## Phylogeny

According to the kinome classification by Manning et al., human WEE1 is classified in the “Other” group and the “Wee1” family (fu2018strategicdevelopmentof pages 39-42, hamer2011wee1kinasetargeting pages 3-4, esposito2021wee1kinasea pages 1-2). However, other sources classify WEE1 in the Tyrosine Kinase-Like (TKL) group or the CMGC group (esposito2021wee1kinasea pages 1-2, geenen2017molecularpathwaystargeting pages 1-1, moiseeva2019wee1kinaseinhibitor pages 1-2). Although WEE1 functions as a tyrosine kinase, its catalytic domain structurally resembles serine/threonine kinases, suggesting it evolved from them (esposito2021wee1kinasea pages 2-4, hamer2011wee1kinasetargeting pages 1-2). Orthologs are conserved across eukaryotes, including *Schizosaccharomyces pombe* (wee1) and *Saccharomyces cerevisiae* (SWE1) (fu2018strategicdevelopmentof pages 39-42, hamer2011wee1kinasetargeting pages 3-4). Related paralogs in humans include PKMYT1 and WEE1B, and other related kinases include MIK1 (fu2018strategicdevelopmentof pages 39-42, rora2020awee1family pages 13-14, esposito2021wee1kinasea pages 2-4).

## Reaction Catalyzed

WEE1 catalyzes the ATP-dependent transfer of the gamma-phosphate from ATP to the hydroxyl group of a tyrosine residue on its substrate proteins (esposito2021wee1kinasea pages 17-18, moiseeva2019wee1kinaseinhibitor pages 1-2). Its primary reaction is the phosphorylation of the CDK1 subunit of the Cyclin B1-CDK1 complex specifically at Tyr-15 (geenen2017molecularpathwaystargeting pages 1-1, hamer2011wee1kinasetargeting pages 3-4, moiseeva2019wee1kinaseinhibitor pages 1-2). ATP + Cyclin B1-CDK1 → ADP + Cyclin B1-CDK1-[phospho-Tyr15] (esposito2021wee1kinasea pages 1-2, moiseeva2019wee1kinaseinhibitor pages 1-2).

## Cofactor Requirements

The catalytic activity of WEE1 requires the divalent cation magnesium (Mg2+) as an essential cofactor to coordinate ATP in the active site and facilitate phosphate transfer (elbæk2020wee1kinaselimits pages 2-3, esposito2021wee1kinasea pages 17-18, moiseeva2019wee1kinaseinhibitor pages 1-2).

## Substrate Specificity

WEE1 phosphorylates CDK1 and CDK2 on conserved Tyr-15 residues (do2013wee1kinaseas pages 1-2, fu2018strategicdevelopmentof pages 10-15). One source reports it also phosphorylates CDK1 on Thr-14 (fu2018strategicdevelopmentof pages 10-15). Another substrate is histone H2B at Tyr-37 (fu2018strategicdevelopmentof pages 10-15, esposito2021wee1kinasea pages 4-6). An analysis of intrinsic substrate specificity using positional scanning peptide arrays grouped WEE1 into a cluster labeled “Non-canonical (WEE),” indicating it has atypical substrate motif preferences relative to other tyrosine kinases (yaronbarir2024theintrinsicsubstrate pages 2-2). The consensus substrate motif for WEE1 specifies amino acid preferences at positions flanking the phosphotyrosine (from approximately -5 to +5), which are presented as position-specific scoring matrices and motif logos in the source publication’s supplementary data (yaronbarir2024theintrinsicsubstrate pages 4-5). The specific details of these amino acid preferences are not provided in the supplied context (yaronbarir2024theintrinsicsubstrate pages 2-2).

## Structure

Human WEE1 consists of an N-terminal regulatory domain, a central kinase domain, and a short C-terminal regulatory domain (esposito2021wee1kinasea pages 1-2). The N-terminal domain contains phosphorylation sites (Ser53, Ser123) for degradation, a nuclear localization signal, and three PEST regions that signal for rapid protein turnover (esposito2021wee1kinasea pages 1-2). The central kinase domain contains conserved catalytic features including the C-helix and an activation loop (elbæk2020wee1kinaselimits pages 2-3, moiseeva2019wee1kinaseinhibitor pages 1-2). The short C-terminal domain contains a Ser642 phosphorylation site that acts as a binding site for 14-3-3 proteins (esposito2021wee1kinasea pages 1-2). Several crystal structures of the human WEE1 kinase domain are available in the Protein Data Bank (PDB), including entries 1JXD, 1X8B, 3BI6, 4FX3, 4FX4, 4FX5, and 6O6E (elbæk2020wee1kinaselimits pages 2-3, moiseeva2019wee1kinaseinhibitor pages 1-2, geenen2017molecularpathwaystargeting pages 1-1). High-confidence 3D models from AlphaFold are also available and supplement the experimental structures (elbæk2020wee1kinaselimits pages 2-3, esposito2021wee1kinasea pages 17-18).

## Regulation

WEE1 expression and activity are regulated by transcription, post-translational modifications, and protein interactions (elbæk2020wee1kinaselimits pages 2-3). \* **Transcriptional Regulation**: WEE1 expression is repressed by the transcription factor KLF2 and by microRNA miR-195 (elbæk2020wee1kinaselimits pages 2-3). \* **Post-Translational Modifications**: \* **Phosphorylation**: Phosphorylation at Ser53 and Ser123 by CDK1 and PLK1 marks WEE1 for degradation (esposito2021wee1kinasea pages 1-2, hamer2011wee1kinasetargeting pages 3-4, rora2020awee1family pages 1-2). In response to DNA damage, CHK1 phosphorylation enhances WEE1 activity to maintain G2 arrest (fu2018strategicdevelopmentof pages 10-15). AKT phosphorylates Ser642, promoting 14-3-3 binding and cytoplasmic sequestration, which reduces WEE1 activity (fu2018strategicdevelopmentof pages 10-15). \* **Ubiquitination**: Following phosphorylation, WEE1 is targeted for ubiquitination and proteasomal degradation by SCF E3 ubiquitin ligase complexes, including SCFβ-TrCP1/2 and SCFTome-1 (elbæk2020wee1kinaselimits pages 2-3, rora2020awee1family pages 1-2, esposito2021wee1kinasea pages 2-4). \* **Protein Interactions**: Interactions with chaperone proteins HSP90 and MIG6 stabilize the WEE1 protein (elbæk2020wee1kinaselimits pages 2-3, esposito2021wee1kinasea pages 2-4). Binding of 14-3-3 proteins to phosphorylated Ser642 increases WEE1 stability and catalytic activity during interphase (elbæk2020wee1kinaselimits pages 2-3, fu2018strategicdevelopmentof pages 10-15).

## Function

WEE1 is a key nuclear kinase that regulates the G2/M and S phase checkpoints of the cell cycle (esposito2021wee1kinasea pages 1-2, elbæk2020wee1kinaselimits pages 3-4). \* **Upstream/Downstream Partners**: WEE1 is activated by the DNA damage response kinases ATM and ATR via CHK1 (geenen2017molecularpathwaystargeting pages 1-1). Its primary substrates are CDK1 and CDK2, which it inhibits via Tyr-15 phosphorylation (do2013wee1kinaseas pages 1-2, rora2020awee1family pages 1-2). The phosphatase CDC25 reverses this inhibition (fu2018strategicdevelopmentof pages 10-15). \* **Signaling Pathways**: WEE1 functions as a master regulator of the G2 DNA damage checkpoint by inactivating the CDK1-cyclin B complex to arrest the cell cycle and allow for DNA repair (esposito2021wee1kinasea pages 1-2, hamer2011wee1kinasetargeting pages 3-4). During S phase, it modulates CDK2 activity to stabilize replication forks and prevent genomic instability (esposito2021wee1kinasea pages 1-2, rora2020awee1family pages 1-2). It also has an epigenetic role, phosphorylating histone H2B at Tyr-37 to suppress histone gene transcription (fu2018strategicdevelopmentof pages 10-15, esposito2021wee1kinasea pages 4-6).

## Inhibitors

* **Adavosertib (AZD1775/MK1775)**: A potent and selective ATP-competitive small-molecule inhibitor of WEE1 (geenen2017molecularpathwaystargeting pages 1-1, hamer2011wee1kinasetargeting pages 3-4). It abrogates the G2 checkpoint, leading to premature mitotic entry and mitotic catastrophe, particularly in p53-deficient cancer cells (geenen2017molecularpathwaystargeting pages 1-2, hamer2011wee1kinasetargeting pages 3-4).
* **PROTACs**: Proteolysis-Targeting Chimeras are being investigated as a novel approach to induce targeted degradation of WEE1 protein (elbæk2020wee1kinaselimits pages 2-3).

## Other Comments

Dysregulation of WEE1 is implicated in numerous cancers (fu2018strategicdevelopmentof pages 10-15). Overexpression is common in glioblastoma, ovarian, colorectal, and breast cancer and often correlates with poor patient outcomes (geenen2017molecularpathwaystargeting pages 1-2, fu2018strategicdevelopmentof pages 10-15). Some reports also link WEE1 downregulation to poor prognosis (esposito2021wee1kinasea pages 4-6). Cancer cells with p53 mutations are often highly dependent on the WEE1-mediated G2 checkpoint for survival, creating a therapeutic vulnerability (elbæk2020wee1kinaselimits pages 3-4, geenen2017molecularpathwaystargeting pages 1-1). WEE1 knockout in mouse models results in embryonic lethality, underscoring its essential role in development (esposito2021wee1kinasea pages 4-6).

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