Structural step forward for NHEJ

Cell Research (2017) 27:1304-1306. doi:10.1038/cr.2017.119; published online 19 Sep 2017

In a recent paper published in *Cell Research*, a cryo-EM structure reveals the interface between DNA-PKcs and the Ku70/80:DNA complex, together forming the DNA-dependent protein kinase holoenzyme in non-homologous DNA end joining. Insight from this structure suggests how an allosteric rearrangement of DNA-PKcs driven by Ku70/80:DNA binding regulates kinase activity in this largest member of a family of structurally homologous phosphoinositide 3-kinase-related protein kinases that includes mTOR, ATR, and ATM.

Repair of double-strand breaks (DSBs) is among the most challenging processes for DNA because the two DNA ends are separated and then must be rejoined. Non-homologous DNA end joining (NHEJ) is the major repair mechanism of DSBs in humans. Much is known about the biochemistry of NHEJ [1, 2], but the positional relationships of the components are paramount to understanding the progression of processing event. Until last year, NHEJ structures included individual components or pieces of them [3-5]. In a recent paper in *Cell Research*, the Xu laboratory reports the cryo-EM structure of DNA-dependent protein kinase (DNA-PK) at 6.6 Å [6]. This is the first structure of sufficient resolution to allow positioning of DNA-PKcs relative to the Ku70/80:DNA complex. This information will be highly important in integrating deeper and broader structural insights with the existing biochemical understanding of the NHEJ pathway.

This year has seen major steps forward for the structural aspects of NHEJ. Earlier in the year, Sibanda *et al.* [7]

published a crystal structure of selenomethionine (Se-Met)-labeled DNA-PKcs complexed with the Ku80ct₁₉₄ (Ku80 residues 539-732) at 4.3 Å. This was the first structure to permit a chain trace of the very large 469-kDa DNA-PKcs. However, the electron density of the Ku80ct₁₉₄ was not observed. Their use of Se-Met-labeled Ku80ct₁₉₄ suggested the location of part of Ku80ct₁₉₄, proposing the Ku-binding sites A and B [7]. Recently, a cryo-EM structure of DNA-PK was reported at 5.8 Å by Sharif *et al.* [8], but their map lacked significant Ku70/80:DNA density, thus this placement within the map remained ambiguous. Until now it was unclear how DNA-PKcs binds to the Ku70/80:DNA complex at all, which is essential to determine the kinase activation mechanism and to what extent the DNA end is protected by DNA-PK during NHEJ. The Xu paper presented the first pseudo-atomic structure of the DNA-PK holoenzyme and begins to address these questions [6].

Yin *et al.* took a novel approach employing recombinant DNA-PKcs for structural studies. They purified DNA-PKcs and Ku70/80 from HEK293F cells, and assembled and purified the 650-kDa DNA-PK holoenzyme for structural analysis. Setting up such a system with recombinant DNA-PKcs production for structural studies will permit them to potentially produce various altered DNA-PKcs structures in the future. Their cryo-EM experimental data was limited to 6.6 Å, but they docked the available crystal structures of DNA-PKcs (at 4.3 Å; PDB: 5LUQ) [7] and Ku70/80:DNA complex (at 2.5 Å; PDB: 1JEY) [9] into the cryo-EM

density, and manually built DNA bases to create their pseudo-atomic model of DNA-PK (Figure 1).

Their structure shows how the double-ring shape of DNA-PKcs, formed by the N-terminal α -solenoid of DNA-PKcs, connects its catalytic domain to the Ku70/80:DNA complex. A DNA-binding tunnel formed by DNA-PKcs and Ku70/80 together cradles ~30 bp within the holoenzyme. The DNA end remains embedded in the N- and M-HEAT regions in the base of DNA-PKcs. Although the Ku70/80 remains over 100 Å from the kinase domain, long range rearrangements in the FAT domain that encircles the kinase catalytic site improve accessibility to the catalytic core. Superimposition between the ‘apo’ DNA-PKcs (molecule B in the crystal structure [7]) and the ‘holo’ DNA-PKcs showed the superimposed M-HEAT domains. However, the N-HEAT domain, which is extended in the apo form of DNA-PKcs, in the holo form folds inward to join with the M-HEAT and FAT domains upon DNA binding. Such a significant difference suggests that Ku70/80:DNA induces conformational changes in the HEAT and FAT repeat, which allosterically stimulates the kinase activation.

Surprisingly, their DNA-PK structure shows that DNA adopts a 30° kink at the DNA-PKcs:Ku70/80 interface with about 16-18 bp of the DNA end extending from the Ku70/80 exit point into DNA-PKcs. Our laboratory determined that dsDNA longer than 26 bp is required for DNA-PKcs and Ku70/80 to form an active complex (i.e., productive mode) [10]. With shorter dsDNA, DNA-PK complex still forms but remains

Figure 1 DNA-PK holoenzyme (with the same color scheme as Yin *et al.* used). **(A)** The approach of DNA-PK structure determination by Yin *et al.* The crystal structures of DNA-PKcs at 4.3 Å (PDB: 5LUQ, the molecule B, one of two DNA-PKcs in the asymmetric unit) [7] and Ku70/80:DNA at 2.5 Å (PDB: 1JEY) [9] were docked into the 6.6-Å cryo-EM density [6]. Both crystal structures are shown in surface representation. Note that three helices of the Ku80ct₁₉₄ are not shown in this representation of DNA-PKcs. The DNA-PKcs is color coded based on the domains as Yin *et al.* used: N-HEAT (pink), M-HEAT (green), FAT (purple), kinase domain (yellow) and FATC (red). Here, the FRB is colored in orange. Also, Ku70 and Ku80 are shown in light blue and blue, respectively. The phosphodiester backbones of dsDNA in Ku70/80:DNA structure were colored in orange and the bases of DNA are shown as sticks. **(B)** Overview of the DNA-PK holoenzyme assembly by Yin *et al.* (PDB: 5Y3R). The DNA-PKcs is shown in surface representation. The Ku70/80 heterodimer is shown in ribbon representation to better see the DNA as it is encircled by the toroidal Ku70/80 protein. The color scheme was the same as **A**. Note that DNA adopts a 30° kink right at the interface between DNA-PKcs and Ku70/80. Ku80ctα2, which was also observed in the crystal structure [7], is shown in blue.

inactive as a kinase (i.e., nonproductive mode) [10]. Thus, their structure represents the productive mode. Large rearrangements driven by the N-HEAT interaction with the DNA and Ku70/80 occur, similar to the DNA-PKcs ‘biting down’ on the DNA. This displacement likely requires the Ku70/80 to anchor one end of the DNA molecule to the side of the N- and M-HEAT domains, providing a spring tension that allows the transduction of the motion through the molecule, clearing the kinase cleft and providing access and thus activation.

Although the density of the C-termini of Ku70 (residues 535–609) and Ku80 (residues 542–732) was missing, they revealed three major intermolecular

interfaces between DNA-PKcs and Ku70/80. Two are new: (1) the M-HEAT and the α/β domains of Ku70 and (2) the N-HEAT and the “bridge” formed by two anti-parallel strands of Ku70/80 and the “pillar” of Ku70. The third interface, a 4-turn helix (designated Ku80ctα2), bound to the concave side of the M-HEAT, was observed near the free DNA end. This is equivalent to the second α-helix of Ku80ct₁₉₄ located in the Ku-binding site B [7]. Intriguingly, two other helices observed in the crystal structure, one of which was located in the Ku-binding site A near the PQR autophosphorylation cluster and contained a Se-Met providing a significant Se anomalous signal [7], were not observed

in this cryo-EM structure.

Many questions arise from this new structural work. Obviously, statistics on the structural analysis remain to be improved. One must keep in mind that their detailed structural information is highly dependent on the quality of the utilized crystal structures. For instance, the DNA-PKcs crystal structure still includes some missing portions, due to disorder, and ambiguity of orientation of some side chains ($R_{\text{crist}}/R_{\text{free}} = 38.6\%/43.7\%$ and Ramachandran/Rotamer outliers = 6.7%/6.0%) [7]. Thus, these regions need to be revealed and validated. Also, at 6.6 Å, locating the exact position of DNA is challenging as Yin *et al.* could not observe extensive

protein-DNA interaction. Moreover, how the CTD of Ku80 interacts with DNA-PKcs is still a mystery.

Nevertheless, this DNA-PK structure by Yin *et al.* has moved the field forward to better understand the human NHEJ mechanism. Further work is required not only to obtain higher resolution DNA-PK structures but also to obtain structures with other NHEJ components. How DNA-PKcs interacts with them and recognizes various substrates to orchestrate the NHEJ process is of great interest. Furthermore, as DNA-PKcs has different phosphorylation states, reflecting various conformational states, it will be interesting to see how a hyper-phosphorylated DNA-PKcs is different from a hypo-phosphorylated DNA-PKcs, which could provide the

intermediate states of the activation mechanism and the mechanism for release of the DNA end. Also, we do not know how DNA-PKcs is activated by DNA, albeit to a lesser extent, in the absence of Ku70/80. Last but not least, how would a dimeric arrangement of two DNA-PK molecules bring two DNA ends together?

Go Watanabe¹, Michael R Lieber¹,
Dewight Williams²

¹USC Norris Comprehensive Cancer Ctr., 1441 Eastlake Ave., Los Angeles, CA 90089-9176, USA;

²LeRoy Eyring Center for Solid State Science, Physical Sciences Bldg A-213, 901 S. Palm Walk, Tempe, AZ 85287-1704, USA

Correspondence: Michael R Lieber
E-mail: lieber@usc.edu

References

- 1 Chang HHY, Pannunzio NR, Adachi N, *et al.* *Nat Rev Mol Cell Biol* 2017; **18**:495-506.
- 2 Mahaney BL, Meek K, Lees-Miller SP. *Biochem J* 2009; **417**:639-650.
- 3 Ochi T, Wu Q, Blundell TL. *DNA Repair* 2014; **17**:98-109.
- 4 Williams DR, Lee KJ, Shi J, *et al.* *Structure* 2008; **16**:468-477.
- 5 Spagnolo L, Rivera-Calzada A, Pearl LH, *et al.* *Mol Cell* 2006; **22**:511-519.
- 6 Yin X, Liu M, Tian Y, *et al.* *Cell Res* 2017; **27**:1341-1350.
- 7 Sibanda BL, Chirgadze DY, Ascher DB, *et al.* *Science* 2017; **355**:520-524.
- 8 Sharif H, Li Y, Dong Y, *et al.* *Proc Natl Acad Sci USA* 2017; **114**:7367-7372.
- 9 Walker JR, Corpina RA, Goldberg J. *Nature* 2001; **412**:607-614.
- 10 West RB, Yaneva M, Lieber MR. *Mol Cell Biol* 1998; **18**:5908-5920.