## Phylogeny

Nemo-like kinase (NLK) is an evolutionarily conserved serine/threonine protein kinase with orthologues in Drosophila (Nemo), Caenorhabditis elegans (LIT-1), Xenopus, zebrafish and mice (Daams & Massoumi, 2020; Ishitani et al., 2003; Kim et al., 2010; Ota et al., 2011). In vertebrates, two phylogenetic forms exist (type I and type II), but mammals and chicken encode only the type II enzyme (Ishitani & Ishitani, 2013). NLK belongs to the CMGC kinase group and, although its catalytic core shares sequence homology with ERK1/ERK5, it is classified as an atypical MAPK because of its distinct exon–intron organisation and the absence of a phosphorylatable tyrosine in the activation loop (Chan, 2011; Daams & Massoumi, 2020; Harada et al., 2002; Johnson et al., 2023).

## Reaction Catalyzed

ATP + [a protein] ⇌ ADP + [a phosphoprotein] (Daams & Massoumi, 2020; Ishitani & Ishitani, 2013; Ishitani et al., 2003).

## Cofactor Requirements

Requires divalent metal ions, Mg²⁺ or Mn²⁺, for catalytic activity (Daams & Massoumi, 2020; Ishitani & Ishitani, 2013; Shi et al., 2010).

## Substrate Specificity

NLK is a proline-directed kinase that preferentially phosphorylates serine or threonine followed by proline (S/T-P motif). Flanking negative-selection elements refine site choice (Johnson et al., 2023). Verified targets include Thr155 and Ser166 of LEF1 (Ishitani & Ishitani, 2013).

## Structure

Human NLK is a 515-residue protein with a central kinase domain (aa 127–415) flanked by low-complexity N- and C-terminal regions; the N-terminus is rich in Ala/Gln/His/Pro and resembles a zinc-finger–type interaction module (Chan, 2011). No crystal structure is available; an AlphaFold model (UniProt Q9UBE8) predicts a canonical kinase fold with β-sheet N-lobe, α-helical C-lobe, conserved C-helix and an atypical activation loop containing a TQE motif in place of the usual TXY (Coulombe & Meloche, 2007; Dahm et al., 2025; Johnson et al., 2023).

## Regulation

• Activation: phosphorylated on Thr286 in the activation loop by the MAP3K TAK1, an event essential for catalytic activity (Ishitani et al., 2011; Ishitani et al., 2003; Chan, 2011).  
• Modulation: p38 MAPK phosphorylates Ser510 in the C-terminus, influencing substrate binding (Chan, 2011).  
• Expression control: down-regulated by miR-181, miR-92b, miR-101, miR-199a-3p, miR-197 and the lncRNA HOTAIR (Huang et al., 2015; Ishitani & Ishitani, 2013).  
• Allosteric: interaction with ZIPK reduces binding to the substrate TCF7L2 (Ishitani & Ishitani, 2013).

## Function

Widely expressed in adult tissues with highest levels in brain (Harada et al., 2002; Ishitani & Ishitani, 2013). NLK phosphorylates multiple regulators, including TCF/LEF transcription factors, SMAD2/3/4, STAT3, c-Myb, A-Myb, FOXO proteins, Notch1 intracellular domain, Pumilio1/2 and CPEB (Daams & Massoumi, 2020; Ishitani & Ishitani, 2013; Liang et al., 2021; Ota et al., 2011; Shi et al., 2010).  
• Signalling pathways: context-dependent modulator of Wnt/β-catenin (negative via TCF/LEF phosphorylation or positive in some settings) and contributor to Notch, TGF-β/Activin, IL-6 and NF-κB cascades (Daams & Massoumi, 2020; Ishitani & Ishitani, 2013; Ishitani et al., 2003; Liang et al., 2021).  
• Biological processes: organ development (lung, heart, skeleton), immune cell differentiation, antiviral defence, neurogenesis, osteogenesis, adipogenesis and oocyte maturation (Daams & Massoumi, 2020; Ishitani & Ishitani, 2013; Ota et al., 2011).

## Inhibitors

Lithium chloride (LiCl) suppresses NLK kinase activity and endogenous activation (Ishitani & Ishitani, 2013).

## Other Comments

NLK is implicated in neurodegenerative disorders such as spinal bulbar muscular atrophy and Huntington’s disease (Daams & Massoumi, 2020). In cancer, it can act either as a tumour suppressor (e.g., prostate cancer, glioma) or oncogenic driver (e.g., hepatocellular carcinoma) and is frequently dysregulated in gallbladder, colorectal, prostate, ovarian, breast and lung cancers (Huang et al., 2015; Ishitani & Ishitani, 2013).

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