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Full Length Research Paper

The systematic characterization of poplar CK2α and its theoretical studies on phosphorylation of P-protein C-terminal domain

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Casein kinase 2 (CK2) is a ubiquitous serine-threonine protein kinase, which of specific substrates are involved in the regulation of several vital physiological processes in plants. Detailed studies of CK2 have focused on the herbaceous species of *Arabidopsis*, maize, however, it has remained largely unexplored in woody trees. In this study, we identified the four genes encoding CK2α subunits in a search for *Populus* genome. Reorganization of *CK2α* genes in *Populus* genome showed that its multiple copies are resulted from segmental duplication and tandem duplication events. Phylogenetic and *in silico* expression analysis indicated that, the divergence was present among poplar *CK2α* genes, dividing into type I and type II, and each type represented by relatively consistent expression profile. Further docking and molecular dynamic simulations revealed that the poplar CK2α specifically recognize the special pentapeptides (XES/T*DD) of natural substrates and then introduce the phosphorylation of C-terminal region of poplar P-protein. This study will provide the insight into the understanding of interacting mechanism of poplar CK2α with their natural substrates, and be of valuable resource for further assessment of the function of phosphorylation of P-protein in woody plants.

Key words: *Populus trichocarpa,* phosphorylation, casein kinase 2, docking, silico simulation.

INTRODUCTION

Phosphorylation of protein mediated by protein kinases is a major post-translational modification mechanism for regulation of a broad spectrum of fundamental cellular processes (Mann et al., 2002; de la Fuente van Bentem and Hirt, 2007). Casein kinase 2 (CK2, EC 2.7.11.1) is a typical and ubiquitous serine-threonine protein kinase with wide substrate profiles, and is highly conserved across all eukaryotes (Litchfield, 2003; Pinna, 2002). In comparison with other protein kinases, CK2 is a constitutively active enzyme, independent of second messengers, and can utilize either ATP or GTP as phosphate donors (Litchfield, 2003; Pinna, 2002). A vast amount of proteins (>300) are known as the substrates of CK2, involved in numerous vital cellular processes, such as cell proliferation, apoptosis and differentiation (Guerra and Issinger, 1999; Litchfield, 2003; Bibby and Litchfield,

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Abbreviations: CK2, Casein kinase 2; **LG,** Linkage Groups; **CVFF,** consistent-valence force-field; **MD,** molecular dynamic.

2005; Lou et al., 2008). In plants, phosphorylation of the specific substrates by CK2 are involved in the regulation of several vital physiological processes, such as seed and plant development (Moreno-Romero et al., 2008; Espunya and Martinez, 2003), auxin-signaling (Marques-Bueno et al., 2011), ABA signaling (Riera et al., 2004), plant defence (Kang and Klessig, 2005), circadian rhythms (Sugano et al., 1999; Sugano et al., 1998; Lee et al., 1999; Daniel et al., 2004), photomorphogenesis (Bu et al., 2011), translation (Aguilar et al., 1998; Dennis and Browning, 2009), transcription (Johnston et al., 2002; Hu et al., 2003) and transport (Lebska et al., 2010).

CK2 is a hetero-tetrameric complex formed by two catalytic subunits (α and α') and two regulatory subunits (β), which enhance the stability, activity and substrate specificity of CK2 hetero-tetramer (Litchfield, 2003). Moreover, previous studies had shown that, the individual catalytic subunits (CK2α and CK2α') could present the bioactivity without any regulatory subunits *in vivo*, and respectively exert different functions (Tripodi et al., 2010; Domanska et al., 2005). With regard to the structure of CK2α, it shares a core architecture, consisting of an N-terminal domain based on a central anti-parallel β-sheet and an α-helical C-terminal. Many eukaryotic *CK2α* catalytic subunits gene had been identified in their individual genome, two genes (*CKA1* and *CKA2*) encoding for catalytic subunits in *Saccharomyces cerevisiae* (Domanska et al., 2005), two in human (Ackermann et al., 2005; Wirkner et al., 1994), two in mouse (Guerra et al., 1999; Chen et al., 2002). In contrast, in plants, genes encoding α subunits seem to be of multigene families, four genes encoding CK2α in *Arabidopsis* (Salinas et al., 2006), three in *Zea mays* (Lebska et al., 2009). Although, genes encoding CK2α catalytic subunits have been described in several plants (Salinas et al., 2006), there is still no systematic and comprehensive characterization of CK2α in woody plant genome, especially for poplar, whose genome had been completely sequenced (Tuskan et al., 2006).

The substrates of phosphorylation by CK2 shared a consensus sequence feature as S/T-x-x-E/D/pS, and C-terminus of the acidic consensus E/D-x-D generally occurs on phosphorylation by CK2 (Meggio and Pinna, 2003; Zschoernig and Mahlknecht, 2009). Thus, several identified phosphorylated sites located on conserved C-terminal region of most poplar acid ribosomal proteins had been proved to be phosphorylated by CK2 *in vivo* (Liu et al., 2010; Niefind et al., 1998). To the best of our knowledge, the interaction mechanism of poplar CK2α catalytic subunits with substrates had not been addressed.

Therefore, the aim of the present study was to evaluate the phosphorylation of the certain substrates actions of poplar CK2α *in vivo* by the method of wide-genomic investigation of poplar CK2α combined with *in silico* simulation. In this study, four genes encoding α subunits were identified across the complete *Populus* genome, and their multiple copies are resulted from segmental duplication and tandem duplication events. On such basis, explicitly solvated docking and molecular dynamic (MD) methods were applied to investigative the interactions involving natural substrate with the poplar CK2α. We proposed that further studies be carried out to understand the interacting mechanism and functions of phosphorylated P-protein in woody plants.

MATERIALS AND METHODS

Identification, chromosomal location and genomic structure of poplar CK2α genes

Poplar CK2α proteins were identified by running PSI-BLAST (position-specific iterated BLAST) iteration 3 with max 500 against protein database of *Populus trichocarpa* available in NCBI (http://www.ncbi.nlm.nih.gov/), and *Arabidopsis* CK2αC sequence was selected as query sequence (Salinas et al., 2006).

CK2α genes were located in the genome of *P. trichocarpa* by NCBI map viewer (http://www.ncbi.nlm.nih.gov/projects/mapview/). Identification of duplicated regions between chromosomes was completed as previous literature (Tuskan et al., 2006). The tandem gene duplication in poplar was determined according to the criteria that five or fewer gene loci occurred within a range of 100kb distance (Hu et al., 2010; Finn et al., 2006).

Gene structure display server (GSDS) program (http://gsds.cbi.pku.edu.cn/) was applied to the illustrate exon/intron organization for individual *CK2α* genes by comparison of the cDNA sequences and their corresponding genomic sequences.

Phylogenetic analysis and microarray analysis

Multiple sequences alignments of the full-length protein sequences were performed using ClustalW program in BioEdit software with default parameters (Hall, 1999). Based on these aligned sequences, the unrooted phylogenetic trees were constructed using MEGA 5.0 software (Tamura et al., 2011), by both Neighbor-joining method (Saitou and Nei, 1987) and Maximum Likelihood method with parameters (p-distance and completed deletion). The reliability of the phylogenetic tree was estimated using bootstrap value with 1000 replicates.

Probe sets corresponding to individual poplar *CK2α* gene were identified using probe match tool available at NetAffx™ Analysis center (http://www.affymetrix.com/analysis/index.affx). For genes with more than one probe sets, non-redundant probe sets were considered as their assigned probe set. The transcript relative abundance value of all poplar *CK2α* genes from various tissues was gained from the poplar transcript abundances datasets (Wilkins et al., 2009) in the website of the *Populus* electronic fluorescent pictograph browser (poplar eFP browser) (http://bar.utoronto.ca/efppop/cgi-bin/efpWeb.cgi). Dendrogram and heat map for display expression pattern were obtained using the Cluster 3.0 (de Hoon et al., 2004) for hierarchical clustering based on Pearson coefficients, and then Java Tree-View 1.1 program (Saldanha, 2004) for visualizing the analyzed datasets.

In silico modeling

All the docking and molecular dynamics (MD) simulations were performed with the different modules implemented under InsightII 2005 software package (InsightII Version 2005, 2005) on Linux workstations, using the consistent-valence force-field (CVFF).

Table 1. Characterization and identification of CK2α subunits genes of poplar.

Gene name	The encoding proteins (GeneBank ID)	RNA (Refseq RNA ID)	Chromosome location
PtrCK2α-1	EEE79291.1	XM_002304276.1	LGIII-NC_008469(8031628-8035845)
PtrCK2α-2	EEE93496.1	XM_002306464.1	LGV-NC_008471(6084281-6089153)
PtrCK2α-3	EEE93497.1	XM_002306465.1	LGV-NC_008471(6097038-6101783)
PtrCK2α-4	EEE78372.1	XM_002303357.1	LGIII-NC_008469(8342118-8348911)

The crystal structures of the catalytic subunit of protein kinase CK2 (CK2α) from *Z. mays* (PDB ID: 1LR4) (Yde et al., 2005) were recovered from the PDB database. All the hetero-atoms were removed from the protein structure (Zhang and Zhong, 2010). On such base, the poplar CK2α proteins were generated with the Biopolymer module (InsightII, 2005) (InsightII Version 2005, 2005). All the obtained protein structures were saturated with hydrogen atoms based on the expected charge distributions of amino acids at physiological pH (InsightII Version 2005, 2005; Yang et al., 2009; 2010a; 2010b; 2011).

The structures of peptide-bougie (Table 2) were generated with the Builder module. Geometry and partial atomic charges of the substrate were conducted using the Discover 3.0 module by applying the BFGS algorithm (Head and Zerner, 1985) with a convergence criterion of 0.01 kcal mol^{-1} $Å^{-1}$. Previous literatures revealed that (Yang et al., 2009; Yang et al., 2010b; Yang et al., 2010a), the docking and molecular dynamics (MD) simulations were performed to explore and understand the interactions between peptide-bougies and poplar CK2α proteins, by the general protocols in the InsightII 2005 software packages (Affinity User Guide, 2005; Yang et al., 2009, 2010a, a). The MD trajectories were generated using a 1.0 fs time step for a total of 5000 ps, saved at 5.0-ps intervals. The interaction energies of peptide-bougies with poplar CK2α proteins and the respective residues at the binding pocket were calculated by the Docking module (Park et al., 2001), over the 1000~5000 ps MD trajectories. More calculated details can refer to elsewhere (Yang et al., 2009).

RESULTS AND DISCUSSION

Identification and characterization of CK2α subunits genes in poplar

Four genes for CK2α subunits (αA, At5g67380; αB, At3g50000; αC, At2g23080; and αcp, At2g23070) in *Arabidopsis* (Salinas et al., 2006), three genes in maize (Lebska et al., 2009), have been characterized previously. However, *CK2α* has not been documented in poplar. In order to identify the *CK2α* subunit genes present in the poplar genome, PSI-BLAST (position-specific iterated BLAST) program was exploited. As a result, four CK2α proteins encoded by four *CK2α* genes were gained from Nr protein database of *P. trichocarpa* in NCBI, respectively named PtrCK2α-1 (EEE79291.1), PtrCK2α-2 (EEE93496.1), PtrCK2α-3 (EEE93497.1) and PtrCK2α-4 (EEE78372.1) (Table 1). The *P. trichocarpa* genome encodes the same numbers of CK2α gene members as *Arabidopsis* (Salinas et al., 2006), the similar as maize (Lebska et al., 2009). It appears that no significant expansion occurred from *CK2α* gene members of poplar. However, previous evidence revealed that many multi-gene families in poplar lineage were expanded and resulted from whole genome duplication events followed by multiple segmental duplication, tandem duplication, and transposition events (Tuskan et al., 2006).

The *CK2α* genes were further *in silico* mapped to loci across all 19 Linkage Groups (LG). A schematic view of the chromosomal location of all genes is shown in Figure 1. It was found that all of the four poplar *CK2α* genes were distributed across the duplicated blocks between the two Linkage Groups (LG), LGIII and LGV (Table 1, Figure 1); and the two genes of *PtrCK2α-2* and *PtrCK2α-3* were considered as the tandem duplication events that they were directly adjacent to each other on LGV without intervening annotated gene (Figure 1). Consistently, the similar segmental duplication and tandem duplication events also occurred in *CK2α* genes of *Arabidopsis* (Salinas et al., 2006). In summary, unlike other multi-genes families of poplar, the expansion that poplar genome usually encodes more gene family members than *Arabidopsis* had not been presented in poplar *CK2α* gene members, since they could possess similar segmental duplication and tandem duplication events.

Phylogenetic analysis of *CK2α* gene members in poplar, *Arabidopsis*, and maize

To determine the phylogenetic relationship among the CK2α proteins of poplar, *Arabidopsis* and maize, an unrooted tree was respectively constructed by both Neighbor-Joining (Saitou and Nei, 1987) and Maximum likelihood methods using MEGA 5.0 (Tamura et al., 2011). The tree topologies generated by the two methods were comparable without modifications at branches, and supported by their high bootstrap values of > 50, suggesting that a reliable unrooted tree topology was constructed.

Phylogenetic analysis identified the presence of 3 distinct CK2α protein groups across these plants: type I, II and III (Figure 2), in which most of plant CK2α proteins (11/15) were assigned to type I, a few (3/15) in type II including AtCK2αcp (AEC07404.1), PtrCK2α-4 and PtrCK2α-3, and only ZmCK2α-4 (ACF80391.1) to type III (Figure 2). The result indicated that all CK2α proteins of poplar were encoded by evolutionarily divergent genes,

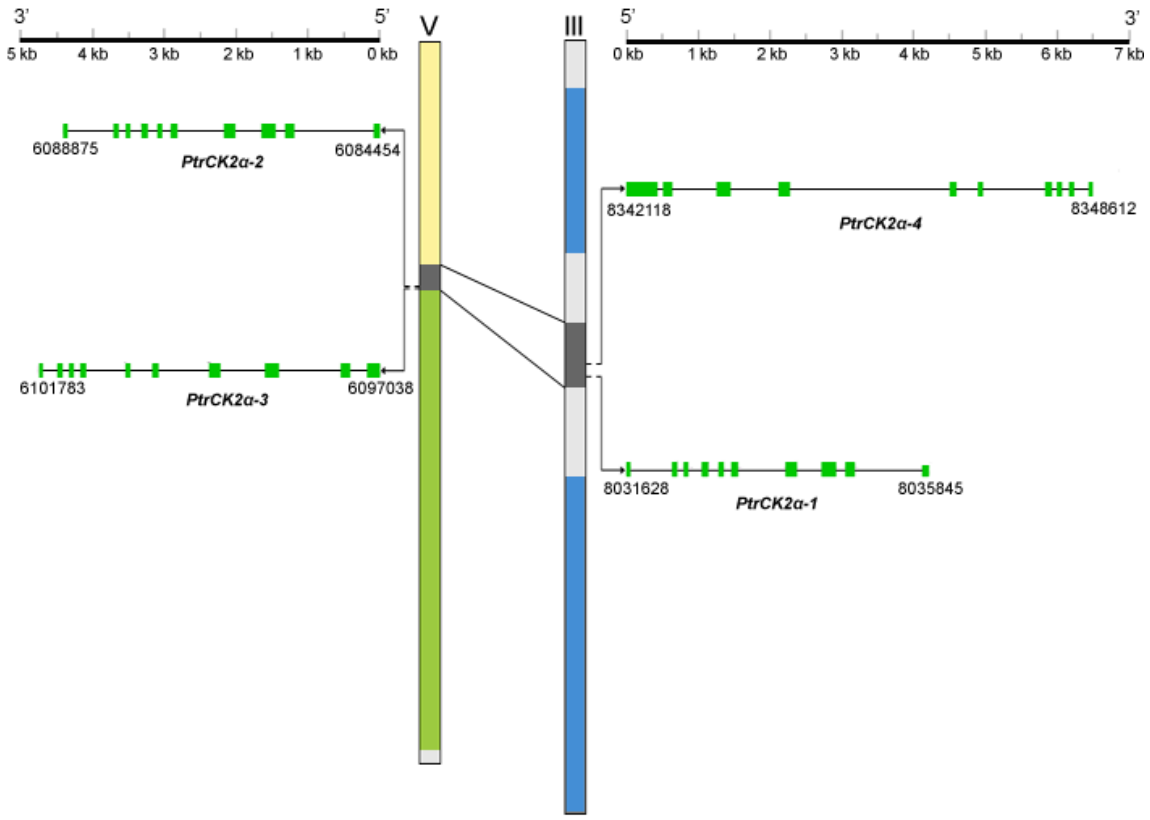

Figure 1. Schematic representation of chromosomal location for the *CK2α* genes over the *Populus trichocarpa* genome, and their Exon/intron structures. Dark grey regions linked by lines were indicated as segmental duplicated homologous blocks between LGIII and LGV chromosomes. Exons and introns of *CK2α* genes are represented by green boxes and black lines, respectively, and their sizes can be estimated by the scale at the top.

and the divergence occurred between two CK2α proteins "PtrCK2α-1" and "PtrCK2α-2", and other two CK2α proteins "PtrCK2α-3" and "PtrCK2α-4", which is consistent with previous report on *Arabidopsis* (Salinas et al., 2006) and mustard (Ogrzewalla et al., 2002). In addition, the divergence in CK2α proteins is supported by apparent differences in amino acid sequences of type I, II and III, especially in their N-terminal extension sequences that are predicted to be putative chloroplast signal peptides in type II, and mitochondrial signal peptides in type III, whereas, no any leader peptides in type I (data not shown). It indicates that independent duplication and divergence of *CK2α* genes are common across plants.

The gene structural display could provide us additional information for evolutionary relationship of multi-gene families (Guo et al., 2007). To further gain novel insight into the phylogenetic relationship of poplar *CK2α* genes, the exon/intron organization was illustrated for individual *CK2α* genes by comparison of the cDNA sequences and their corresponding genomic sequences (Figure 1). As a result, the closely related *CK2α* genes members from the same type in poplar, respectively exhibited a highly conserved distribution of exon/intron, and the presence of some extent of variation between type I and type II. The similar gene architecture of *CK2α* genes in the same type might provide additional supports to the phylogenetic analysis. On the other hand, the differences of gene organization between different types might also support the divergence in *CK2α* genes of poplar.

Differential expression profile of poplar *CK2α* genes

Publicly available microarray data was often considered as a reliable means of studying gene expression profiles (Ohlrogge and Benning, 2000). To gain the expression pattern of all poplar *CK2α* genes, the poplar Affymetrix microarray data (Wilkins et al., 2009) were reorganized. All 4 poplar *CK2α* genes have their corresponding probe sets in the dataset and the expression profile was displayed as indicated Figure 3. Poplar *CK2α* genes showed distinct tissue-specific expression patterns that were preferentially expressed in c), root (R), and seedling under specific conditions, while few in mature leaf (ML), young leaf (YL) and xylem (X). Combined with the high

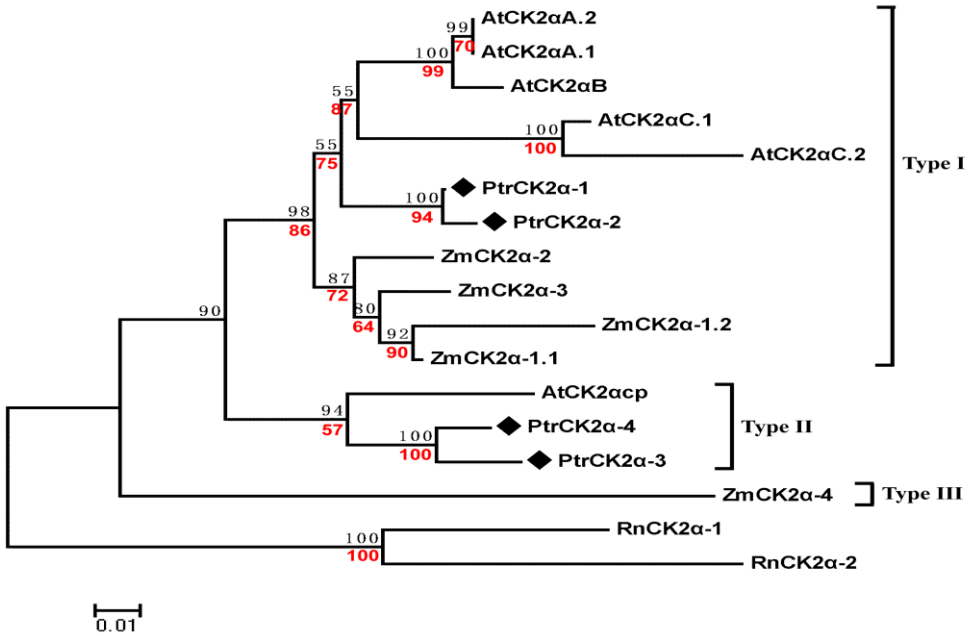

Figure 2. Phylogenetic analysis of CK2α proteins. Multiple sequence alignment of 17 full-length amino acid sequences of CK2α were performed by ClustalW and a phylogenetic tree was constructed using MEGA version 5.0 (Tamura et al., 2011) by both Neighbor-joining method (Saitou and Nei, 1987) and Maximum Likelihood method with 1000 bootstrap replicates. Branch lengths are proportional to the amino acid distances along each branch. Neighbor-joining bootstrap values for clans supported above the 50% level are indicated in black font above branches, whereas Maximum Likelihood bootstrap values above the 50% level are showed in red font below the branches. The black diamonds are highlighted in the front of all CK2α protein subtypes from poplar. All CK2α protein names and their individual corresponding ID number for phylogenetic analysis are listed as follow: ZmCK2α-1.2 (AAF76187.1); ZmCK2α-1.1 (P28523.1); ZmCK2α-3 (AAG36872.1); ZmCK2α-2 (CAA72290.1); ZmCK2α-4 (ACF80391.1); AtCK2αA.1 (AED98335.1); AtCK2αA.2 (AED98334.1); AtCK2αB (AEE78615.1); AtCK2αC.1 (AEC07406.1); AtCK2αC.2 (AEC07405.1); AtCK2αcp (AEC07404.1); PtrCK2α-1 (EEE79291.1); PtrCK2α-2 (EEE93496.1); PtrCK2α-3 (EEE93497.1); PtrCK2α-4 (EEE78372.1); RnCK2α-1 (AAH91130.1); RnCK2α-2 (EDL87283.1).

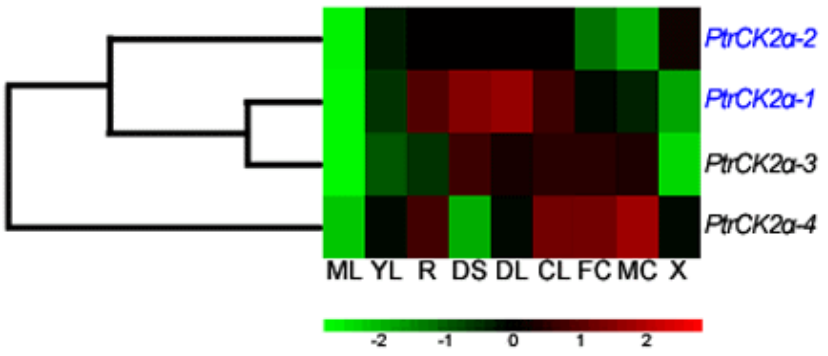

Figure 3. Relative transcript abundance profiles of popar *CK2α* genes across different tissues. A heat map displaying the transcript abundance is produced using the genome-wide microarray data generated by Wilkins and coworkers (Wilkins et al., 2009). The transcript abundance levels for the popar *CK2α* genes were clustered using hierarchical clustering based on Pearson correlation. Color scale at the bottom of each dendrogram represents log2 expression values, green color represents low level and red color represents high level of transcript abundances. The same type of poplar *CK2α* genes are marked in the same color. Symbols represent as follows: ML, mature leaf; YL, young leaf; R, root; DS, dark-grown seedlings; DL, etiolated dark-grown seedling transferred to light for 3h; CL, continuous light-grown seedling; FC, female catkins; MC, male catkins; X, xylem.

Table 2. The two selected phosphopeptides for docking study on their interaction with poplar CK2α subunits.

Poplar P-Proteins	JGI Protein ID	Identified phosphopeptides	Residue	Segment	M_r (Da)	Charge	Score
RPP2B	832971	KEEKVEEKEES*DDDMGFSLFD	S103	92-113	2585.04	2	170
RPP2C	836661		S104	93-114			
RPP2A	567964	KEEKVEEKEDT*DDDLGFSLFD	T100	90-110	2070.77	2	150

*Phosphorylated residues. The two identified phosphorylation peptides are referred from our previous identification (Liu et al., 2010).

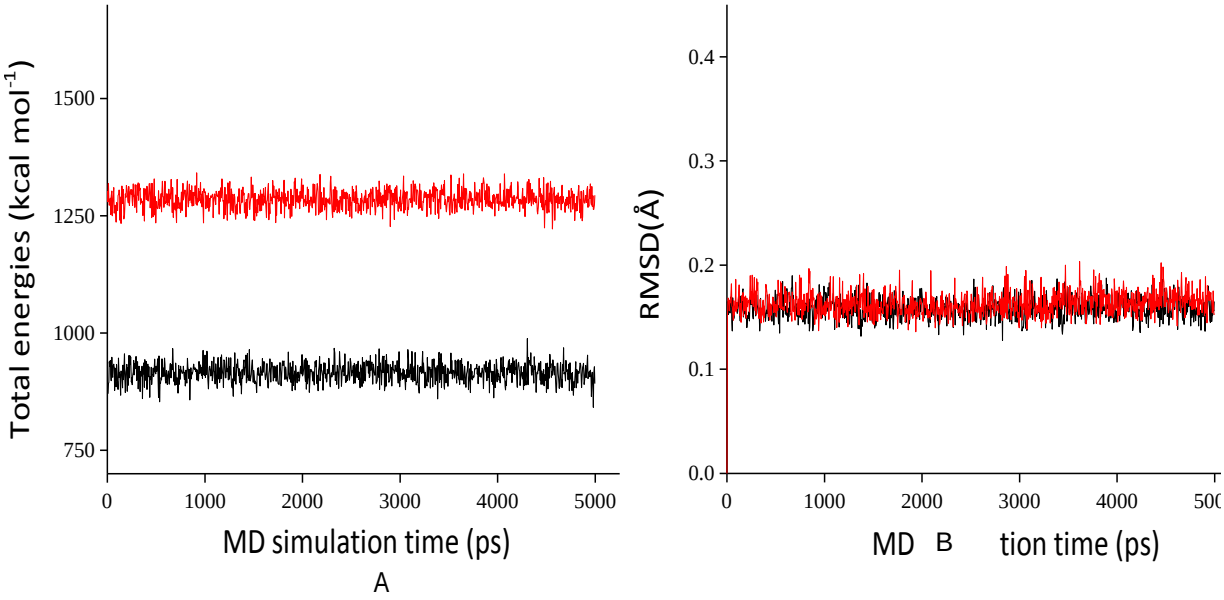

Figure 4. The time-evolution total energies (A) and backbone-atom root mean square deviations (RMSD, B) for the docked complexes during the MD simulations. The values of KEEKVEEKEES[a]DDDMGFSLFD-CK2α and KEEKVEEKEDT[a]DDDLGFSLFD-CK2α were represented by black and red lines, respectively.

activities of CK2 present in chloroplasts from *Arabidopsis* leaves (Salinas et al., 2006), it indicates that some translated CK2 proteins after transcription should be transported to chloroplasts. *PtrCK2α-3* and *PtrCK2α-4* genes from type II in phylogenetic tree have significantly similar expression pattern that high-level expression are mostly present in FC, MC, and continuous light-grown seedling (CL). The expression profile of *PtrCK2α-1* and *PtrCK2α-2* genes from type I in phylogenetic tree also showed relatively consistent that transcript accumulation focused on continuous light-grown seedling (CL), etiolated dark-grown seedling transferred to light 3h (DL), dark-grown seedlings (DS), and root (R). The fact that poplar *CK2α* genes from the same type share similar expression patterns across tissues, provide an additional evidence for their phylogenetic relationship.

Previous studies on *Arabidopsis* implicated that CK2 play an important role in regulating light signaling and circadian rhythms (Sugano et al., 1999). However, little is known whether light signaling could regulate the expression of *CK2α* genes. In the current study, it was found that the transcript abundance of *PtrCK2α-1* and *PtrCK2α-4* were induced by light, while *PtrCK2α-2* and *PtrCK2α-3* were negatively modulated by light. It seems that the mechanism should contribute to quick start phosphorylation of their extensive substrates under the conditions of dark and light transition.

In silico simulation on the interaction of CK2α with the substrate

In order to evaluate the interaction of these identified CK2α catalytic subunits with C-terminal regions of poplar acidic ribosomal P-protein (Liu et al., 2010), two identified phosphopeptides (Table 2) from the C-terminal regions with high confidence are selected to explore the interaction with poplar CK2α using explicit solvent docking and molecular dynamic (MD) simulations.

As shown in Figure 4, total energies and backbone-atom RMSDs indicated that the two docked complexes reached equilibrium after about 1000 ps and remained rather stable afterwards. Accordingly, the geometric and energetic analyses were performed on the average

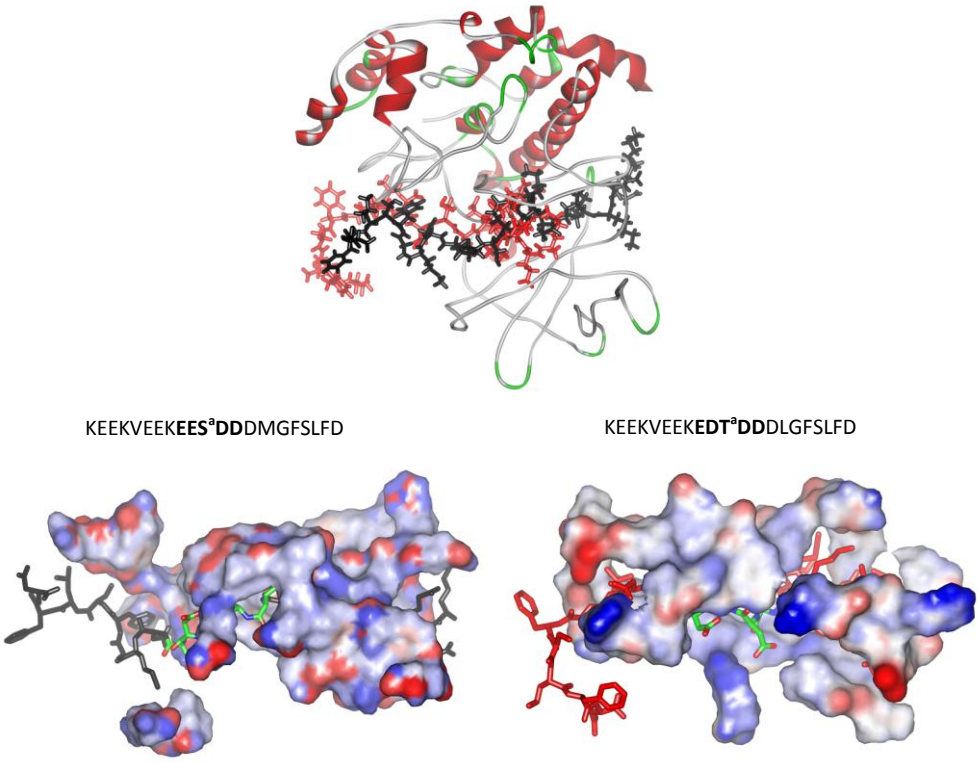

Figure 5. The modeled structures of KEEKVEEKEES[a]DDDMGFSLFD (black) and KEEKVEEKEDT[a]DDDLGFSLFD (red) bound to the CK2α active site. The protein is in ribbon. The Connolly surfaces of the CK2α binding pocket are created using the InsightII 2005 scripts. The ligands are represented by stick models. Ribbon colors: Helices, beta sheets, turns and random coils are in red, cyan, green and white, respectively. The hydrogens were avoided for readability.

structures of 1000~5000 ps MD trajectories. The superposed structures in Figure 5 showed that the two identified phosphopeptides occupy the proximity space at the binding pocket of PtrCK2α-1, which is mapped to the loop region on the top of helix region within the protein (Dlakic, 2005). In addition, there are some common characteristics of the binding modes. The interaction energies (E_{inter}) of KEEKVEEKEES*DDDMGFSLFD or KEEKVEEKEDT*DDDLGFSLFD with PtrCK2α-1 were calculated to be -1833.05 and -1676.66 kcal mol^{-1}. Electrostatic interactions rather than van der Waals interactions played a dominant role for the binding processes, contributing to more than 89%. As Figure 5 shows, the EES*DD of peptide 1 and EDT*DD of peptide 2 were buried in the binding pocket of enzyme, involving strong electrostatic interactions with them. As these fully or partially conserved residues are general for the phosphopeptides (Tchórzewski, 2002; Chang et al., 2005; Liu et al., 2010; Meggio and Pinna, 2003; Zschoernig and Mahlknecht, 2009; Dlakic, 2005), it commendably support a viewpoint that the CK2α identifies the phosphopeptides through the XES/T*DD sequences and then introduces the phosphorylation at the site S/T, which is in good agreement with the previous experimental reports (Riera et al., 2001; Meggio and Pinna, 2003; Liu et al., 2010). Taken together, it is likely that the special pentapeptides (XES/T*DD) are the identified sites for the CK2α and therefore, further phosphorylation studies in the poplar are urgently needed to support this point of view.

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REFERENCES

Ackermann K, Neidhart T, Gerber J, Waxmann A, Pyerin W (2005). The catalytic subunit alpha' gene of human protein kinase CK2 (CSNK2A2): genomic organization, promoter identification and determination of Ets1 as a key regulator. Mol. Cell. Biochem., 274(1-2): 91-101.

Affinity User Guide (2005). Accelrys Inc., San Diego, USA.
Aguilar R, Montoya L, Sanchez de Jimenez E (1998). Synthesis and Phosphorylation of Maize Acidic Ribosomal Proteins Implications in Translational Regulation. Plant Physiol., 116(1): 379-385.
Bibby AC, Litchfield DW (2005). The multiple personalities of the regulatory subunit of protein kinase CK2: CK2 dependent and CK2 independent roles reveal a secret identity for CK2beta. Int. J. Biol. Sci., 1(2): 67-79.
Bu Q, Zhu L, Dennis MD, Yu L, Lu SX, Person MD, Tobin EM, Browning KS, Huq E (2011). Phosphorylation by CK2 Enhances the Rapid Light-induced Degradation of Phytochrome Interacting Factor 1 in Arabidopsis. J. Biol. Chem., 286(14): 12066-12074.
Chang I, Szick-Miranda K, Pan S, Bailey-Serres J (2005). Proteomic Characterization of Evolutionarily Conserved and Variable Proteins of Arabidopsis Cytosolic Ribosomes 1 [w]. Plant Physiol., 137(3): 848-862.
Chen XW, Liu XG, Zheng KQ, Liang NC (2002). Cloning, sequencing, expression, and primary identification of recombinant mouse protein kinase CK2 alpha subunit. Ai Zheng, 21(7): 751-756.
Daniel X, Sugano S, Tobin EM (2004). CK2 phosphorylation of CCA1 is necessary for its circadian oscillator function in Arabidopsis. Proc. Natl. Acad. Sci. USA., 101(9): 3292-3297.
de Hoon M, Imoto S, Nolan J, Miyano S (2004). Open source clustering software. Bioinformatics (Oxford, England), 20(9): 1453-1454.
de la Fuente van Bentem S, Hirt H (2007). Using phosphoproteomics to reveal signalling dynamics in plants. Trends Plant Sci., 12(9): 404-411.
Dennis MD, Browning KS (2009). Differential phosphorylation of plant translation initiation factors by Arabidopsis thaliana CK2 holoenzymes. J. Biol. Chem., 284(31): 20602-20614.
Dlakic M (2005). The ribosomal subunit assembly line. Genome Biol., 6(10): 234.
Domanska K, Zielinski R, Kubinski K, Sajnaga E, Maslyk M, Bretner M, Szyszka R (2005). Different properties of four molecular forms of protein kinase CK2 from Saccharomyces cerevisiae. Acta Biochim. Pol., 52(4): 947-951.
Espunya MC, Martinez MC (2003). In situ hybridization analysis of protein kinase CK2 expression during plant development. Physiol. Plant, 117(4): 573-578.
Finn R, Mistry J, Schuster-Bockler B, Griffiths-Jones S, Hollich V, Lassmann T, Moxon S, Marshall M, Khanna A, Durbin R (2006). Pfam: clans, web tools and services. Nucleic. Acids Res., 34(Database Issue): D247-D251.
Guerra B, Issinger OG (1999). Protein kinase CK2 and its role in cellular proliferation, development and pathology. Electrophoresis, 20(2): 391-408.
Guerra B, Siemer S, Boldyreff B, Issinger OG (1999). Protein kinase CK2: evidence for a protein kinase CK2 beta subunit fraction, devoid of the catalytic CK2 alpha subunit, in mouse brain and testicles. FEBS. Lett., 462(3): 353-357.
Guo AY, Zhu QH, Chen X, Luo JC (2007). GSDS: a gene structure display server. Yi Chuan, 29(8): 1023-1026.
Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In, 1999. Nucleic Acids Symposium Series, pp. 95-98.
Head JD, Zerner MC (1985). A Broyden—Fletcher—Goldfarb—Shanno optimization procedure for molecular geometries. Chem. Phys. lett., 122(3): 264-270.
Hu P, Wu S, Hernandez N (2003). A minimal RNA polymerase III transcription system from human cells reveals positive and negative regulatory roles for CK2. Mol. Cell, 12(3): 699-709.
Hu R, Qi G, Kong Y, Kong D, Gao Q, Zhou G (2010). Comprehensive analysis of NAC domain transcription factor gene family in Populus trichocarpa. BMC Plant Biol., 10(1): 145.
Insight II Version 2005 (2005). 2005 edn. Accelrys Inc., San Diego, USA.
Johnston IM, Allison SJ, Morton JP, Schramm L, Scott PH, White RJ (2002). CK2 forms a stable complex with TFIIIB and activates RNA polymerase III transcription in human cells. Mol. Cell Biol., 22(11): 3757-3768.
Kang HG, Klessig DF (2005). Salicylic acid-inducible Arabidopsis CK2-like activity phosphorylates TGA2. Plant Mol. Biol., 57(4): 541-557.
Lebska M, Ciesielski A, Szymona L, Godecka L, Lewandowska-Gnatowska E, Szczegielniak J, Muszyńska G (2010). Phosphorylation of maize eukaryotic translation initiation factor 5A (eIF5A) by Casein Kinase 2. J. Biol. Chem., 285(9): 6217-6226.
Lebska M, Szczegielniak J, Dobrowolska G, Cozza G, Moro S, Muszynska G (2009). A novel splicing variant encoding putative catalytic alpha subunit of maize protein kinase CK2. Physiol. Plant, 136(3): 251-263.
Lee Y, Lloyd AM, Roux SJ (1999). Antisense expression of the CK2 alpha-subunit gene in Arabidopsis. Effects on light-regulated gene expression and plant growth. Plant Physiol., 119(3): 989-1000.
Litchfield DW (2003). Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochem. J., 369(Pt 1): 1-15.
Liu CC, Lu TC, Li HH, Wang HX, Liu GF, Ma L, Yang CP, Wang BC (2010). Phosphoproteomic identification and phylogenetic analysis of ribosomal P-proteins in Populus dormant terminal buds. Planta, 231(3): 571-581.
Lou DY, Dominguez I, Toselli P, Landesman-Bollag E, O'Brien C, Seldin DC (2008). The alpha catalytic subunit of protein kinase CK2 is required for mouse embryonic development. Mol. Cell Biol., 28(1): 131-139.
Mann M, Ong SE, Gronborg M, Steen H, Jensen ON, Pandey A (2002). Analysis of protein phosphorylation using mass spectrometry: deciphering the phosphoproteome. Trends Biotechnol., 20(6): 261-268.
Marques-Bueno MD, Moreno-Romero J, Abas L, De Michele R, Martinez MC (2011). A dominant negative mutant of protein kinase CK2 exhibits altered auxin responses in Arabidopsis. Plant J..
Meggio F, Pinna L (2003). One-thousand-and-one substrates of protein kinase CK2? The FASEB J., 17(3): 349-368.
Moreno-Romero J, Espunya MC, Platara M, Arino J, Martinez MC (2008). A role for protein kinase CK2 in plant development: evidence obtained using a dominant-negative mutant. Plant J., 55(1): 118-130.
Niefind K, Guerra B, Pinna LA, Issinger OG, Schomburg D (1998). Crystal structure of the catalytic subunit of protein kinase CK2 from Zea mays at 2.1 A resolution. EMBO J., 17(9): 2451-2462.
Ogrzewalla K, Piotrowski M, Reinbothe S, Link G (2002). The plastid transcription kinase from mustard (Sinapis alba L.). A nuclear-encoded CK2-type chloroplast enzyme with redox-sensitive function. Eur. J. Biochem., 269(13): 3329-3337.
Ohlrogge J, Benning C (2000). Unraveling plant metabolism by EST analysis. Curr. Opin. Plant Biol., 3(3): 224-228.
Park JI, Nou IS, Lee SS, Kang KK, Watanabe M (2001). Identification of S-genotypes by PCR-RFLP in breeding lines of Brassica. Mol. Cells, 12(2): 227-232.
Pinna LA (2002). Protein kinase CK2: a challenge to canons. J. Cell. Sci., 115(Pt 20): 3873-3878.
Riera M, Figueras M, Lopez C, Goday A, Pages M (2004). Protein kinase CK2 modulates developmental functions of the abscisic acid responsive protein Rab17 from maize. Proc. Nat. Acad. Sci. U.S.A., 101(26): 9879-9884.
Riera M, Peracchia G, Pages M (2001). Distinctive features of plant protein kinase CK2. Mol. Cell. Biochem., 227(1-2): 119-127.
Saitou N, Nei M (1987). The Neighbor-joining Method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4(4): 406-425.
Saldanha AJ (2004). Java Treeview-extensible visualization of microarray data. Bioinformatics, 20(17): 3246-3248.
Salinas P, Fuentes D, Vidal E, Jordana X, Echeverria M, Holuigue L (2006). An extensive survey of CK2 alpha and beta subunits in Arabidopsis: multiple isoforms exhibit differential subcellular localization. Plant Cell Physiol., 47(9): 1295-1308.
Sugano S, Andronis C, Green RM, Wang ZY, Tobin EM (1998). Protein kinase CK2 interacts with and phosphorylates the Arabidopsis circadian clock-associated 1 protein. Proc. Nat. Acad. Sci. U.S.A., 95(18): 11020-11025.
Sugano S, Andronis C, Ong MS, Green RM, Tobin EM (1999). The protein kinase CK2 is involved in regulation of circadian rhythms in Arabidopsis. Proc. Nat. Acad. Sci. USA, 96(22): 12362-12366.
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol., 28(10): 2731-2739.

Tchórzewski M (2002). The acidic ribosomal P proteins. Int. J. Biochem. Cell. Biol., 34(8): 911-915.
Tripodi F, Cirulli C, Reghellin V, Marin O, Brambilla L, Schiappelli MP, Porro D, Vanoni M, Alberghina L, Coccetti P (2010). CK2 activity is modulated by growth rate in Saccharomyces cerevisiae. Biochem. Biophys. Res. Commun., 398(1): 44-50.
Tuskan G, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A (2006). The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). Science, 313(5793): 1596-1604.
Wilkins O, Nahal H, Foong J, Provart NJ, Campbell MM (2009). Expansion and Diversification of the Populus R2R3-MYB Family of Transcription Factors. Plant Physiol., 149(2): 981-993.
Wirkner U, Voss H, Lichter P, Pyerin W (1994). Human protein kinase CK2 genes. Cell Mol. Biol. Res., 40(5-6): 489-499.
Yang Z, Nie Y, Yang G, Zu Y, Fu Y, Zhou L (2010a). Synergistic effects in the designs of neuraminidase ligands: Analysis from docking and molecular dynamics studies. J. Theor. Biol., 267(3): 363-374.
Yang Z, Wu X, Yang G, Zu Y, Zhou L (2011). Understanding the chiral recognitions between neuraminidases and inhibitors: Studies with DFT, docking, and MD methods. Int. J. Quantum. Chem.. DOI: 10.1002/qua.23046
Yang Z, Yang G, Zu Y, Fu Y, Zhou L (2010b). Computer-Based De Novo Designs of Tripeptides as Novel Neuraminidase Inhibitors. Int. J. Mol. Sci. 11(12): 4932-4951.
Yang ZW, Yang G, Zu YG, Fu YJ, Zhou LJ (2009). The conformational analysis and proton transfer of the neuraminidase inhibitors: a theoretical study. Phys. Chem. Chem. Phys., 11: 10035-10041.
Yde CW, Ermakova I, Issinger OG, Niefind K (2005). Inclining the purine base binding plane in protein kinase CK2 by exchanging the flanking side-chains generates a preference for ATP as a cosubstrate. J. Mol. Biol., 347(2): 399-414.
Zhang N, Zhong R (2010). Docking and 3D-QSAR studies of 7-hydroxycoumarin derivatives as CK2 inhibitors. Eur. J. Med. Chem., 45(1): 292-297.
Zschoernig B, Mahlknecht U (2009). Carboxy-terminal phosphorylation of SIRT1 by protein kinase CK2. Biochem. Biophys. Res. Commun., 381(3): 372-377.