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Is Casein Kinase 2 Able to Phosphorylate Plant α-Tubulin?

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Abstract—Results of classical and structural bioinformatical research allow to predict casein kinase 2 dependent phosphorylation of conservative residues of Ser94 and Ser419 in *Trypanosoma* and *Arabidopsis* α-tubulin. Location of these residues in the region of internal contact of α-/β-tubulin heterodimer has been demonstrated. It is hypothesized that phosphorylation of Ser94 can affect dimerization of α-/β-tubulin in *Trypanosoma* and *Arabidopsis*. Most likely, potential phosphorylation of Ser419 does not have a direct effect on microtubule structure but is related to interaction with associated proteins, in particular with kinesins.

Keywords: casein kinase 2, tubulin, phosphorylation, *Trypanosoma*, *Arabidopsis*

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INTRODUCTION

Casein kinase 2 (CK2) is a ubiquitous enzyme of eukaryotes. In general it is a tetramer containing two isozyme catalytic (CK2α and CK2α') and two regulatory subunits [1]. Moreover, an additional catalytic subunit isoform, CK2α'', was found in mammals [2, 3]. Only one isoform of the regulatory subunit of this enzyme was found in humans. However, additional isoforms of CK2β were found in other organisms (*Saccharomyces cerevisiae* for example) [1].

CK2 is involved in the regulation of various cellular processes, such as cell cycle, circadian rhythm, apoptosis, malignant transformation, carcinogenesis, etc. [4, 5]. Moreover, data of many studies indicate that CK2 is involved in control of cell morphology, polarity, and regulation of microfilaments and microtubules as well [6, 7]. The last fact was demonstrated on various model systems from yeast to mammals [8–13]. Thus, the role of CK2 in regulation of cell morphology, polarity, and cytoskeleton organization and function is evident.

The first data indicating relations between CK2 and mammalian tubulin were obtained in experiments on intact microtubules isolated from rabbit brain [12, 14]. Later coimmunoprecipitation and western blot demonstrated that catalytic CK2α and CK2α' subunits are capable for direct interaction with animal tubulin, whereas colocalization of free regulatory CK2β subunits and tubulin was not observed [15]. The RNA interference demonstrate that CK2 associate with microtubules and mediate their integrity [10]. Incubation of rat retina with CK2 inhibitors caused disintegration of microtubules and arrest nucleus migration in juvenile cells of retinal precursors [16]. Incubation of culture of human astrocytes and vesicular endothelial cells in the presence of CK2 inhibitors caused alteration of cell shapes and damages in cytoskeleton organization [17]. This indicates that phosphorylation of microtubule proteins by CK2 favors assembly of microtubules and affects their dynamics directly [18].

In recent experiments with tubulin and casein kinase 2 (CK2α) carrying radioactive labels on the one hand and experiments with tubulin- and CK2α-specific antibodies on the other hand, colocalization of CK2 and microtubules was demonstrated. Moreover, the capability of this enzyme to phosphorylate α-tubulin in *Trypanosoma equiperdum* was discovered [18]. Immunoblotting of radioactively labeled tubulin and CK2 revealed only one phosphorylated fraction identified with CK2α- and tubulin-specific antibodies [18]. This indicates the physical association between pools of tubulin and CK2 in *T. equiperdum*. However, despite evidence of this interaction, CK2-dependent phosphorylation sites in α-tubulin has not yet been identified. Data obtained in experiments on *Trypanosoma* indicate the possible involvement of CK2-dependent phosphorylation of tubulin in stabilization, integrity control, and dynamics of microtubule rearrangement [18].

The α-chain of plant CK2 contains a conservative catalytic site, and tetramer holoenzyme of CK2 is composed of two α- and β-subunits [19]. As in animals and fungi, plant CK2 phosphorylates casein in vitro and its activity is typical for other kingdoms [20]. Moreover, it was detected that plant CK2 phosphorylate transcriptional factor GBFl. This triggers its interaction with DNA [21]. For plant transcription factor PIF1 it was demonstrated light-dependent phosphor-

ylation by CK2. This induces proteasome degradation of PIF1 and photomorphogenesis activation [22]. Since plant CK2 phosphorylates transcription initiation factors, it is possible CK2 involvement in translation initiation is hypothesized [23, 24]. Casein kinase 2 is involved in the regulation of plant circadian rhythms and phosphorylate transcription factor CCA1 (Circadian Clock Associated 1). The last is the major oscillator in angiosperms, and phosphorylation of CCA1 stabilizes it [25]. It was demonstrated that CK2 is important for maintenance and stability control of genome and chromatin structure [26]. Moreover, CK2 has ectokinase activity and phosphorylates a number of extracellular proteins [19]. In experiments with *Solanum berthaultii*, plant CK2 colocalized with microtubules and phosphorylate SB401. The last interacts with microtubules and F-actin [27]. Phosphorylation by CK2 inhibits SB401 interaction with microtubules. Scientists hypothesize that CK2 regulates microtubules and actin filaments via phosphorylation of SB401 [27]. Nevertheless, literature contains only data suggesting an indirect interaction between CK2 and tubulin, and there are no data on direct interaction between CK2 and tubulin.

That is why we focus our study on the search of probable CK2-specific phosphorylation sites in α-tubulin from *Trypanosoma equiperdum* and analysis of the possibility of similar interactions in *Arabidopsis thaliana*. The probability of such interactions is quite high considering significant interspecies similarity of tubulin molecules and conservative structure of CK2 subunits in *Trypanosoma* and *Arabidopsis*.

MATERIALS AND METHODS

Amino acid sequences used in this study were taken from UniProtKB (Protein KnowledgeBase, www.uniprot.org) [28]. Homology search for *A. thaliana* was performed using SIB BLAST Network Service BLASTp algorithm (BLASTP ver. 2.2.31+). The following parameters were used: BLOSUM62 comparison matrix, expectation value threshold E-value = 10 (number of expected matches in a random database) with filtration for low-complexity regions or numerous gaps in alignment (http://web.expasy.org/blast/; http://blast.ncbi.nlm.nih.gov) [29, 30]. Potential plant homologs were selected based on percentage of identity and sequence similarity, percentage of gaps, and E-value [29]. Multiple alignments of amino acid sequences were done in ClustalX (v. 2.0.10) (www.clustal.org) [31] using BLOSSUM substitution matrixes [31].

Cladistics analysis of tubulin molecules and clustering of interaction sites were based on coclustering of amino acid sequences using neighbor-joining algorithm [32–34]. Dendrograms were built and analyzed using MEGA7 software (http://www.mega-software.net/) [34].

Potential tubulin phosphorylation sites were identified using a local version of GPS 3.0 software (http://gps.biocuckoo.org) [35, 36].

Our 3D-models of α-tubulin molecules from *T. equiperdum* (A0A1G4I5D2_TRYEQ) and the nearest homolog from *A. thaliana* (TBA4_ARATH, Q0WV25) were built using the Swiss-Model server (https://swissmodel.expasy.org/) [37]. 3D-models of A0A1G4I5D2_TRYEQ and TBA4_ARATH was built out using template PDB structures: 5kx5.1 (chain A—Tubulin alpha chain, 2,5 Å)—Crystal structure of tubulin-stathmin-TTL-Compound 11 complex (89.07% identity) [38]; and 3e22.1 (chain A—Tubulin alpha-1C chain, 3,8 Å)—Tubulin-colchicine-soblidotin: Stathmin-like domain complex (85.97% identity) [39], respectively.

Topology of phosphorylation sites identified for α-tubulin was detected via structural superposition and comparison with the chimeric complex 5KX5 (Crystal structure of tubulin-stathmin-TTL-Compound 11 complex, X-Ray diffraction, 2,5 Å) from RCSB Protein Data Bank: Tubulin alpha chain (UniProtKB: D0VWZ0_ SHEEP) and Tubulin beta chain (UniProtKB: D0VWY9_SHEEP) from *Ovis aries*, Stathmin-4 (UniProtKB: STMN4_RAT, P63043) from *Rattus norvegicus* [38].

Visualization, superposition of structural models, and analysis of protein complexes and phosphorylation site topologies were performed using PyMOL v.1.5.0.5 software (www.pymol.org).

RESULTS AND DISCUSSION

The search for *T. equiperdum* α-tubulin sequences in a database was the starting point of our study. Proceeding from data of Boscán et al. [18], we chose sequence A0A1G4I5D2 (A0A1G4I5D2_TRYEQ, Last modified June 7, 2017) from the UniProtKB database.

To identify possible phosphorylation sites, GPS 3.0 local version software with CK2a1 group limits was used. Two scanning modes—medium and high hit reliability thresholds (Table 1)—were used. Three possible phosphorylation sites in the α-tubulin molecule were identified using the average threshold limit for the search. Those sites correspond to five various CK2 profiles (GPS 3.0 software library). Subsequent elevation of the threshold value up to the maximum reduced the number of the potential sites to two (Ser94 and Ser419). Those sites correspond two alternative CK2-specific phosphorylation sites in the α-tubulin molecule (Table 1, Fig. 1).

BLASTp-search of plant homologs for A0A1G4I5D2_TRYEQ against the full UniProtKB collection of *A. thaliana* amino acid sequences confirmed quite complete identity between α-tubulin from *T. equiperdum* and six α-tubulin isotypes from *Arabidopsis* (Table 2). TBA4_ARATH (Tubulin alpha-4 chain) and TBA2_ARATH (Tubulin alpha-2 chain)

Table 1. Potential phosphorylation sites of α-tubulin from *T. equiperdum* (according to GPS 3.0 software analysis data)

Site	Profile match	Consensus peptide	Score	Cutoff
		Medium threshold		
Ser94	CMGC/CK2/CK2a1	FHPEQLI**S**GKEDAAN	3.056	1.947
Ser419	CMGC/CK2	GMEEGEF**S**EAREDLA	11.121	7.389
	CMGC/CK2/CK2a1	GMEEGEF**S**EAREDLA	2.583	1.947
Ser439	CMGC/CK2	YEEVGAE**S**ADMDGEE	7.628	7.389
	CMGC/CK2/CK2a1	YEEVGAE**S**ADMDGEE	2.104	1.947
		High threshold		
Ser94	CMGC/CK2/CK2a1	FHPEQLI**S**GKEDAAN	3.056	2.848
Ser419	CMGC/CK2	GMEEGEF**S**EAREDLA	11.121	9.894

Position of sites in amino acid sequence

>tr|A0A1G4I5D2|A0A1G4I5D2_TRYEQ Tubulin alpha chain OS=Trypanosoma equiperdum GN=TEOVI_000891200 PE=3 SV=1
MREAICIHIGQAGCQVGNACWELFCLEHGIQPDGAMPSDKTIGVEDDAFNTFFSETGAGKHVPRAV
FLDLEPTVVDEVRTGTYRQLFHPEQLI**S**GKEDAANNYARGHYTIGKEIVDLCLDRIRKLADNCTGL
QGFLVYHAVGGGTGSGLGALLLERLSVDYGKKSKLGYTVYPSPQVSTAVVEPYNSVLSTHSLLEHT
DVAAMLDNEAIYDLTRRNLDIERPTYPTLNRLIGQVVSSLTASLRFDGALNVDLTEFQTNLVPYPR
IHFVLTSYAPVISAEKAYHEQLSVSEISTAVFEPASMMTKCDPRHGKYMACCLMYRGDVVPKDVNA
AVATIKTKRTIQFVDWSPTGFKCGINYQPPTVVPGGDLAKVQRAVCMIANSTAIAEVFARIDHKFD
LMYSKRAFVHWYVGEGMEEGEF**S**EAREDLAALEKDYEEVGAE**S**ADMDGEEDVEEY

Score is calculated according to a GPS algorithm and reflects phosphorylation probability (the higher the score value, the higher the phosphorylation probability). Cutoff is the threshold value (the threshold value reflects resolution, sensitivity, and specificity).

appeared to be the closest homologs. Alignment between A0A1G4I5D2_TRYEQ and TBA4_ARATH confirmed high similarity between them and complete identity of sequences at the regions of Ser94 and Ser419 sites, which we identified as most probable CK2-dependent phosphorylation sites (Fig. 1).

```
A0A1G4I5D2_TRYEQ          MREAICIHIGQAGCQVGNACWELFCLEHGIQPDGAMPSDKTIGVEDDAFNTFFSETGAGK
sp|Q0WV25|TBA4_ARATH      MRECISIHIGQAGIQVGNACWELYCLEHGIQPDGQMPSDKTVGGGDDAFNTFFSETGAGK
                          ***.*.******* *********:********** ******:*  ***************
                                                        S94
A0A1G4I5D2_TRYEQ          HVPRAVFLDLEPTVVDEVRTGTYRQLFHPEQLISGKEDAANNYARGHYTIGKEIVDLCLD
sp|Q0WV25|TBA4_ARATH      HVPRAVFVDLEPTVIDEVRTGTYRQLFHPEQLISGKEDAANNFARGHYTIGKEIVDLCLD
                          *******:******:****************************:****************

A0A1G4I5D2_TRYEQ          RIRKLADNCTGLQGFLVYHAVGGGTGSGLGALLLERLSVDYGKKSKLGYTVYPSPQVSTA
sp|Q0WV25|TBA4_ARATH      RIRKLADNCTGLQGFLVFNAVGGGTGSGLGSLLLERLSVDYGKKSKLGFTVYPSPQVSTS
                          *****************::***********:*****************:**********:

A0A1G4I5D2_TRYEQ          VVEPYNSVLSTHSLLEHTDVAAMLDNEAIYDLTRRNLDIERPTYPTLNRLIGQVVSSLTA
sp|Q0WV25|TBA4_ARATH      VVEPYNSVLSTHSLLEHTDVSILLDNEAIYDICRRSLSIERPTYTNLNRLVSQVISSLTA
                          ********************: :********: **.*.******..****:.**:*****

A0A1G4I5D2_TRYEQ          SLRFDGALNVDLTEFQTNLVPYPRIHFVLTSYAPVISAEKAYHEQLSVSEISTAVFEPAS
sp|Q0WV25|TBA4_ARATH      SLRFDGALNVDVTEFQTNLVPYPRIHFMLSSYAPVISAEKAFHEQLSVAEITNSAFEPAS
                          ***********:***************:*:**********:******:**:.:.******

A0A1G4I5D2_TRYEQ          MMTKCDPRHGKYMACCLMYRGDVVPKDVNAAVATIKTKRTIQFVDWSPTGFKCGINYQPP
sp|Q0WV25|TBA4_ARATH      MMAKCDPRHGKYMACCLMYRGDVVPKDVNAAVGTIKTKRTIQFVDWCPTGFKCGINYQPP
                          **:*****************************.*************.*************
                                                                                S419
A0A1G4I5D2_TRYEQ          TVVPGGDLAKVQRAVCMIANSTAIAEVFARIDHKFDLMYSKRAFVHWYVGEGMEEGEFSE
sp|Q0WV25|TBA4_ARATH      TVVPGGDLAKVQRAVCMISNSTSVAEVFSRIDHKFDLMYAKRAFVHWYVGEGMEEGEFSE
                          ******************:***::****:**********:******************

A0A1G4I5D2_TRYEQ          AREDLAALEKDYEEVGAESADMDGEEDVEEY
sp|Q0WV25|TBA4_ARATH      AREDLAALEKDYEEVGAEGGDDEDDEGEEY-
                          *****************..* :.:*. *
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Fig. 1. Alignment between α-tubulin from *Trypanosoma equiperdum* (A0A1G4I5D2_TRYEQ) and closest homologs from *Arabidopsis thaliana* (TBA4_ARATH, Q0WV25). Positions of two probable CK2 phosphorylation sites identified via profile search are emphasized.

Table 2. Results of the search for *A. thaliana* A0A1G4I5D2_TRYEQ homologs via SIB BLASTP

UniProtKB Identifier	UniProtKB		BLASTP	
	protein name	recommended name	alignment score	*E*-value
Q0WV25	TBA4_ARATH	Tubulin alpha-4 chain	807	0.0
B9DGT7	TBA2_ARATH	Tubulin alpha-2 chain	807	0.0
P29511	TBA6_ARATH	Tubulin alpha-6 chain	806	0.0
B9DHQ0	TBA5_ARATH	Tubulin alpha-5 chain	806	0.0
Q56WH1	TBA3_ARATH	Tubulin alpha-3 chain	806	0.0
P11139	TBA1_ARATH	Tubulin alpha-1 chain	784	0.0
P12411	TBB1_ARATH	Tubulin beta-1 chain	365	2e-121
Q9ASR0	TBB3_ARATH	Tubulin beta-3 chain	361	4e-120
Q56YW9	TBB2_ARATH	Tubulin beta-2 chain	361	4e-120
P29515	TBB7_ARATH	Tubulin beta-7 chain	361	5e-120
P29513	TBB5_ARATH	Tubulin beta-5 chain	360	1e-119
P29514	TBB6_ARATH	Tubulin beta-6 chain	359	3e-119
P29516	TBB8_ARATH	Tubulin beta-8 chain	358	8e-119
P29517	TBB9_ARATH	Tubulin beta-9 chain	357	1e-118
P24636	TBB4_ARATH	Tubulin beta-4 chain	352	1e-116
P38558	TBG2_ARATH	Tubulin gamma-2 chain	223	9e-67
P38557	TBG1_ARATH	Tubulin gamma-1 chain	221	5e-66

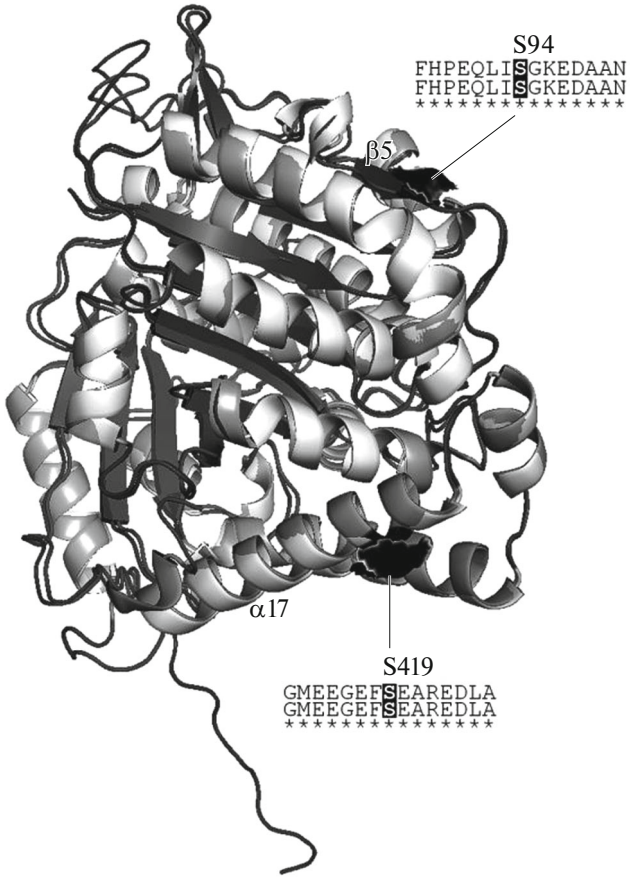

Fig. 2. (On the left). Superposition of structure models of A0A1G4I5D2_TRYEQ and TBA4_ARATH. Yellow surfaces demonstrate identity of localization of Ser94 and Ser419 in homologs from *Trypanosoma* and *Arabidopsis*. Surface topology of the residues confirms their accessibility to modifications.

To analyze topology of Ser94 and Ser419 residues in α-tubulin molecules from *Trypanosoma* and *Arabidopsis*, reconstruction of their 3D structure was performed using initial amino acid sequences A0A1G4I5D2_TRYEQ and TBA4_ARATH (see Materials and Methods). Structural superposition of the models and subsequent visualization of the possible phosphorylation sites indicate that Ser94 located in the β5 sheet and Ser419 located in the α17 helix. Both residues located at the surface of the globule and have outward orientation. Thus, selected residues are accessible for posttranslational modifications (Fig 2).

To clarify functional significance of α-tubulin molecule modifications at Ser94 and Ser419, we have carried out molecular modeling using the experimentally confirmed 5KX5 complex (Crystal structure of tubulin-stathmin-TTL-Compound 11 complex) from the RCSB Protein Data Bank. The structure of the 5KX5 complex is chimeric and includes two α-tubulin subunits from *O. aries* (UniProtKB: D0VWZ0), two β-tubulin subunits from *O. aries* (Uni-ProtKB: D0VWY9), stathmin-4 from *R. norvegicus* (UniProtKB: P63043), and tubulin-tyrosine ligase (TTL) from *Gallus gallus* (UniProtKB: E1BQ43) [38].

Structural superposition of previously built 3D models of A0A1G4I5D2_TRYEQ from *T. equiperdum* and TBA4_ARATH from *A. thaliana* and α-tubulin subunits of the 5KX5 complex demonstrated that root mean square values were less than 1 in both cases. This indicates similarity of spatial coordinates and correctness of complex reconstruction.

Analysis of the complex demonstrates location of Ser94 and Ser419 residues at the proximity to the con-

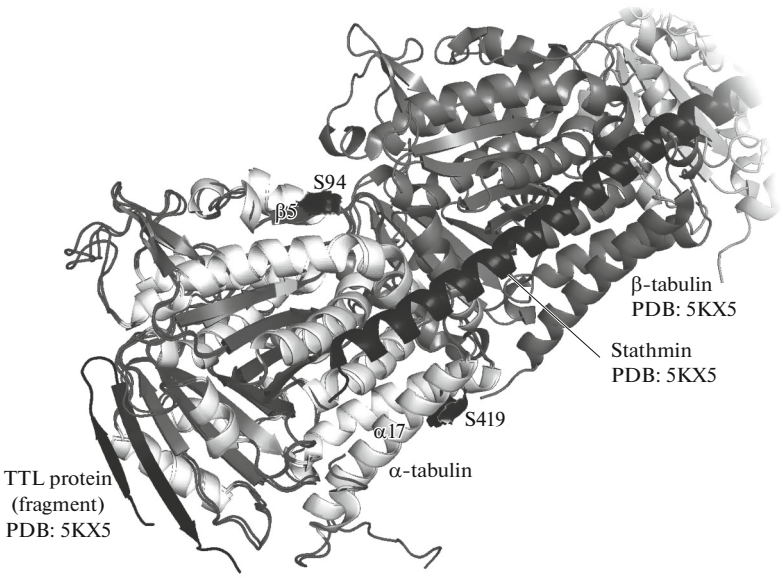

Fig. 3. (On the right). Topology of Ser94 and Ser419 amino acid residues revealed via structural alignment between α-tubulin from *Trypanosoma* and *Arabidopsis* and chimeric complex 5KX5 from the Protein Data Bank [38]: α-tubulin—models of α-tubulin from *T. equiperdum* and *A. thaliana*; β-tubulin—β-tubulin from *Ovis aries*; Stathmin—Stathmin-4 from *Rattus norvegicus*; TTL protein—fragment of tubulin-tyrosine ligase (TTL) from *Gallus gallus*. Positions of the residues indicate localization of CK2-specific sites in the region of inner contact of α-/β-tubulin.

```
>TBG1_ARATH_S422
MFADNDLSEFDESRD
>TBG1_ARATH_S129
IDREADGSDSLEGFV
>TBB2_ARATH_T409
GMDEMEFTEAESNMN
>TBB2_ARATH_T429
YQQYQDATADEEGDY
>TBB2_ARATH_S40
TGRYTGDSDLQLERI
>TBB2_ARATH_S322
AMFRGKMSTKEVDEQ
>TBB8_ARATH_T409
GMDEMEFTEAESNMN
>TBB8_ARATH_T429
YQQYQDATADEEEGY
>TBB8_ARATH_S322
AMFRGKMSTKEVDEQ
>TBG2_ARATH_S422
MFADNDLSEFDESRD
>TBA5_ARATH_S419
GMEEGEFSEAREDLA
>TBA5_ARATH_S94
FHPEQLISGKEDAAN
>TBB5_ARATH_S323
AIFRGQMSTKEVDEQ
>TBB5_ARATH_T410
GMDEMEFTEAESNMN
>TBB5_ARATH_T430
YQQYQDATADEEGEY
>TBB5_ARATH_S323
AIFRGQMSTKEVDEQ
>TBB9_ARATH_T409
GMDEMEFTEAESNMN
>TBB9_ARATH_T40
TGQSCGDTDLQLERI
>TBB9_ARATH_T429
YQQYQDATVGEEEYE
>TBB9_ARATH_S322
AVFRGKMSTKEVDEQ
>TBA1_ARATH_S419
GMEEGEFSEAREDLA
>TBA1_ARATH_S94
FHPEQLISGKEDAAN
>TBA6_ARATH_S419
GMEEGEFSEAREDLA
>TBA6_ARATH_S94
FHPEQLISGKEDAAN
>TBB4_ARATH_T409
GMDEMEFTEAESNMN
>TBB4_ARATH_T429
YQQYQDATAGEEEYE
>TBB6_ARATH_T409
GMDEMEFTEAESNMN
>TBB6_ARATH_T429
YQQYQDATADDEGEY
>TBB6_ARATH_S322
AMFRGKMSTKEVDEQ
>TBB7_ARATH_T409
GMDEMEFTEAESNMN
>TBB7_ARATH_T429
YQQYQDATADEEGEY
>TBB7_ARATH_S322
AMFRGKMSTKEVDEQ
>TBA2_ARATH_S419
GMEEGEFSEAREDLA
>TBA2_ARATH_S94
FHPEQLISGKEDAAN
>TBA3_ARATH_S419
GMEEGEFSEAREDLA
>TBA3_ARATH_S94
FHPEQLISGKEDAAN
>TBA4_ARATH_S419
GMEEGEFSEAREDLA
>TBA4_ARATH_S94
FHPEQLISGKEDAAN
>TBB1_ARATH_T410
GMDEMEFTEAESNMN
>TBB1_ARATH_T430
YQQYQDATADEEDEY
>TBB1_ARATH_S323
AMFRGKMSTKEVDEQ
>TBB3_ARATH_S40
TGRYTGDSDLQLERI
>TBB3_ARATH_S322
AMFRGKMSTKEVDEQ
>TBB3_ARATH_T409
GMDEMEFTEAESNMN
>TBB3_ARATH_T429
YQQYQDATADEEGDY
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Fig. 4. Potential CK2 phosphorylation sites in *Arabidopsis* tubulin (*.fasta format). Fragments of sequences of all *A. thaliana* tubulin isotypes that revealed varying similarity with a number of canonical sites of CK2. Fragments were saved in S/T ± 7 format.

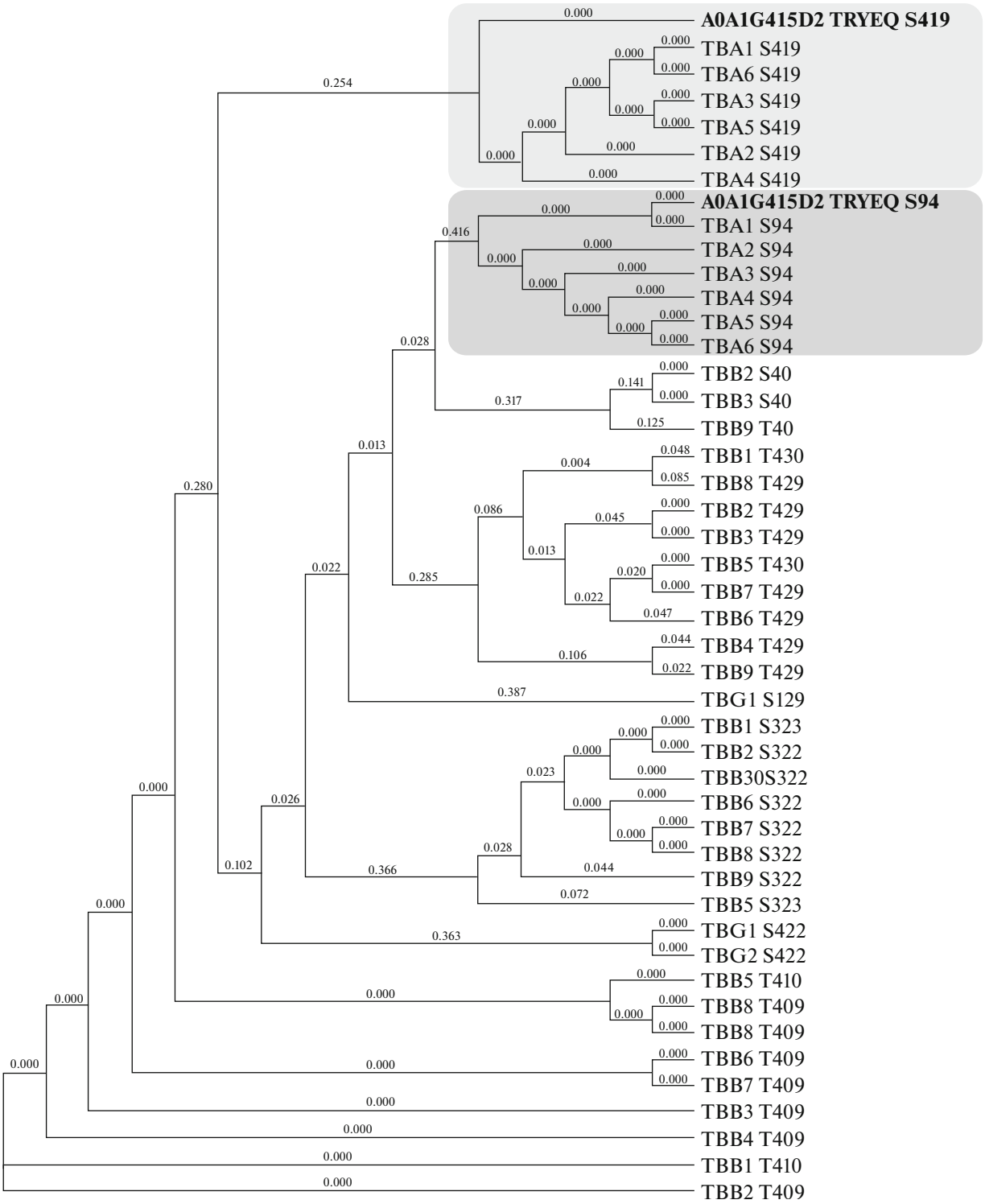

Fig. 5. Clusterization of potential CK2-dependent phosphorylation sites identified in plant tubulins based on profile analysis and control fragments from *T. equiperdum* α-tubulin.

tact region of α-tubulin with β-tubulin in the heterodimer. Thus, modifications of these amino acid residues might affect assembly/disassembly of the heterodimer.

In general, ninety potential sites that more or less correspond to phosphorylation profiles of CK2 were identified proceeding from the profile search for 17 *A. thaliana* tubulin isotypes. To make a final conclu-

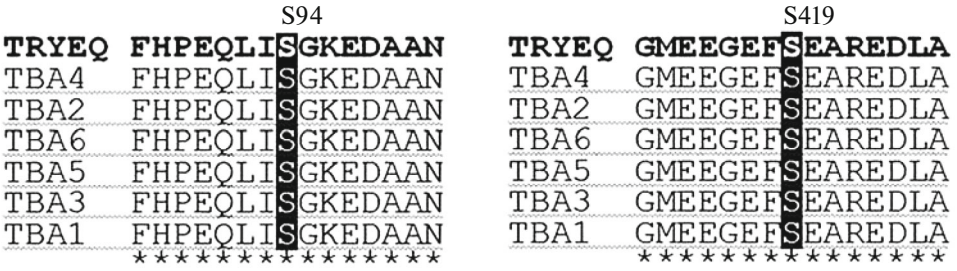

Fig. 6. Alignment of control sequences of Ser94 and Ser419 sites from *T. equiperdum* (TRYEQ) α-tubulin and all *A. thaliana* α-tubulin isotypes. Complete identity of the sites is demonstrated.

sion about isotype specificity of sites at Ser94 and Ser419, the sequences of all sites we represented in S/T ± 7 form and saved in *.fasta format (Fig. 4). This let us make clusterization of the sequences. Fragments of *T. equiperdum* α-tubulin sequences corresponding to Ser94 and Ser419 phosphorylation sites were used as control. Clusterization of the sequences revealed two distinct clads, including control sequences (Fig. 5). Six α-tubulin isotypes were clustered for Ser419 and Ser94:TBA1, TBA2, TBA3, TBA4, TBA5, and TBA6. At the same time, phylogenetic distance between control sequences of *T. equiperdum* and *A. thaliana* was equal to zero (Fig. 5). Alignments of peptide sequences from the clads confirmed their complete identity (Fig. 6). Thus, it could be hypothesized that phosphorylation of Ser94 and Ser419 are characteristic of all α-tubulin isotypes if these residues are indeed phosphorylated.

The results of the search for peptide GMEEGEFSEAREDLA in the PhosphoSitePlus database demonstrate that S419-p is a phosphorylation site for a number of human α-tubulin isotypes (TUBA8, TUBA3C, and TUBA3E). On the basis of a number of experiments, the S419-p site was annotated as a phosphorylation site [40]. However, studies of effects of mutations in TUBA1A (I188L, I238V, P263T, L286F, V303G, R402C, R402H, and S419L) on cytoskeleton did not reveal any anomalies in the formation of the α/β-tubulin dimer, which could be associated with S419L replacement; no deviations from wild type were registered. Moreover, α-tubulin from the cell line carrying S419L mutation composes the interphase network of microtubules without any visible anomalies; the interphase network of microtubules is identical to the wild type [41]. Nevertheless, there are data on the importance of S419 for interaction with kinesin, and mutations in that position can affect the development of the α-tubulin-kinesin complex [41, 42]. At the same time, FHPEQLISGKEDAAN (S94-p) motive coincide with the conservative T94-p ((FHPEQLITGKEDAAN)) site of human α-tubulin (TUBA1A, TUBA1B, TUBA1C, TUBA3C, TUBA4A, TUBA4B, and TUBA8). The site was identified many times via mass spectrometry. It is annotated as the casein kinase 2 phosphorylation site (information curated by PhosphoSitePlus) [40, 43, 44]. It is well known that phosphorylation of serine residues prevails over phosphorylation of threonine residues and there are more serine sites for the same serine/threonine-specific protein kinase [45]. It could be hypothesized that Ser/Thr exchange at position 94 of *T. equiperdum* and *A. thaliana* α-tubulin was appropriate as the CK2 recognition pattern.

Our analysis indicates that phosphorylation of the S94 residue is an important factor affecting development of α/β-tubulin dimer in *Trypanosoma* and *Arabidopsis*. We believe that direct phosphorylation of α-tubulin by CK2 in *T. equiperdum* [18] and *A. thaliana* is definitely associated with the S94 residue. Moreover, the possibility of phosphorylation of S419 cannot be excluded; however, this modification does not directly affect the microtubule structure and is related to interaction with associated proteins.

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