## Phylogeny

Classification based on kinase domain sequence places CDC7 as an atypical kinase outside of the main conventional groups (AGC, CAMK, CK1, CMGC) (johnson2023anatlasof pages 3-4). However, other analyses have grouped CDC7 within the CMGC kinase family (sawa2009drugdesignwith pages 2-3). Clustering based on substrate specificity motifs places CDC7 in a group with MOS and KHS1/KHS2 (johnson2023anatlasof pages 4-5). CDC7 is a conserved kinase with known orthologs across species, such as Hsk1 in fission yeast (matsumoto2013regulationofchromosome pages 7-7). Its regulatory subunit Dbf4 also has orthologs, including ASK in humans and Dfp1 in fission yeast (gillespie2022ddktheoutsourced pages 14-15, masai2000regulationofdna pages 4-7).

## Reaction Catalyzed

ATP + a protein <=> ADP + a phosphoprotein (dick2020structuralbasisfor pages 4-5). CDC7 is a serine/threonine kinase that catalyzes the transfer of the gamma-phosphate from ATP to specific serine or threonine residues on its protein substrates (dick2020structuralbasisfor pages 1-3, gillespie2022ddktheoutsourced pages 14-15).

## Cofactor Requirements

Catalytic activity requires magnesium (Mg²⁺), which coordinates the nucleotide within the kinase active site (dick2020structuralbasisfor pages 4-5).

## Substrate Specificity

A comprehensive atlas of substrate specificities for 303 human serine/threonine kinases, including CDC7, was generated using a phosphosite-centric peptide array (PSPA) experimental workflow (johnson2023anatlasof pages 3-4). The precise consensus motifs detailing preferred or disfavored amino acids at positions -3 to +4 for each kinase, including CDC7, are reported in the supplementary data of that publication (johnson2023anatlasof pages 2-3, johnson2023anatlasof pages 6-7). Structural and biochemical data show that CDC7 has a strong substrate preference for a phosphorylated or acidic residue (Asp/Glu) at the P+1 position, immediately C-terminal to the target phosphorylation site (dick2020structuralbasisfor pages 1-3, dick2020structuralbasisfor pages 4-5). This specificity is mediated by invariant CDC7 residues Arg373 and Arg380, which engage the P+1 acidic or phosphate group and are essential for substrate recognition (dick2020structuralbasisfor pages 1-3, dick2020structuralbasisfor pages 5-6).

## Structure

Human CDC7 is a 574-amino acid protein that adopts a canonical bilobal kinase fold (sawa2009drugdesignwith pages 3-5, dick2020structuralbasisfor pages 1-3). The kinase domain is interrupted by three kinase insert (KI) sequences: KI-1, KI-2, and KI-3 (sawa2009drugdesignwith pages 3-5). The KI-2 region contains a critical zinc-finger (ZF) domain that is essential for kinase activity (dick2020structuralbasisfor pages 1-3, dick2020structuralbasisfor pages 4-5). This ZF domain anchors the activation loop to the CDC7 C-lobe and its regulatory subunit DBF4, a conformation required to order the substrate-binding platform and open the active site for catalysis (dick2020structuralbasisfor pages 1-3). The ATP-binding pocket contains methionine 134 as the gatekeeper residue (sawa2009drugdesignwith pages 3-5). CDC7 is catalytically inactive in isolation and requires heterodimerization with a regulatory subunit, either DBF4 or DRF1, for activation (dick2020structuralbasisfor pages 1-3, gillespie2022ddktheoutsourced pages 14-15). DBF4 binds to CDC7 via its conserved M and C motifs; motif C interacts with the CDC7 N-lobe to stabilize the active kinase conformation, while motif M binds the C-lobe (dick2020structuralbasisfor pages 4-5, unknownauthors2014characterizingtheassociations pages 27-32).

## Regulation

The primary mechanism for CDC7 activation is its association with a regulatory subunit, Dbf4 (also called ASK) or its paralog Drf1 (dick2020structuralbasisfor pages 1-3, gillespie2022ddktheoutsourced pages 3-5). The expression of both CDC7 and Dbf4 is transcriptionally regulated by E2F family factors, leading to a rise in their protein levels at the G1/S transition (gillespie2022ddktheoutsourced pages 3-5, gillespie2022ddktheoutsourced pages 5-6). Dbf4 levels are further regulated by proteolysis; it is targeted for degradation by the Anaphase-Promoting Complex (APC/C) at the onset of anaphase and in mid-G1, and by the SCFβTRCP ubiquitin ligase (gillespie2022ddktheoutsourced pages 3-5, unknownauthors2014characterizingtheassociations pages 27-32). In response to replication stress, the checkpoint kinase Rad53 (in yeast) phosphorylates the CDC7-Dbf4 complex (DDK), causing its dissociation from chromatin and subsequent inhibition of replication initiation (larasati2016mechanismsgoverningddk pages 1-3). The activity of DDK is counteracted by the phosphatase PP1 (Glc7 in yeast), which is recruited by the protein Rif1 to dephosphorylate DDK substrates such as Mcm4 (gillespie2022ddktheoutsourced pages 3-5, larasati2016mechanismsgoverningddk pages 1-3).

## Function

CDC7 is an essential serine/threonine kinase that plays a pivotal role in the initiation of eukaryotic DNA replication (masai2000regulationofdna pages 4-7, montagnoli2010targetingcelldivision pages 1-2). In complex with its regulatory subunit Dbf4, it forms the active Dbf4-dependent kinase (DDK), which phosphorylates multiple subunits of the MCM2-7 complex, the core helicase of the pre-replicative complex (pre-RC) (larasati2016mechanismsgoverningddk pages 1-3, sawa2009drugdesignwith pages 3-5). Key phosphorylation targets include the N-terminal domains of MCM2, MCM4, and MCM6 (unknownauthors2014characterizingtheassociations pages 69-76, sawa2009drugdesignwith pages 3-5). This phosphorylation event, particularly the hyperphosphorylation of MCM4, relieves an autoinhibitory activity within the MCM complex, which is a critical step for origin firing (gillespie2022ddktheoutsourced pages 5-6). DDK-mediated activation of the MCM helicase facilitates the recruitment of additional initiation factors, including Cdc45 and the GINS complex, to assemble the active CMG (Cdc45-MCM-GINS) helicase that unwinds DNA (gillespie2022ddktheoutsourced pages 5-6). CDC7 also phosphorylates other replication and checkpoint-related proteins, including Claspin, Treslin, and RecQ4, thereby integrating DNA replication with S-phase checkpoint control (montagnoli2010targetingcelldivision pages 2-4, gillespie2022ddktheoutsourced pages 3-5).

## Inhibitors

Multiple classes of experimental CDC7 inhibitors have been identified. These include XL413 (BMS-863233) and TAK-931, which are potent and selective inhibitors (montagnoli2010targetingcelldivision pages 2-4, dick2020structuralbasisfor pages 7-8). Drug repositioning screens identified the antimicrobial agents Dequalinium chloride and Clofoctol as inhibitors that target the CDC7-Dbf4 interaction (cheng2018identificationofnovel pages 9-10). Other potent, ATP-competitive chemical classes include pyrrolopyridinones (e.g., PHA-767491, IC50 = 10 nM; NMS-354, IC50 = 3 nM), tricyclic pyridothienopyrimidines (Ki = 2 nM), 4-(1H-Indazol-5-yl)-6-phenylpyrimid-2(1H)-ones, thienopyrazoles, and imidazolones (sawa2009drugdesignwith pages 6-8, sawa2009drugdesignwith pages 5-6, zhao2009synthesisandevaluation pages 3-4, montagnoli2010targetingcelldivision pages 5-6).

## Other Comments

CDC7 is frequently overexpressed in a wide range of human cancers, including ovarian, breast, oral squamous cell, and diffuse large B-cell lymphomas (cheng2018identificationofnovel pages 9-10, montagnoli2010targetingcelldivision pages 2-4). Elevated CDC7 expression often correlates with poor prognosis, advanced tumor stage, and resistance to DNA-damaging chemotherapies (cheng2018identificationofnovel pages 9-10, montagnoli2010targetingcelldivision pages 2-4). CDC7 overexpression is also associated with p53 inactivation, and gain-of-function p53 mutants can enhance CDC7-dependent replication initiation (cheng2018identificationofnovel pages 9-10). While CDC7 overexpression is a common feature in tumors, disease-related somatic mutations in the CDC7 gene are reported to be rare (montagnoli2010targetingcelldivision pages 1-2). Inhibition of CDC7 induces p53-independent apoptosis in cancer cells, whereas normal cells undergo a reversible cell-cycle arrest at the G1/S boundary, suggesting a potential therapeutic window for CDC7-targeted drugs (montagnoli2010targetingcelldivision pages 2-4).

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