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HOMOLOGY MODELING OF YEAST CYCLIN-DEPENDENT PROTEIN KINASE

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The important functions that CDKs perform in cell division and cell cycle regulation made central protein kinase of *Saccharomyces cerevisiae* CDC28 a target model for structural and functional analysis. The 3D models of CDC28 protein kinase using molecular modeling techniques will enlarge our understanding of the phosphorylation mechanism and the structural changes of mutant kinases. Structural template for *S. cerevisiae* CDC28 was identified from PDB (Protein Databank) using BLASTP (basic local alignment search tool for proteins). Template-target alignments were generated for homology modeling and checked manually for error. The models were then generated using MODELLER and validated using PROCHECK followed by energy minimization and molecular dynamics calculations in AMBER force field.

Важные функции, которые выполняют циклинзависимые протеинкиназы (cyclin-dependent kinases, CDKs) в делении клетки и регуляции клеточного цикла, превратили CDC28 *Saccharomyces cerevisiae* в центральную мишень и привлекательную модель для исследования их структурных и функциональных свойств. Построение адекватных 3D-моделей протеинкиназы CDC28 с использованием современных методов компьютерного молекулярного моделирования позволяет расширить наши знания о функциональных протеинкиназах, механизмах фосфорилирования и структурных перестройках мутантных аллелей. В данной работе на основе гомологичного моделирования восстановлена структура протеинкиназы *S. cerevisiae* CDC28 при помощи пакета MODELLER. Шаблон для искомой структуры *S. cerevisiae* CDC28 был идентифицирован из базы данных PDB (Protein Databank) с помощью программных модулей PROCHECK и BLASTP (basic local alignment search tool for proteins). Далее проводилось МД-моделирование с использованием пакета AMBER.

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INTRODUCTION

The 3D structural information for CDC28 proteins helps us to understand the mechanism transfer of γ-phosphate from adenosine triphosphate (ATP) to a protein substrate [1]. With the use of homology modeling techniques we constructed modeled structures for ScCDC28 protein kinase. Modeling of ScCDC28 protein kinase is generated followed by molecular dynamics calculations and model assessment is described in this work.

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1. MATERIALS AND METHODS

1.1. *Saccharomyces cerevisiae CDC28* **Protein Sequence.** The aminoacid sequence of ScCDC28 is obtained from *Saccharomyces* Genome Database [2].

1.2. Homology Modeling. Modeling of the ScCDC28 (target) was performed using MODELLER (protein modeling by satisfaction of spatial restraints) [3].

1.3. Template Selection for *Saccharomyces cerevisiae CDC28.* The ScCDC28 annotated with known structural templates in PDB (Protein Databank) was identified using BLASTP (Basic Local Alignment Search Tool for proteins). The query sequence found 64.11% homology with the PDB entry: 1QMZ (phosphorylated CDK2-cyclin A-substrate peptide complex) (see Fig. 1).

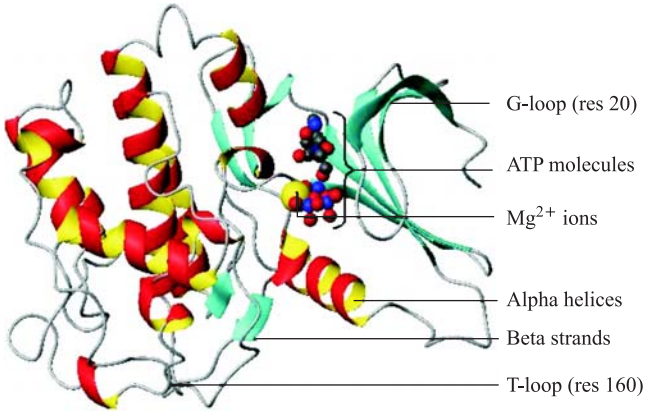

Fig. 1. PDB entry: 1QMZ (phosphorylated CDK2-cyclin A-substrate peptide complex)

1.4. Alignment of Target and Template Sequences. Target-template sequences alignment was executed by the ALIGN-2D (sequence alignment module in MODELLER). This command executes a global dynamic programming method for comparison between the target-template sequences [3]. Gaps with variable gap penalty function are included for structural loops and core regions, in order to get maximum correspondence between the sequences.

1.5. Energy Refinement. The calculated 3D-model structure was energy minimized in AMBER force field for 2 ns time steps using steepest descent and conjugate gradient minimization algorithms [4]. Parameters included the covalent bond distances and angles, stereochemical validation, atom nomenclature were validated using PROCHECK [5]. Target model is then superimposed with the template and RMSD (root-mean-square deviation) was calculated. The modeled structures thus constructed can be viewed as visualization tools such as RasMol [6] and MOLMOL [7].

2. RESULTS AND DISCUSSIONS

2.1. Target-Template Sequence Comparison. Three-dimensional structures of structurally conserved regions (scr's) were very similar between the ScCDC28 and HCDK2 models (see Fig. 2). The generated 3D model of ScCDC28 protein was checked by PROCHECK (see Fig. 3). The torsion angles of ϕ and ψ in the generated model are shown in the Ramachandran plot as shown in Fig. 3.

Fig. 2. Target-template alignment. «*» shows the conserved regions between the sequences and «–» shows the gaps

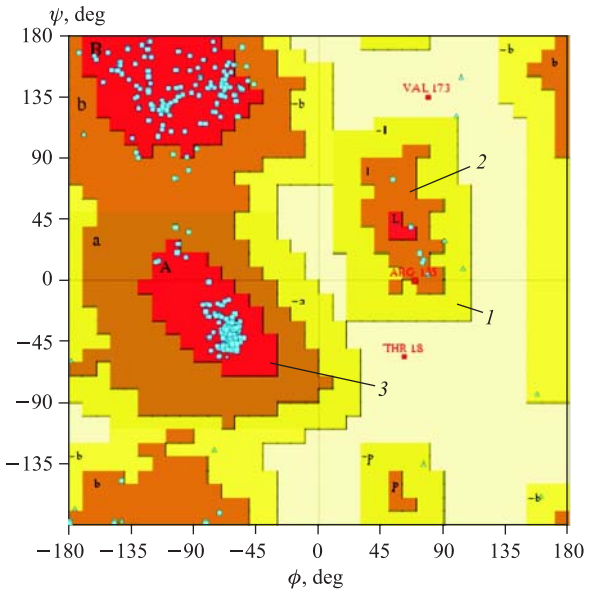

Fig. 3. The Ramachandran plot of yeast CDC28 protein kinase structure at 15 ps (*1*), 1 ns (*2*) and 2 ns (*3*) obtained by PROCHECK

The torsion angles of 91.5% of the residues had values in the most favored regions. Since X-ray structure of HCDK2 at 2.0 Å has 91.1% of the residues with values in the most favored regions, it can be said that our CDC28 structure satisfies example of a good model.

2.2. RMSD and RMSF — Reliable Indicators to Check Variability. During the MD simulation, the changes in molecular conformation were monitored in terms of a Root Mean Square Displacement (RMSD) and Root Mean Square Fluctuation (RMSF) [3]. During the course of MD trajectory the beta strands are prone to have more fluctuations than the alpha helices. These beta strands are more flexible due to the presence of hydrogen bonds. In the X-ray structure (1QMZ) regions of HCDK2 (shown in black blocks in Fig. 4) corresponding to the residues Phe5 to Glu13 (FQKVEKIGE), Val18 to Asn24 (VVYKARN), Val30 to Lys34 (VVALK), Leu67 to Ile71 (LLDVI), Tyr78 to Glu82 (YLVFE), Val124 to Leu125, Leu134 to Asn137 (LLIN), Ala141 to Leu144 (AIKL), Arg151 to Ala152 (RA) are the regions of beta strands which showed fluctuation during the trajectory. Similarly, in the structure of modeled ScCDC28 (shown in grey blocks in Fig. 4) the corresponding residues are between the Tyr8 to Glu16 (YKRLEKVGE), Val21 to Asp27 (VVYKALD), Val36 to Ile42 (VVALKKI), Leu73 to Val77 (LYDIV), Leu84 to Glu89 (LYLVFE), Leu93 to Asp94 (LD), Ile132 to Leu133, Leu143 to Asn145 (LIN), Asn149 to Lys151 (NLK), Arg159 to Ala160 (RA).

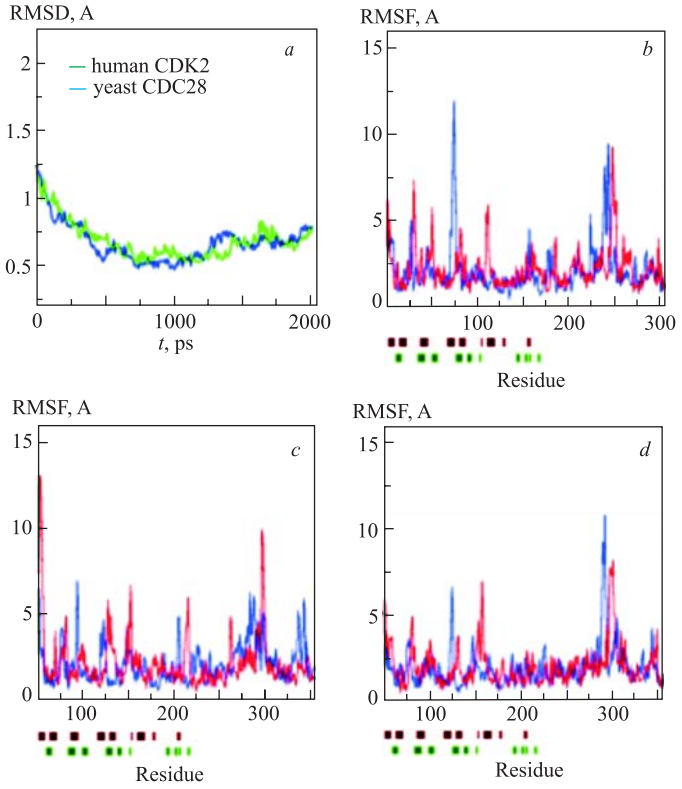

Fig. 4. Analyzing structural variability between target-template structures using RMSD (*a*), RMSF at 15 ps (*b*), at 1 ns (*c*) and at 2 ns (*d*), respectively

The RMSD and RMSF plots between ScCDC28 and the reference protein suggest that the main chain fold was conserved and it remained undistorted (see Fig. 4). Positions of the important conserved regions including the G- and T-loops of kinases, ATP–Mg^{2+} ion complex and substrate component are highly conserved.

2.3. Activation Triangle around of ATP. To characterize the structural features of kinases we use structural elements, which have important role in regulation. They are T-loop and G-loop and positions of ATP. The T160 in T-loop, ATP and G16 in G-loop positions of HCDK2 structure and the T169, ATP and G20 positions of ScCDC28 structure in the final (2-ns) state are analyzed with an «activation triangle» (see Fig. 5).

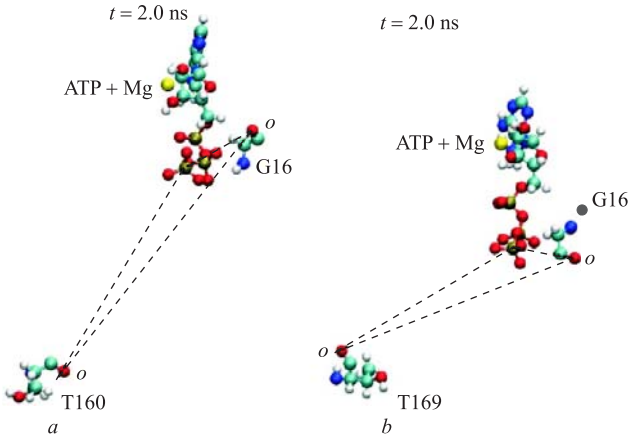

Fig. 5. The T160, ATP and G16 positions of HCDK2 structure (*a*) and the T169, ATP and G20 positions of ScCDC28 structure (*b*) in the final (2-ns) state. The ATP molecule, residues T160 and G20 are represented by ball models

The ATP-res16 and ATP-res20 distances in the CDK2 and CDC28 structures, respectively, evidently lie within ~ 5.0 and ~ 5.5 Å during the all 2-ns dynamical changes. Thus, all the hydrogen bond network in the ATP-res20 for the binding site varies between the CDK2 and CDC28 structures.

2.4. Aminoacid Residues around Phosphorylated Regulatory Site. The CDK2, CDC28/ATP' dynamical peculiarities in the neighbor of phosphorylation site (T160 in HCDK2, T169 in ScCDC28) were analyzed in detail. From the «activation triangle» described above, the T160 (T169)-res16 distances for the HCDK2-G16/ATP and ScCDC28-G20/ATP were estimated.

The distances between the res16-ATP, T160/T169-ATP, res16-T160/169 in the HCDK2 and ScCDC28 are 19 and 21 Å, 20 and 25 Å, correspondingly, showed similar behavior that confirms good homology prediction (see Fig. 6).

CONCLUSIONS

Detailed analysis of the data obtained from structure prediction methods and molecular dynamics calculations confirms high degree of similarity between yeast protein kinase CDC28 and human kinase CDK2 justified using HCDK2 as model for ScCDC28 [8, 9]. Through this

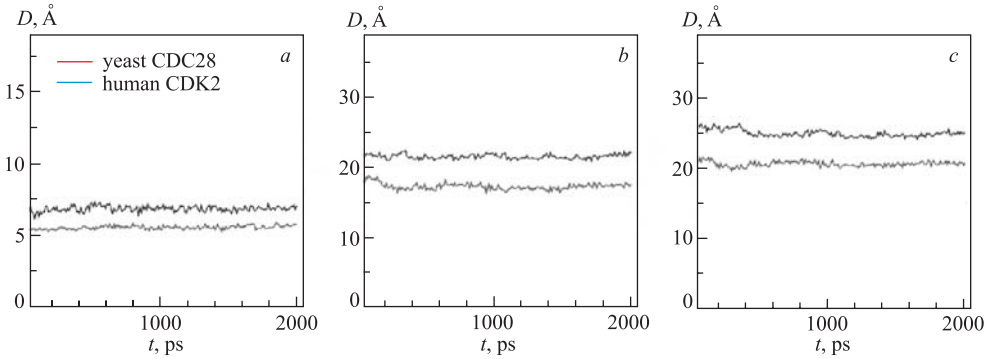

Fig. 6. The time dependences of the res16-ATP, res20-ATP (*a*), T160-ATP, T169-ATP (*b*), T160-res16, T169-res20 (*c*) distances are shown for the HCDK2 and ScCDC28, respectively, in accordance with the «activation triangle»

In Silico approach one can understand the conformation behavior [8, 9] between the important conserved regions including the G- and T-loops of kinases, ATP–Mg^{2+} ion complex and substrate component in correlation with the physiological properties between these structures.

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