## Phylogeny

PRKDC (DNA-PKcs) belongs to the phosphatidylinositol-3-kinase-related kinase (PIKK) family of atypical serine/threonine protein kinases, which also comprises ATM, ATR and mTOR (Bartlett & Lees-Miller, 2018; Chen et al., 2021; Smith & Jackson, 1999). It is the largest PIKK member (Camfield et al., 2024). Orthologues are conserved throughout vertebrates (human, mouse, chicken, dog, horse, amphibian) and a putative homologue exists in the slime mould Dictyostelium discoideum, whereas clear orthologues are absent from Caenorhabditis elegans, Arabidopsis thaliana, Drosophila melanogaster and yeasts (Bartlett & Lees-Miller, 2018).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + O-phospho-L-seryl/threonyl-[protein] (Puustinen et al., 2020).

## Cofactor Requirements

• Double-stranded DNA ends are required for activation (Bartlett & Lees-Miller, 2018; Puustinen et al., 2020).  
• Mg²⁺ is essential for catalysis (Chen et al., 2021; Smith & Jackson, 1999).  
DNA with single-stranded overhangs stimulates activity more strongly than blunt ends (Bartlett & Lees-Miller, 2018).

## Substrate Specificity

PRKDC preferentially phosphorylates serine or threonine followed by glutamine (consensus [S/T]Q) (Johnson et al., 2023; Yue et al., 2020). While this SQ/TQ preference is shared with ATM and ATR, DNA-PKcs can also modify non-SQ/TQ motifs (e.g., S/T-L or S/T-Y) and substrates lacking obvious consensus sites, such as the C-terminal domain of RNA polymerase II (Dylgjeri & Knudsen, 2022).

## Structure

A >4000-residue protein organised into three regions (Baretić et al., 2019; Sibanda et al., 2017):  
1. N-terminal solenoid (res. 1–2801) built from HEAT/armadillo repeats that form a cradle encircling a central DNA-binding channel and engaging the Ku70/80 heterodimer.  
2. Central “Circular Cradle” (within N-terminal region) continuing the solenoid architecture.  
3. C-terminal head (res. 2802–4128) containing FAT, bilobal kinase domain, FRB-like insertion, LBE, PRD and FATC domains. FAT/FATC clamp the kinase domain, restricting access to its active site; the FRB-like insert acts as a gatekeeper (Baretić et al., 2019; Camfield et al., 2024).  
High-resolution cryo-EM and X-ray structures reveal allostery between the solenoid body and the catalytic head (Sibanda et al., 2017; Williams et al., 2008).

## Regulation

• Allosteric activation upon Ku70/80 binding to DNA ends; Ku80’s C-terminal tail induces conformational changes that open the catalytic cleft (Bartlett & Lees-Miller, 2018; Jette & Lees-Miller, 2015).  
• Extensive autophosphorylation on PQR (e.g., S2056) and ABCDE (e.g., T2609) clusters further modulates activity and promotes dissociation from DNA (Bartlett & Lees-Miller, 2018; Sibanda et al., 2017).  
• Phosphorylation by ATM, ATR and AKT adds additional regulatory layers (Dylgjeri & Knudsen, 2022; Yue et al., 2020).  
• Additional PTMs include PARylation, acetylation, ubiquitination, neddylation, nitrosylation and glycosylation (Dylgjeri & Knudsen, 2022).

## Function

Highly expressed in human cells, PRKDC safeguards genome stability (Yue et al., 2020).  
• DNA repair: core component of the non-homologous end joining (NHEJ) pathway, forming the DNA-PK holoenzyme with Ku70/80 (Wu et al., 2024).  
• V(D)J recombination: activates the nuclease Artemis to process DNA hairpins (Bartlett & Lees-Miller, 2018).  
• Additional roles: regulation of transcription, mitosis, cell migration and autophagy (Puustinen et al., 2020; Yang et al., 2020).

Key substrates/interactors include Ku70/80, Artemis, XRCC4, histone H2AX, XLF, p53, Hsp90 and RNA polymerase II (Bartlett & Lees-Miller, 2018; Chen et al., 2021).

## Inhibitors

Broad-spectrum PI3K/PIKK inhibitors wortmannin and LY294002; more selective compounds NU-7441, VX-984, AZD7648, and M3814; dual DNA-PKcs/mTOR inhibitor CC-115 (Wu et al., 2024; Bartlett & Lees-Miller, 2018; Unknown Authors, 1999).

## Other Comments

Loss-of-function PRKDC mutations cause severe combined immunodeficiency with radiosensitivity and neurological defects (Woodbine et al., 2013). Dysregulation contributes to tumorigenesis, and DNA-PKcs inhibitors are under evaluation to sensitise cancers to DNA-damaging therapies (Chen et al., 2021).

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