## Phylogeny

Casein kinase 1 isoform epsilon (CK1ε) is a member of the casein kinase 1 (CK1) family, which constitutes a distinct branch of the serine/threonine protein kinases within the eukaryotic kinome (francisco2022caseinkinase1 pages 1-2, fulcher2020functionsandregulation pages 1-2). According to the kinome classification by Manning et al., CK1ε is assigned to the CK1 group, which is part of the CMGC kinase group (schittek2014biologicalfunctionsof pages 13-13, fulcher2020functionsandregulation pages 16-17). The CK1 family includes several isoforms; sources variously report six isoforms (α, δ, ε, γ1, γ2, γ3) or seven (α, β, γ1, γ2, γ3, δ, ε) (francisco2022caseinkinase1 pages 1-2, schittek2014biologicalfunctionsof pages 1-2). These isoforms share high sequence homology within their N-terminal kinase domains but have highly variable C-terminal regions (fulcher2020functionsandregulation pages 2-4). CK1ε shares over 96-98% sequence identity with the CK1δ isoform in the kinase domain and 53% identity in the C-terminal regulatory domain (francisco2022caseinkinase1 pages 1-2, schittek2014biologicalfunctionsof pages 1-2). Orthologs of CK1ε have been studied in *Xenopus*, mouse, and fly models (unknownauthors2007regulationofcasein pages 40-45).

## Reaction Catalyzed

CK1ε is a serine/threonine kinase that catalyzes the transfer of the γ-phosphate group from ATP to the hydroxyl group of serine or threonine residues on substrate proteins (fulcher2020functionsandregulation pages 14-15, schittek2014biologicalfunctionsof pages 13-13). ATP is the exclusive phosphate donor (fulcher2020functionsandregulation pages 2-4). While CK1ε primarily targets serine/threonine residues, some CK1 orthologs and isoforms have demonstrated dual-specificity activity toward tyrosine residues on synthetic substrates, though this is less established for human CK1ε (fulcher2020functionsandregulation pages 1-2, fulcher2020functionsandregulation pages 15-16).

## Cofactor Requirements

The catalytic activity of CK1ε requires the presence of divalent cations (francisco2022caseinkinase1 pages 2-3, schittek2014biologicalfunctionsof pages 17-18). It can utilize either Mg²⁺ or Mn²⁺ as a cofactor (leya2025caseinkinase1 pages 12-14, fulcher2020functionsandregulation pages 10-11, fulcher2020functionsandregulation pages 14-15).

## Substrate Specificity

CK1ε is an acidophilic kinase, preferentially phosphorylating substrates containing acidic residues near the target site (johnson2023anatlasof pages 4-4). The consensus motif often involves a priming phosphorylation, targeting serine/threonine residues located three residues downstream of a phosphorylated serine or threonine (pS/pT-X-X-S*/T*) (francisco2022caseinkinase1 pages 1-2, fulcher2020functionsandregulation pages 2-4, fulcher2020functionsandregulation pages 14-15). Acidic residues can substitute for the priming phosphosite (fulcher2020functionsandregulation pages 2-4). CK1ε exhibits fine substrate discrimination through negative-selectivity elements that flank the phosphorylation site, preventing phosphorylation by other kinases (johnson2023anatlasof pages 4-4). While it often acts via a priming mechanism, some evidence indicates it can phosphorylate both primed and unprimed substrates; for instance, it phosphorylates an unprimed Ser45 on β-catenin (francisco2022caseinkinase1 pages 2-3). It also recognizes an F-X-X-X-F motif on substrates such as PER1 (fulcher2020functionsandregulation pages 4-5).

## Structure

CK1ε consists of a conserved N-terminal kinase domain and a variable C-terminal regulatory domain of approximately 124 amino acids that contains multiple phosphorylation sites and has an autoinhibitory function (francisco2022caseinkinase1 pages 1-2, schittek2014biologicalfunctionsof pages 1-2, unknownauthors2007regulationofcasein pages 32-36). The kinase domain contains a putative nuclear localization signal (unknownauthors2007regulationofcasein pages 40-45). X-ray crystallography has revealed that the activation loop of CK1ε can adopt two distinct conformations, termed “Loop Up” and “Loop Down,” which influences substrate selection (francisco2022caseinkinase1 pages 2-3). The kinase also features anion binding pockets that are modulated by phosphorylation events within its C-terminal tail, thereby controlling kinase activation (francisco2022caseinkinase1 pages 2-3).

## Regulation

The primary mechanism for CK1ε regulation is autophosphorylation of its C-terminal tail, which is generally autoinhibitory (francisco2022caseinkinase1 pages 1-2, schittek2014biologicalfunctionsof pages 1-2). Specific autoinhibitory phosphorylation sites include Ser408, Ser343, Ser354, Ser362, Ser363, and Ser389 (francisco2022caseinkinase1 pages 2-3, schittek2014biologicalfunctionsof pages 1-2). This inhibition can be relieved by protein phosphatases or limited proteolysis (unknownauthors2007regulationofcasein pages 24-28, unknownauthors2007regulationofcasein pages 40-45). CK1ε activity is also modulated by upstream signals, including Wnts and AMPK pathways, which alter tail phosphorylation (francisco2022caseinkinase1 pages 1-2). Interacting proteins provide another layer of regulation; the DEAD-box RNA helicase DDX3 can allosterically activate CK1ε in a Wnt-dependent context, whereas Axin can act as a competitive inhibitor by preventing CK1ε from binding to DVL (fulcher2020functionsandregulation pages 2-4, fulcher2020functionsandregulation pages 4-5, schittek2014biologicalfunctionsof pages 12-13).

## Function

CK1ε is ubiquitously expressed but is enriched in the brain, heart, and skeletal muscle (fulcher2020functionsandregulation pages 2-4, unknownauthors2007regulationofcasein pages 40-45). It is a central component of the circadian clock, where it regulates the stability of PERIOD proteins (PER1, PER2) through a “phosphoswitch” mechanism (francisco2022caseinkinase1 pages 1-2). CK1ε executes a slow priming phosphorylation on PER2 Ser662, which is followed by rapid phosphorylation of downstream serines, leading to PER2 stabilization (francisco2022caseinkinase1 pages 1-2). Conversely, it can also phosphorylate the phosphodegron region of PER2 (S480, S484), which targets it for proteasomal degradation (francisco2022caseinkinase1 pages 2-3). In the Wnt signaling pathway, CK1ε phosphorylates DVL proteins, β-catenin (at S45), APC, and Axin, thereby modulating both canonical and non-canonical pathways (francisco2022caseinkinase1 pages 2-3, schittek2014biologicalfunctionsof pages 1-2, unknownauthors2007regulationofcasein pages 24-28, unknownauthors2007regulationofcasein pages 32-36). Its functions also extend to the Hedgehog, Hippo, NFκB, and p53 signaling pathways, as well as the regulation of cell-cell communication via phosphorylation of Connexin-43 (schittek2014biologicalfunctionsof pages 1-2, francisco2022caseinkinase1 pages 2-3).

## Inhibitors

Several experimental inhibitors target CK1ε. IC261 is a selective inhibitor of both CK1δ and CK1ε that induces G2/M cell cycle arrest and apoptosis in cancer cells (fulcher2020functionsandregulation pages 17-18, yang2008inhibitionofcasein pages 7-9). PF-670462 is another potent and selective inhibitor of CK1δ and CK1ε, with a reported IC50 of 14 nM for CK1ε (unknownauthorsUnknownyearregulatoryproteinproteininteractions pages 67-70). D4476 is a broader CK1 family inhibitor that also affects CK1ε activity (schittek2014biologicalfunctionsof pages 12-13). Currently, no inhibitors with high specificity for only the CK1ε isoform are widely available (fulcher2020functionsandregulation pages 2-4).

## Other Comments

Dysregulation and mutations of CK1ε are associated with several human diseases. It is prominently linked to circadian rhythm disorders, including Familial Advanced Sleep Phase Syndrome (FASPS) and Delayed Sleep Phase Syndrome (francisco2022caseinkinase1 pages 1-2, francisco2022caseinkinase1 pages 2-3, schittek2014biologicalfunctionsof pages 1-2). The FASPS-associated T44A mutation in CK1ε alters kinase function and circadian period (unknownauthors2007regulationofcasein pages 40-45). Similarly, the S662G mutation in its substrate PER2 disrupts CK1ε-mediated phosphorylation and causes FASPS (francisco2022caseinkinase1 pages 1-2). The S408N polymorphism in CK1ε increases kinase activity and is associated with delayed sleep phase disorders (francisco2022caseinkinase1 pages 2-3). CK1ε is also implicated in tumorigenesis, including breast and colon cancer, and its levels are elevated in multiple cancer types (fulcher2020functionsandregulation pages 10-11, unknownauthors2007regulationofcasein pages 24-28, yang2008inhibitionofcasein pages 7-9). Further associations include chronic liver diseases and neurodegenerative disorders (leya2025caseinkinase1 pages 12-14).

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