## Phylogeny

HUNK (Hormonally up-regulated neu tumor-associated kinase) is a serine/threonine protein kinase classified as a member of the SNF1/AMPK family of kinases (ramossolis2022hunkgenealterations pages 1-2, unknownauthors2024characterizationofa pages 58-63, unknownauthors2006hormonallyupregulatedneutumorassociated pages 23-24). Phylogenetically, it shows highest homology to the *Saccharomyces cerevisiae* SNF1 family of kinases and is related to kinases conserved across species including yeast (SNF1), plants, and mammals (AMPK) (unknownauthors2006hormonallyupregulatedneutumorassociated pages 31-32, unknownauthors2006hormonallyupregulatedneutumorassociated pages 23-24). HUNK represents a new branch of the SNF1 family tree and does not fit within its most recognized subfamilies (unknownauthors2006hormonallyupregulatedneutumorassociated pages 31-32). The murine Hunk protein shares approximately 95% homology with its human homolog (unknownauthors2006hormonallyupregulatedneutumorassociated pages 29-30). According to the Manning et al. 2002 classification, HUNK is placed within the CAMK (Calcium/Calmodulin-dependent protein kinase) group due to its relationship with SNF1/AMPK-related kinases (unknownauthors2006hormonallyupregulatedneutumorassociated pages 23-24, unknownauthors2006hormonallyupregulatedneutumorassociated pages 31-32). A hierarchical clustering analysis based on amino acid motif selectivity also places HUNK in a group related to the CAMK or NEK kinase subfamilies (johnson2023anatlasof pages 4-5).

## Reaction Catalyzed

HUNK catalyzes the ATP-dependent transfer of the gamma-phosphate group from ATP to the hydroxyl group of serine or threonine residues on substrate proteins (unknownauthors2006hormonallyupregulatedneutumorassociated pages 37-38, unknownauthors2006hormonallyupregulatedneutumorassociated pages 23-24, manning2002theproteinkinase pages 1-2).

## Cofactor Requirements

The catalytic activity of HUNK requires ATP as a phosphate donor (unknownauthors2006hormonallyupregulatedneutumorassociated pages 23-24, unknownauthors2006hormonallyupregulatedneutumorassociated pages 37-38). The kinase also requires divalent metal ions such as Mg2+ or Mn2+ to assist in phosphate transfer (unknownauthors2006hormonallyupregulatedneutumorassociated pages 39-40, unknownauthors2006hormonallyupregulatedneutumorassociated pages 23-24, unknownauthors2006hormonallyupregulatedneutumorassociated pages 31-32).

## Substrate Specificity

A specific consensus phosphorylation motif for HUNK has not been identified (unknownauthors2024characterizationofa pages 58-63, unknownauthors2019targetinghunkto pages 39-43). In an atlas of substrate specificities for the human serine/threonine kinome, HUNK ranked 7th (98.86 percentile) for phosphorylation of the substrate PDHA1 at Ser293, indicating high specificity for the sequence motif RYHGHSMSDP (johnson2023anatlasof pages 21-23).

## Structure

HUNK is an approximately 80 kDa protein composed of 714 amino acids (unknownauthors2006hormonallyupregulatedneutumorassociated pages 30-31, ramossolis2022hunkgenealterations pages 1-2). Its domain organization includes an N-terminal domain, a central 260-amino acid kinase catalytic domain, and a C-terminal domain (unknownauthors2006hormonallyupregulatedneutumorassociated pages 30-31, unknownauthors2019targetinghunkto pages 26-32). The catalytic domain contains motifs typical of serine/threonine kinases, including a DLKPEN motif in subdomain VIB, and lacks conserved residues found in tyrosine kinases (unknownauthors2006hormonallyupregulatedneutumorassociated pages 29-30). A conserved SNF1 homology (SNH) domain of about 45 amino acids, which may have an autoinhibitory function, is located C-terminal to the kinase domain (unknownauthors2006hormonallyupregulatedneutumorassociated pages 31-32). Within the kinase domain, there is a region of positively charged amino acids that matches the phosphatidylinositol 4,5-bisphosphate (PIP2) consensus binding site (unknownauthors2019targetinghunkto pages 26-32).

No experimentally determined 3D structures have been published (unknownauthors2024characterizationofa pages 58-63). Homology and AlphaFold models reveal a canonical bilobed kinase fold with the ATP-binding site located in the cleft between the lobes (unknownauthors2019targetinghunkto pages 51-57). Key regulatory features include a smaller N-terminal lobe containing the C-helix, which is critical for regulatory conformational changes, and a larger C-terminal lobe containing the activation loop, which controls catalytic activity (unknownauthors2019targetinghunkto pages 51-57, unknownauthors2024characterizationofa pages 58-63). The hydrophobic spine, a set of conserved hydrophobic residues spanning both lobes, is expected to maintain structural integrity for catalysis (unknownauthors2019targetinghunkto pages 51-57, unknownauthors2024characterizationofa pages 58-63).

## Regulation

HUNK is subject to post-translational modifications, including phosphorylation, acetylation, carboxylation, and glycosylation (unknownauthors2006hormonallyupregulatedneutumorassociated pages 31-32). Phosphorylation sites have been identified at S65, S360, S368, Y378, S561, S585, and T618 (unknownauthors2024characterizationofa pages 63-67, unknownauthors2024characterizationofa pages 58-63). However, the upstream kinases responsible for these modifications and the functional consequences remain undetermined (unknownauthors2024characterizationofa pages 63-67, unknownauthors2024characterizationofa pages 58-63, unknownauthors2019targetinghunkto pages 39-43). HUNK binds to the Nedd4 E3 ubiquitin ligase, but direct ubiquitination sites have not been reported (unknownauthors2024characterizationofa pages 63-67).

HUNK kinase activity is critical for its function, as a kinase-inactive K91M mutant impairs tumor growth and alters HUNK localization (unknownauthors2019targetinghunkto pages 39-43). The SNH domain is speculated to have an autoinhibitory regulatory function (unknownauthors2006hormonallyupregulatedneutumorassociated pages 31-32). HUNK expression is upregulated by the steroid hormones 17β-estradiol and progesterone, as well as by the oncogenes HER2/neu and Akt (unknownauthors2024hunkasana pages 39-44, ramossolis2022hunkgenealterations pages 1-2).

## Function

HUNK kinase regulates cellular processes including cell survival, proliferation, autophagy, and metastasis (ramossolis2022hunkgenealterations pages 1-2). In adult mice, HUNK is expressed in the ovary, lung, brain, mammary gland, uterus, and prostate (unknownauthors2006hormonallyupregulatedneutumorassociated pages 31-32, unknownauthors2006hormonallyupregulatedneutumorassociated pages 39-40). Its expression is highly regulated during murine development (unknownauthors2006hormonallyupregulatedneutumorassociated pages 31-32).

Upstream regulators that increase HUNK expression include HER2/neu and Akt (ramossolis2022hunkgenealterations pages 1-2). HUNK phosphorylates several substrates, including EGFR at T654, Rubicon at S44 and S92, ITM2A at T35, GEF-H1 at S645, and AGAP3 at S396 (unknownauthors2024characterizationofa pages 63-67, ramossolis2022hunkgenealterations pages 1-2). These phosphorylation events promote breast cancer metastasis, switch Rubicon from an autophagy inhibitor to a promoter, inhibit epithelial-mesenchymal transition, and influence endocytosis, respectively (unknownauthors2024characterizationofa pages 63-67, ramossolis2022hunkgenealterations pages 1-2). HUNK also phosphorylates histone H1 and myelin basic protein in vitro (unknownauthors2006hormonallyupregulatedneutumorassociated pages 39-40). Non-substrate interacting partners of HUNK include the Nedd4 E3 ubiquitin ligase, the Beclin-1 complex, cofilin-1 (CFL-1), and Rabaptin-5 (unknownauthors2024characterizationofa pages 63-67, unknownauthors2024hunkasana pages 39-44, unknownauthors2019targetinghunkto pages 39-43).

## Inhibitors

Staurosporine can pharmacologically inhibit HUNK kinase activity (ramossolis2022hunkgenealterations pages 1-2). In contrast, another source states that no pharmacological inhibitors of HUNK exist (unknownauthors2019targetinghunkto pages 39-43).

## Other Comments

HUNK is implicated in breast cancer, particularly HER2+ and triple-negative subtypes, where its overexpression correlates with poorer clinical outcomes and it promotes tumorigenesis, metastasis, and drug resistance (ramossolis2022hunkgenealterations pages 2-4, unknownauthors2024hunkasana pages 39-44). Gene alterations are rare (approx. 1.3% mutation frequency in breast cancer), but include missense mutations in the kinase domain, copy number gains, and fusions (HUNK-MRAP and EVA1C-HUNK) (ramossolis2022hunkgenealterations pages 2-4). The human HUNK gene is located on chromosome 21q22, a region associated with Down syndrome and Alzheimer’s disease (unknownauthors2006hormonallyupregulatedneutumorassociated pages 29-30).

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