1. Phylogeny  
   WEE1‐like protein kinase is an evolutionarily conserved enzyme found throughout eukaryotes. Orthologs have been identified in organisms ranging from yeasts to mammals, and the protein is a critical member of the cell cycle checkpoint machinery. Its catalytic domain is highly conserved and, although WEE1 phosphorylates a tyrosine residue, its overall fold exhibits strong similarity to that of serine/threonine kinases. Comparative analyses indicate that WEE1 shares a common evolutionary origin with other CDK‐regulatory kinases such as Myt1, which, despite differences in substrate specificity and cellular localization, together constitute a subgroup of kinases essential for maintaining the G2/M checkpoint. The presence of WEE1 orthologs across diverse species supports the view that this kinase emerged early in eukaryotic evolution and has been maintained as an integral regulator of cell cycle progression (squire2005structureandinhibition pages 2-3, schmidt2017regulationofg2m pages 1-3).
2. Reaction Catalyzed  
   WEE1 functions by catalyzing the transfer of a phosphate group from ATP to a specific tyrosine residue on its substrate protein. In this case, the substrate is the cyclin B1‐bound form of CDK1, and the targeted phosphoacceptor is the tyrosine residue at position 15. The chemical reaction can be represented as:  
     ATP + [cyclin B1–CDK1] → ADP + [cyclin B1–CDK1]–pTyr15 + H⁺  
   This phosphorylation event renders CDK1 inactive and is critical for delaying the onset of mitosis until cell cycle conditions are appropriate (squire2005structureandinhibition pages 7-8, lloyd2013cancerbiomarkerdiscovery pages 110-112).
3. Cofactor Requirements  
   The catalytic activity of WEE1 is strictly dependent on ATP as the phosphate donor, and the reaction requires the presence of divalent metal ions. Mg²⁺ is the primary cofactor that stabilizes ATP binding within the active site, thereby facilitating the transfer of the phosphate group to the substrate tyrosine residue (squire2005structureandinhibition pages 2-3, wang2023targetingcdk1in pages 2-3).
4. Substrate Specificity  
   WEE1 displays a narrow substrate specificity that is confined to the cyclin B1‐CDK1 complex. The enzyme phosphorylates CDK1 exclusively on tyrosine 15, and this modification is not observed on monomeric CDK1. Structural features within the kinase domain of WEE1 form a binding pocket that specifically recognizes the sequence context surrounding Tyr15 in CDK1. Although a full linear consensus sequence for substrate recognition has not been explicitly defined in the literature provided, the phosphorylation event is highly selective and is achieved via docking interactions that favor the cyclin‐bound conformation of CDK1 (squire2005structureandinhibition pages 7-8, lloyd2013cancerbiomarkerdiscovery pages 110-112, wang2023targetingcdk1in pages 8-9).
5. Structure  
   WEE1 is composed predominantly of a central catalytic kinase domain that exhibits the canonical two‐lobed structure typical of protein kinases. The N-terminal lobe is mainly comprised of β-sheets, while the C-terminal lobe is dominated by α-helices. The interface between these lobes forms the active site cleft where ATP binds. Structural studies, including high-resolution crystallographic analyses, have demonstrated that despite its role in phosphorylating a tyrosine residue, WEE1 shares considerable structural similarity with serine/threonine kinases. The enzyme contains a glycine-rich loop (P-loop) that facilitates ATP binding and an activation segment that remains in an ordered conformation in the active state. Notable within the structure is the conserved catalytic aspartate (Asp426), which is essential for the phosphoryl transfer reaction. Unique adaptations in WEE1 include a substitution within the glycine-rich loop, where a glutamate residue (Glu309) is positioned to sterically hinder the phosphorylation of threonine residues, thereby favoring tyrosine specificity. Additionally, residues in the activation loop, such as Arg481, create a shallow groove that assists in precisely docking the cyclin B1–CDK1 substrate so that Tyr15 is optimally positioned for phosphorylation. This combination of conserved kinase motifs with distinct structural modifications underlines the atypical nature of WEE1’s catalytic mechanism (squire2005structureandinhibition pages 3-5, squire2005structureandinhibition pages 7-8, schmidt2017regulationofg2m pages 5-9).
6. Regulation  
   The activity of WEE1 is subject to stringent control throughout the cell cycle. Under normal conditions, WEE1 levels and kinase activity are elevated during the S and G2 phases to enforce the G2/M checkpoint by maintaining CDK1 in an inactive, phosphorylated state. At the transition from G2 to M phase, WEE1 becomes hyperphosphorylated, a modification that correlates with a decrease in its activity. This hyperphosphorylation is closely associated with its proteolytic degradation during the M/G1 transition, resulting in a significant drop in protein levels that permits the activation of CDK1 and progression into mitosis. Additionally, feedback mechanisms exist in which active CDK1 can contribute to the inhibition of WEE1, thus creating a tightly regulated loop that ensures the timely entry into mitosis. Although the precise kinases responsible for WEE1 phosphorylation have not been exhaustively detailed, the regulation appears to involve a combination of autophosphorylation events and phosphorylation by other cell cycle-regulatory enzymes. These post-translational modifications are critical for modulating both the catalytic activity and stability of WEE1 (squire2005structureandinhibition pages 7-8, schmidt2017regulationofg2m pages 1-3).
7. Function  
   WEE1 functions as an essential negative regulator of the cell cycle by controlling the timing of mitotic entry. By phosphorylating CDK1 exclusively on Tyr15, WEE1 ensures that the cyclin B1–CDK1 complex remains inactive during the S and G2 phases, thereby allowing cells time to complete DNA replication and repair any accumulated damage before undergoing mitosis. This checkpoint control is vital for maintaining genomic integrity and preventing the propagation of damaged DNA. The expression of WEE1 is cell cycle–dependent, with its activity peaking during S and G2 and declining as cells progress through mitosis. The precise control of CDK1 activation by WEE1 is integral to the proper coordination of cell division and is a critical component of the DNA damage response network. In cellular contexts where DNA damage is detected, sustained WEE1 activity contributes to the enforcement of the G2/M checkpoint, thereby preventing premature mitotic entry and the potential onset of genomic instability (squire2005structureandinhibition pages 7-8, lloyd2013cancerbiomarkerdiscovery pages 110-112, wang2023targetingcdk1in pages 2-3).
8. Other Comments  
   The clinical relevance of WEE1 is underscored by the development of several small molecule inhibitors that target its kinase activity. Notably, AZD1775 (also known as MK-1775) is a well-characterized inhibitor that has been investigated in clinical trials for its ability to abrogate the G2/M checkpoint, thereby sensitizing tumor cells—especially those with p53 deficiencies—to DNA-damaging agents. These inhibitors demonstrate that interfering with WEE1 activity can force cells into premature mitosis, a strategy that has significant therapeutic potential in oncology. In addition to its role in enforcing cell cycle checkpoints, aberrant regulation or dysregulation of WEE1 has been associated with cancer progression. While the available literature does not elaborate on specific disease mutations altering WEE1’s function, its pivotal role in controlling CDK1 activity makes it a potential target in cancers characterized by unchecked cell division. Research in this area continues to focus on improving the selectivity and potency of WEE1 inhibitors to maximize their therapeutic benefit while minimizing off-target effects (matheson2016targetingwee1kinase pages 4-6, wang2023targetingcdk1in pages 9-10, lloyd2013cancerbiomarkerdiscovery pages 110-112).
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