## Phylogeny

• Ser/Thr-protein kinase N3 (PKN3) is classified within the AGC kinase group, PKN/PRK subfamily, which is evolutionarily closest to conventional and novel protein kinase C isoforms (collazos2011siterecognitionand pages 11-13).  
• Orthologs have been identified in Mus musculus, Rattus norvegicus, Danio rerio and Drosophila melanogaster, underscoring conservation across vertebrates and invertebrates (asquith2022identificationof4‐anilinoquin(az)oline pages 1-3).  
• The catalytic domain shares high sequence identity with PKN1 and PKN2, whereas N-terminal regulatory regions diverge, accounting for isozyme-specific control (hutchinson2013differentialbindingof pages 36-40).

## Reaction Catalyzed

• ATP + protein-L-Ser/Thr ⇌ ADP + protein-L-Ser/Thr-phosphate (collazos2011siterecognitionand pages 11-13).

## Cofactor Requirements

• Catalytic activity requires Mg²⁺ to coordinate ATP phosphates (collazos2011siterecognitionand pages 11-13).

## Substrate Specificity

• Kinase-substrate atlas data assign a consensus motif R-X-R-X-X-[S/T]-ϕ (ϕ = hydrophobic) with strict arginine at −5 and −3 and hydrophobic preference at +1 (sophocleous2021thestructureand pages 19-19).  
• Additional profiling confirms preference for basic residues at −2/−3 positions relative to the phospho-acceptor (collazos2011siterecognitionand pages 11-13).

## Structure

• Domain organisation: N-terminal C2-like domain, three tandem HR1 repeats that bind Rho GTPases, central poly-proline segment, and C-terminal bilobal kinase domain (hutchinson2013differentialbindingof pages 36-40).  
• Crystal structure of an HR1 repeat in complex with RhoA (PDB 1W4E) defines the GTPase interaction interface (collazos2011siterecognitionand pages 9-11).  
• Full-length AlphaFold model AF-Q6P5Z2-F1 depicts the ordered C-helix, hydrophobic spine, HRD catalytic triad and DFG Mg²⁺-binding motif characteristic of active AGC kinases (asquith2022identificationof4‐anilinoquin(az)oline pages 1-3).  
• Unique feature: centrally located poly-proline motif (P500PPKPPRL) that mediates SH3-domain binding to p130Cas (unknownauthorsUnknownyear5.4.the4th pages 158-160).

## Regulation

• Phosphorylation of Thr774 in the activation loop by PDK1 primes catalytic activity (asquith2022identificationof4‐anilinoquin(az)oline pages 1-3).  
• Subsequent phosphorylation of the turn-motif Thr860 by mTORC2 completes activation (unsalkacmaz2012theinteractionof pages 1-2).  
• HR1-mediated binding of RhoA, RhoB or RhoC allosterically stimulates kinase activity (hutchinson2013differentialbindingof pages 36-40).  
• Ubiquitination and lipid interactions further modulate stability and membrane localisation; the responsible enzymes are not yet defined (asquith2022identificationof4‐anilinoquin(az)oline pages 1-3).  
• PI3K pathway hyperactivation up-regulates PKN3 transcription in PTEN-deficient contexts (leenders2004pkn3isrequired pages 1-2).

## Function

• Basal expression is confined to skeletal muscle, heart and liver, whereas strong up-regulation is observed in prostate, pancreatic, breast and T-cell leukaemia cell lines (asquith2022identificationof4‐anilinoquin(az)oline pages 1-3).  
• Acts downstream of class I PI3K, largely independent of Akt, to promote cytoskeletal reorganisation and invasive migration (leenders2004pkn3isrequired pages 1-2).  
• Interacts with RhoC, phosphorylates adaptor p130Cas at Ser432 and binds α-actinin, integrating PI3K and Rho signalling to drive motility (unsalkacmaz2012theinteractionof pages 1-2, asquith2022identificationof4‐anilinoquin(az)oline pages 1-3).  
• Pkn3-null mice are viable but display impaired fibroblast migration, reduced endothelial sprouting and diminished metastatic colonisation, indicating roles in angiogenesis and tumour dissemination (mukai2016pkn3isthe pages 1-2).

## Inhibitors

• 4-Anilinoquin(az)oline derivatives constitute the first reported small-molecule chemotype with cell-active PKN3 inhibition (asquith2022identificationof4‐anilinoquin(az)oline pages 1-3).  
• Atu027, a liposomal siRNA targeting PKN3 mRNA, suppresses tumour growth and metastasis in pre-clinical models (asquith2022identificationof4‐anilinoquin(az)oline pages 1-3).

## Other Comments

• Elevated PKN3 expression correlates with aggressive disease in prostate, pancreatic and breast cancers (asquith2022identificationof4‐anilinoquin(az)oline pages 1-3, leenders2004pkn3isrequired pages 1-2).  
• No recurrent pathogenic coding variants have been reported to date (asquith2022identificationof4‐anilinoquin(az)oline pages 1-3).

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