaction analysis, Arabidopsis interaction viewer was used (Popescu et al., 2009). Subcellular localization for each protein was predicted and confirmed by WoLF PSORT program (Horton, et al., 2007).

Arabidopsis Interactions Viewer V.2.0 was used for the protein- protein interaction in order to find the interactome for all AtRFC subunits (Popescu et al., 2009).

3 Results and Discussion

3.1 Modeling of 3D structure in Tubulin α subfamily

The three-dimensional (3-D) structure details of proteins are of major importance in providing insights into their molecular functions. 3-D structure of all six subunits showed highly structural similarities. Additionally outputs have been confirmed by Ramachandran plots (Fig. 1a and 1b). The modelled enzymes are monomers folded into α/β domains. AtTUA family consists of minimum 15 central stranded β sheet flanked by 11α helices. The similar structure is shared by other tubulin alpha enzymes from mammalian origins but the number of helices and β sheets differ from one species to another. The Ramachandran plots in Fig. 1a and 1b indicate the region of possible angle formations by w (phi) and c (psi) angles. The conventional terms represent the torsion angles on either side of alpha carbon in peptides. The plot was divided into three regions: favoured (91.5%), allowed (6%) and outlier (2.5%). This result is significant since the high percentage of residues in favoured region (90%). This indicates that the built model is of good quality, as shown in Fig. 1a and Fig. 1b.



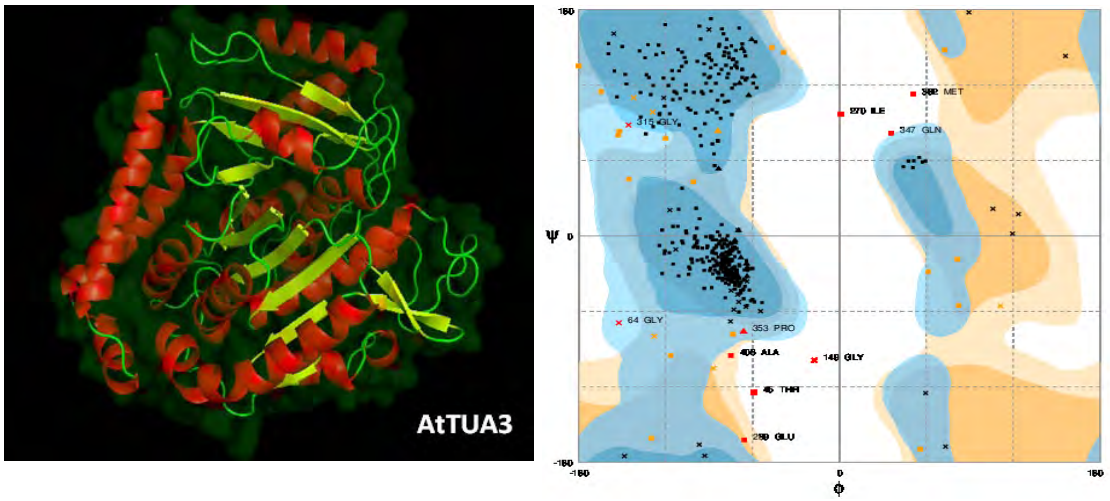
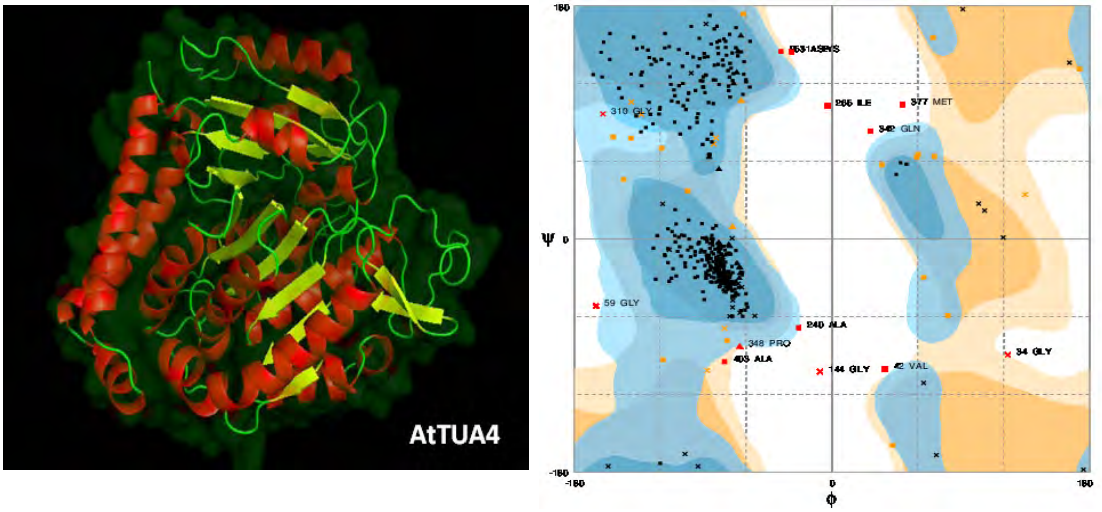
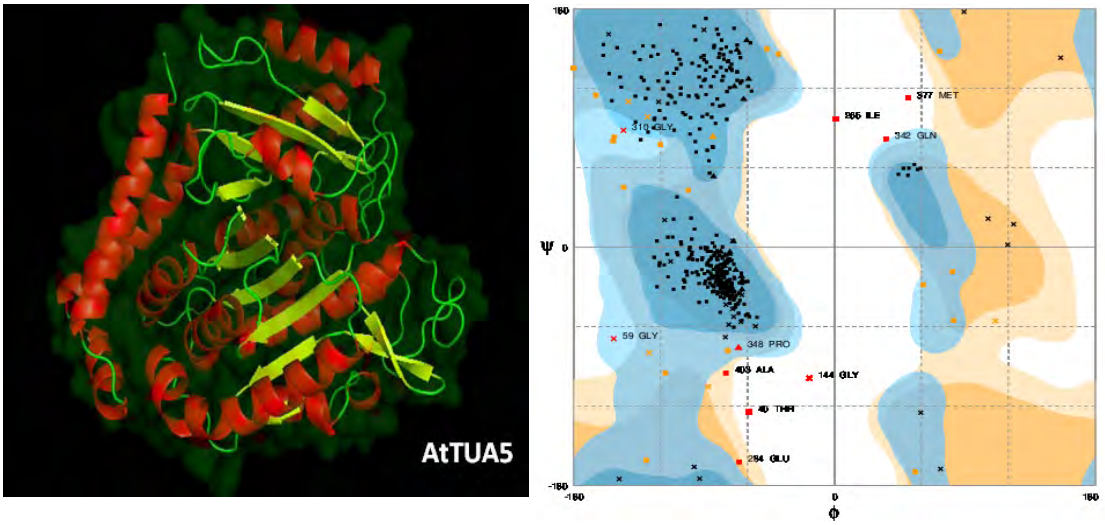


Fig. 1a 3D modelsand Ramachadran plots of AtTUA1/2/3.





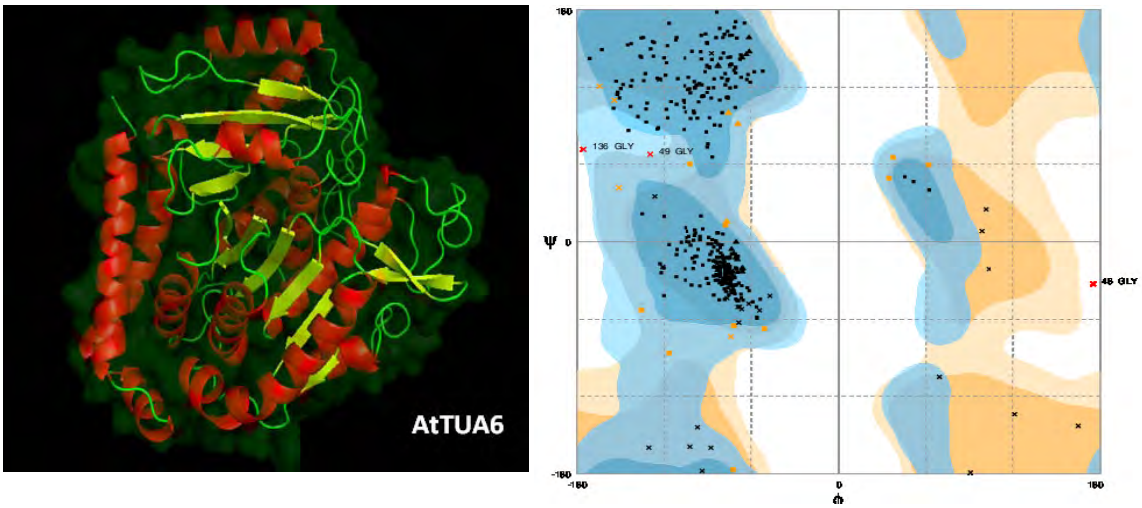


Fig. 1b 3D structure and Ramachadran plots of AtTUA4/5/6.

3.2 Multiple sequence alignment and phylogenetic tree analysis

Aligned sequences showed substantially significant homology, and constructed phylogenetic tree representing the already proven relationship between proteins in this family which play their major role as *GTPase* binding proteins.

The protein sequence of TUA1, TUA2, TUA3, TUA4, TUA5 and TUA6 were obtained from the NCBI sequence database (Table 1). The amino acid sequences are highly similar to the sequences of the homologous human TUA subunits, respectively, and also show amino acid sequence similarity to functionally homologous proteins of other organisms.

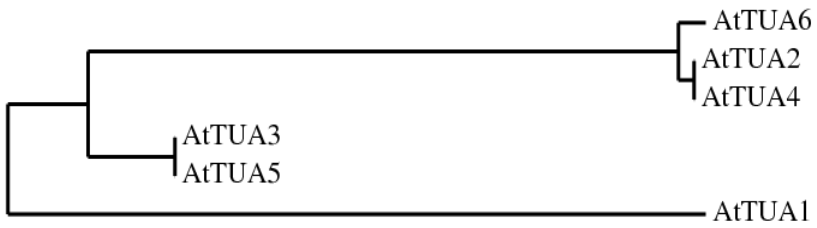


Fig. 2 Tubulin α subfamily phylogenetic tree.

The phytogenic relationships among the five subunits revealed that the two proteins (AtTUA3 and AtTUA5) are identical and that they diverged from AtTUA1. AtTUA2 and AtTUA4 evolved together with AtTUA6 with reserved evolutionary relationships (Fig. 2).This confirm the similarity in function of AtTUA subunits along the sequence alignment results. The sequence alignment scores in the TUA subfamily are shown in Table 2.

Table 2 ClustaW2 alignment result.

SeqA	Name	Length	SeqB	Name	Length	Score
1	AtTUA1	450	2	AtTUA2	450	88.6667
1	AtTUA1	450	3	AtTUA3	450	91.7778
1	AtTUA1	450	4	AtTUA4	450	88.6667
1	AtTUA1	450	5	AtTUA5	450	91.7778
1	AtTUA1	450	6	AtTUA6	450	88.4444
2	AtTUA2	450	3	AtTUA3	450	93.5556
2	AtTUA2	450	4	AtTUA4	450	100.0
2	AtTUA2	450	5	AtTUA5	450	93.5556
2	AtTUA2	450	6	AtTUA6	450	99.5556
3	AtTUA3	450	4	AtTUA4	450	93.5556
3	AtTUA3	450	5	AtTUA5	450	100.00
3	AtTUA3	450	6	AtTUA6	450	93.3333
4	AtTUA4	450	5	AtTUA5	450	93.5556
4	AtTUA4	450	6	AtTUA6	450	99.5556
5	AtTUA5	450	6	AtTUA6	450	93.3333

3.3 Domain search in Pfam database

Pfam domain search shows that all members in the TUA subfamily contains the Tubulin GTPase domain and the Tubulin C-terminal domain, confirming the sequence alignment results. Tubulin is homologous to FtsZ protein, responsible for bacterial cell division (alpha-beta tubulin structure). FTsZ and tubulins are GTPases, having the GTPase domain. FtsZ can polymerise into tubes, sheets, and rings in vitro and is ubiquitous in bacteria and archaea. FtsZ is found at the C-terminal domain (Nogales et al., 1998).

In all AtTUA the ATPase domain extends from 49 to 246 amino acid residues, while C-terminal domain extends from 248 to 393 residues, shown in Table 3.

All six subunits show conserved regions characteristic of Tubulin/FtsZ family GTPase binding domain and the Tubulin C-terminal domain (Nogales et al., 1998).

Table 3 AtTUA family domains.

Domains	**Tubulin/FTSZfamily, GTPase domain**		**Tubulin c-terminal domain**	
Proteins	*Start*	*End*	*Start*	*End*
TUA1	49	246	248	393
TUA2	49	246	248	393
TUA3	49	246	248	393
TUA4	49	246	248	393
TUA5	49	246	248	393
TUA6	49	246	248	393

3.4 Subcellular localization

The Subcellular localizations of AtTUAs are analysed with using WoLF PSORT program. Results showed good and significant reliabilities that all Tubulin alpha members are located in cytoskeleton and/or cytosol. TAIR software confirmed those localization results (Table 4).

Table 4 AtRFC family localization results.

Protein	Subcellular localization
AtTUA1	Cytostol
	Cytoskeleton
AtTUA2	Cytostol
AtTUA3	Cytoskeleton
	Cytostol
AtTUA4	Cytostol
	Cytoskeleton
AtTUA5	Cytostol
	Cytoskeleton
AtTUA6	Cytostol
	Cytoskeleton

3.5 Interactions

According to data obtained from the Arabidopsis Interactions Viewer (TAIR) we found several interactions belonging to all six subunits of AtTUA family. The interactome network of TAIR analysis revealed that AtTUA1, AtTUA4 and AtTUA6 subunits showed strongly interaction with Tubulin folding cofactor B (Fig. 3). Additionally AtTUA4 and AtTUA6interact with Prefoldin 6 protein and EDA10 protein, whereas only AtTUA6 interacts with Prefoldin 3 protein.

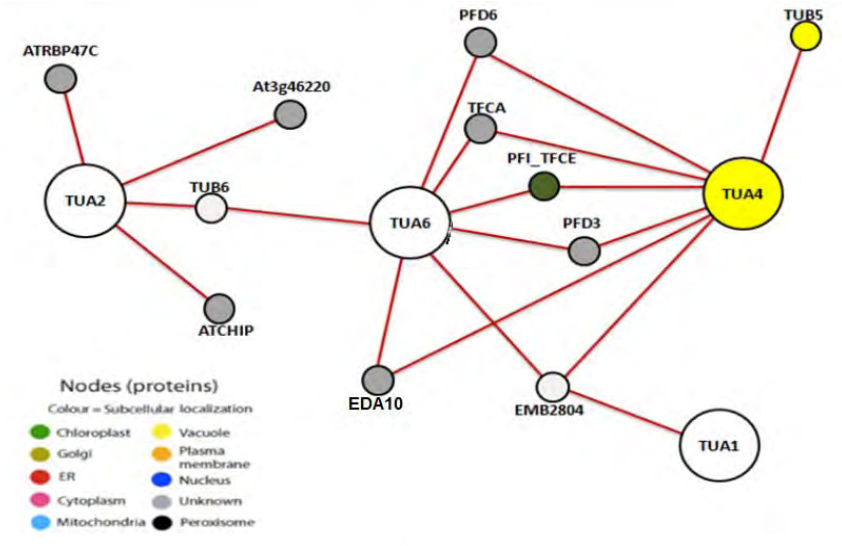


Fig. 3 AtTUAfamilyinteraction network (only siginifcant interactions are shown).

Tubulin cofactor B is involved in embryo development and cell division (Du et al., 2010). Prefoldin 3 and 6, members of the PFD complex, are thought to function along with the TCP ring complex to properly fold microtubule proteins. Additionally, Prefoldin 3 is shown to play essential role in Arabidopsis tolerance is salt stress (Rodríguez-Milla and Salinas, 2009). Embryo sac development arrest10 protein (EDA10) is involved in guanyl-nucleotide exchange factor activity by stimulating of the exchange of guanyl nucleotides associated with a GTPase (Pagnussat et al., 2005).

The common partner for AtTUA2 and AtTUA4 are tubulin folding factors A, E and B. Tubulin folding factors are mostly involved in the control of the correct balance of α- and β-tubulin monomers (Victor et al., 2002). AtTUA3 and AtTUA5 show no significant interactions (Table 5 and Fig. 3).

Table 5 Interactomes of AtRFC subunits.

AtRFCs	Interactom ID	Interactome name and function
TUA1	EMB2804	Tubulin folding cofactor B
TUA2	ATRBP47C	RNA-binding protein 47C
TUA2	ATCHIP	Carboxyl terminus of HSC70-interacting protein
TUA2	At3g46220	Unknown name and function
TUA2	TUB6	Tubulin beta-6 chain
TUA4	EDA10	Embryo sac development arrest 10
TUA4	TUB5	Tubulin beta-5 chain
TUA4	PFD6	Prefoldin 6 chain
TUA4	PFI_TFC E	Tubulin folding cofactor E Pfifferling (PFI)
TUA4	TFCA	Tubulin folding cofactor A (KIESEL)
TUA4	PFD3	Prefoldin 3 chain
TUA4	EMB2804	Tubulin folding cofactor B
TUA6	PFD6	Prefoldin 6
TUA6	PFI_TFC E	Tubulin folding cofactor E / Pfifferling (PFI)
TUA6	TFCA	KIS_tubulin folding cofactor A (KIESEL)
TUA6	EMB2804	Tubulin folding cofactor B
TUA6	EDA10	Embryo sac development arrest 10
TUA6	TUB6	Tubulin beta-6 chain
TUA6	PFD3	Prefoldin 3

4 Conclusions

Tubulin family is a multi protein complex consisting of several subunits. Tubulin α subfamily is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain. This subfamily includes six tubilin α proteins. The 3D structure and inteactome analysis confirmed their similarity and functions.Additinally, through the strong interaction between AtTUA6 and Embryo sac development arrest10 (EDA10), AtTUA may additionaly functions in the exchange of guanyl nucleotides associated with a GTPase. It was expected that AtTUA1, AtTUA3 and AtTUA4 interacts with various tubulin coding factors, confirming previous research. Interestingly, AtTUA4 and AtTUA6 are shown to interact with Prefoldin2 and Profoldin 6 protein that supports the proper folding of microtubules and have important role in Arabidopsis tolerance to salt stress.

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