Supplementary information, Data S1

Materials and Methods

Reagents

Anti-Flag M2 affinity agarose gel was from Sigma; Mono S and Superose 6 were from GE Healthcare; Polyethylenimine (PEI) was from polysciences (23966); HEK293 cells were from Invitrogen. Inc. and culture medium was from Union-Biotech Co., Ltd. (Shanghai, China); Anti-phospho-p53 (Ser15) was purchased from Abclonal; Horseradish peroxidase-labeled anti-rabbit secondary antibody was from AbMart.

Protein purification

Plasmids containing full-length ORF of DNA-PKcs, KU70 and KU80 were transfected into HEK293F cells in suspension culture at density of 2.0×10^6/ml. After culturing for 66 h at 37 °C, cells were harvested by centrifugation at 4000 rpm for 15 min. Cells were lysed using buffer containing 50 mM HEPES-NaOH, pH 7.4, 300 mM NaCl, 0.5% CHAPS, 10% glycerol, 5 mM $MgCl_2$, 5 mM ATP, 0.5 mM EDTA, 2 mM DTT and 1× protease inhibitor cocktail. The supernatant was applied to Anti-Flag affinity agarose gel followed by HRV-3C digestion and ion-exchange chromatography. Biotinylated Y-shaped dsDNA were synthesized and annealed as described previously (Supplementary information, Table S2) [1]. KU70/80-DNA was purified to homogeneity using Superose 6 (Increase 5/150 GL, GE healthcare). DNA-PKcs and KU70/80-DNA were mixed and DNA-PK complex was obtained by gel filtration through Superose 6 (Increase 5/150 GL, GE healthcare). Fractions were subjected to SDS-PAGE.

Pull-down assays

To detect DNA binding properties of DNA-PKcs, KU70/80 and DNA-PK complex, 8 μg DNA-PKcs or 4.5 μg KU70/80 was incubated with 1.5μg Biotinylated DNA in buffer contained 25 mM HEPES-NaOH, pH 7.4, 150 mM NaCl, 0.05% Triton X-100, 5% glycerol, 0.5 mM EDTA, 2 mM DTT. The mixture was applied to streptavidin beads and the bound proteins were subjected to SDS-PAGE and visualized by coomassie brilliant blue staining.

To detect the interaction between DNA-PKcs and KU70/80, 8 μg DNA-PKcs and 7.5 μg KU70/proteinA-KU80 were incubated with or without 2 μg DNA in the same binding buffer as mentioned above. The mixture was applied to IgG beads and the bound proteins were subjected to SDS-PAGE and visualized by coomassie brilliant blue staining.

EM data acquisition

Negative staining was used to evaluate the protein quality, in brief, 5 μl of freshly purified DNA-PK complex (~0.02 mg/ml) were applied onto copper grids supported by a thin layer of glow-discharged carbon film (Zhongjingkeyi Technology Co., Ltd). After adsorption for 1 min, uranyl formate (2% w/v) was used for negative staining at room temperature. The negative stained grid was checked using FEI Tecnai T12 operating at 120 kV at Tsinghua University (Beijing, China).

Cryo-grid preparation was performed using FEI Vitrobot mark IV operated at 10 °C and 100% humidity. Aliquots of 4 μl of freshly purified DNA-PK complex (~0.5 mg/ml) were applied to glow-discharged holey carbon grids (Quantifoil Au, R1.2/1.3, 300 mesh). The grids were blotted for 3.5 s and flash-plunged into liquid ethane, pre-

cooled in liquid nitrogen. The cryo-grids were examined using FEI Arctica operating at 200 KV equipped with Falcon II direct electron detector at Tsinghua University, and then good cryo-grids were dry-shipped to the National Center for Protein Science, Shanghai (NCPSS) for data collection. At NCPSS, pre-examined cryo-grids were loaded to FEI Titan Krios electron microscope equipped with a Cs corrector. All the cryo-images were recorded on Gatan K2 Summit camera operated in super-resolution mode using SerialEM. The magnification is 18 000, corresponding to a final pixel size of 1.3 Å by binning 2 of the original micrographs. For each image stack, a total dose of about 50 electrons were equally fractioned into 32 frames with a total exposure time of 8 s. Defocus values ranged from 1.7 to 4.7 μm.

Image processing

For cryo-EM data sets, beam-induced motion correction was performed using the MotionCor2 through all frames after 2 × 2 binned of the original micrographs [2]. The contrast transfer function parameters were estimated by CTFFIND3 [3] and other procedures of cryo-data processing were performed within RELION 1.4 or RELION 2.0 [4, 5]. We manually picked about 5 000 particles to generate the templates for particle auto-picking and about 900 000 particles were then auto-picked from 3 496 micrographs for further processing. Ice contaminations and noise were successfully removed from first round 2D classification. As shown in Supplementary information, Figure S2, Angular distribution analysis shows obvious orientation preference of the data set. To reduce the orientation preference, another round of 2D classification was

performed and 150 000 particles from dominant views were removed. 319 477 particles were then subjected to 3D classification with previously reconstructed map (EMDB: 1209) as the initial model after low-pass filtered to 60 Å. And 53 451 particles with all four components were used for first reconstruction and a reported 7.9 Å map was generated (gold-standard FSC 0.143 criteria) [6]. After B-factor sharpening (Post-processing in RELION), the reported resolution was 6.6 Å (corrected gold-standard FSC 0.143 criteria). We also performed further refinement with different local mask but did not get better density map [7]. Chimera [8] was used to visualize and evaluate of the 3D maps, and the local resolution map was calculated using ResMap [9].The procedures for structure determination are summarized in Supplementary information, Figure S2.

Model building into the cryo-EM map

The crystal structures of DNA-PKcs (PDB ID: 5LUQ) [10] and KU70/80-DNA (PDB ID: 1JEY) [11] were manually docked into the cryo-EM map of DNA-PK holoenzyme using UCSF Chimera [8]. The DNA bases were manually built in COOT. DNA-PKcs, KU70/80 and DNA models were flexibly fitted into the cut-off individual component cryo-EM maps with MDFF [12], respectively. We set the scaling factor ξ to lower value in order to model the large conformational changes of N-HEAT of DNA-PKcs in the holoenzyme. Then the whole model was refined in MDFF against the holoenzyme cryo-EM with the higher value of scaling factor to fit more precisely into the map. Finally, the correlation coefficent between the final model and the cryo-EM map was calculated with PHENIX [13].

References

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