Structural insights into Non-Homologous End Joining (NHEJ)

Susan P. Lees-Miller
Department of Biochemistry and Molecular Biology
Robson DNA Science Center
Arnie Charbonneau Cancer Institute
Cumming School of Medicine
University of Calgary
Alberta
Canada

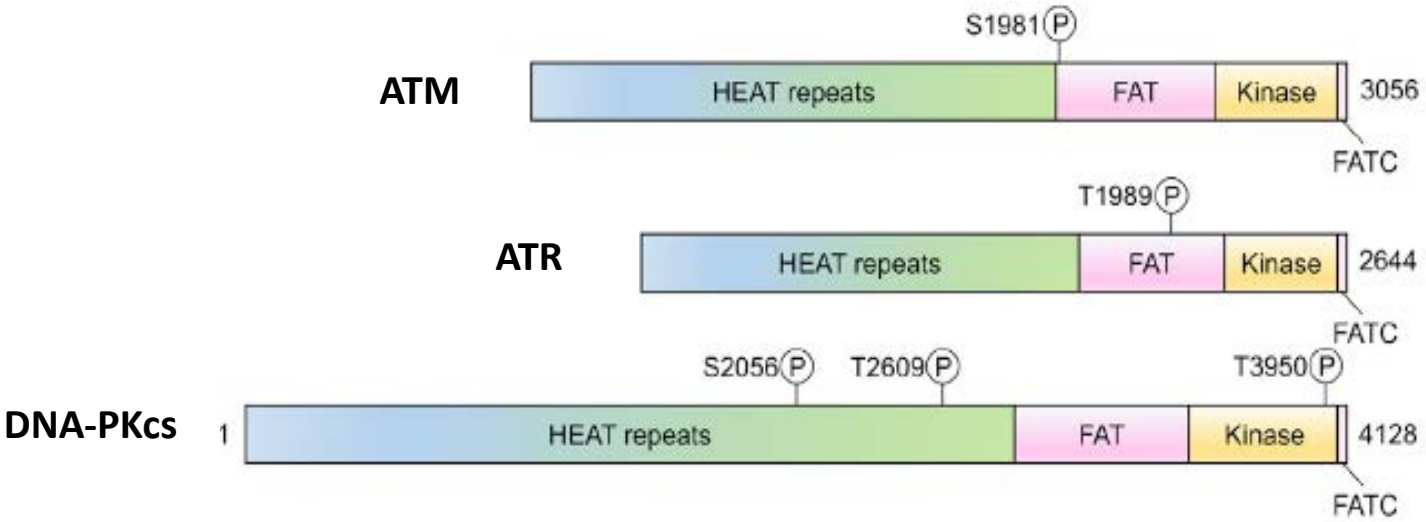
NIH DNA Repair Group April 20th 2021

rdsc Robson DNA Science Centre

UNIVERSITY OF CALGARY | Alberta Health Services | Arnie Charbonneau Cancer Institute

Phosphatidyl inositol 3-kinase-like protein kinases PIKKs

DNA-PKcs	DNA-dependent protein kinase catalytic subunit (PRKDC)
ATM	Ataxia-Telangiectasia Mutated
ATR	ATM and Rad3-related



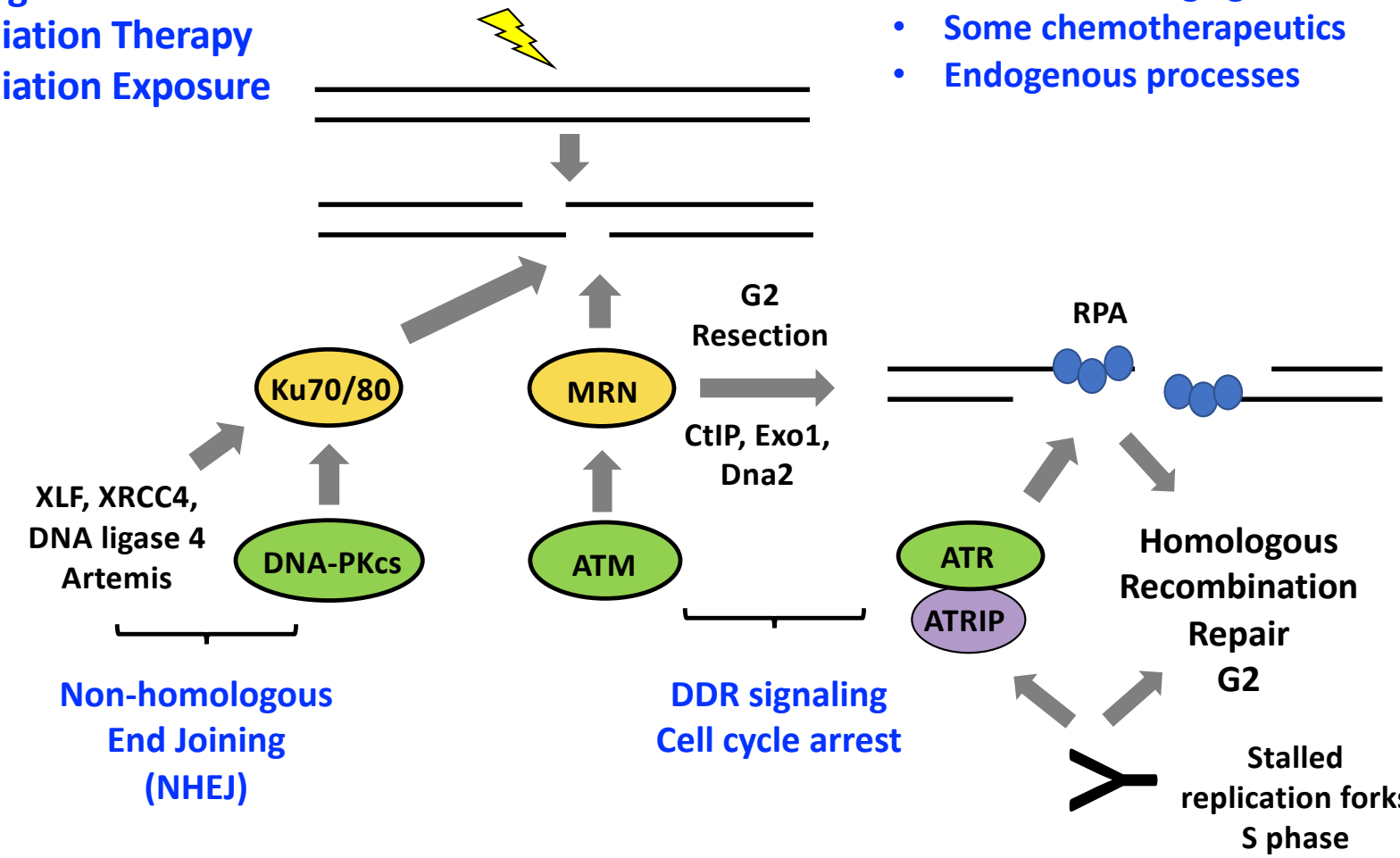
Serine/threonine protein kinases
Activated in response to DNA damage
Phosphorylate substrates on SQ/TQ and other motifs

From: Blackford and Jackson, Molecular Cell, 2017

Cellular response to IR-induced DNA double strand breaks (DSBs)

Ionizing Radiation
- Radiation Therapy
- Radiation Exposure

Other DSB inducing agent
- Some chemotherapeutics
- Endogenous processes



1985- Human cells contain a DNA-activated protein kinase

Walker et al, EMBO J 4(1) 139-145 (1985)

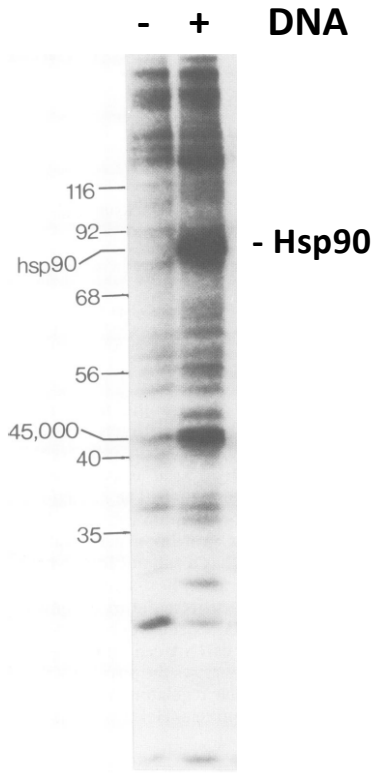
The EMBO Journal vol.4 no.1 pp.139–145, 1985

Double-stranded DNA induces the phosphorylation of several proteins including the 90 000 mol. wt. heat-shock protein in animal cell extracts

Anthony I.Walker[1], Tim Hunt, Richard J.Jackson and Carl W.Anderson[2]

Department of Biochemistry, Cambridge University, Tennis Court Road, Cambridge CB2 1QW, UK

[1]Present address: ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY, UK
[2]On sabbatical leave from: The Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA
Communicated by T.Hunt



Carl W. Anderson Ph.D
Brookhaven National Laboratory, NY

1989

Identification of DNA-PK phosphorylation sites in Hsp90 PEETQTQ

First example of SQ/TQ motif for PIKK

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 264, No. 29, Issue of October 15, pp. 17275–17280, 1989
Printed in U.S.A.

The Human Double-stranded DNA-activated Protein Kinase Phosphorylates the 90-kDa Heat-shock Protein, hsp90α at Two NH_2-terminal Threonine Residues*

(Received for publication, May 19, 1989)

Susan P. Lees-Miller and Carl W. Anderson‡
From the Biology Department, Brookhaven National Laboratory, Upton, New York 11973

The 90-kDa heat-shock protein, hsp90, is an abundant cytoplasmic protein that can be phosphorylated *in vitro* by a double-stranded (ds) DNA-activated protein kinase found in cells from several species. Here we show that the dsDNA-activated protein kinase from human HeLa cells phosphorylates 2 threonine residues in the sequence PEETQTQDQPME at the amino terminus of human hsp90α. Hsp90β, which is 97% identical to hsp90α but lacks both amino-terminal threonines, is not phosphorylated by the dsDNA-activated protein kinase. Mouse hsp86 and rabbit hsp90α are homologous to human hsp90α; both heterologous proteins are phosphorylated at the same amino-terminal threonines by the human dsDNA-activated protein kinase.

the biochemical function of hsp90 is not known, hsp90 is complexed with nonactivated steroid hormone receptors (Catelli *et al.*, 1985; Sanchez *et al.*, 1985; Baulieu, 1987), tyrosine oncogene kinases (Brugge, 1986), and actin (Koyasu *et al.*, 1986). Humans and mice have at least two functional hsp90 genes which encode very closely related hsp90 polypeptides. The human genes have been designated hsp90α and hsp90β (Simon *et al.*, 1987), and cDNAs corresponding to both genes have been sequenced (Rebbe *et al.*, 1987; Hickey *et al.*, 1989). Hsp90 purified from HeLa suspension cells contains nearly equal amounts of the α and β gene products (Lees-Miller and Anderson, 1989). The two forms of hsp90 are distinguished primarily by two short segments in the amino-terminal domain of hsp90α that are absent from hsp90β. One of these is at the amino terminus; PEEVHHG of hsp90β is replaced by

1990

Preliminary purification and characterization of DNA-PKcs (p350) and co-purification with Ku

MOLECULAR AND CELLULAR BIOLOGY, Dec. 1990, p. 6472–6481 Vol. 10, No. 12
0270-7306/90/126472-10$02.00/0

Human Cells Contain a DNA-Activated Protein Kinase that Phosphorylates Simian Virus 40 T Antigen, Mouse p53, and the Human Ku Autoantigen

SUSAN P. LEES-MILLER, YUH-RU CHEN, AND CARL W. ANDERSON*
Biology Department, Brookhaven National Laboratory, Upton, New York 11973

Received 27 June 1990/Accepted 5 September 1990

HeLa cells contain a serine/threonine protein kinase (DNA-PK) that is strongly activated in vitro by low concentrations of double-stranded DNA (dsDNA). Activation was specific for dsDNA; both natural DNAs and synthetic oligonucleotides functioned as kinase activators. The fact that DNA-PK activity was rapidly inhibited by incubation with dsDNA and ATP suggests that DNA-PK activity also may be regulated by autophosphorylation. During gel filtration, DNA-PK activity behaved as a 350-kDa protein, and highly purified DNA-PK contained a dsDNA-binding, 350-kDa polypeptide that was phosphorylated in a dsDNA-dependent manner. We conclude that this 350-kDa polypeptide is likely to be DNA-PK. Previously we showed that the dsDNA-activated kinase phosphorylates two threonines at the N terminus of hsp90α (S. P. Lees-Miller and C. W. Anderson, J. Biol. Chem. 264:17275–17280, 1989). Here we show that DNA-PK also phosphorylates the simian virus 40 large tumor antigen, the mouse tumor-suppressor protein p53, the human Ku autoantigen, and two unidentified HeLa DNA-associated polypeptides of 52 and 110 kDa. Identification of these and other newly identified DNA-binding substrates suggest that the dsDNA-activated kinase may regulate transcription, DNA replication, or cell growth.

Simultaneous discovery of DNA-PKcs
Tim Carter, William Dynan, Steve Jackson

MOLECULAR AND CELLULAR BIOLOGY, Dec. 1990, p. 6460–6471 Vol. 10, No. 12
0270-7306/90/126460-12$02.00/0

A DNA-Activated Protein Kinase from HeLa Cell Nuclei

TIMOTHY CARTER,* IVANA VANČUROVÁ, IVON SUN, WILLARD LOU, AND SUSAN DELEON
Department of Biological Sciences, St. John's University, Jamaica, New York 11439

Received 12 June 1990/Accepted 29 August 1990

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11920–11924, December 1992
Biochemistry

Ku autoantigen is the regulatory component of a template-associated protein kinase that phosphorylates RNA polymerase II

ARIK DVIR*, SCOTT R. PETERSON*, MARK W. KNUTH†‡, HUA LU§, AND WILLIAM S. DYNAN*¶

*Department of Chemistry and Biochemistry, Campus Box 215, University of Colorado, Boulder, CO 80309; †McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706; and §Department of Biochemistry, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, NJ 08544

Communicated by Joan A. Steitz, September 21, 1992

Cell, Vol. 63, 155–165, October 5, 1990, Copyright © 1990 by Cell Press

GC Box Binding Induces Phosphorylation of Sp1 by a DNA-Dependent Protein Kinase

Stephen P. Jackson,* Judy J. MacDonald,*
Susan Lees-Miller,† and Robert Tjian*
*Howard Hughes Medical Institute
Department of Molecular and Cell Biology
Division of Biochemistry and Cell Biology
University of California at Berkeley
Berkeley, California 94720
†Department of Biology
Brookhaven National Laboratory
Upton, New York 11973

Cell, Vol. 72, 131–142, January 15, 1993, Copyright © 1993 by Cell Press

The DNA-Dependent Protein Kinase: Requirement for DNA Ends and Association with Ku Antigen

Tanya M. Gottlieb and Stephen P. Jackson
Wellcome/CRC Institute
Tennis Court Road
Cambridge
CB2 1QR
England

The Phosphatidyl Inositol 3-kinase-like family of serine/threonine protein kinases, PIKKs

1995

A Single Ataxia Telangiectasia Gene with a Product Similar to PI-3 Kinase

Kinneret Savitsky,* Anat Bar-Shira,* Shlomit Gilad, Galit Rotman, Yael Ziv, Lina Vanagaite, Danilo A. Tagle, Sara Smith, Tamar Uziel, Sharon Sfez, Maya Ashkenazi, Iris Pecker, Moshe Frydman, Reli Harnik, Sankhavaram R. Patanjali, Andrew Simmons, Gregory A. Clines, Adam Sartiel, Richard A. Gatti, Luciana Chessa, Ozden Sanal, Martin F. Lavin, N. G. J. Jaspers, A. Malcolm R. Taylor, Colin F. Arlett, Toru Miki, Sherman M. Weissman, Michael Lovett, Francis S. Collins, Yosef Shiloh†

SCIENCE • VOL. 268 • 23 JUNE 1995

Cell, Vol. 82, 849–856, September 8, 1995,

DNA-Dependent Protein Kinase Catalytic Subunit: A Relative of Phosphatidylinositol 3-Kinase and the Ataxia Telangiectasia Gene Product

Katharine O. Hartley,* David Gell,* Graeme C. M. Smith,* Hong Zhang,† Nullin Divecha,‡ Margery A. Connelly,† Arie Admon,§ Susan P. Lees-Miller,†‖ Carl W. Anderson,† and Stephen P. Jackson*

Carl W. Anderson Ph.D

BA	Harvard 1966
PhD	Washington University 1970
PDF	Cold Spring Harbour 1971-1974
1975	Biology Department, Brookhaven National Laboratory, NY
	Chair, 1999-2011
2011	Senior Scientist Emeritus
	Visiting scientist at National Institute of Environmental Health Sciences (NIEHS), NC

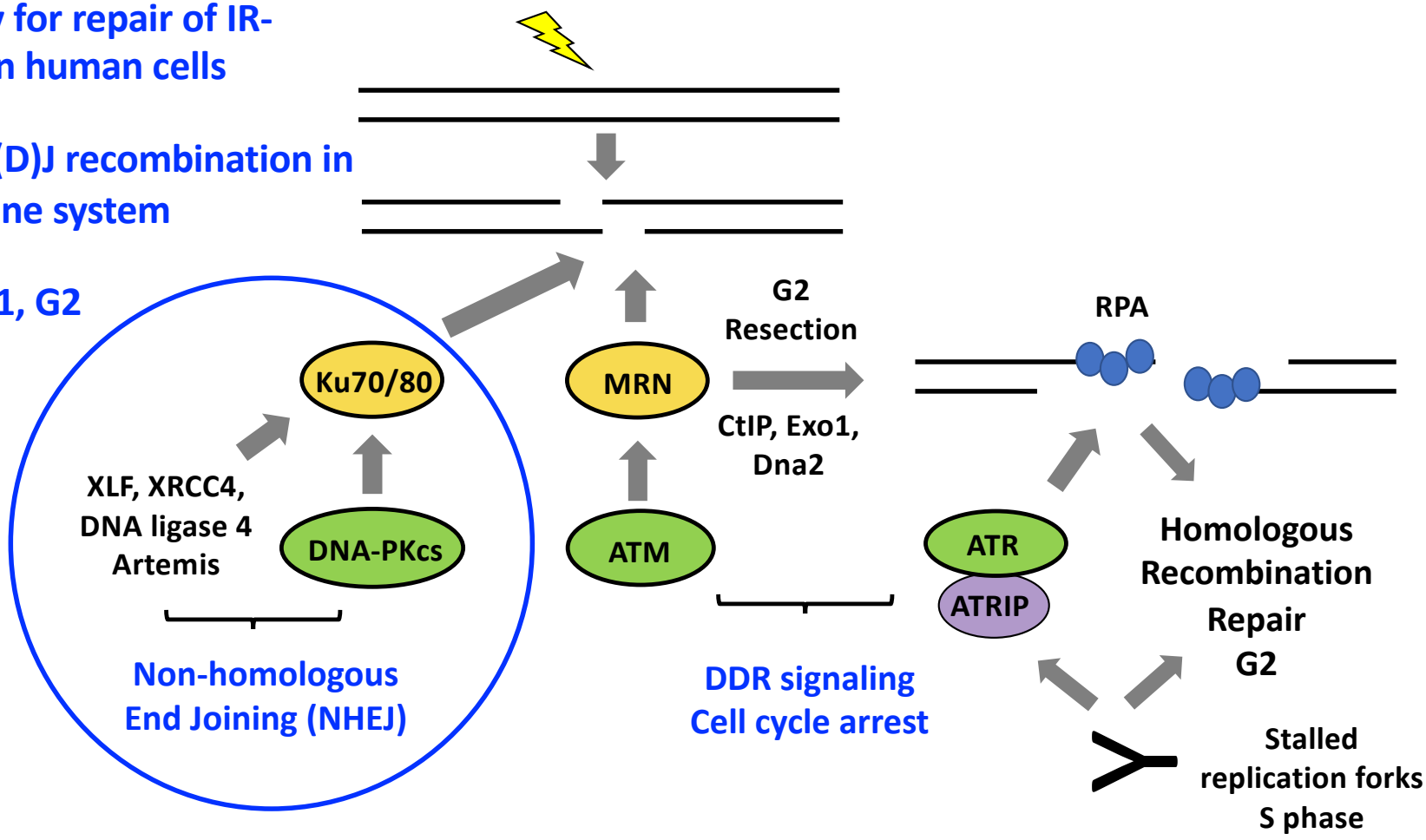
October 21 2020
Age 76

Research interests:

- Poliovirus genome
- Discovery of DNA-PK, early work on DNA-PK purification, phosphorylation, and cloning.
- Post-translational modification of p53 with Ettore Appella NIH.
- Founded the International Association for Protein Structure Analysis and Proteomics

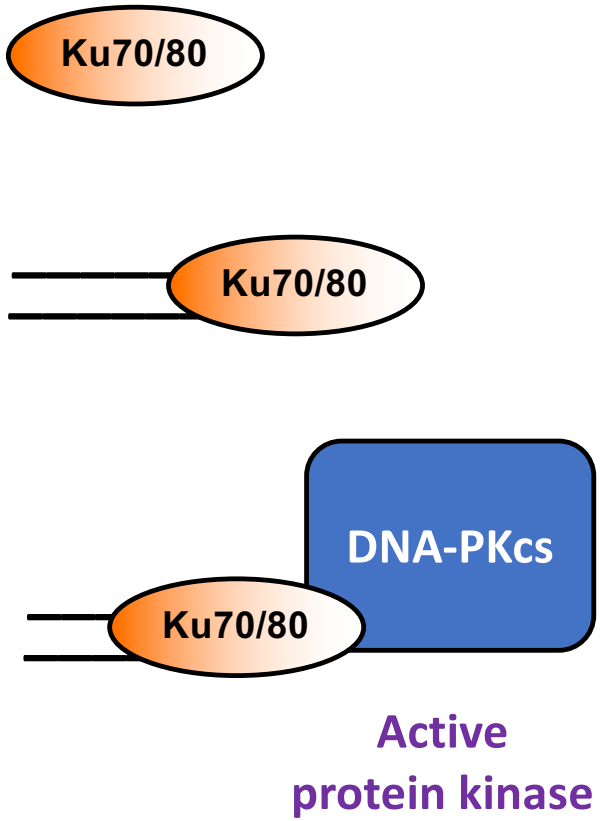
Mechanism of Non-Homologous End Joining (NHEJ):

- Major pathway for repair of IR-induced DSBs in human cells
- Required for V(D)J recombination in adaptive immune system
- Active in G0, G1, G2
- Rapid repair
- Error prone



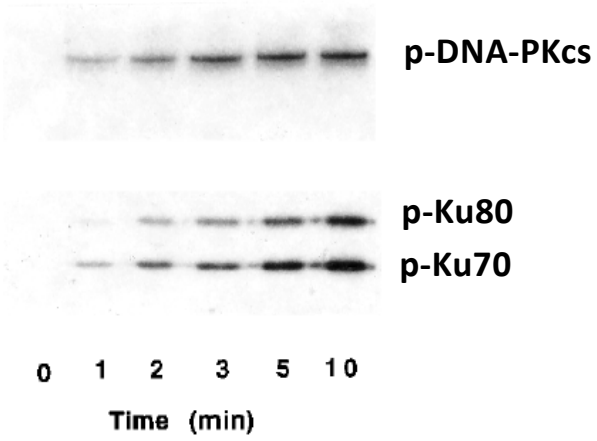
DNA-PKcs and Ku70/80 assemble on ends of dsDNA to form DNA-PK

- Ku binds with high affinity to ends of dsDNA
- In the presence of DNA-PKcs, Ku translocates inwards so that DNA-PKcs now occupies the extreme ends of the DNA *(Yoo and Dynan, NAR, 1999)*
- DNA-PKcs plus Ku assembled on dsDNA = DNA-PK

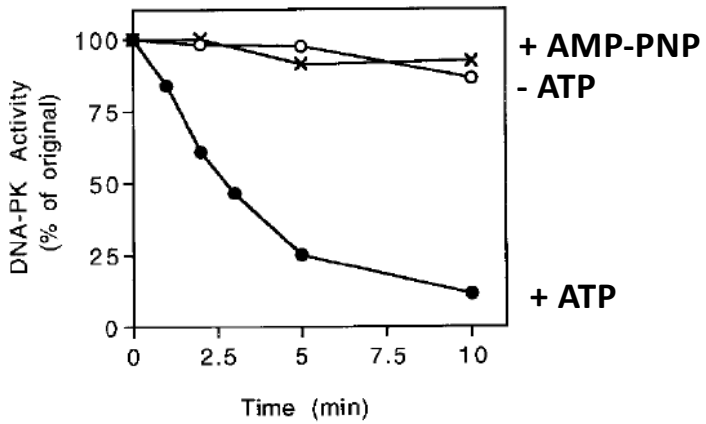


Autophosphorylation of DNA-PK results in disruption of the Ku-DNA-PKcs complex and loss of activity

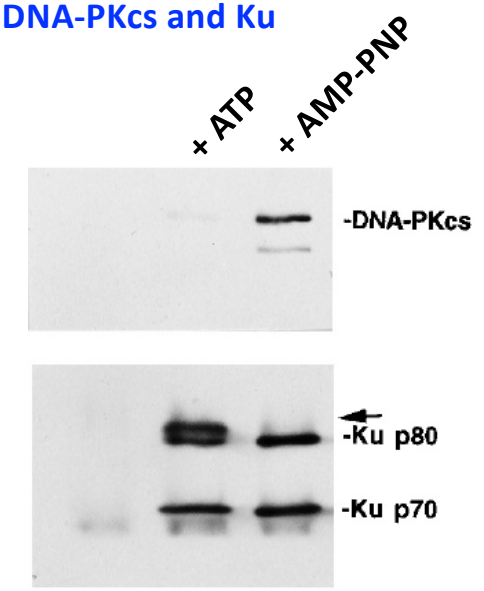
Autophosphorylation of purified DNA-PKcs, Ku70 and Ku80



Pre-incubation with ATP results in loss of DNA-PK activity



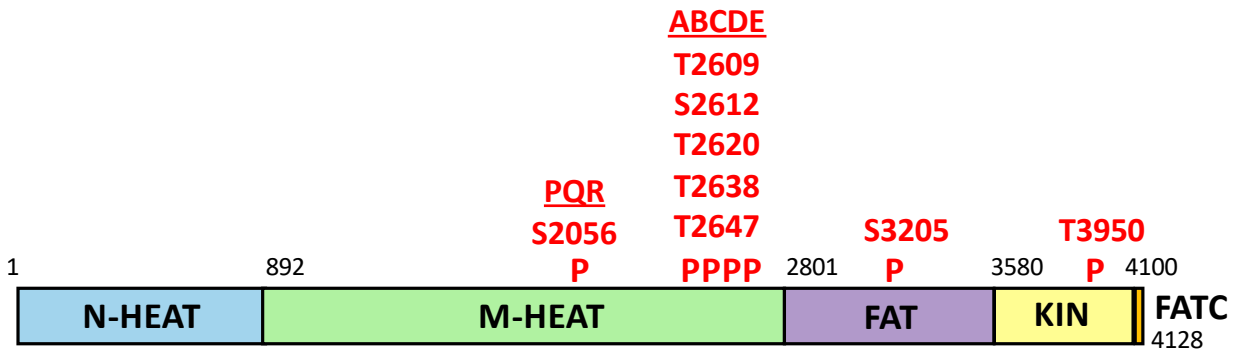
Autophosphorylation disrupts the interaction of DNA-PKcs and Ku



Purified: DNA-PKcs, Ku70/80
IP: Ku70/80
WB: DNA-PKcs and Ku

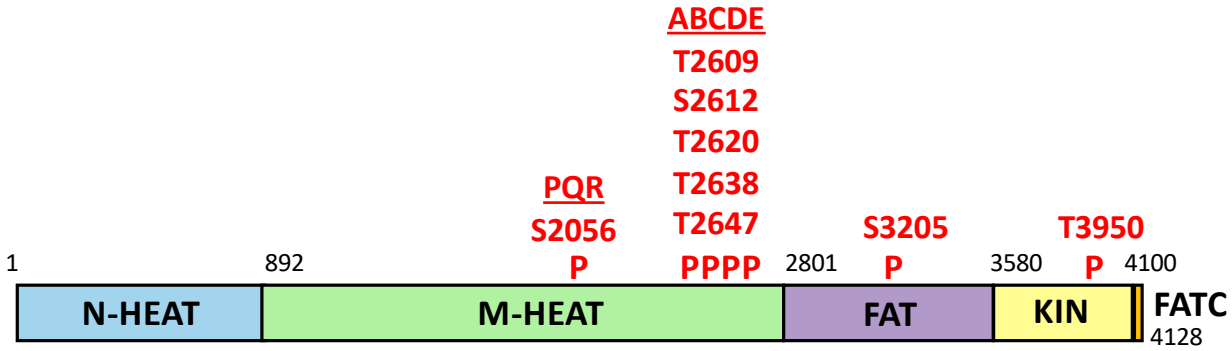
Chan and Lees-Miller, JBC, 1996

Identification of DNA-PKcs autophosphorylation sites

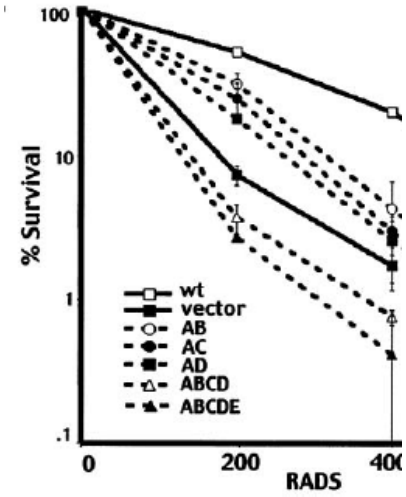


ABCDE and S3205	*Douglas et al, BJ, 2002*
T2609	*Chan et al, G&D, 2002*
2056 and PQR	*Cui et al MCB 2005; Chen et al, JBC, 2005*
T3950	*Douglas et al, MCB, 2007*

Autophosphorylation of ABCDE and PQR phosphorylation sites are important for DSB repair and VDJ recombination



Kathy Meek
Michigan State University



+ DNA-PKcs

- DNA-PKcs

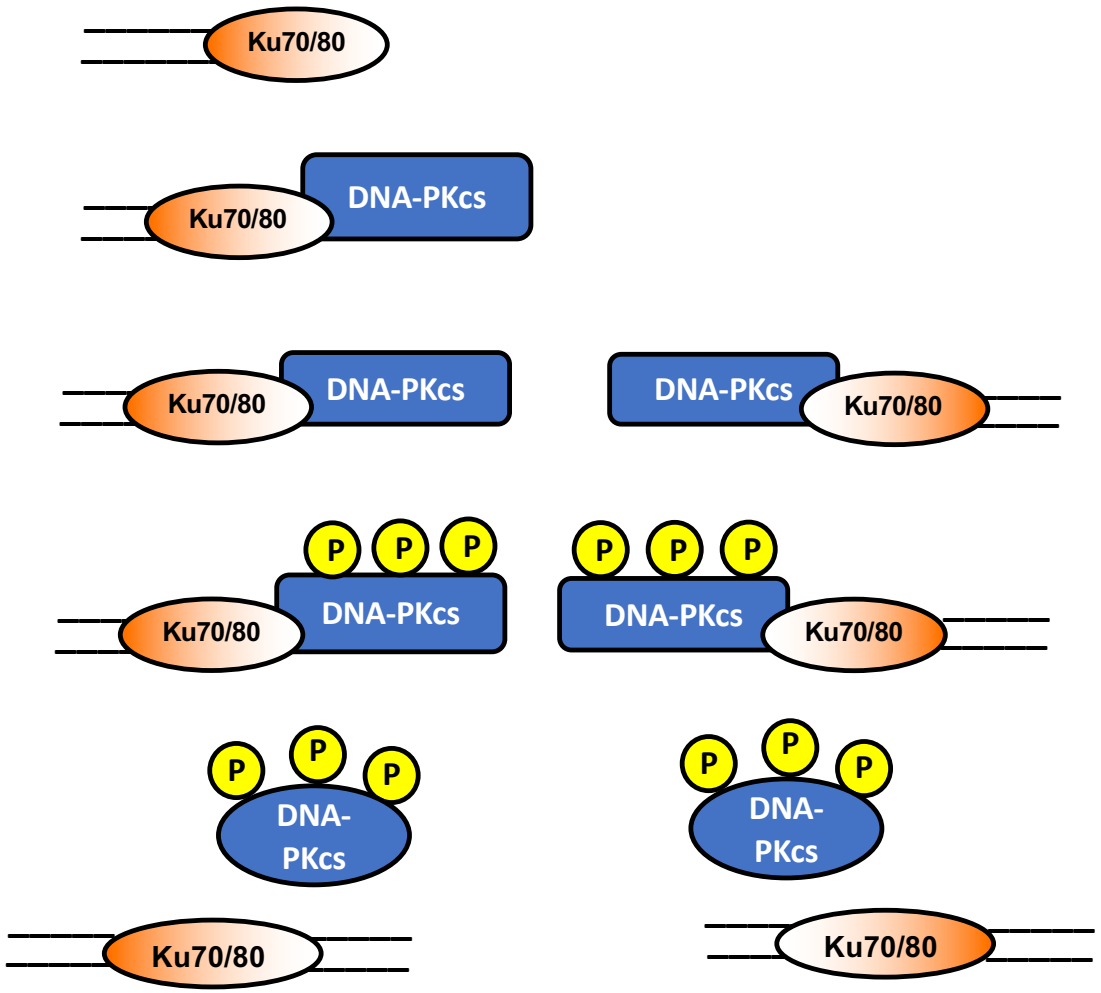
- ABCDE>A

DNA-PKcs null cells re-expressing DNA-PKcs-ABCDE>A are more sensitive to IR than cells lacking DNA-PKcs

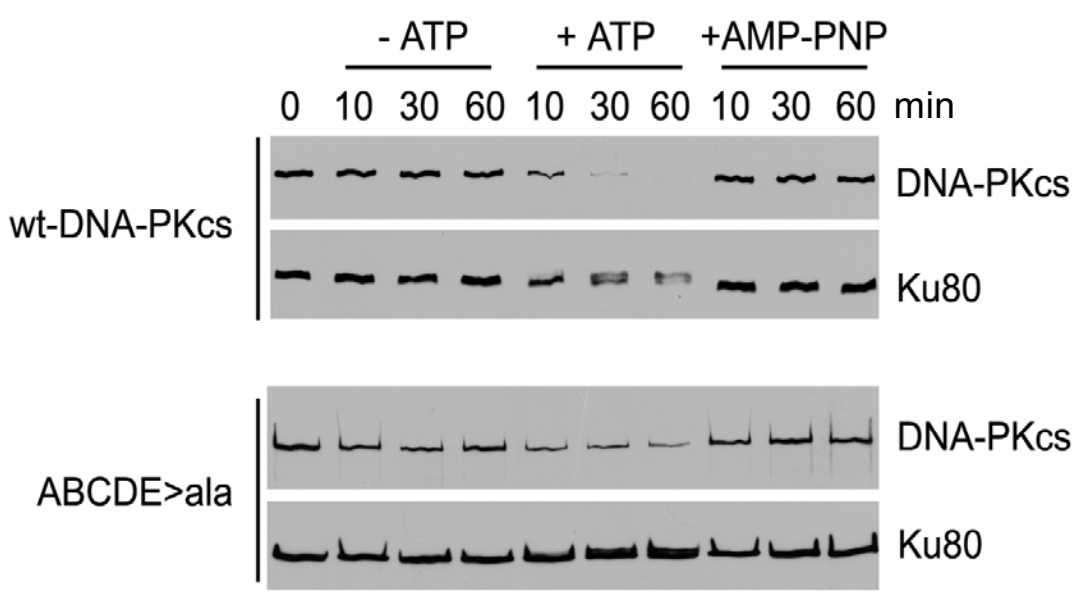
Ding et al, MCB 2003

Model for assembly of Ku and DNA-PKcs on dsDNA ends

- Ku binds DSB
- Recruits DNA-PKcs
- Assembly of a synaptic complex on both ends of the DSB (active DNA-PK)
- Autophosphorylation of DNA-PKcs (in trans?)
- Dissociation of autophosphorylated DNA-PKcs



Ablation of ABCDE sites impairs release of DNA-PKcs from DNA-bound Ku in vitro

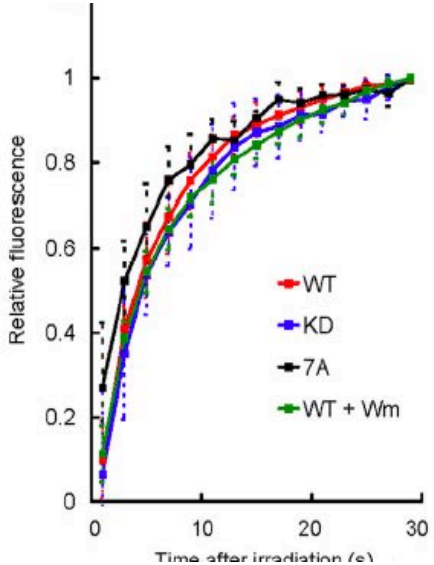


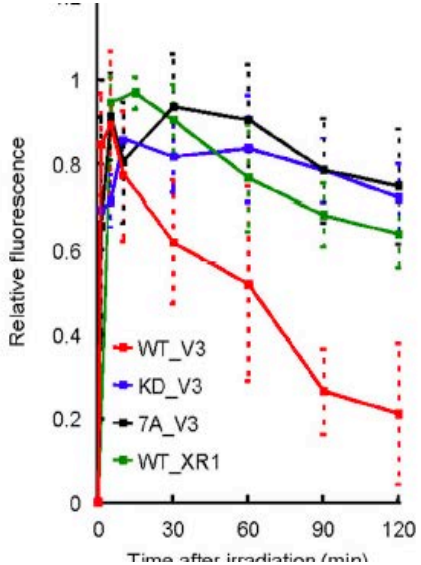
Purified DNA-PKcs and Ku
dsDNA-Biotin streptavidin-bead pull down assays
Western blot DNA-PKcs and Ku

Hammel et al, J. Biol. Chem. 2010
Jette et al, Prog. Biophys. Mol. Biol. 2015

In vivo evidence for autophosphorylation induced dissociation of DNA-PKcs from DSBs

UV laser microbeam irradiation - GFP-DNA-PKcs





Wt-DNA-PKcs rapidly dissociates from UV laser induced DNA damage

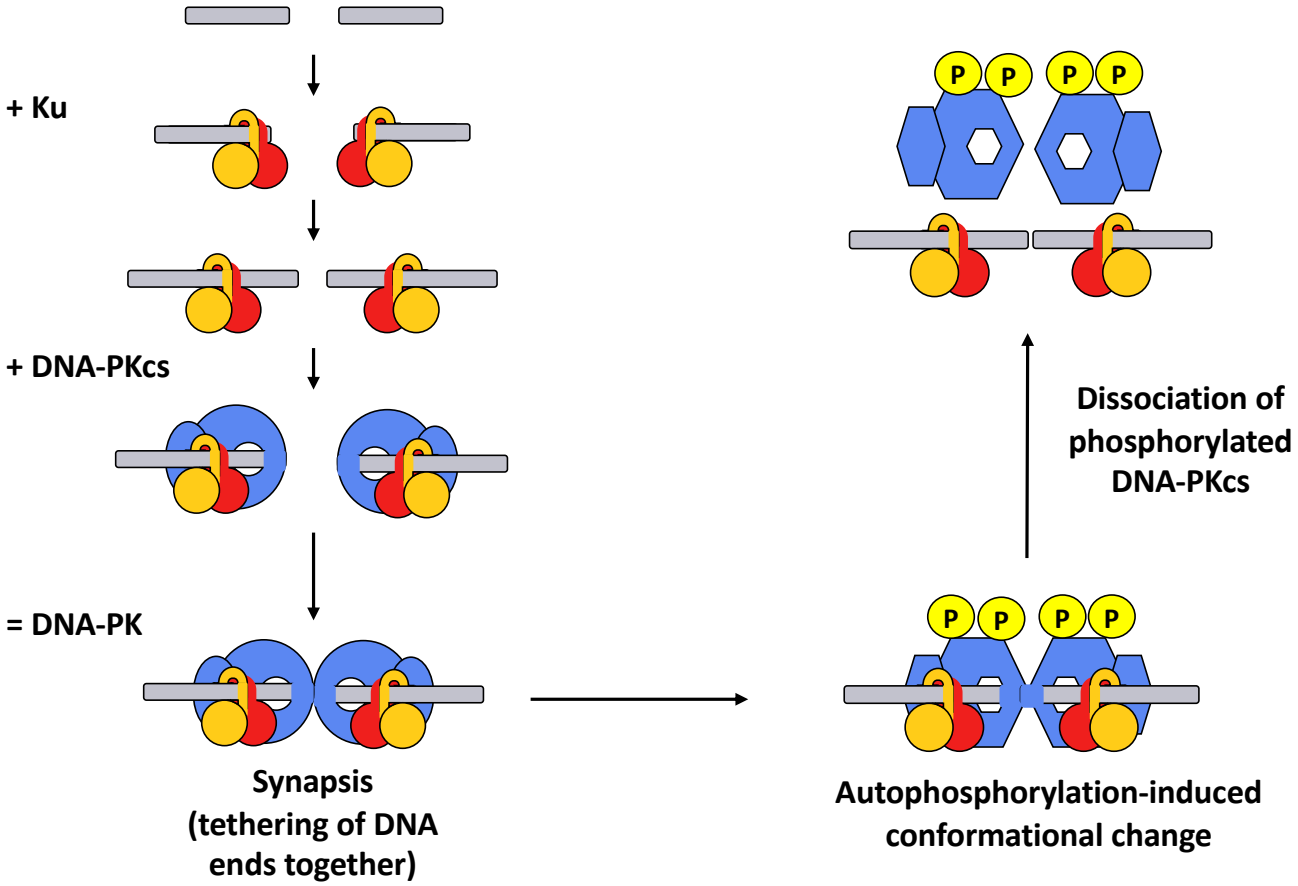
Phosphorylation defective (ABCDE: T to A)

and kinase dead

are retained

Uematsu et al, J Cell Biol. 2007 (David Chen)

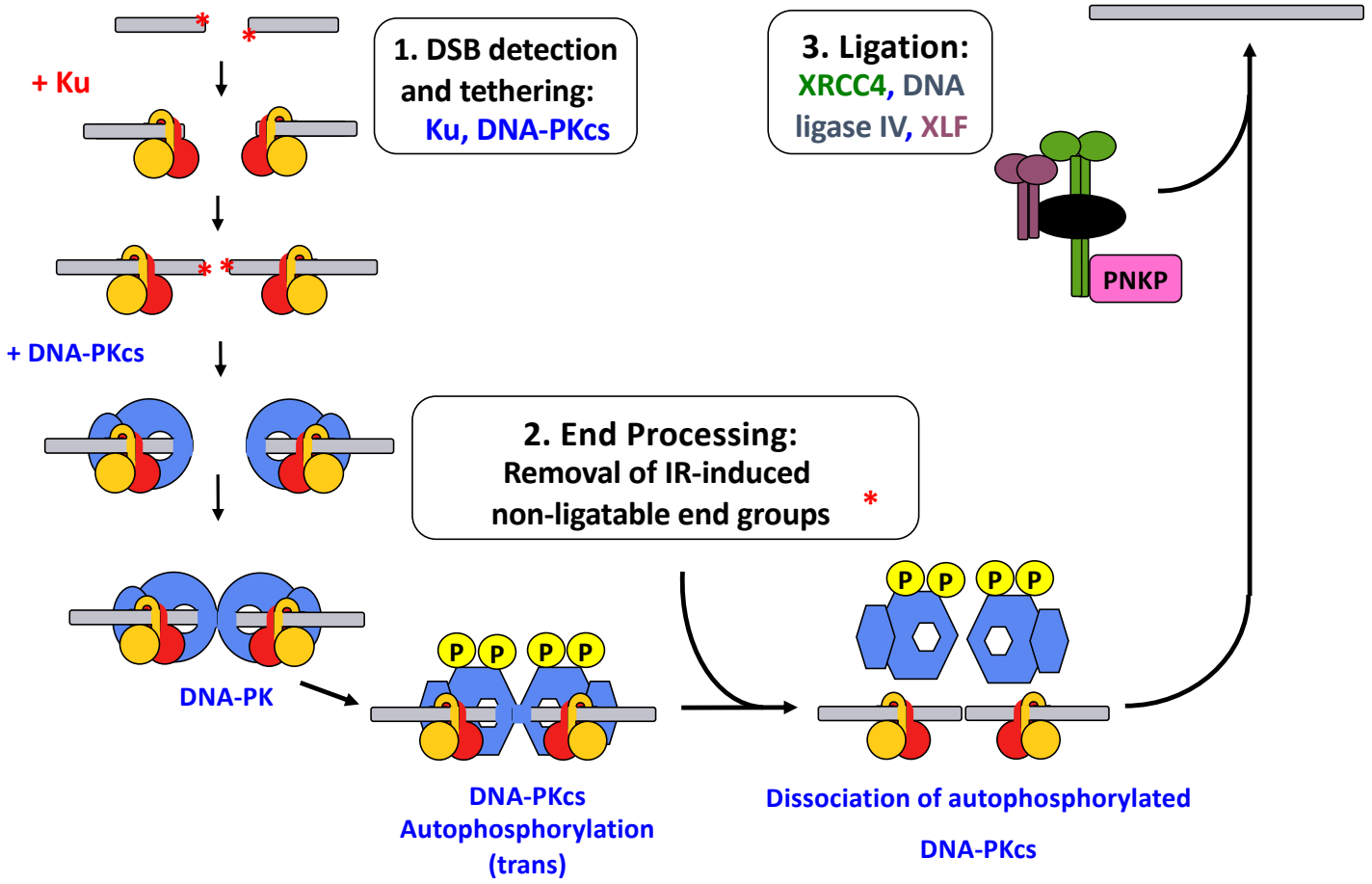
Model for assembly and autophosphorylation induce disassembly of the DNA-PK complex in the initial stages of NHEJ:



Model for NHEJ incorporating detection of DSB by Ku, recruitment and autophosphorylation of DNA-PKcs, removal of non-ligatable end-groups and ligation

1. *IR induces DSBs with non-ligatible end groups*
2. *Detection of DSB*
3. *Tethering of DSB ends*

- *Autophosphorylation of DNA-PKcs*
- *Processing (removal of non-ligatable end-groups produced by IR)*
- *Ligation of DSB ends*
- *Removal of NHEJ machinery*



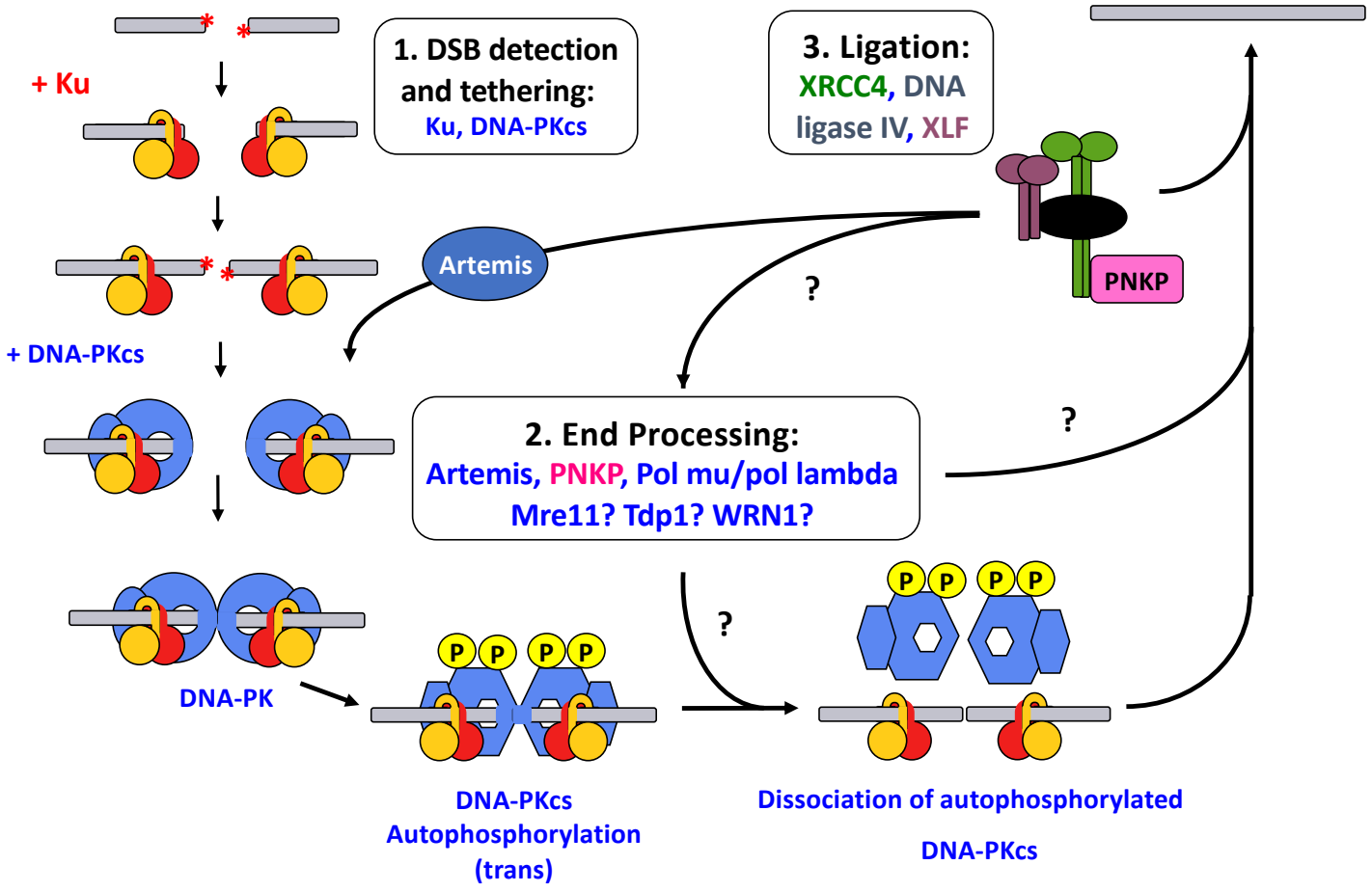
Model for NHEJ incorporating detection of DSB by Ku, recruitment and autophosphorylation of DNA-PKcs, removal of non-ligatable end-groups and ligation

Order of events?

Order of recruitment?

Artemis interacts with DNA-PKcs and DNA ligase IV. How/when is Artemis recruited?

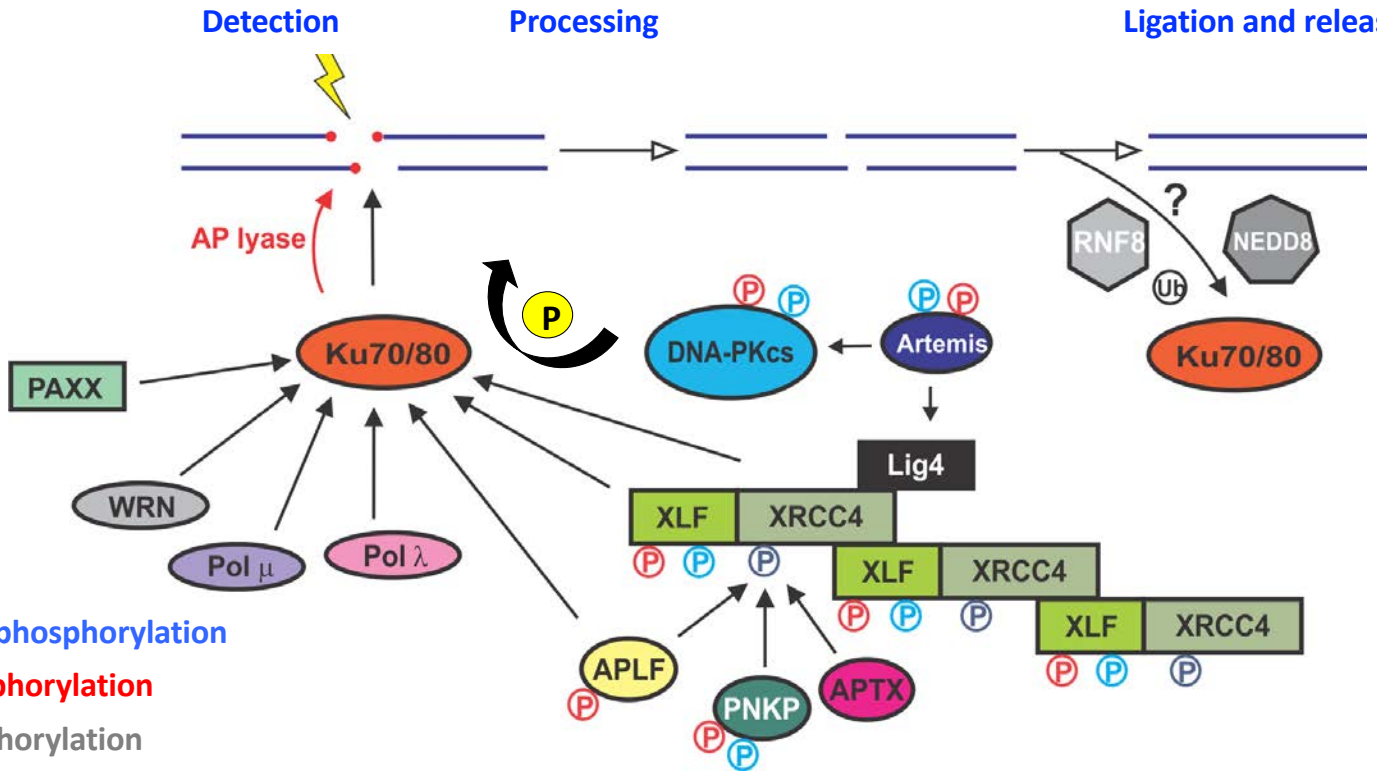
PNKP interacts with XRCC4. When is XRCC4 recruited? When does processing occur?



Assembly of a molecular machine

Detection, tethering, end-processing and ligation all occur within one dynamic molecular assembly

DSB ends always enclosed within assembly, hand-off from one stage to another



Adapted from Williams et al, DNA Repair, 2014
Yano and Chen, Cell Cycle 2008

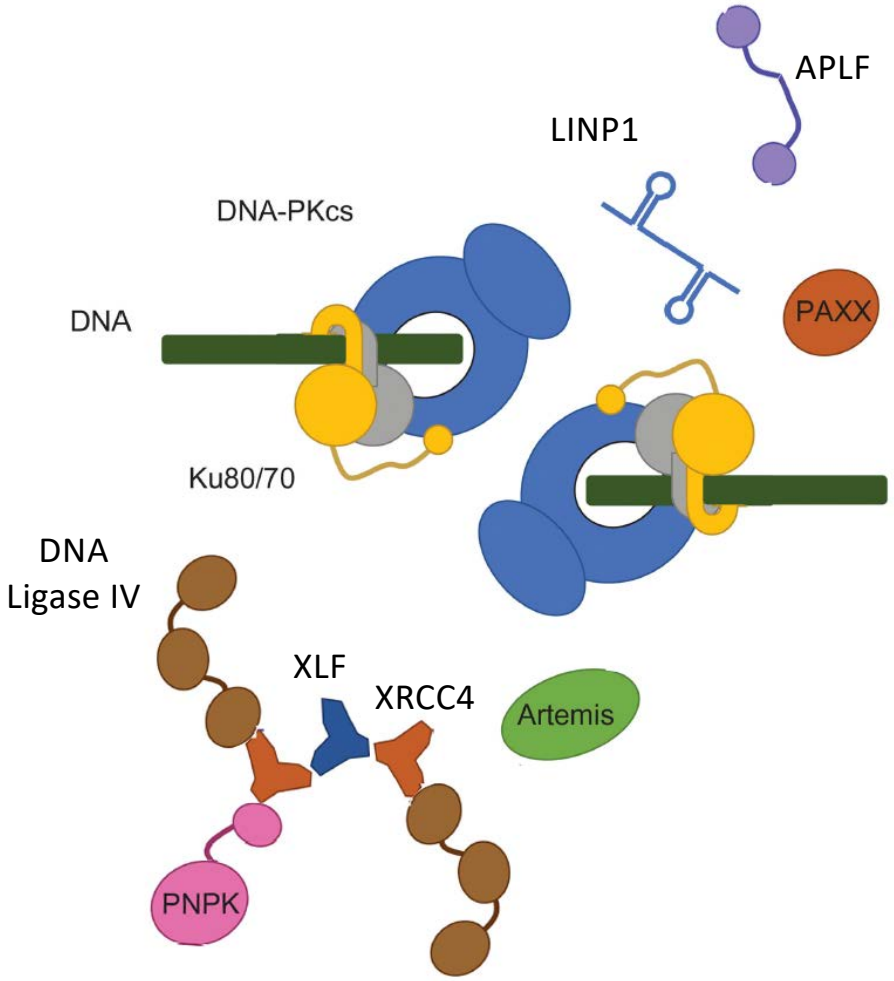
John Tainer
LBNL/MD Anderson

How does the NHEJ machinery assemble and carry out NHEJ?

- **How does the XLF-XRCC4-DNA ligase IV complex interact with the Ku-DSB-DNA-PKcs complex?**

- **How does Artemis interact with DNA-PKcs and DNA ligase IV?**

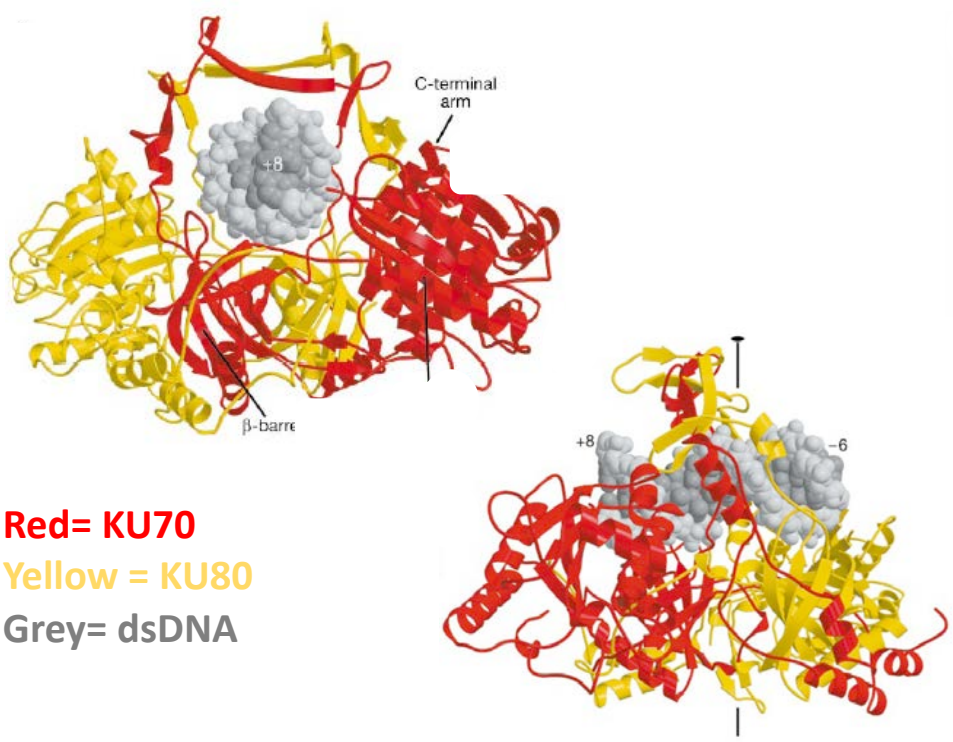
- **How do PAXX, APLF and LINP1 stabilize the synaptic complex?**



Hammel et al, JBC, 2016
Brosey et al, Methods Enzymol, 2017
Wang et al, Nature Struc. Mol. Biol. 2018
Thapar et al, NAR 2020

Structures of NHEJ components

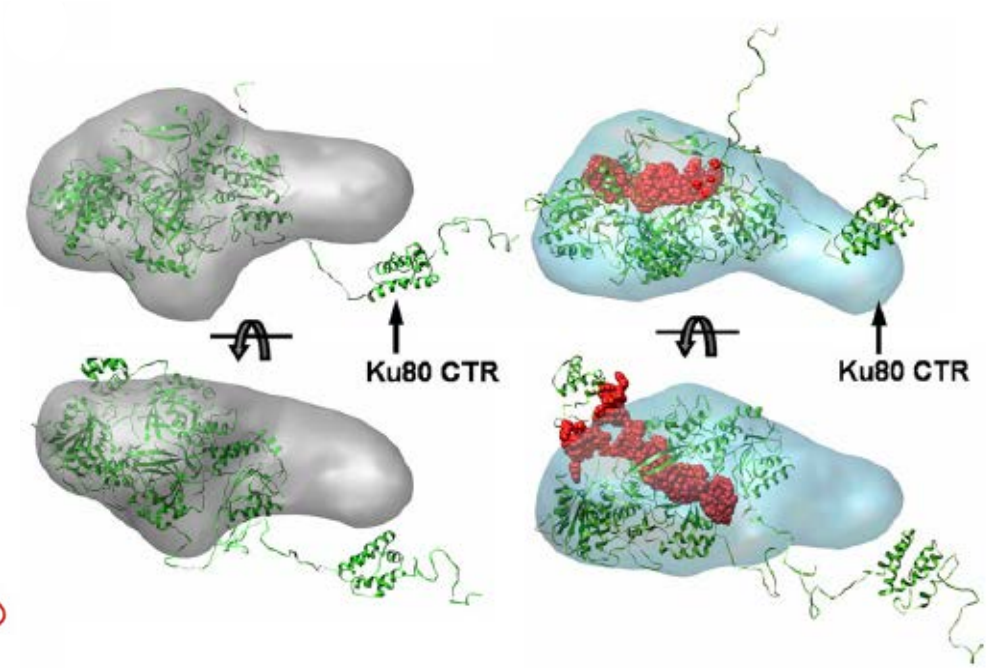
Structure of the Ku70/80 heterodimer, core DNA binding domain



Red= KU70
Yellow = KU80
Grey= dsDNA

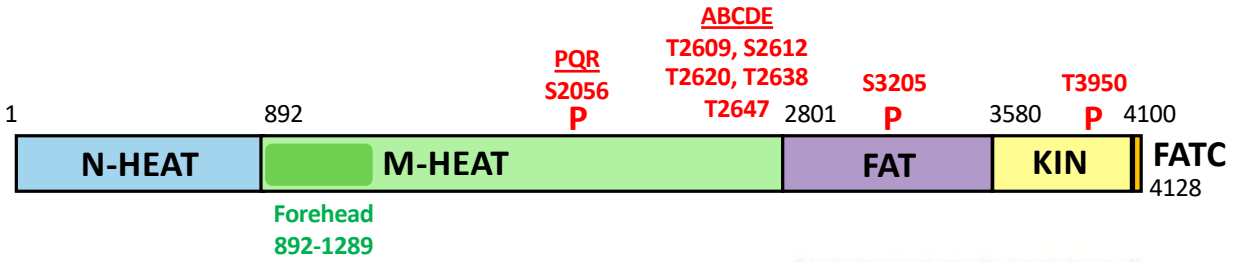
Walker et al, Nature 2001

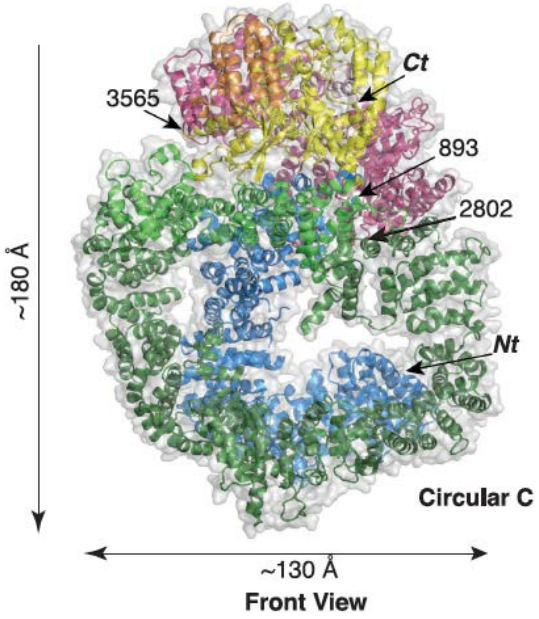
KU80 contains a flexible C-terminal "tail" that interacts with DNA-PKcs

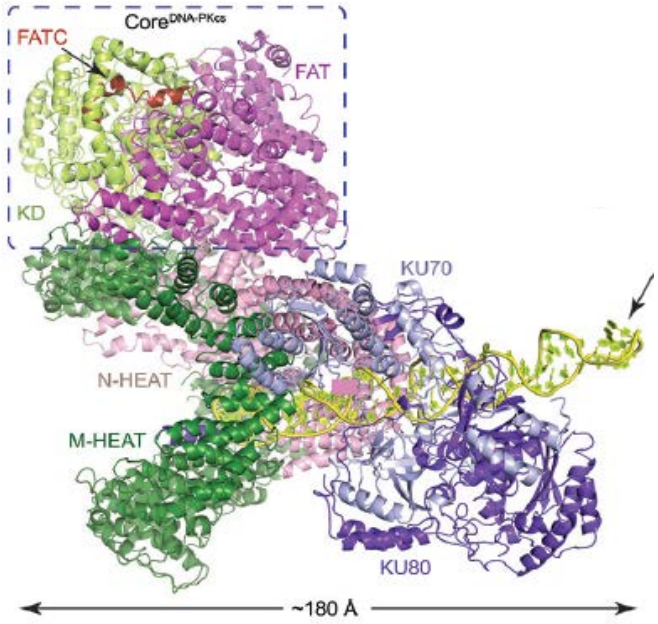


Hammel et al, JBC, 2010
Gell and Jackson, NAR, 1999
Harris et al, J. Mol. Biol. 2004

Structures of DNA-PKcs and DNA-PKcs-Ku-dsDNA (DNA-PK)







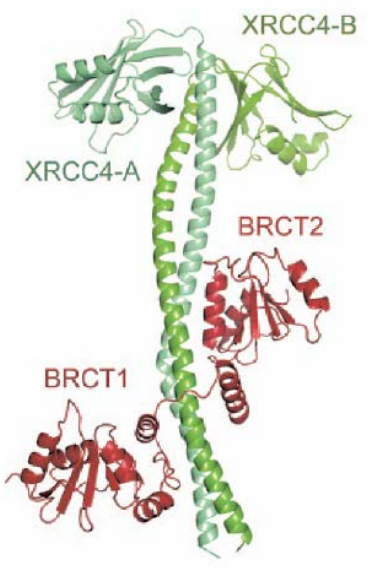
Sibanda et al, Nature, 2010
Sharif et al, PNAS, 2017
Chen et al, Molec Cell, 2021
Chaplin et al, Nat. Struc. Molec. Biol. 2021

Sibanda et al, Science, 2017

Yin et al, Cell Reports, 2017

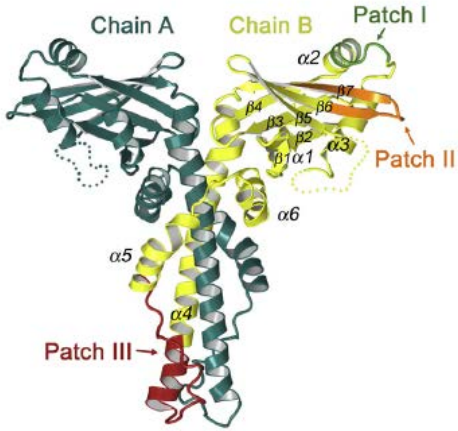
Structures of NHEJ components

XRCC4
Minus C terminal tail

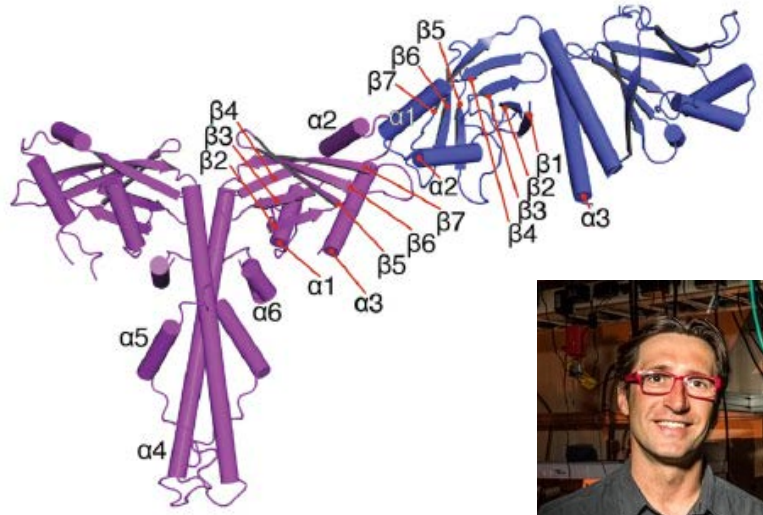


Junop et al, EMBO J, 2000
Sibanda et al, NSMB, 2001
Wu et al, MCB, 2009
Andres et al, Mol. Cell, 2007

XLF (XRCC4-like factor)
Minus C terminal tail



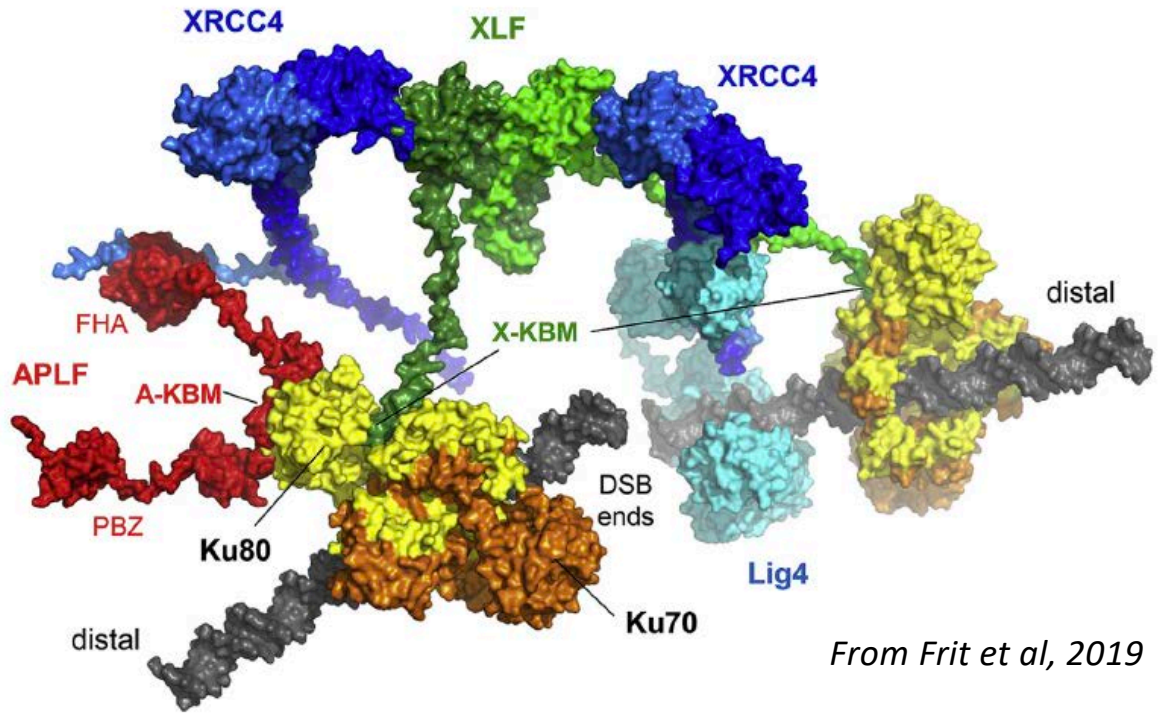
XRCC4 (truncated, blue) and XLF (truncated, magenta) interact via their head domains



Michal Hammel

Hammel et al, JBC, 2011

Models for how the NHEJ proteins may interact at DSBs based on individual structures



From Frit et al, 2019

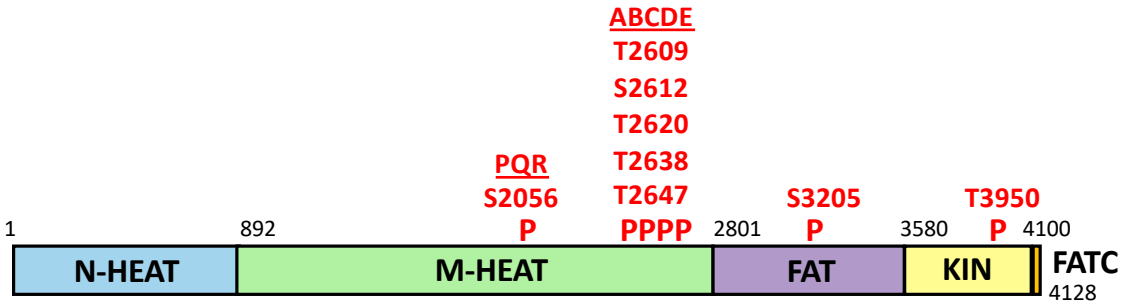
Hammel et al, JBC, 2011, 2016
Frit et al, Prog Biophys Mol Biol. 2019
Liang et al, Prog Biophys Mol Biol. 2020
Graham et al, Molec. Cell 2016

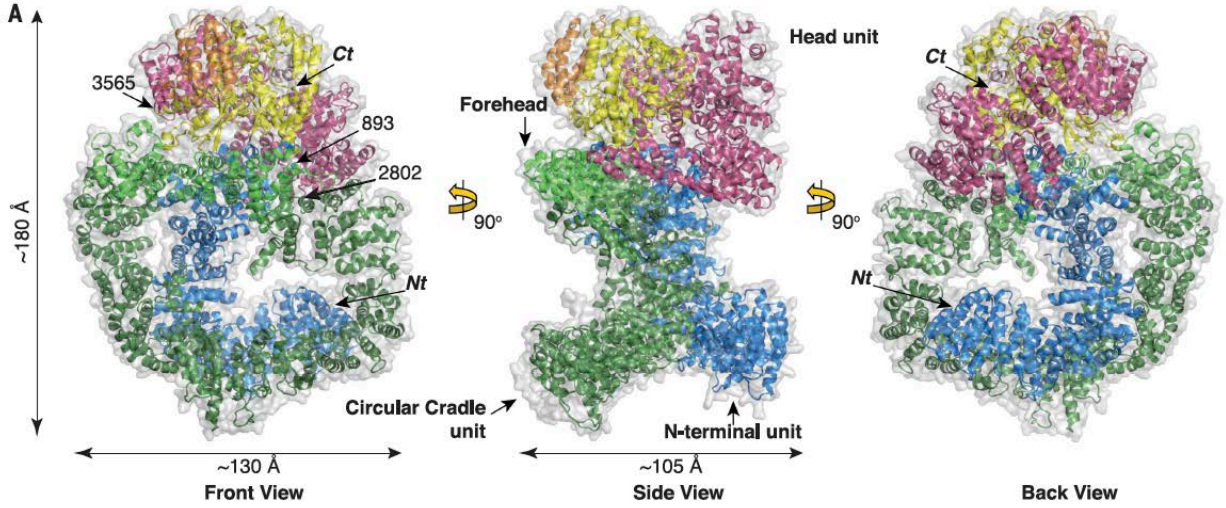
Challenges with understanding the function of DNA-PKcs

Its BIG

4128 amino acids

180Å x 130Åx 105Å





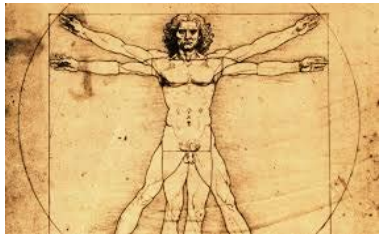
Sibanda et al, Science, 2017

What's the difference between these model organisms?

Contain DNA-PKcs/PRKDC

"DNA-PKcs is a vertebrate-specific PIKK"

Human



Mouse

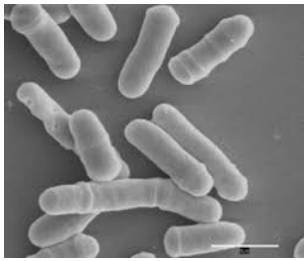
Danio rerio (Zebrafish)

No DNA-PKcs/PRKDC

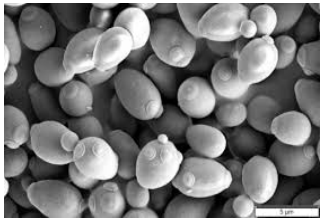
Drosophila melanogaster

Caenorhabditis elegans

Arabidopsis thaliana

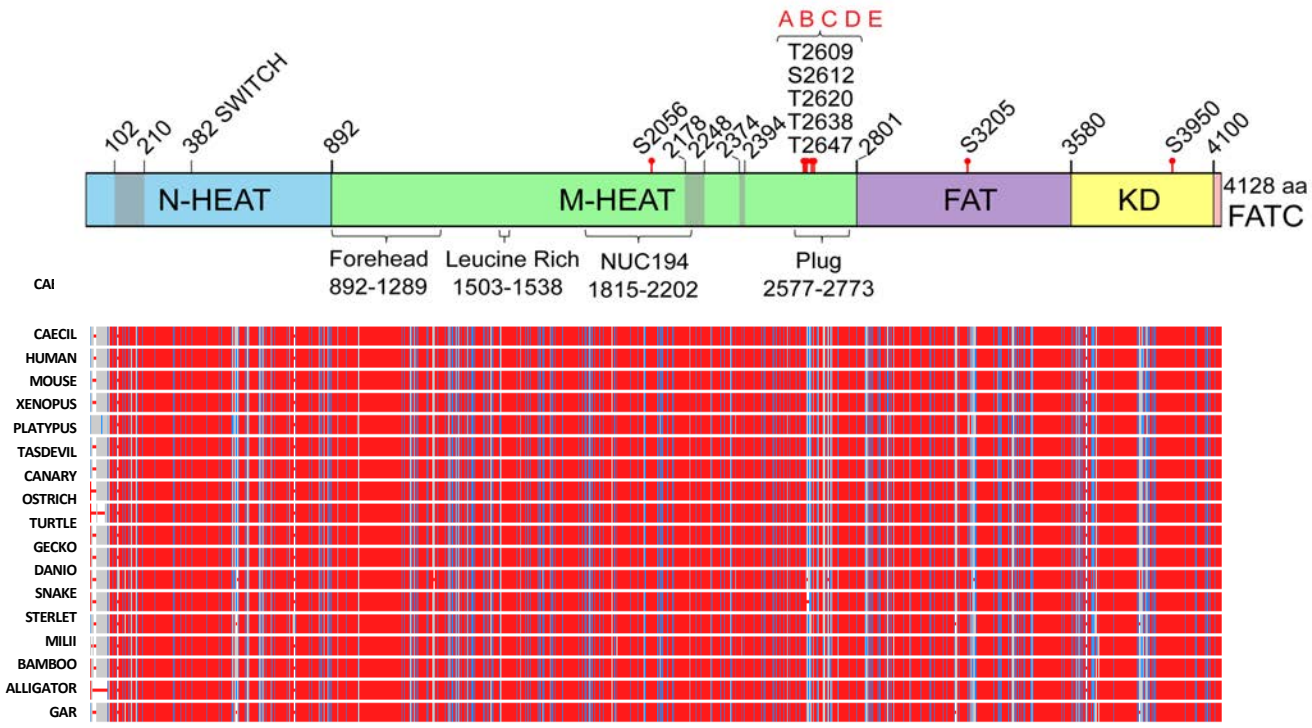


S. pombe



S. cerevisiae

DNA-PKcs is highly conserved in vertebrates



COBALT

Red = highly conserved

Blue = less conserved

Grey = no conservation

Lees-Miller et al, Prog. Biophys. Mol. Biol. 2020

DNA-PKcs is highly conserved from human to sponge (metazoa/vertebrates/invertebrates)

COBALT

Red = highly conserved
Blue = less conserved
Grey = no conservation

Lancelet

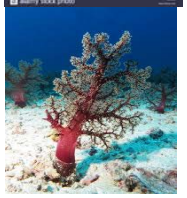
Annelid worm
Polychaete
Alvinella pompejana

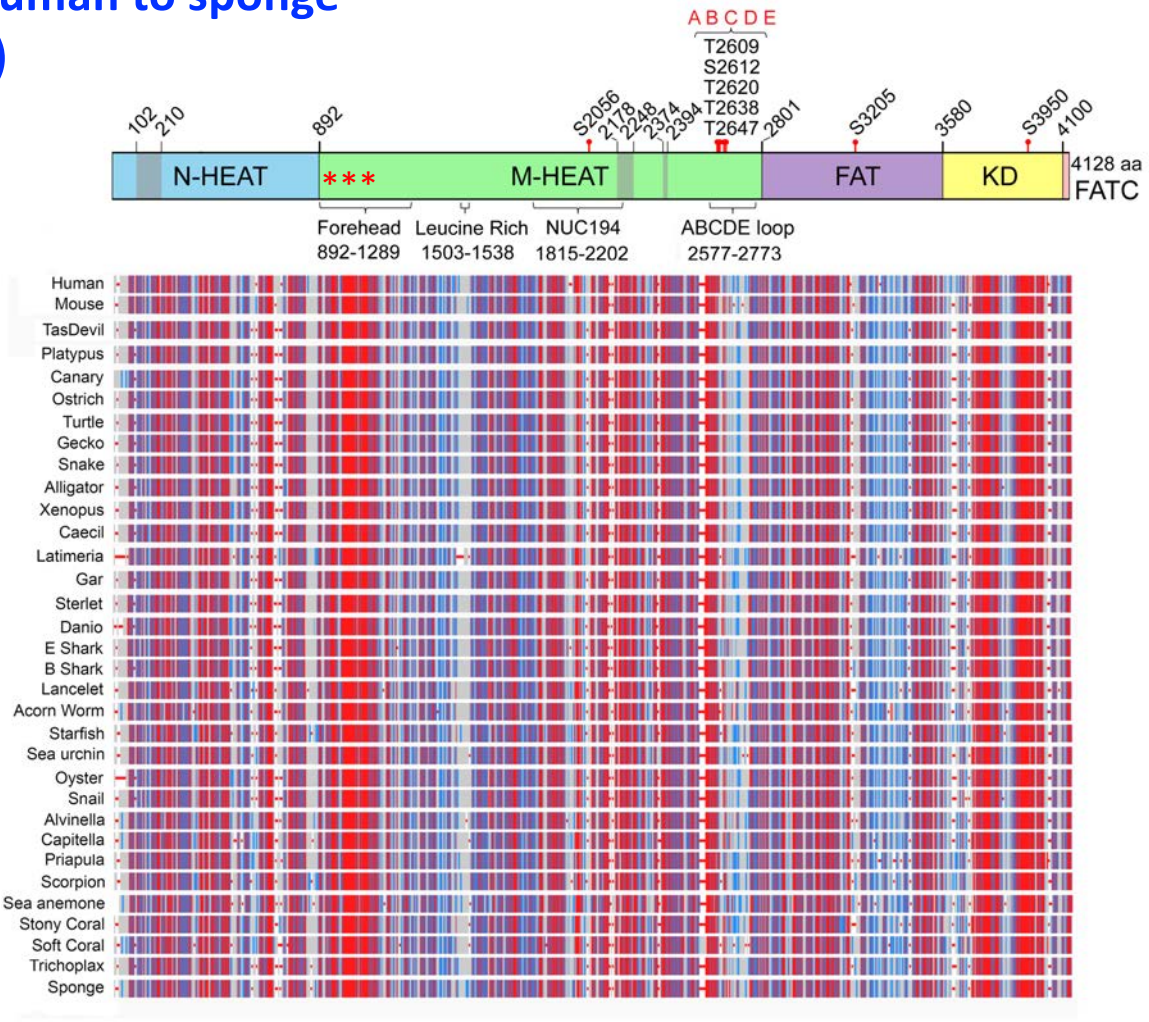
Scorpion

Trichoplax
Placazoa

Sea urchin

Coral





Lees-Miller et al, Prog. Biophys. Mol. Biol. 2020

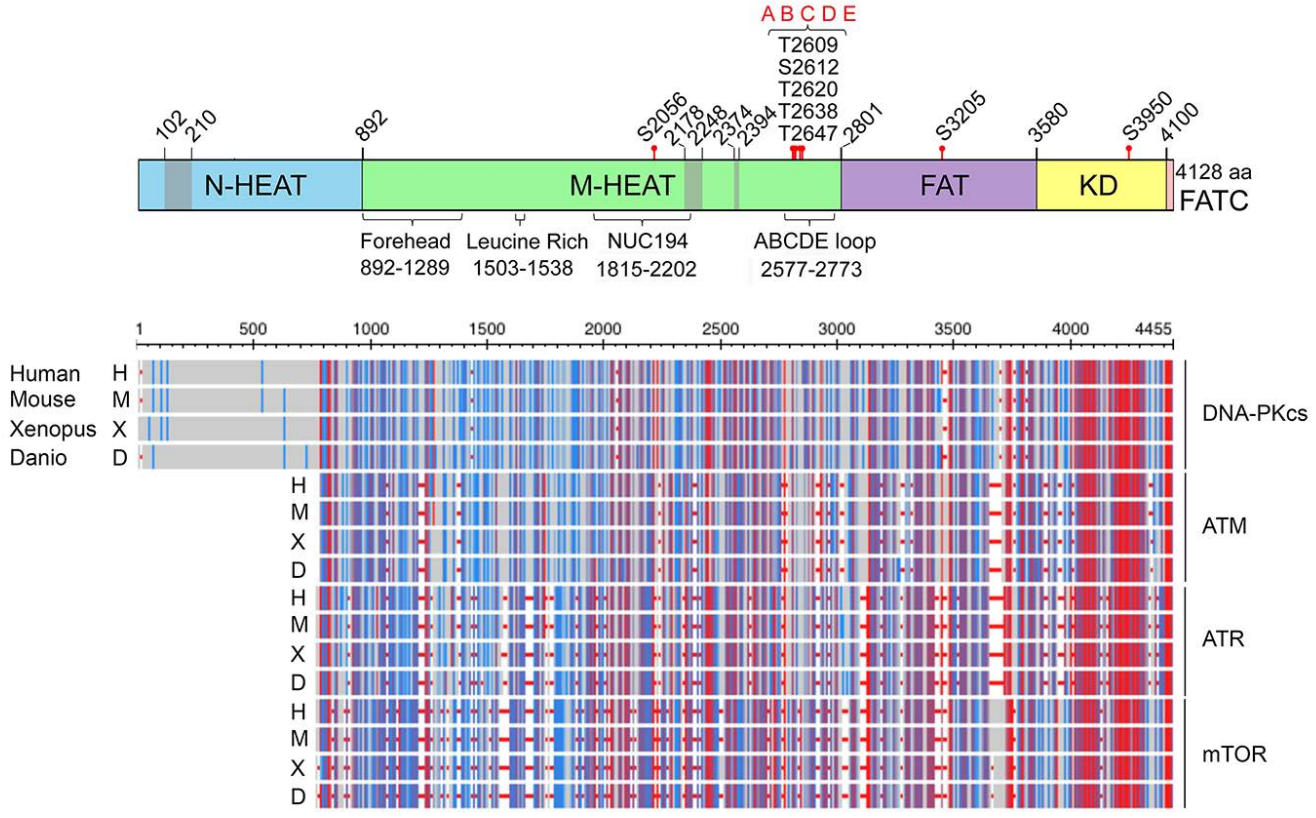
Weak amino acid similarity between DNA-PKcs and other PIKKs

Amino acid identity between DNA-PKcs ATM, ATR and mTOR From Clustal Omega

DNA-PKcs

- **Human: 100%**
- **Mouse: 80%**
- **Xenopus 64%**
- **Danio 58%**

- **ATM ~18%**
- **ATR ~ 17%**
- **mTOR ~16%**



COBALT

Red = highly conserved
Blue = less conserved
Grey = no conservation

DNA-PKcs conservation in representatives from all major phyla

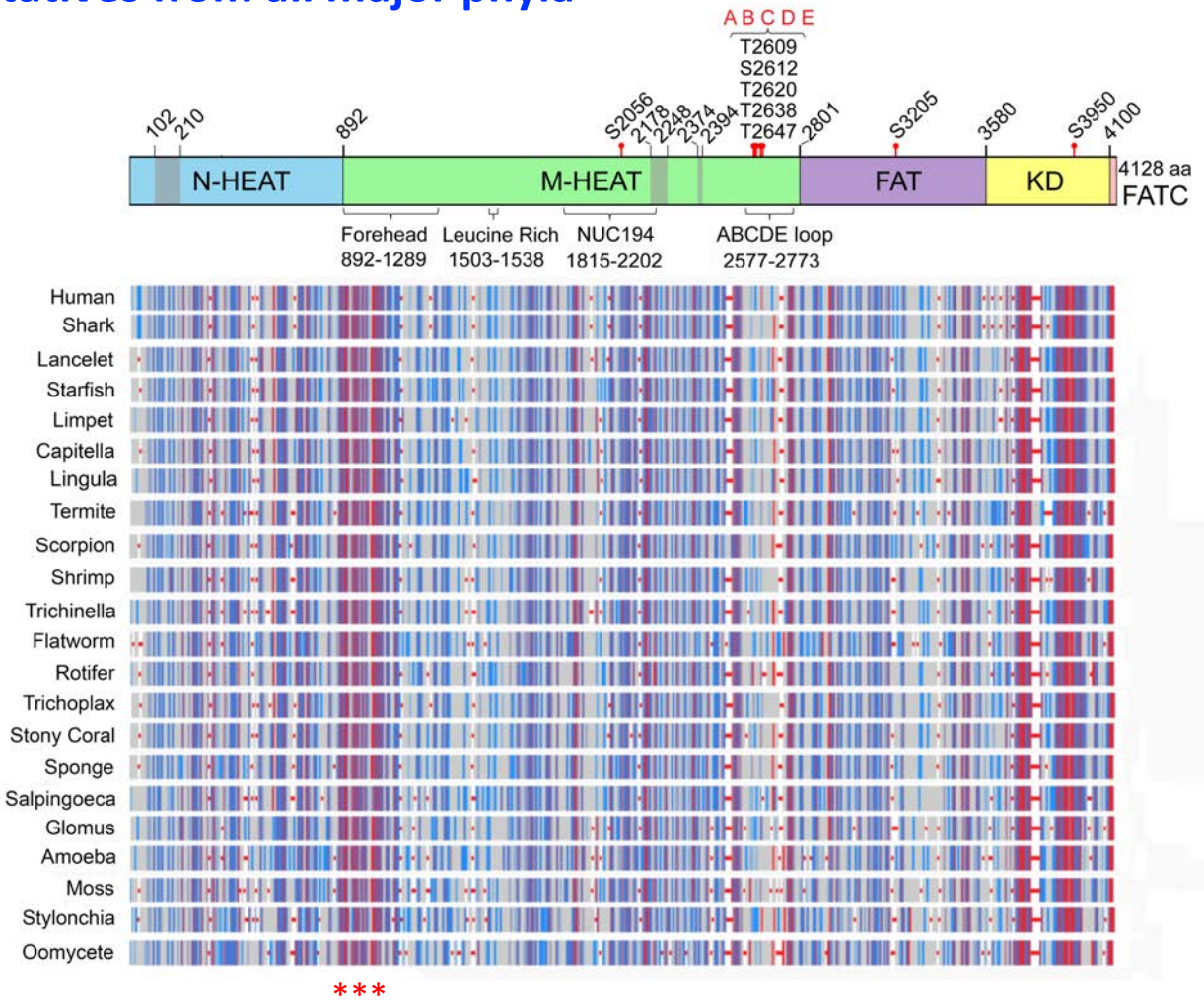
COBALT **Red = highly conserved**
Blue = less conserved
Grey = no conservation

Rotifer
Brachionus plicatilis

Nematode
Roundworm
Trichinella pseudospiralis

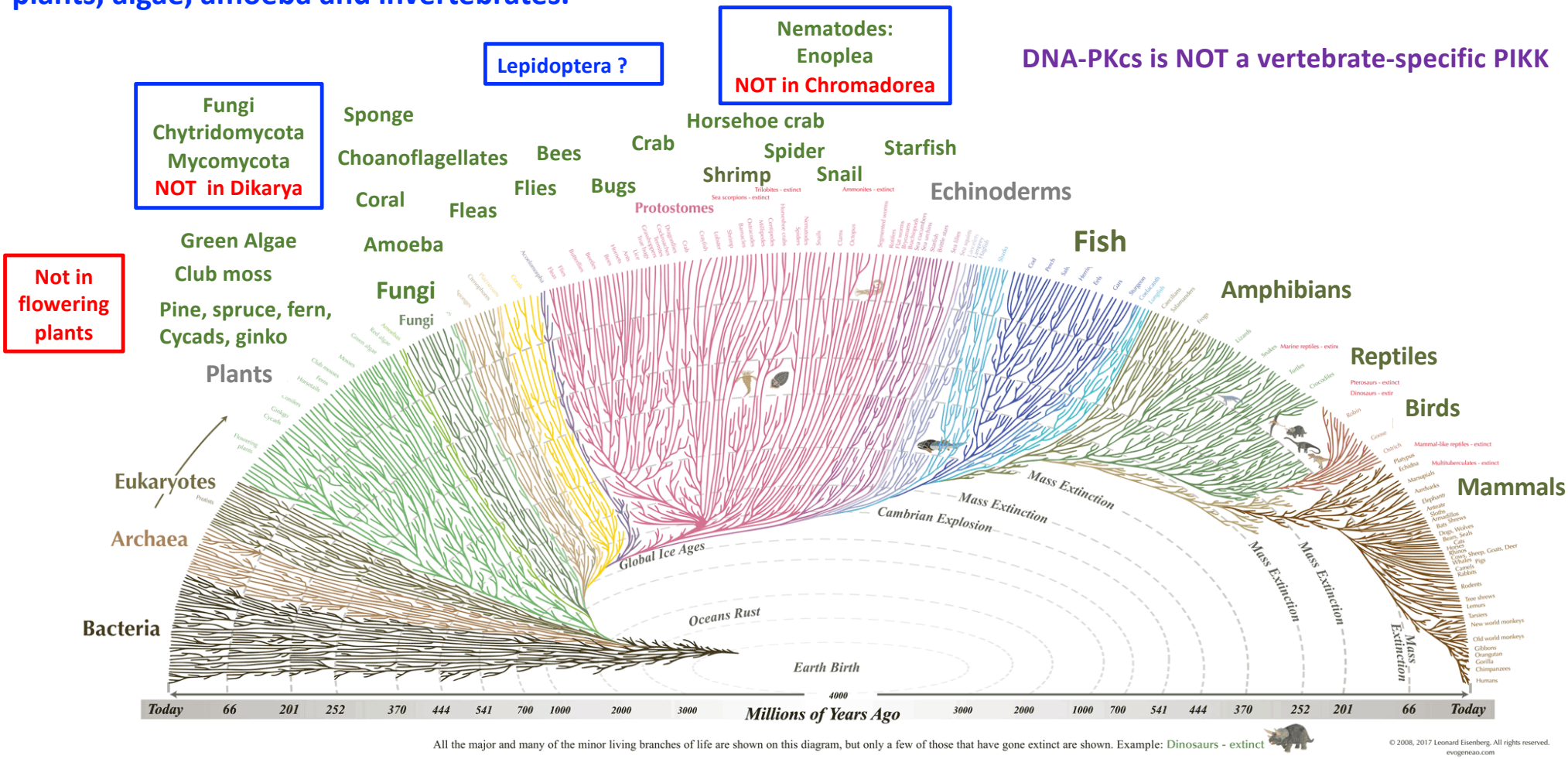
Plant
Moss
Physcomitrella patens

Oomycete
Leaf mold
Plasmopara halstedii



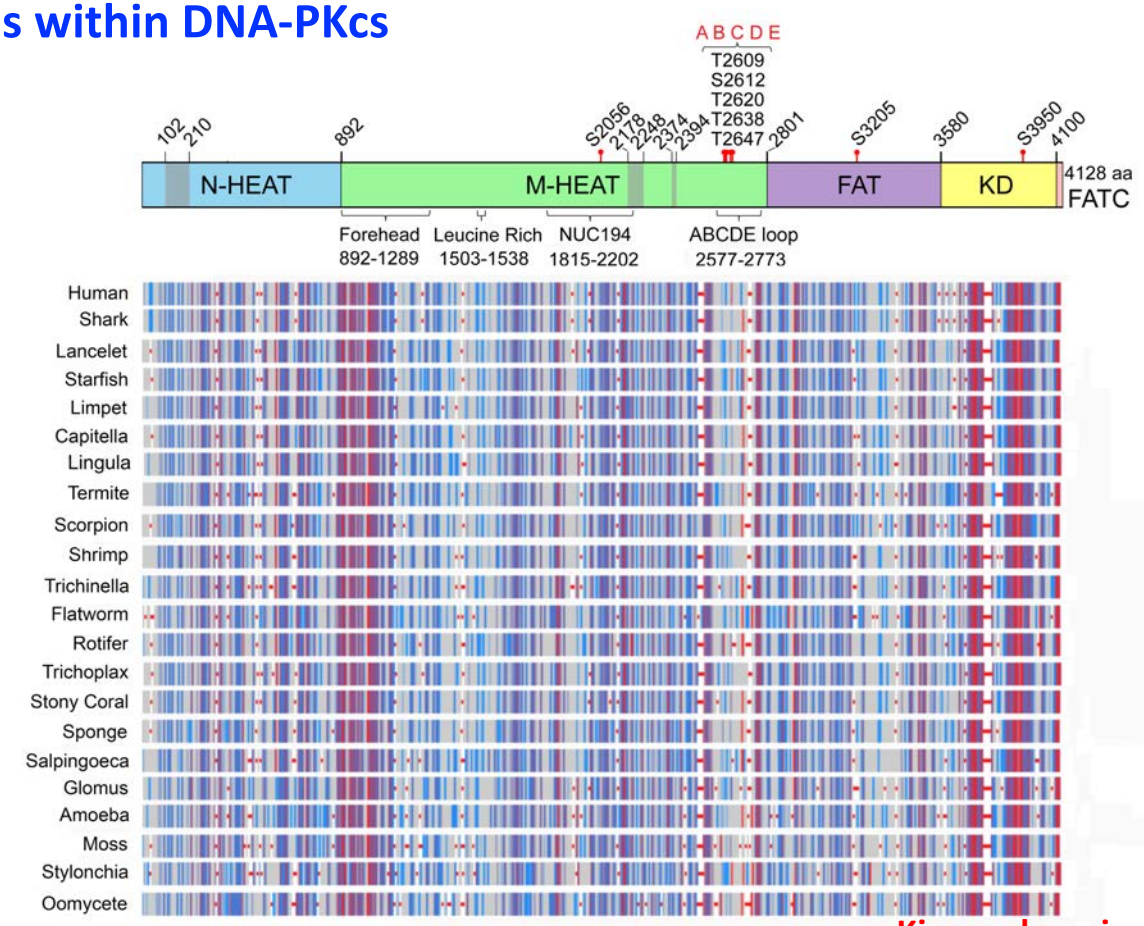
Lees-Miller et al, Prog. Biophys. Mol. Biol. 2020

With a few exceptions, DNA-PKcs/PRKDC is present in all major eukaryotic phyla including molds, non-flowering plants, algae, amoeba and invertebrates.



https://evogeneao.s3.amazonaws.com/images/tree_of_life/tree-of-life_2000.png

Highly conserved regions within DNA-PKcs



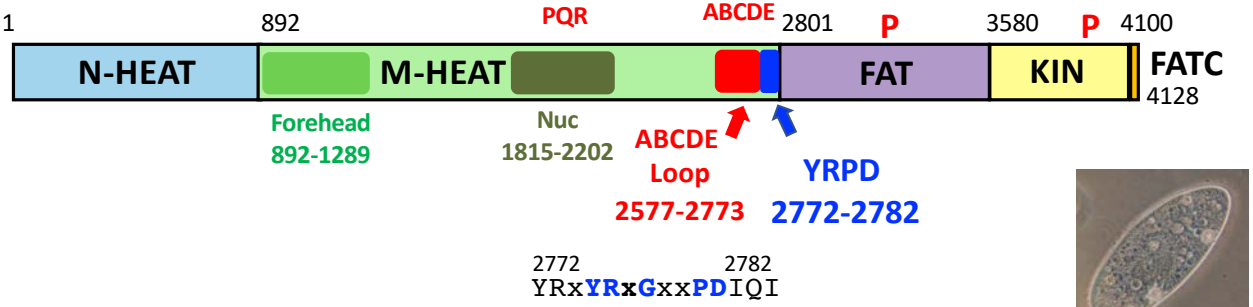
Forehead
892-1289

Kinase domain
3580-4099

FATC
4100-4128

Lees-Miller et al, Prog. Biophys. Mol. Biol. 2020

Conservation of the YRxGxxPD motif in DNA-PKcs from humans to molds



2772 2782
YRxYRxGxxPDIQI

HUMAN	DAQVVLYRSYRHGDLPDIQIKHSSLITPLQAVAQ
ESHARK	DAKVTLYRSYRFGDLPDIQIKYSSLIAPLQALAQ
LANCELET	GNLVTLYRQYRVGDLPDIQIKYSYIIAPLQALAQ
STARFISH	FSTVTMYRRYRTGDLPDIQIKYSDLIAPLQALAQ
LIMPET	ENQVTMYRKYRTGDLPDIQIKHSYIIAPLQALAH
ANNELID WORM	EHQVTMYRQYRTGDFPDIQIKYSFLIAPLQALAQ
BRACHIOPOD	EAQVTMYRKYRTGDLPDIQIKYSYVIAPLQALAQ
TERMITE	EANIASFREYRSGDFPDIEISYSAIIKPMQELAK
SCORPION	EGQVTIYRQYRIGDLPDIEINYSSIIVPLQALAQ
SHRIMP	EAQVTMYRQYRVGELPDVQIPHRALIGPLQALSQ
ROUND WORM	QQSLDLRGKYRRGDFPDIQIPYSAVIITLQAVAE
PLATYHELMINTH	EHRVTLRRYRVGDLPDVQMEQGALVTTLAGLAQ
ROTIFER	ENHVEKYRNYRIGDFPDIQIKHSELIAPMQALAQ
TRICHOPLAX	ENKVVMYRKYRDGELPDIQIKHSELIAPFQALAQ
STONYCORAL	EGAIVMYRKYRSGDLPDIQIKYSELIAPLQALAQ
SPONGE	QSYVVMYRKYRMGELPDIQIKHSELIRPLQALAQ
CHOANOFLAGELLATE	AAQVTLFRSYRTGELPDIQIKQRELLQPLQGLAQ
FUNGUS	PKNISMLRQYRVGELPDIGINHGDLINPLQTLAQ
AMOEBA	ENQVTMFRKYRSGELPDIQIKLQEILRPLQSLCQ
MOSS	ANKVHMMRQYRTGELPDIEIKHADIVGPLAALAE
CILIATE	KHQVGLYRRYRVGELPDIQISYEDVLKPLQAITK
OOMYCETE	QEHVRIKRAYRAGEFPDIQITQQDIVDPIMALCE
	: # ** *::**:#: :: #: #: .

Cnidaria
Coral
Dendronephthya gigantea

Sponge
Amphimedon queenslandica

Starfish
Acanthaster planci

Paramecium

Platyhelminth
Flatworm
Macrostomum lignano

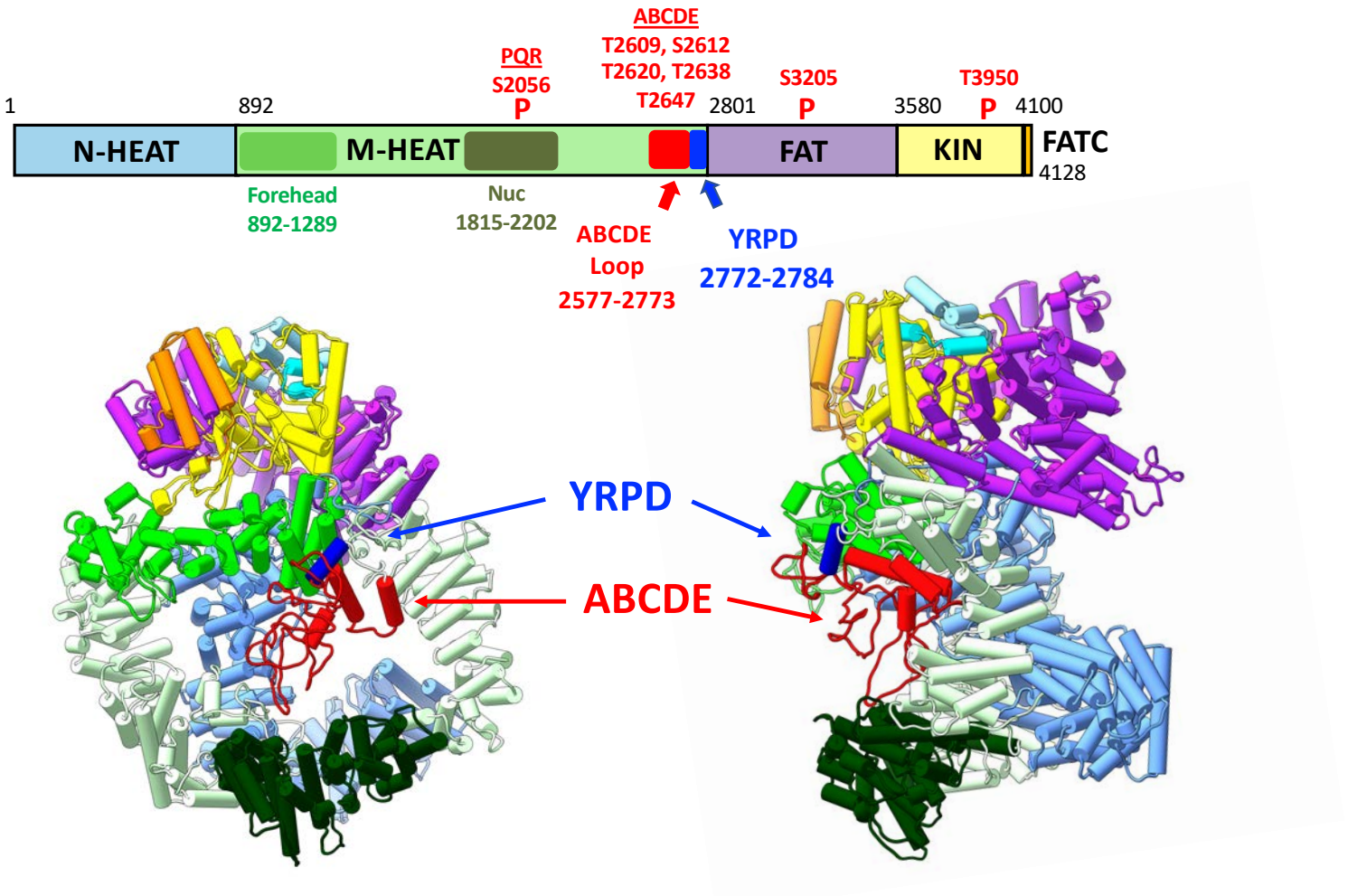
Plants
Ginko biloba

Amoeba

Nematode
Roundworm
Trichinella pseudospiralis

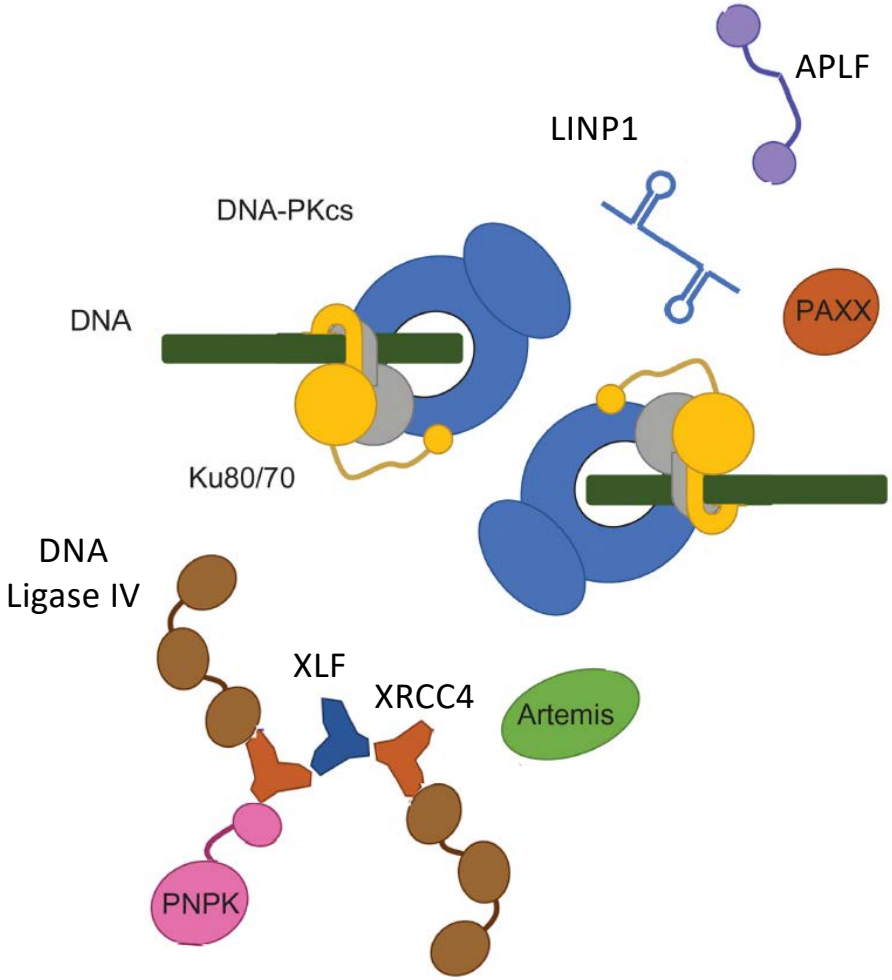
Oomycete
Leaf mold
Plasmopara halstedii

YRPD motif is located between ABCDE loop and FAT domain



Hammel et al, Prog. Biophys Mol. Biol. 2020; Tsutakawa in Lees-Miller et al, Prog. Biophys Mol. Biol. 2020

Assembly of the NHEJ machine?

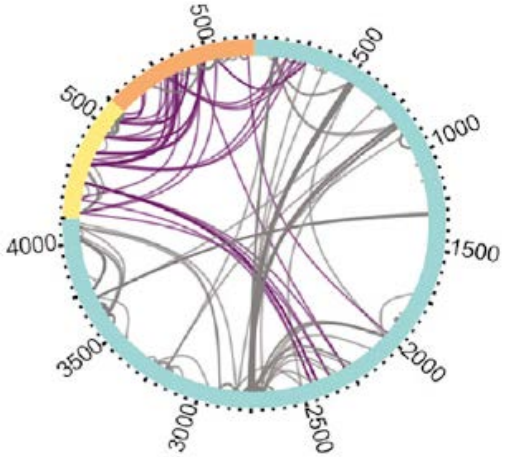


Hammel et al, JBC, 2016
Brosey et al, Methods Enzymol, 2017
Wang et al, Nature Struc. Mol. Biol. 2018
Thapar et al, NAR 2020

Assembly of DNA-PKcs-Ku-DNA on dsDNA in a synaptic complex

Hydrogen/Deuterium Exchange Mass Spectrometry
Cross-linking mass spectrometry

DNA-PKcs
Ku70
Ku80



Hepburn et al, Structure, 2020

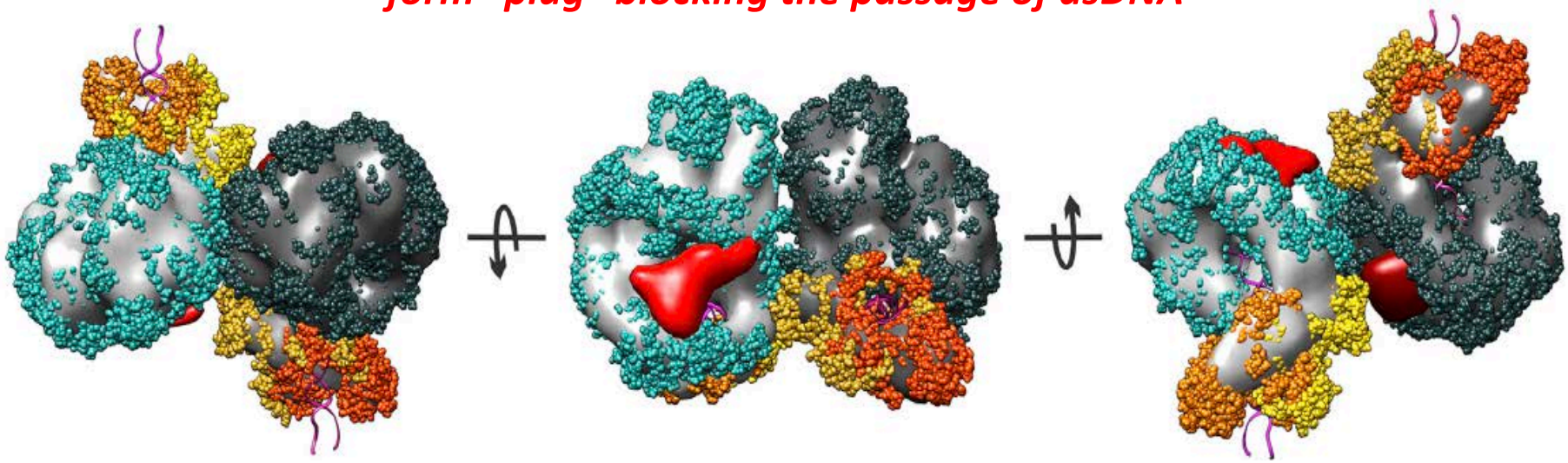
Dave Schriemer
University of Calgary

DNA-PKcs-Ku-DNA synaptic complex at ~ 13 Å

DSB ends offset by 115Å

Long-Range Synaptic complex: *Graham et al, 2016*

DNA-PKcs residues 2577-2773 (disordered loop containing ABCDE phosphorylation sites) form "plug" blocking the passage of dsDNA

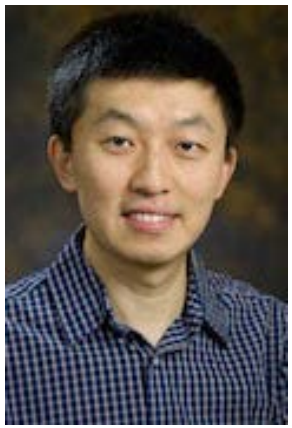


Hepburn et al, Structure, 2020

Cryo-EM structures of NHEJ synaptic complexes

Long-Range Synaptic Complex: dsDNA, Ku, DNA-PKcs, XRCC4-DNA ligase IV, XLF

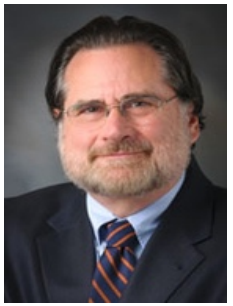
Short-Range Synaptic Complex: dsDNA, Ku, XRCC4-DNA ligase IV, XLF



Yuan He
Northwestern University
Siyu Chen

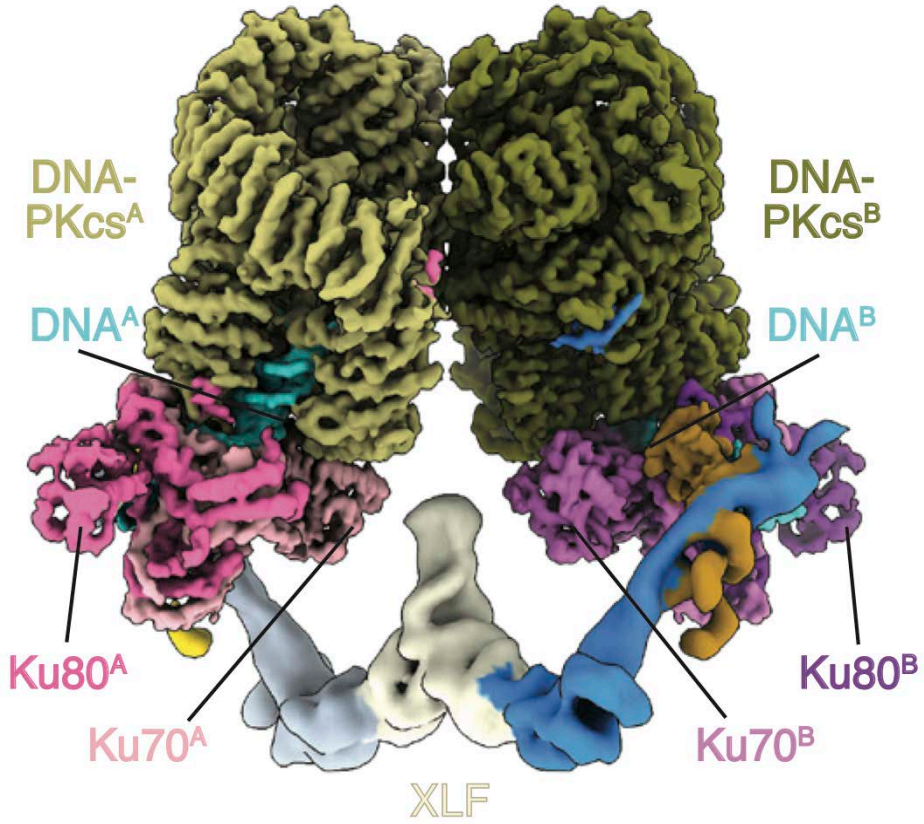
Alan Tomkinson
University of New Mexico
Comprehensive Cancer Centre

NCI Program Project
Grant P01 CA092584
Structural Biology of
DNA Repair Machines
SBDR

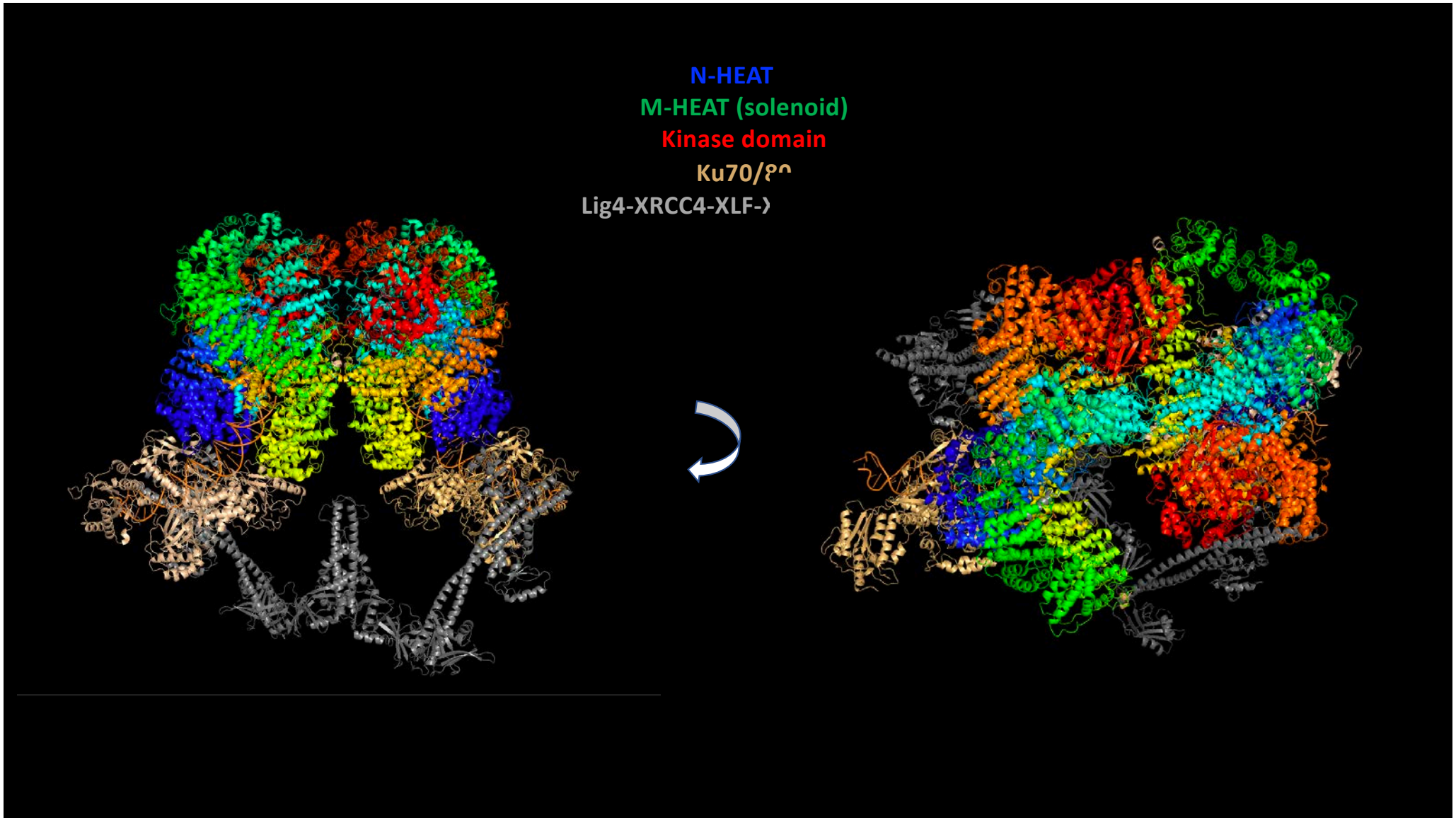


John Tainer
LBNL
MD Anderson
Cancer Centre

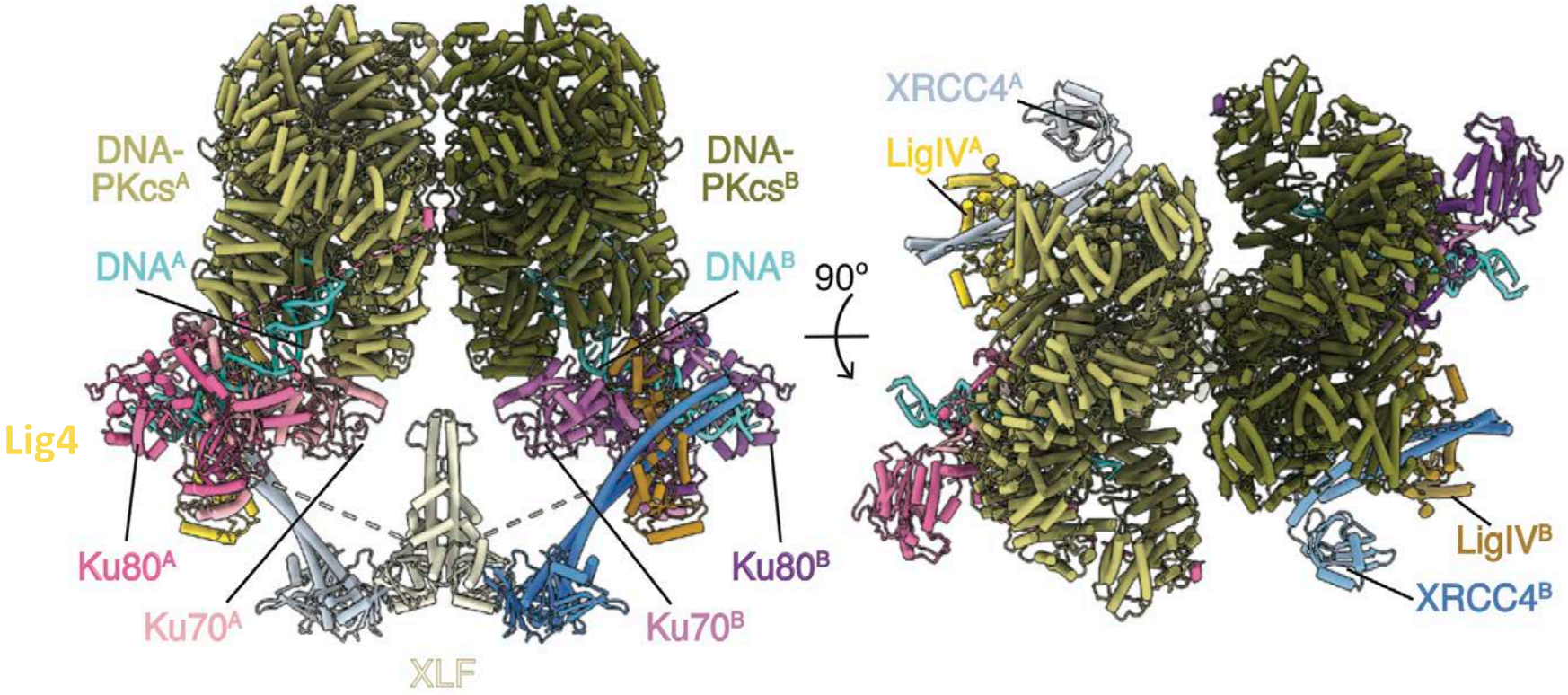
Long-Range Synaptic Complex
dsDNA, Ku70/80, DNA-PKcs, XLF, XRCC4-Lig4



Chen et al, Nature, 2021

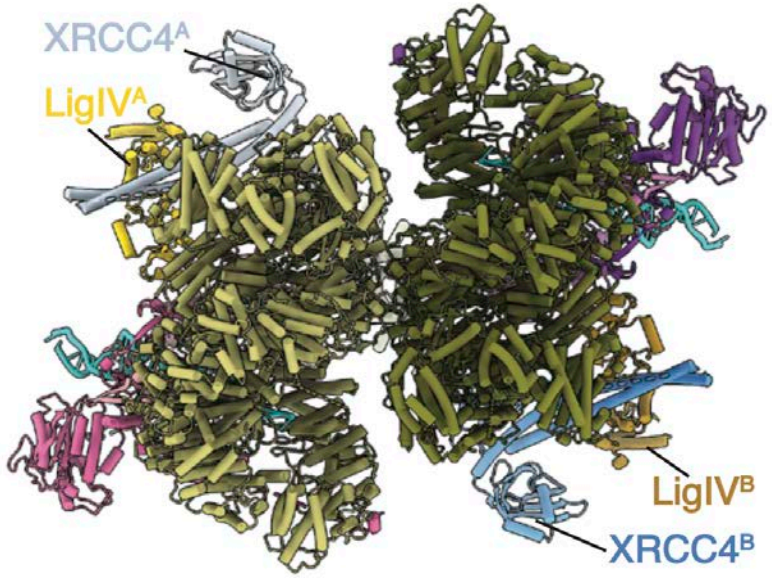

N-HEAT
M-HEAT (solenoid)
Kinase domain
Ku70/80
Lig4-XRCC4-XLF-

Long-Range Synaptic Complex
dsDNA, Ku70/80, DNA-PKcs, XLF, XRCC4-Lig4



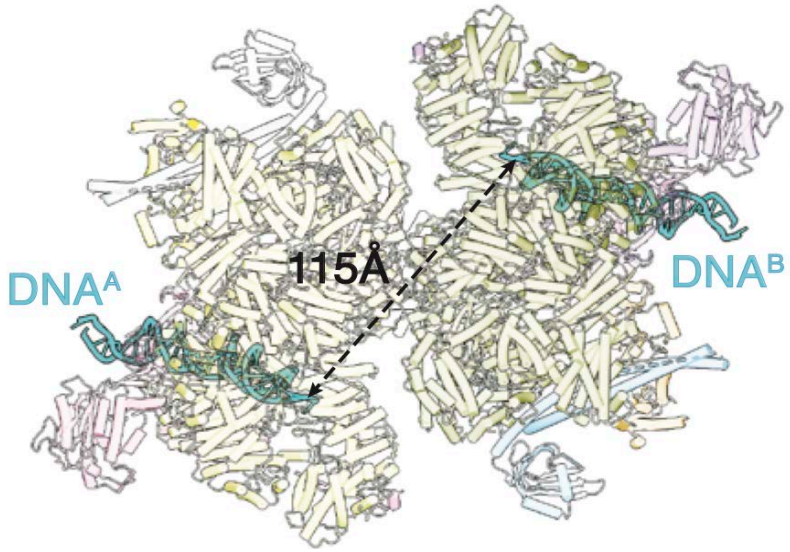
Chen et al, Nature, 2021

Long-Range Synaptic Complex
dsDNA, Ku70/80, DNA-PKcs, XLF, XRCC4-Lig4



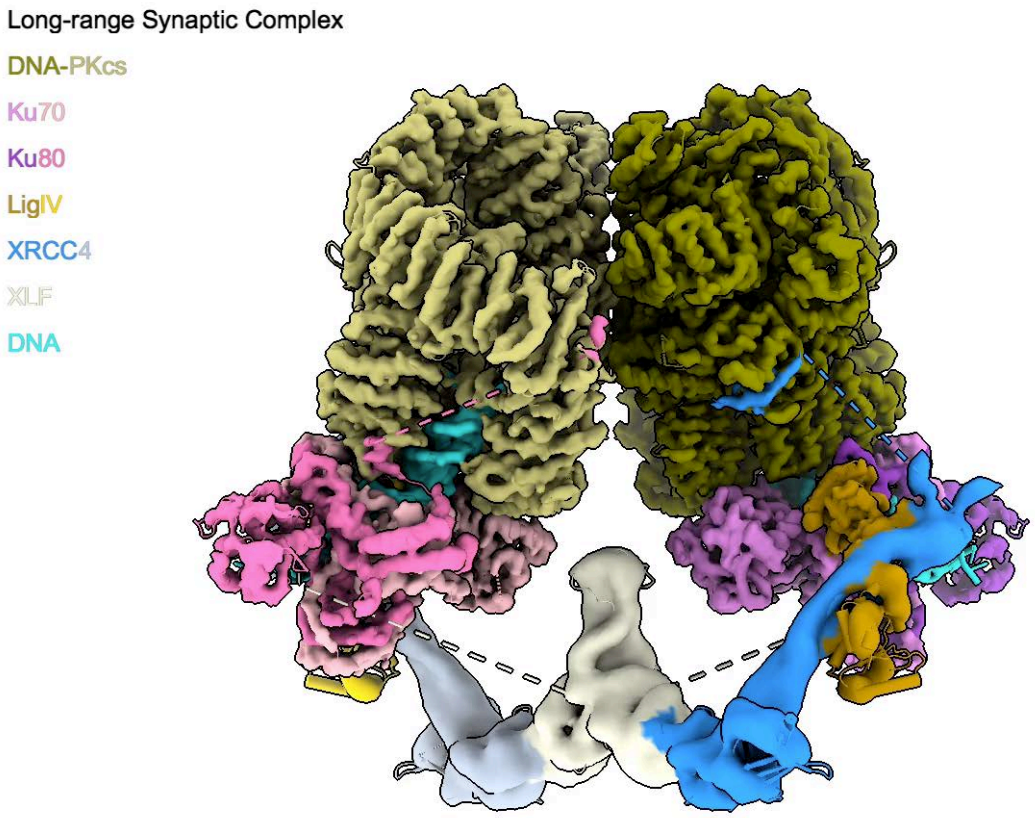
Chen et al, Nature, 2021

DSB ends offset by ~ 115 Å



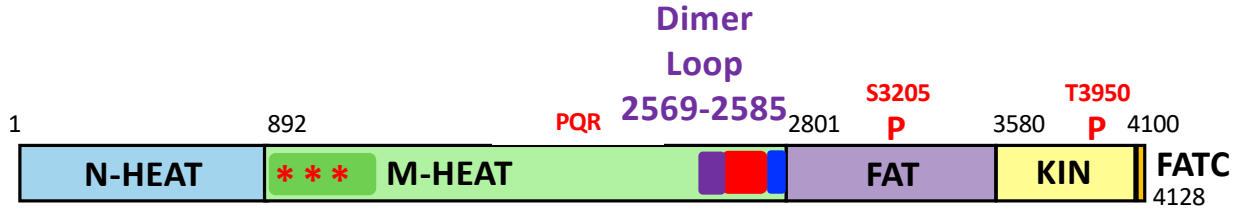
As seen in
Graham et al, Molecular Cell, 2016
Hepburn et al, Structure, 2020

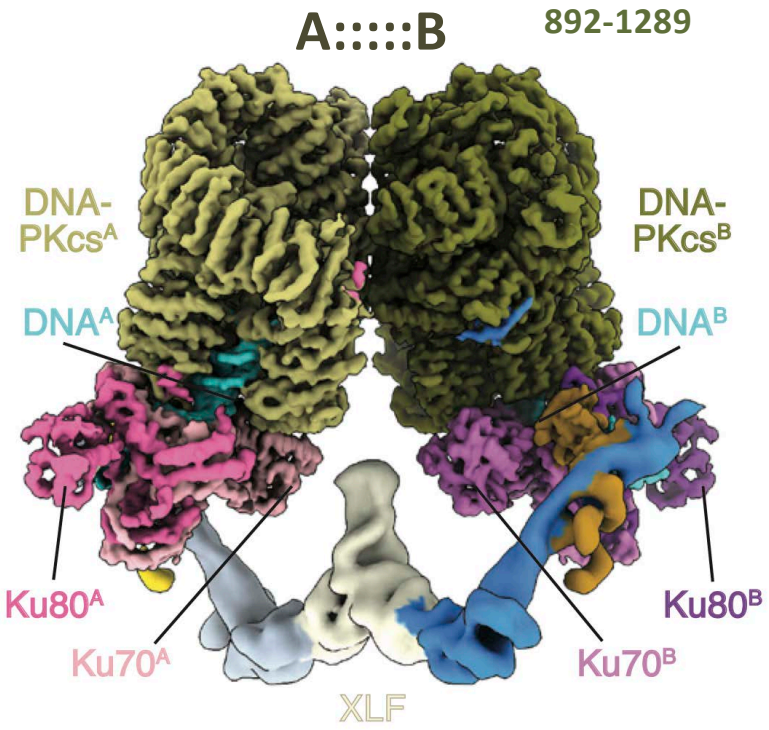
Long-Range Synaptic Complex (movie)

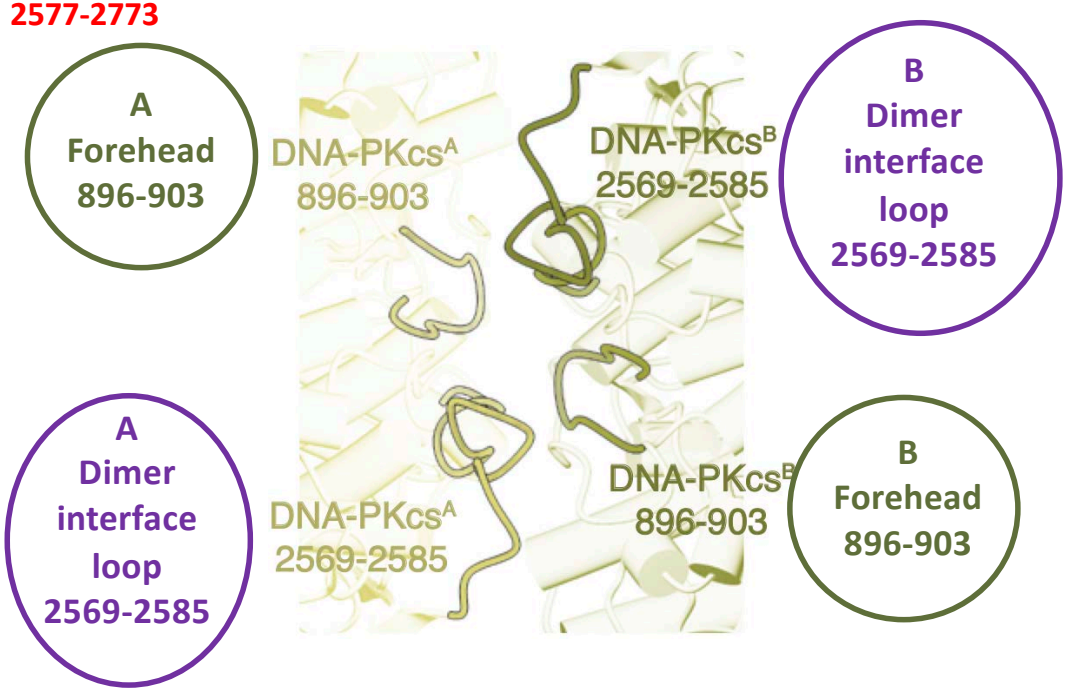


Chen et al, Nature, 2021

DNA-PKcs interaction interface within the Long-Range synaptic complex



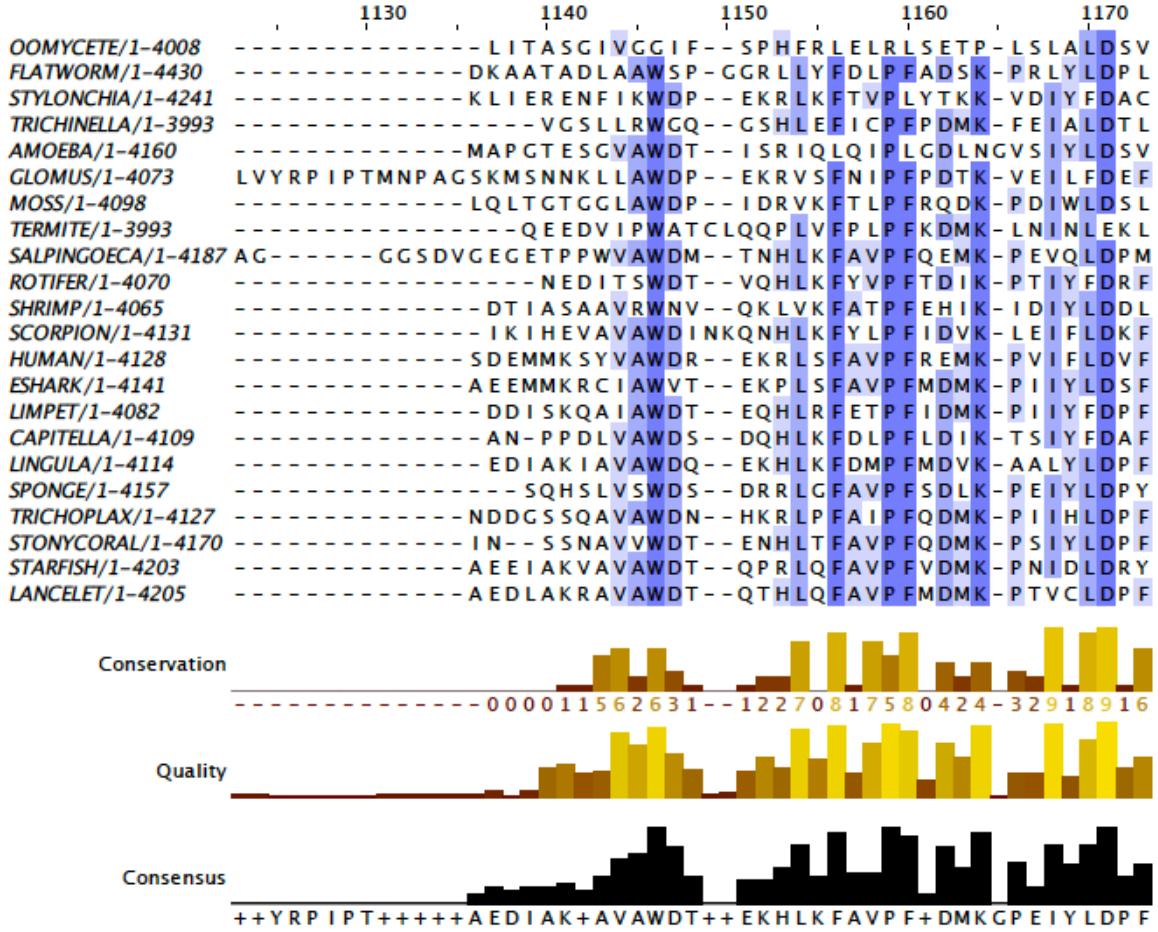




DNA-PKcs dimerization interface

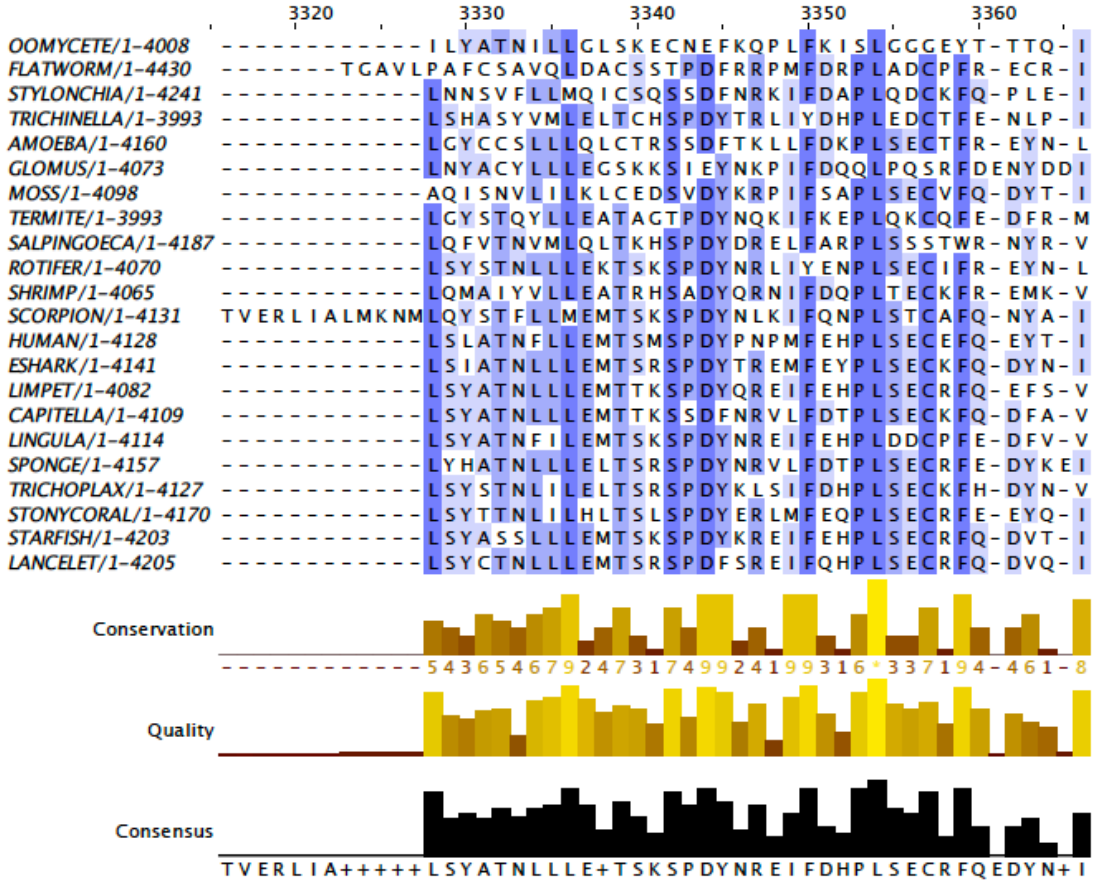
Forehead Loop

Human
896-AVPFREMKP-903



DNA-PKcs dimerization interface
Dimerization loop 2569-2586

Human **2569-SPDYPNPMFEHPLSECEF–2586**



Dimerization interface:

Dimerization Loop
(2569-2585)

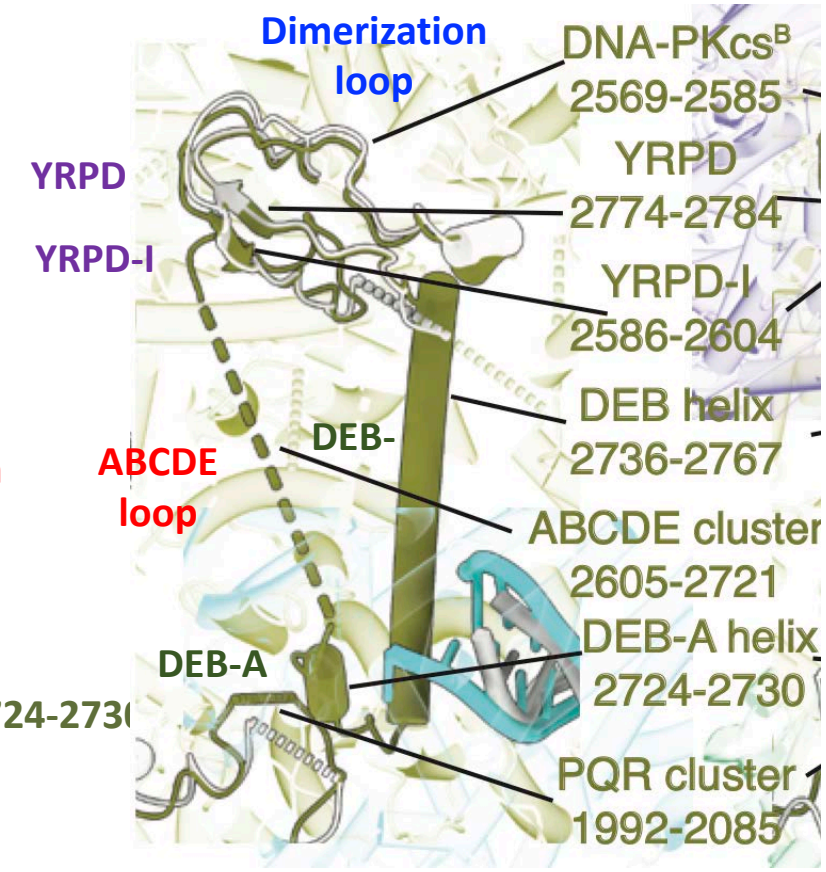
YRPD (2774-2784)

YRPD-Interacting loop
YRPD-I (2586-2604)
Antiparallel beta sheets

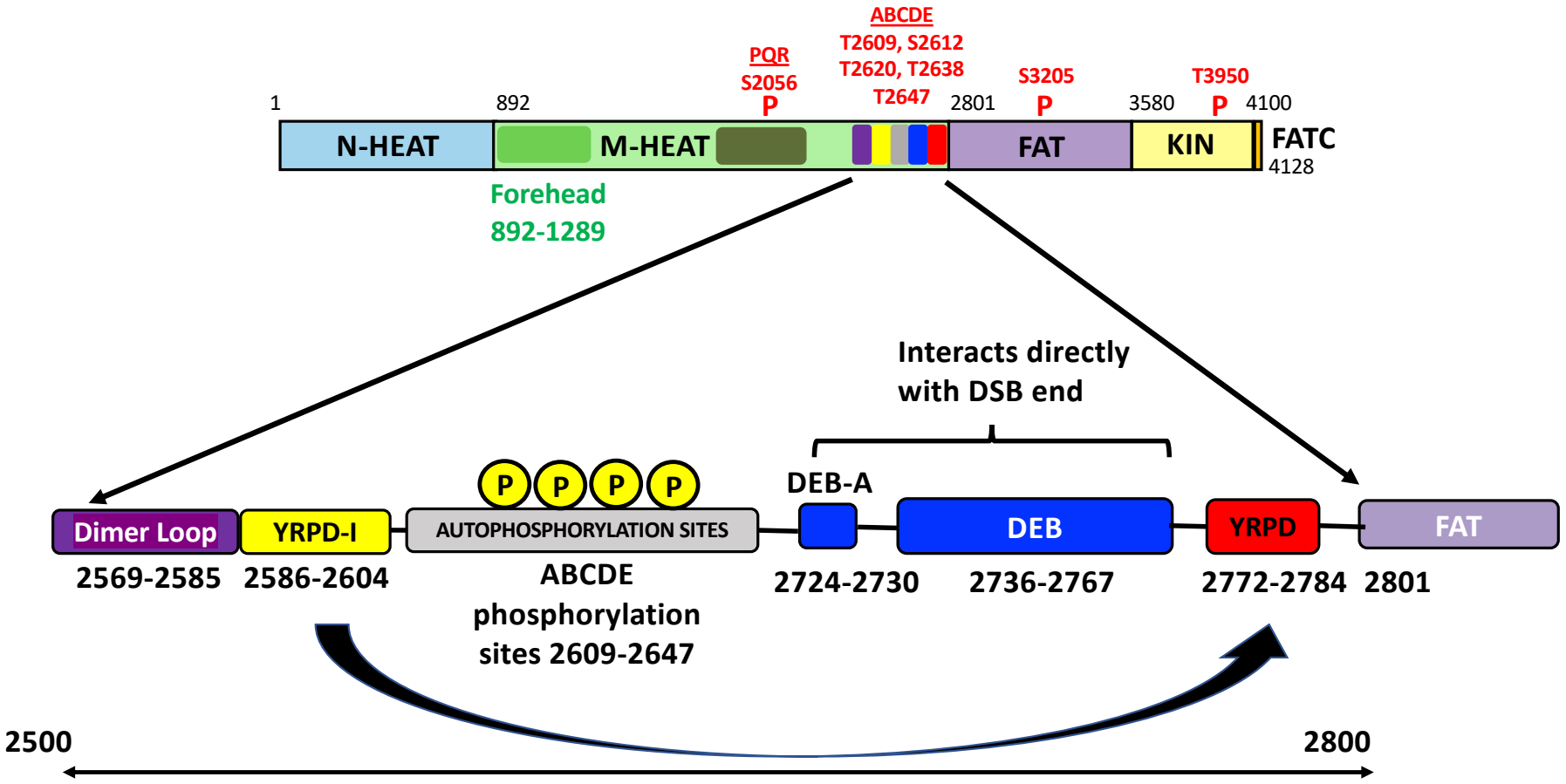
ABCDE
autophosphorylation
loop 2605-2721

DNA-end binding helix DEB (2736-2767)
Linker
DNA-end binding helix-appended DEB-A (2724-273

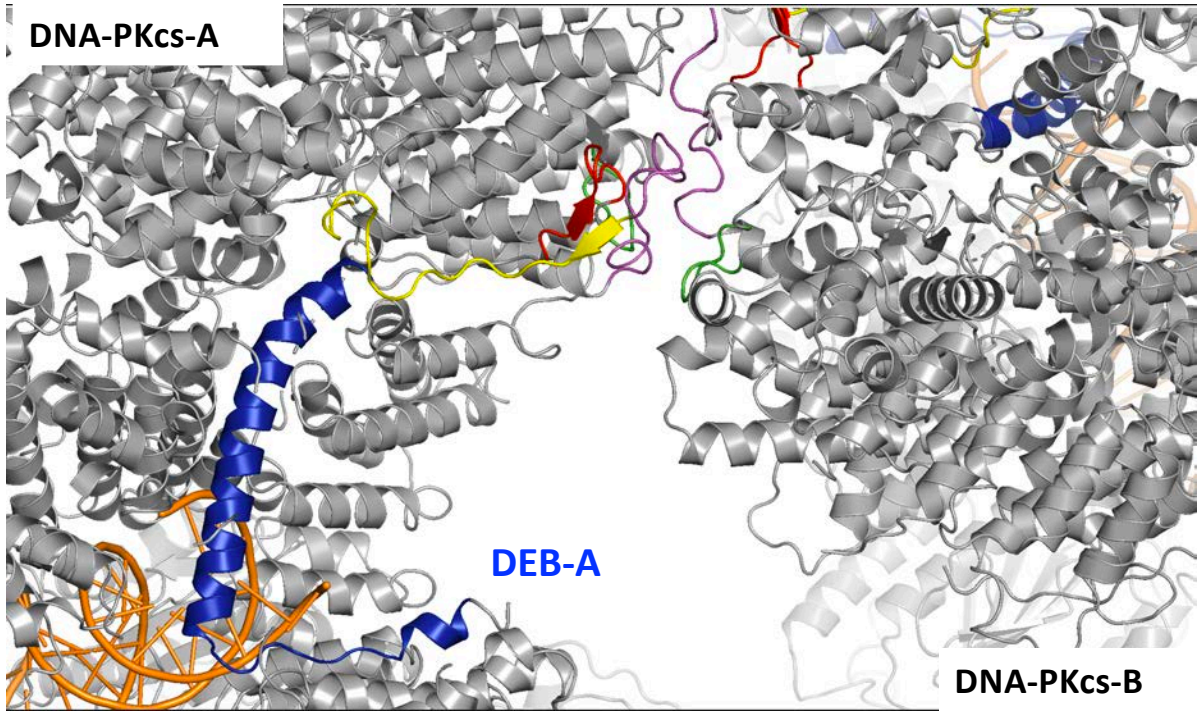
DEB-A-linker-DEB
interact directly with DSB end



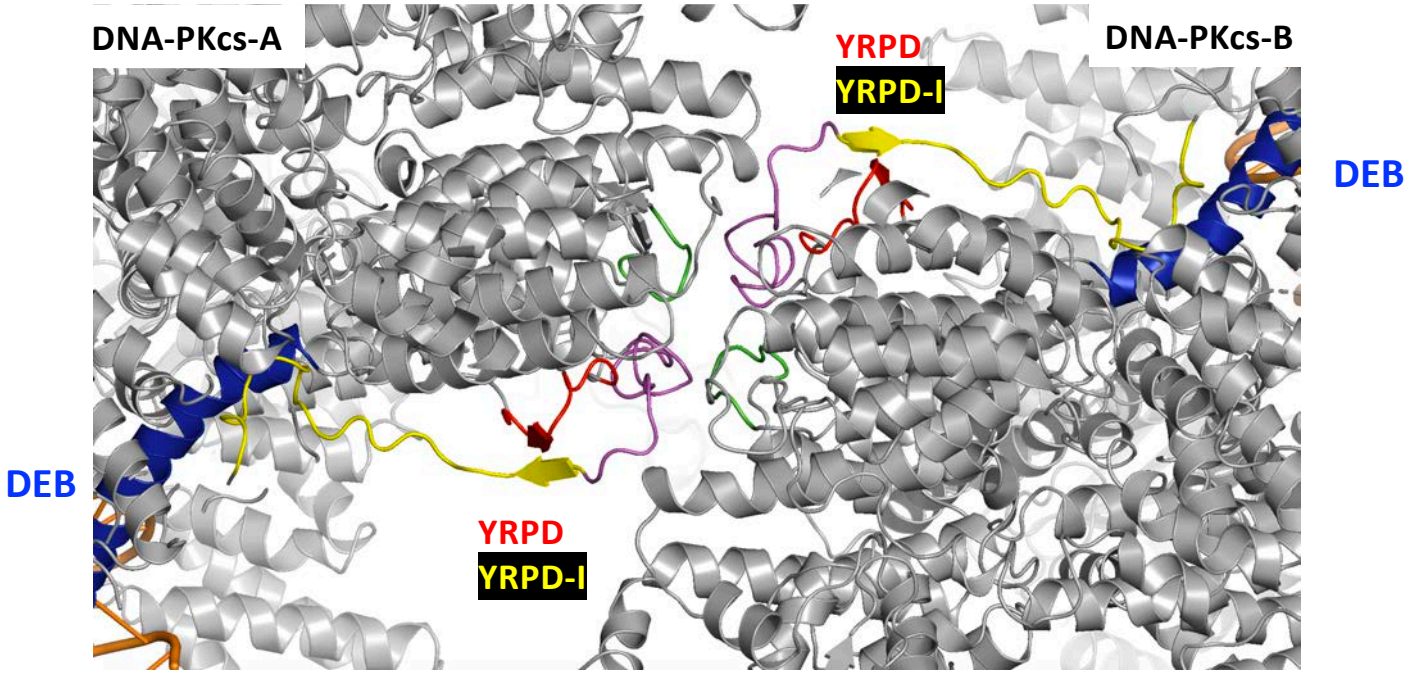
DNA-PKcs residues 2569-2784 are important for dimerization of the synaptic complex



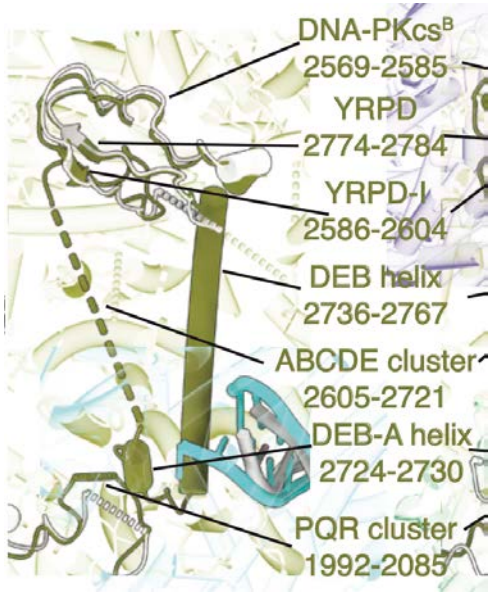
Forehead loop	Green	896-903
Dimerization loop	Magenta	2569-2585
YRPD-I	Yellow	2586-2604
DEB	Blue	2724-2767
YRPD	Red	2774-2784

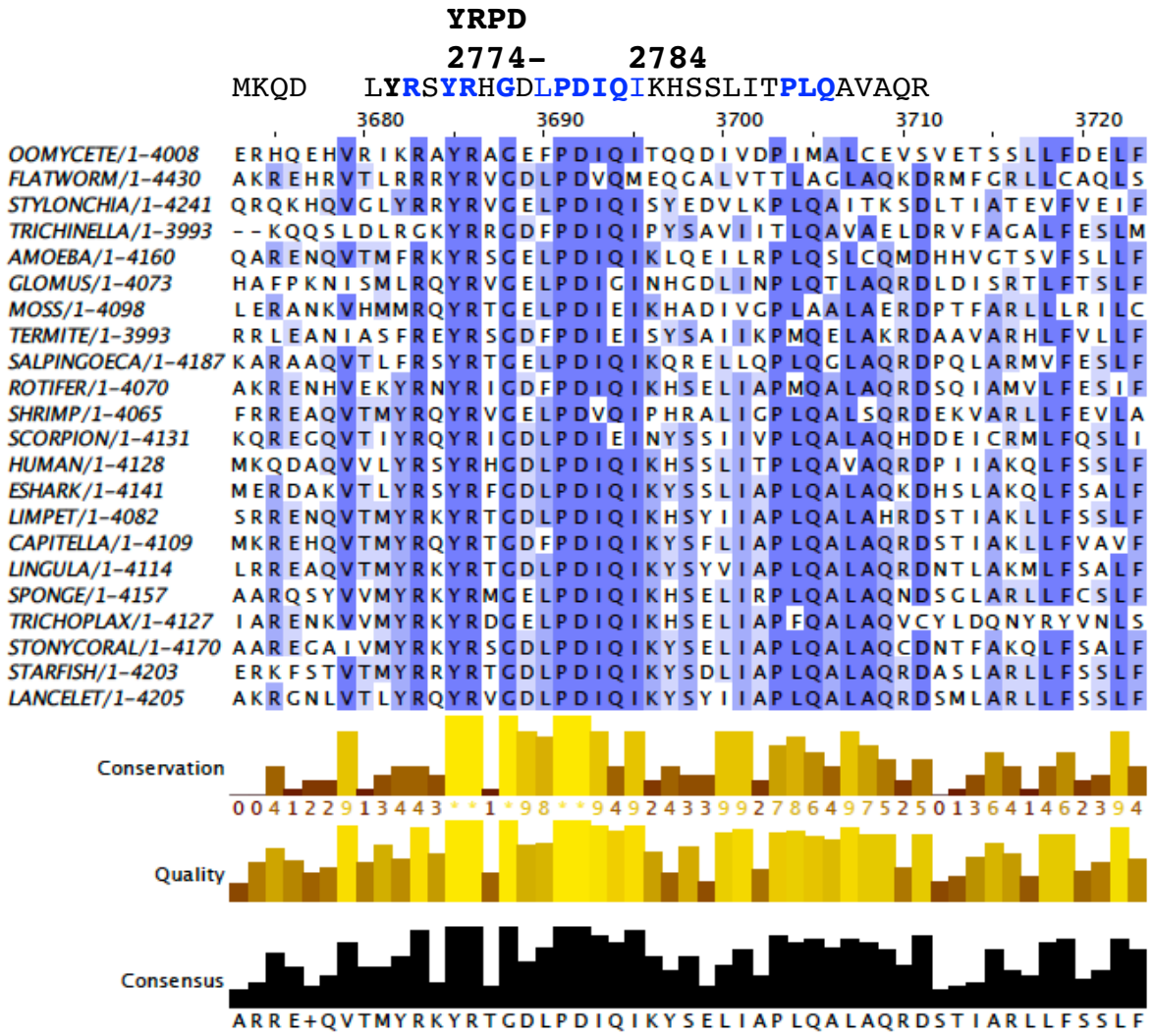


Forehead loop	Green	896-903
Dimerization loop	Magenta	2569-2585
YRPD-I	Yellow	2586-2604
DEB	Blue	2724-2767
YRPD	Red	2774-2784

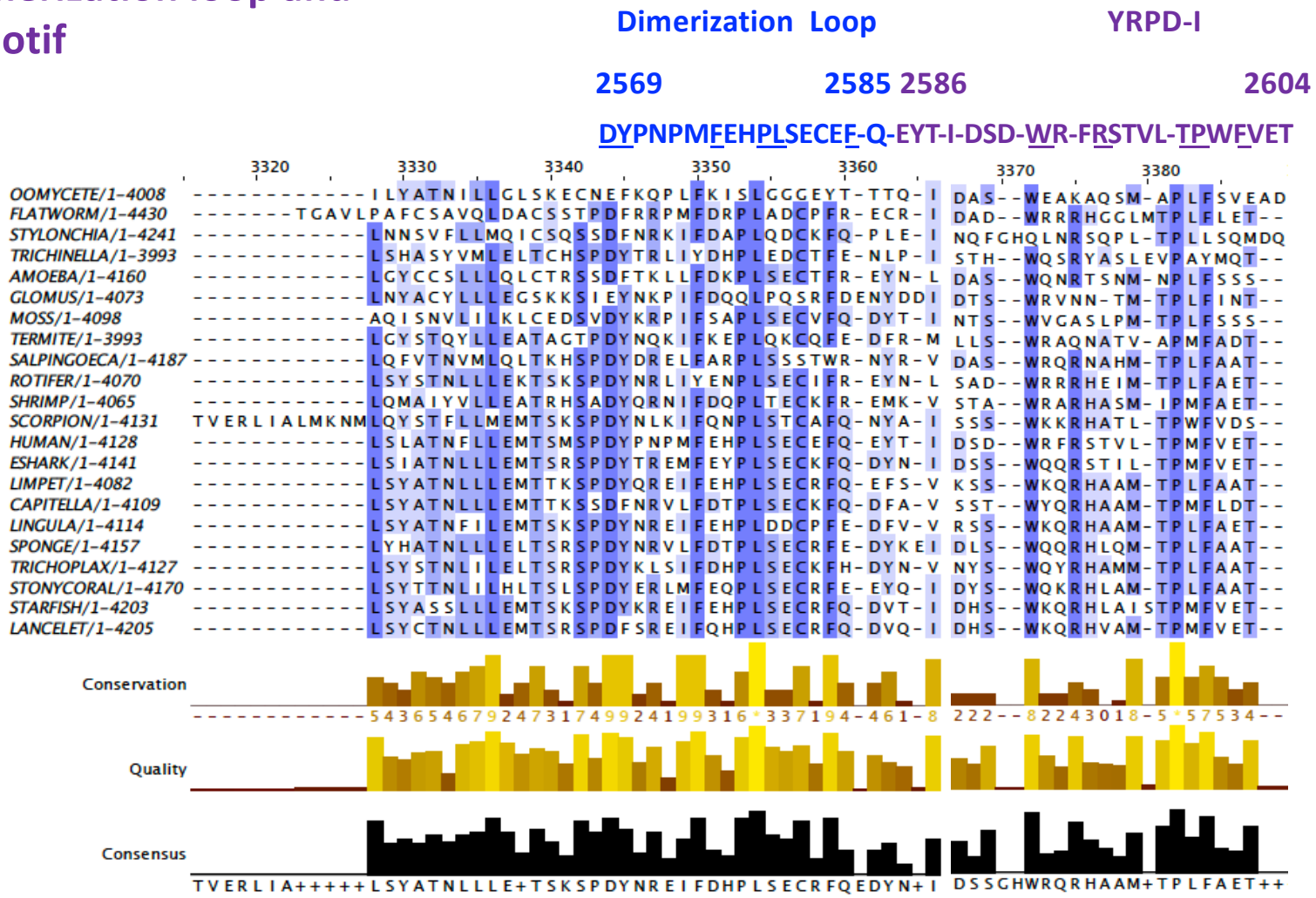


Conservation of YRPD

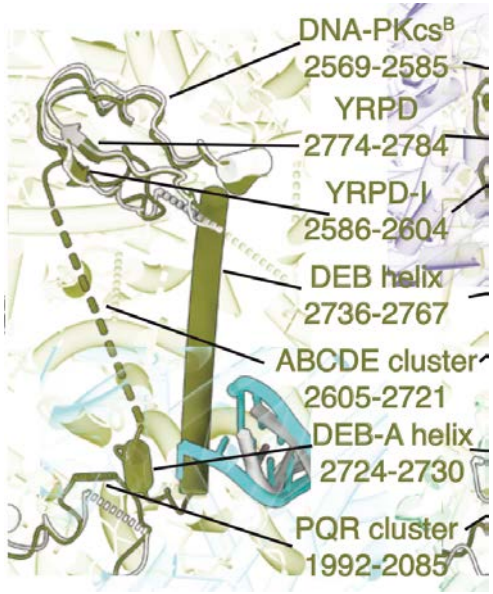


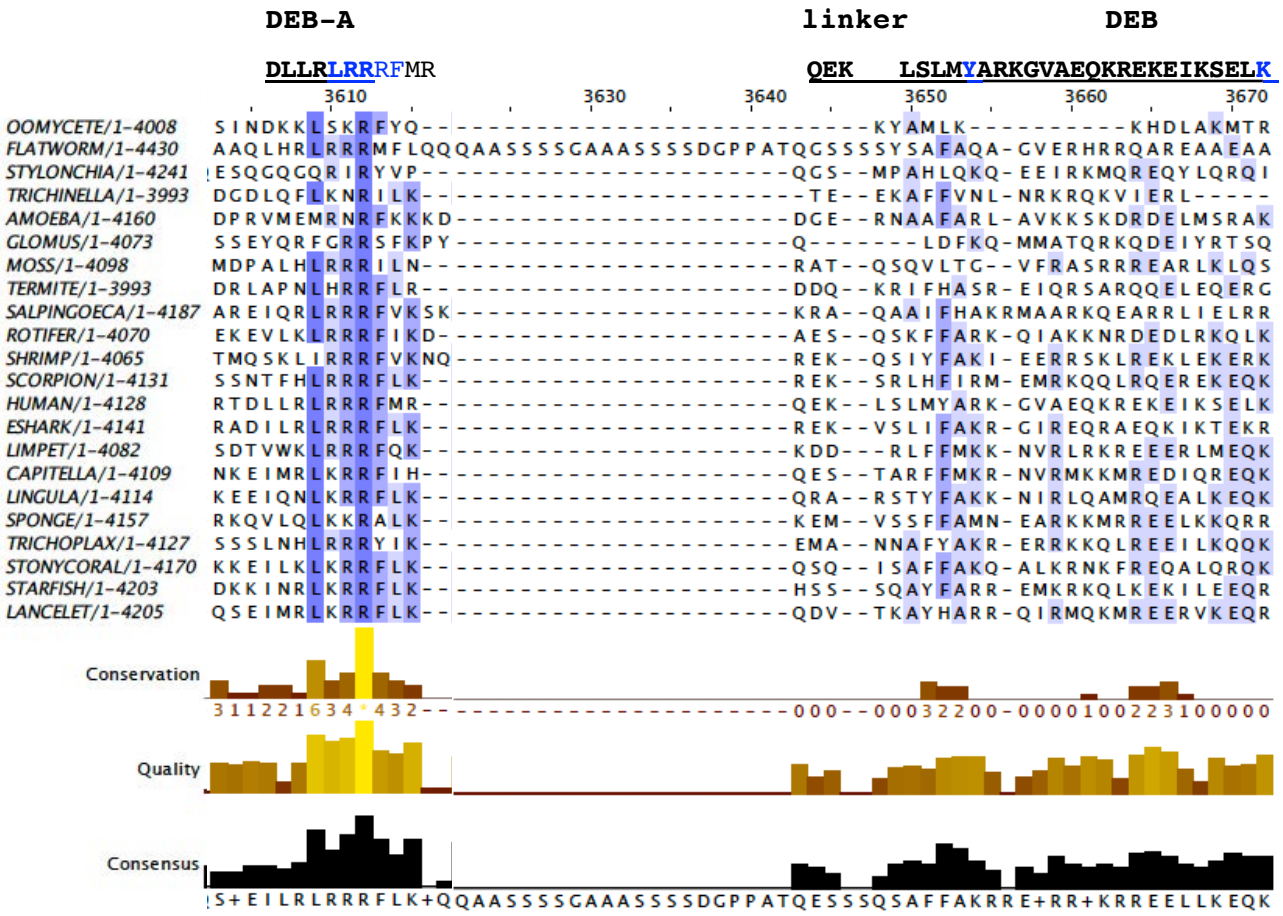


Conservation of dimerization loop and YRPD-interacting motif



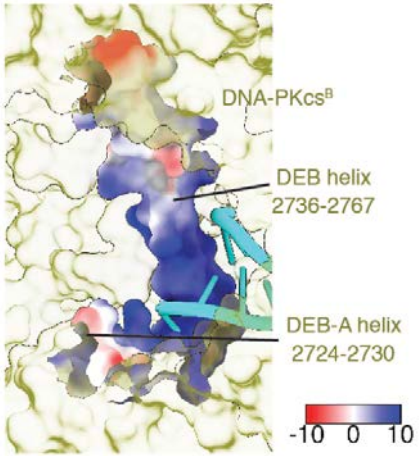
Conservation of basic amino acids in DEB-A helix, consistent with interaction with DNA



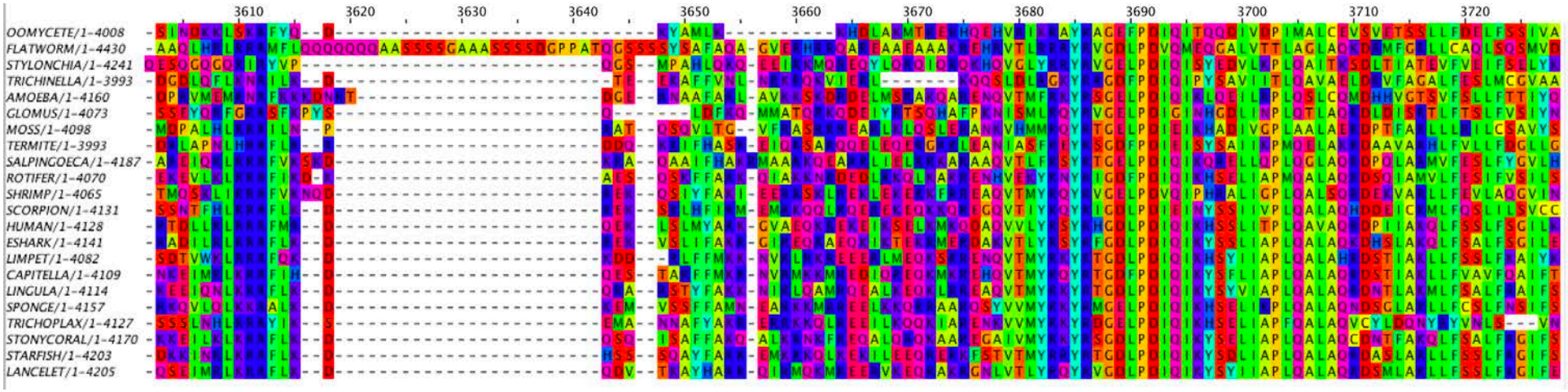


Conservation of basic charge (dark blue) in DEB-A/DEB

Positively charged DEB helix in LR Synaptic complex



DEB-A **DEB** **YR YR G PD**



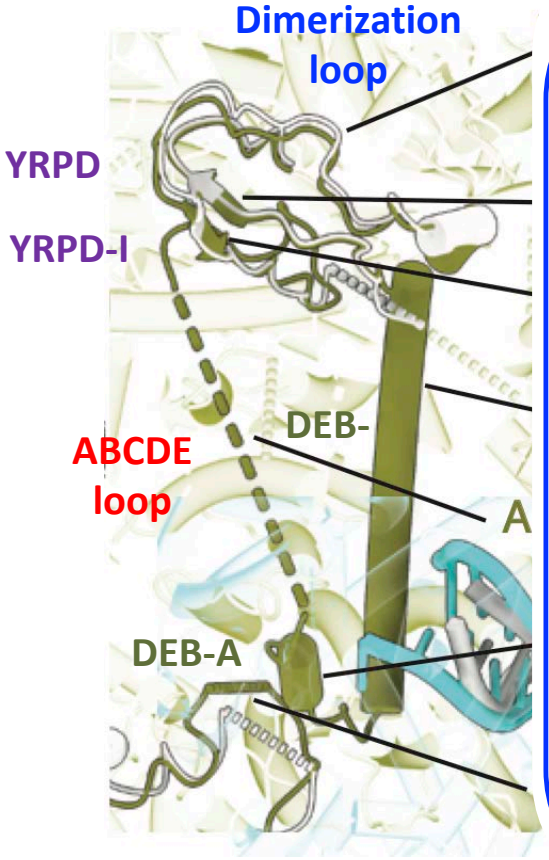
Dimerization Loop (2569-2585)

YRPD (2774-2784)

YRPD-Interacting loop
YRPD-I (2586-2604)

ABCDE
autophosphorylation
loop 2605-2721

DEB (2736-2767)
Linker
DEB-A (2724-2730



DEB-A-linker-DEB
interacts directly with DSB end

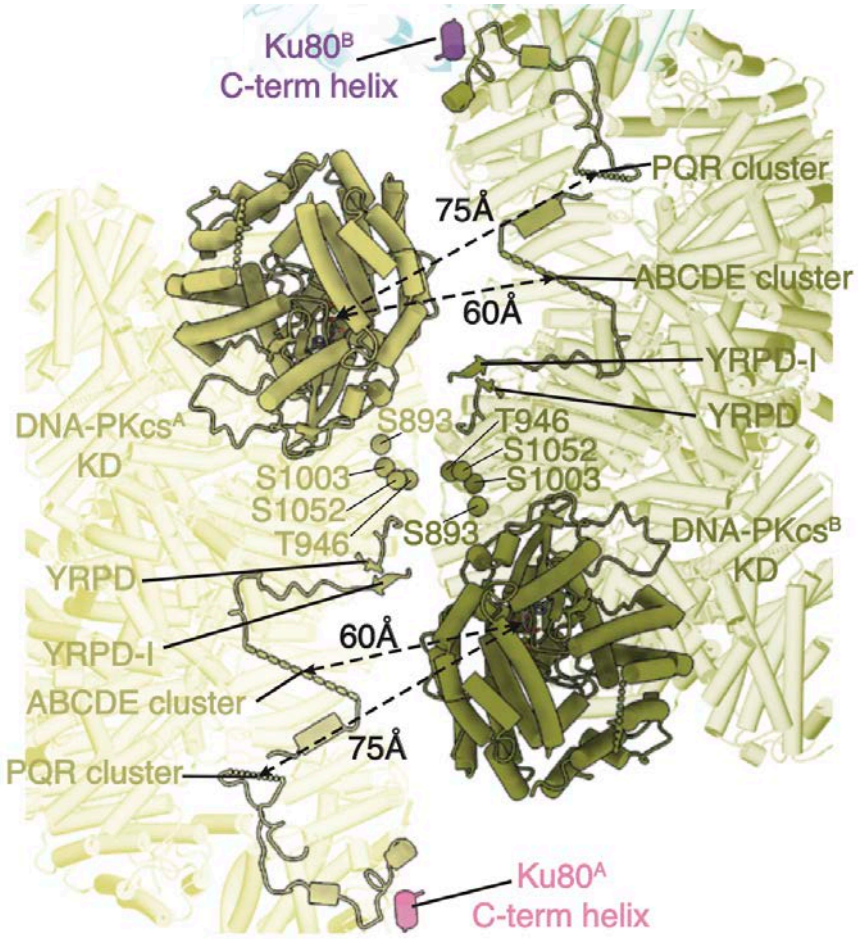
Model for Autophosphorylation - induced dissociation

- Disruption of YRPD/YRPD-I
- Autophosphorylation of ABCDE in trans: negative charge
- Disruption of DEB-A/DEB with DSB
- Release of DNA-PKcs from the DSB end and synaptic complex

DNA-PKcs interaction interface

Autophosphorylation of ABCDE and PQR clusters at the synaptic dimer interface

Active site of DNA-PKcs (A) positioned to phosphorylate ABCDE and PQR clusters of DNA-PKcs (B) in trans

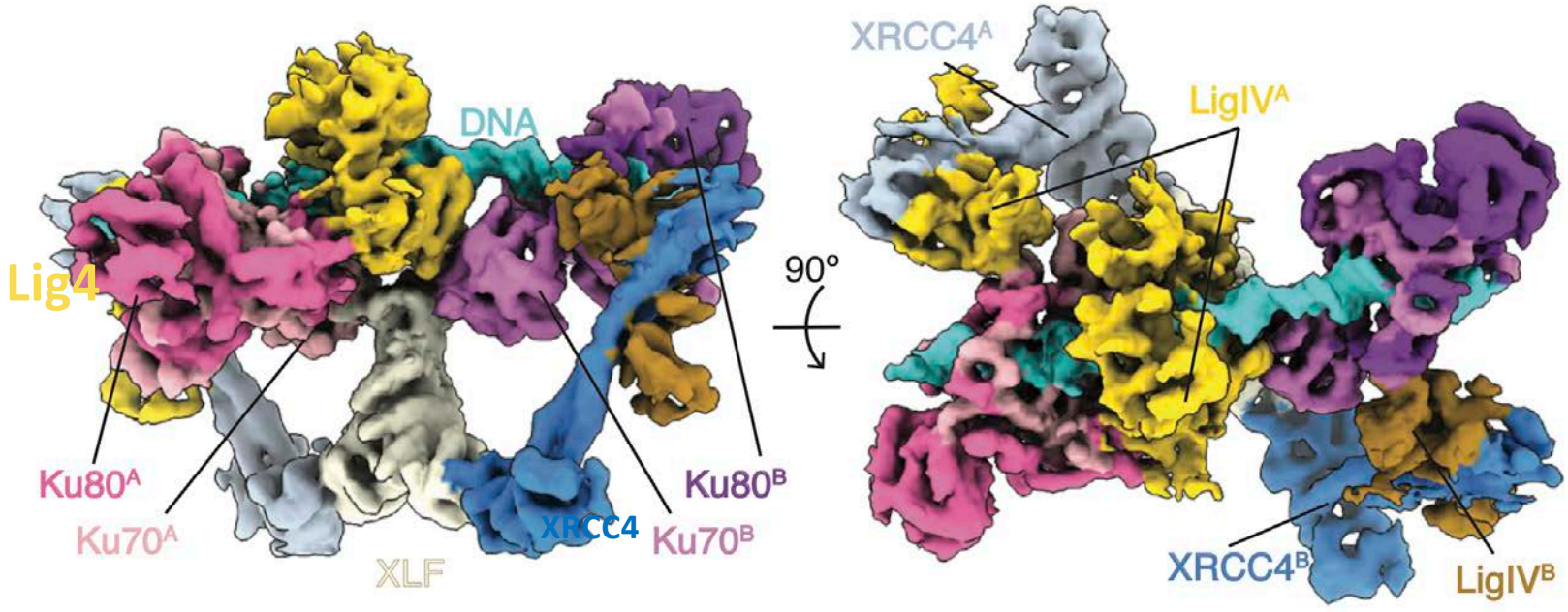


Short-Range Synaptic Complex

dsDNA, Ku70/80, XLF, XRCC4-Lig4 (minus DNA-PKcs)

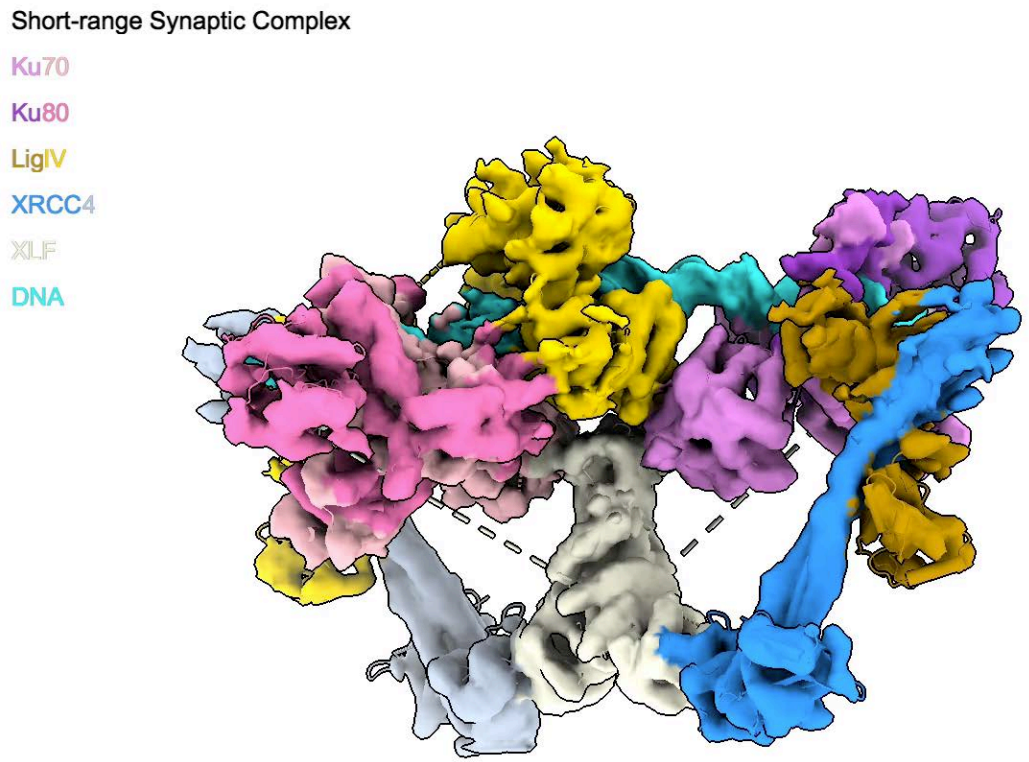
XRCC4-Ku conformational change, DNA horizontal, DSB ends in close proximity

Lig4 catalytic domain engaged with nick/SSB on one DNA strand



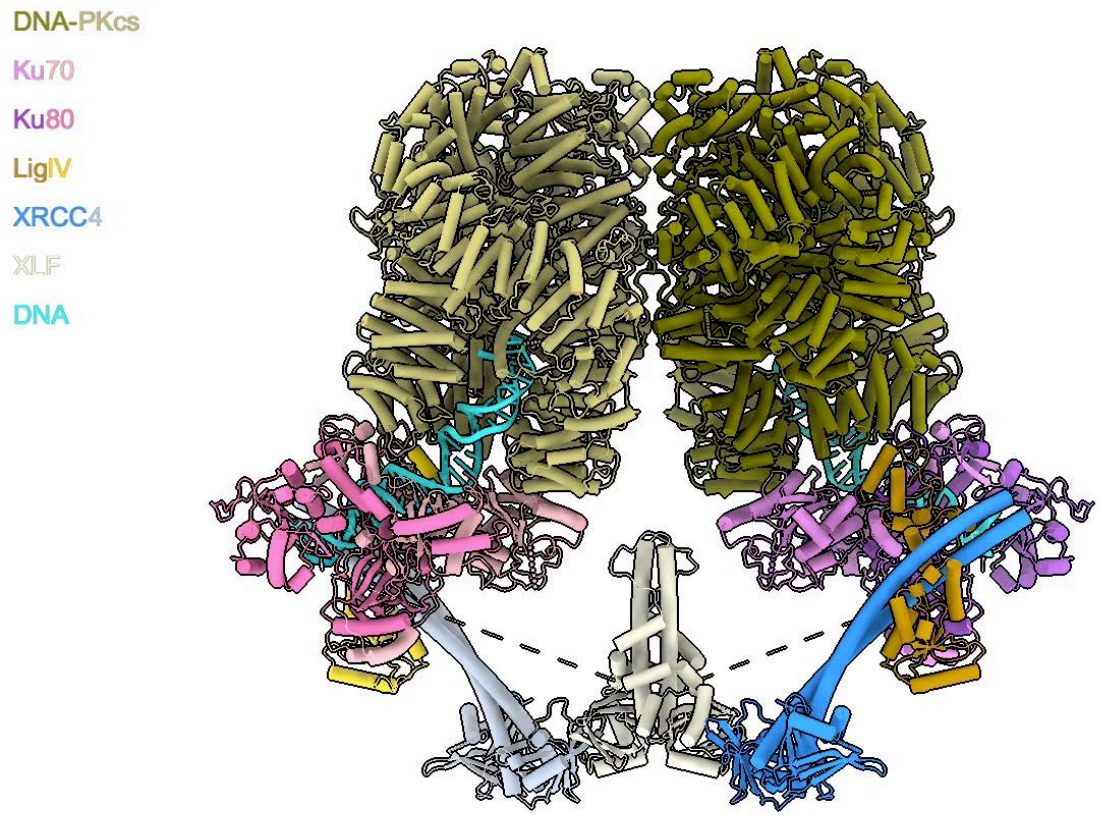
Chen et al, Nature, 2021

Short-Range Synaptic Complex



Chen et al, Nature, 2021

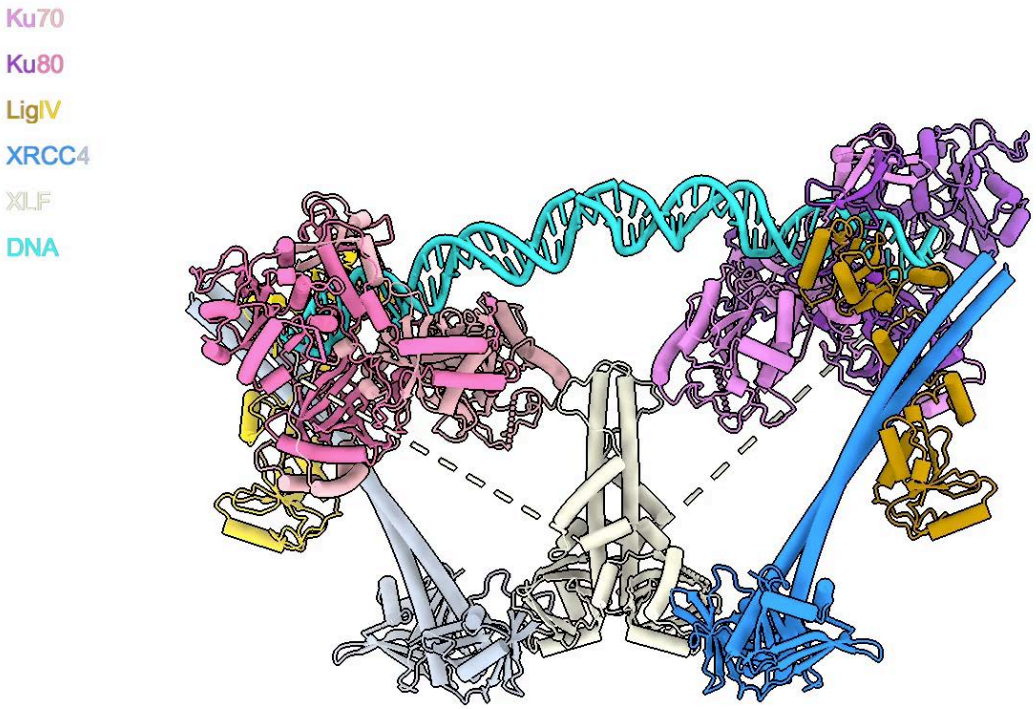
Model for transition from Long Range complex to Short Range complex



Long-range Synaptic Complex

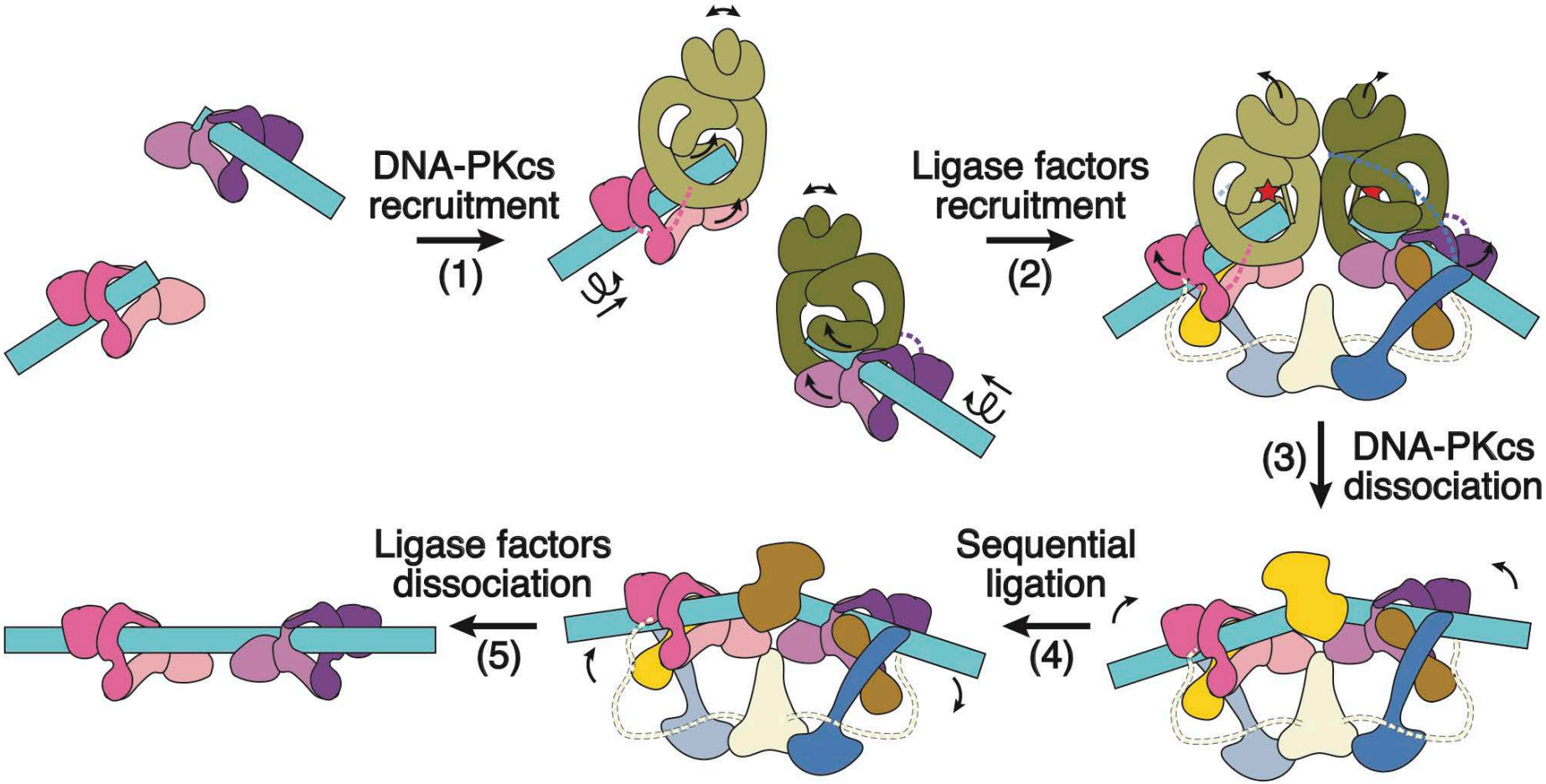
DNA ligase IV single turnover enzyme

1 DNA Lig4 joins 1 nick/SSB: Two-step Ligation Process



Chen et al, Nature, 2021

Model for NHEJ and transition from Long-Range Synaptic Complex to Short-Range Synaptic Complex



Summary

- Autophosphorylation of DNA-PKcs regulates its association with dsDNA in vitro and in vivo
- High-resolution structure of Long-range synaptic complex with XLF, XRCC4-Lig4, Ku and DNA-PKcs
- Positively charged DNA end binding helix (DEB) that interacts with DSB end
- Model for how phosphorylation of ABCDE cluster could lead to disruption of the DNA-PKcs synaptic dimer interface
- High-Resolution structure of Short-range range synaptic complex with XLF, XRCC4-Lig4, Ku revealing how DNA ligase IV interacts sequentially with 2 nicks/SSBs
- DNA-PKcs is not vertebrate specific.
- Although it is absent from many model organisms it is conserved through evolution:
- invertebrates, protists, non-flowering plants
- DEB-A and YRPD motifs are highly conserved through evolution indicating conservation of DNA-PKcs' function in DSB repair

Acknowledgements

Carl W Anderson,
Brookhaven National Laboratory

Lab members:
Linda Lee
Alexander Cobban
Jonathan Roveredo

Past Lab Members:
Yaping Yu
Shujuan Fang
Pauline Douglas

Collaborators

Yuan He	Northwestern University
Siyu Chen	
John Tainer	MD Anderson, LBNL
Michal Hammel	LBNL
Susan Tsutakawa	LBNL
Kathy Meek	Michigan State University
Dave Schriemer	University of Calgary
James Lees-Miller	University of Calgary
Terence Strick	Institut de Biologie de l'Ecole Normale Supérieure, Paris

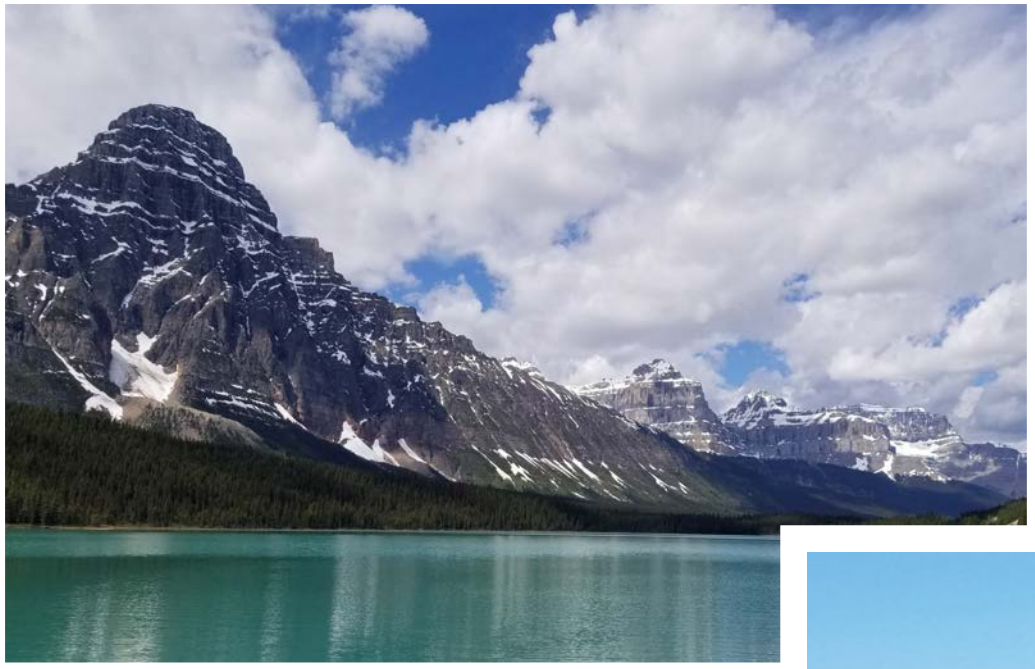
NIH NATIONAL CANCER INSTITUTE

PO1 CA092584 (Tainer)

NIH National Institutes of Health

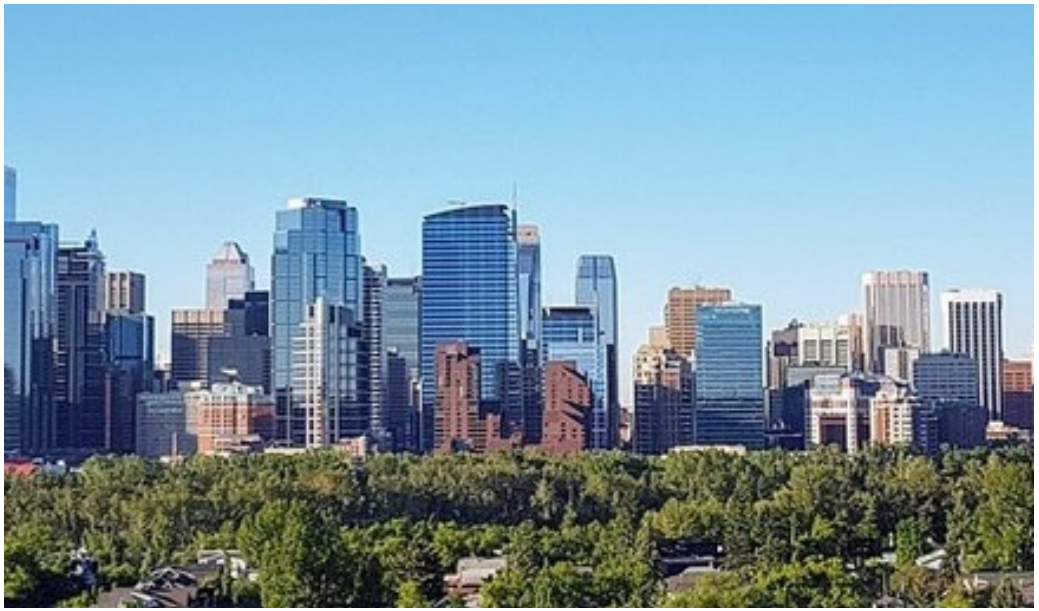
1R01GM135651-01 (He)

NSERC CRSNG

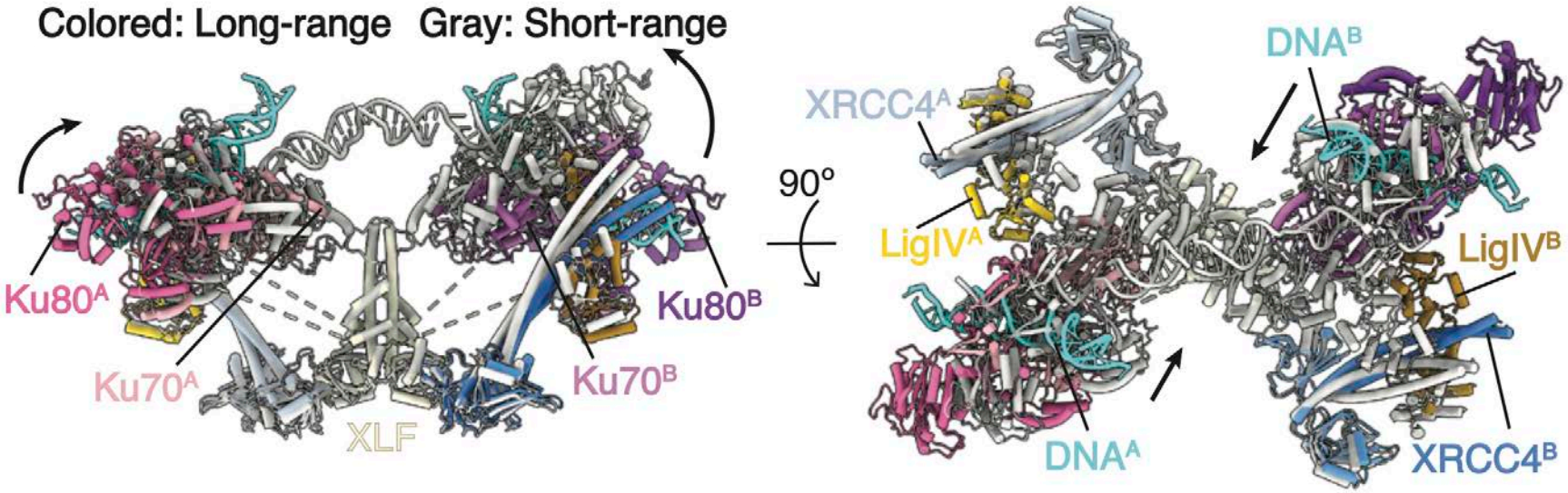


Thank you for your attention!

PDF positions available
leesmill@ucalgary.ca



Model for transition from Long range to Short Range complex

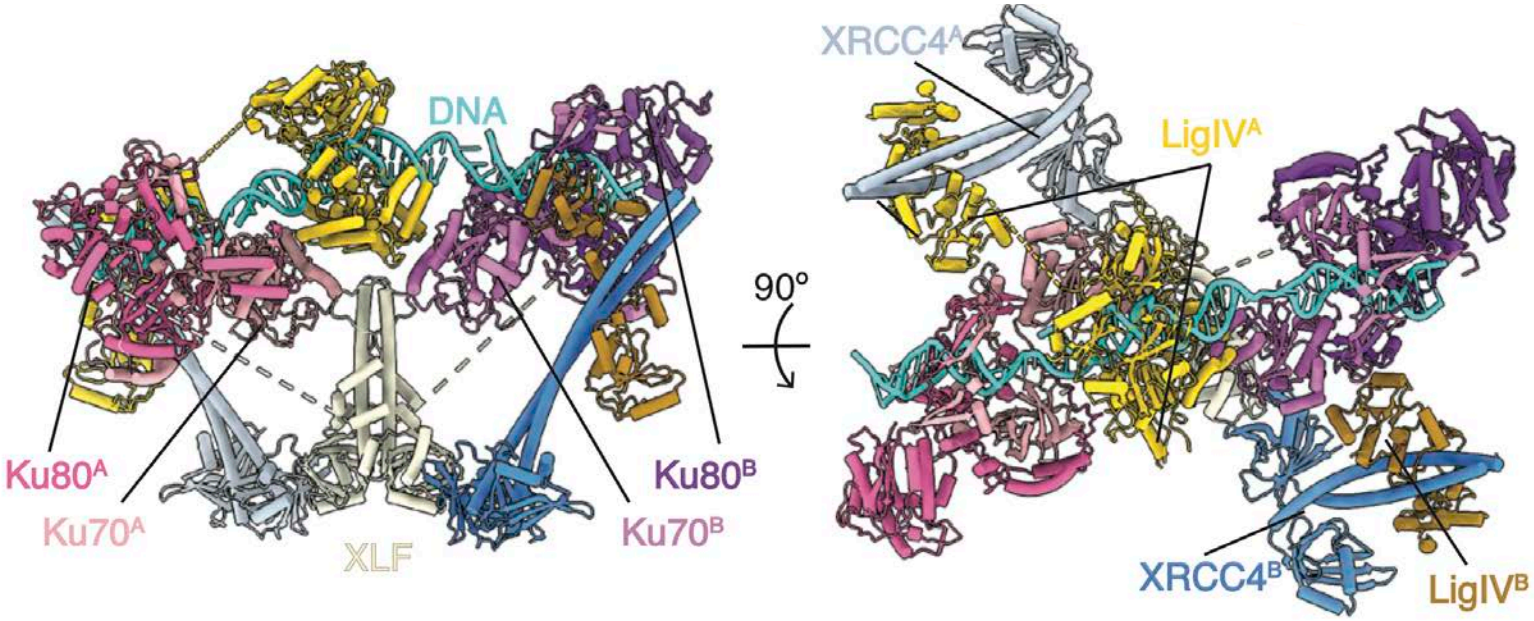


Chen et al, Nature, 2021

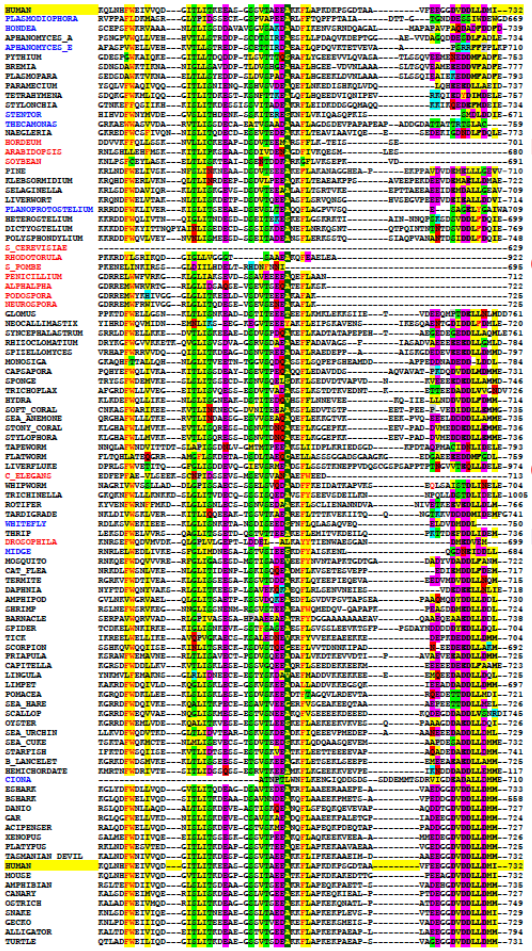
Short-Range Synaptic Complex

dsDNA, Ku70/80, XLF, XRCC4-Lig4 (minus DNA-PKcs)

Single Lig4 catalytic domain engaged with the DSB ends



Chen et al, Nature, 2021



Barley, Arabodopsis, Soybean

Cereviseae, pombe other Dikarya yeast

C. elegans

Drosophila-no PRKDC/partial Ku80CTR

In general there is a good correlation between presence of Ku80 CTR and presence of DNA-PKcs.

Exception is insects for example, Drosophila has no PRKDC but has a partial Ku80CTR. More work needed to understand insects.