## Phylogeny

• Member of the STE group, mixed-lineage kinase (MLK) subfamily; shares ~40 % sequence identity with MLK1 and DLK, placing it on the STE11 branch of the human kinome (mathea2016structureofthe pages 1-3, mathea2016structureofthe pages 14-16).  
• Vertebrate orthologs are reported in Mus musculus, Rattus norvegicus, Gallus gallus, Xenopus laevis and Danio rerio; zebrafish encodes separate genes for the α- and β-type isoforms (mathea2016structureofthe pages 14-16, nordgaard2022zakβisactivated pages 10-12).  
• A single nematode homolog, zak-1, functions upstream of the p38 ortholog pmk-1 in Caenorhabditis elegans (vind2020zakαrecognizesstalled pages 11-13).  
• No clear ortholog is present in Saccharomyces cerevisiae or Drosophila melanogaster, indicating lineage-restricted expansion (vind2020ribosomalstresssurveillancethree pages 10-10).

## Reaction Catalyzed

Protein-Ser/Thr + ATP ⇄ Protein-Ser/Thr-P + ADP (mathea2016structureofthe pages 1-3).

## Cofactor Requirements

Catalysis is ATP-dependent; the cited literature does not specify divalent metal requirements (mathea2016structureofthe pages 13-14).

## Substrate Specificity

• Positional-scanning peptide libraries defined a consensus W-H-T-ϕ-H-ϕ motif (ϕ = A,V,I,F,Y,W,Q) centered on a threonine phosphoacceptor; histidine is preferred at –1 and +2 (mathea2016structureofthe pages 6-8).  
• “ZAKtide” (ANHWHTVHLRA) is an optimized reporter substrate; KM,ATP ≈ 140 µM (mathea2016structureofthe pages 6-8).  
• Activation-segment peptides from MAP2K4 and MAP2K7 are poor substrates, indicating a requirement for supplemental docking interactions in full-length targets (mathea2016structureofthe pages 8-10).  
• Phosphoproteomics of skeletal-muscle lysate identified 48 direct substrates and ~1 200 putative targets enriched at the Z-disc and focal adhesions, including FLNC, SYNPO2 and BAG3 (stonadge2023myofibrillarmyopathyhallmarks pages 6-7).

## Structure

• Domain organisation – ZAKα: N-terminal kinase domain (KD, residues 5-309), leucine-zipper (LZ), sterile-α motif (SAM) and a C-terminal ribosome-binding module composed of semi-redundant S and CTD domains (vind2020zakαrecognizesstalled pages 13-14). ZAKβ: identical KD + LZ followed by a unique stress-fiber-binding domain (SFBD) in place of the SAM/CTD cassette (nordgaard2022zakβisactivated pages 6-8).  
• Crystal structure of KD-LZ (PDB 5HES) bound to vemurafenib reveals a bilobed fold with αC displaced outward, breaking the Lys45–Glu53 ion pair and locking the kinase in an inactive DFG-in state (mathea2016structureofthe pages 8-10).  
• The P-loop is kinked; Phe27 π-stacks with the inhibitor, while Cys24 lies adjacent to the chlorophenyl group, providing a potential covalent anchor site (mathea2016structureofthe pages 10-13).  
• Activation loop: Thr161 is phosphorylated in the crystal; Ser165 remains disordered, underscoring the requirement for dual phosphorylation for full activity (mathea2016structureofthe pages 8-10).  
• The LZ packs against αG forming a hydrophobic patch yet does not mediate dimerisation in the crystal (mathea2016structureofthe pages 16-19).  
• The SAM domain functions as an autoinhibitory element restricting ribosome engagement until relieved by phosphorylation (vind2020zakαrecognizesstalled pages 13-14).

## Regulation

• Autophosphorylation of Thr161 and Ser165 in the activation loop is necessary for maximal catalytic output (mathea2016structureofthe pages 8-10).  
• Protein kinase N1 promotes trans-autophosphorylation of these sites under osmotic stress (mathea2016structureofthe pages 1-3).  
• ZAKα undergoes additional autophosphorylations flanking the ribosome-binding region that modulate sensor engagement (vind2020zakαrecognizesstalled pages 11-13).  
• ZAKβ is phosphorylated at Ser335 and Ser339 during cellular compression or in contracting muscle (nordgaard2022zakβisactivated pages 8-10).  
• Isoform-specific stimuli – stalled/damaged ribosomes activate ZAKα, whereas stress-fiber perturbation and mechanical compression activate ZAKβ (vind2020zakαrecognizesstalled pages 13-14, nordgaard2022zakβisactivated pages 6-8).

## Function

• Acts upstream of MAP2K7/MAP2K4→JNK and MAP2K3/MAP2K6→p38 in stress-activated cascades (mathea2016structureofthe pages 1-3).  
• ZAKα binds 18S rRNA helix 14 and initiates the ribotoxic-stress response leading to p38/JNK activation (vind2020zakαrecognizesstalled pages 13-14).  
• ZAKβ localises to Z-discs and stress fibres, senses cellular compression, and is indispensable for contraction-induced p38/JNK activation in skeletal muscle (nordgaard2022zakβisactivated pages 6-8).  
• Highly expressed in adult human cardiomyocytes where it controls hypertrophic and doxorubicin-induced stress signalling (mathea2016structureofthe pages 1-3).  
• Phosphorylates Z-disc proteins FLNC, SYNPO2, BAG3 and others, linking kinase activity to myofibrillar integrity (stonadge2023myofibrillarmyopathyhallmarks pages 6-7).

## Inhibitors

• Vemurafenib: KD ≈ 29 nM by ITC; IC₅₀ ≈ 23 nM in enzymatic assay; crystal structure defines binding mode (mathea2016structureofthe pages 10-13, mathea2016structureofthe pages 16-19).  
• Ponatinib, nilotinib, sorafenib, dabrafenib and rebastinib produce ΔT\_m up to 15 °C and inhibit the kinase with low-nanomolar potency in biochemical assays (mathea2016structureofthe pages 6-8).  
• PLX4720 blocks ZAKβ in cell-based actin-ruffling assays (stonadge2023myofibrillarmyopathyhallmarks pages 5-6).  
• DHP-2 is an experimental inhibitor that suppresses ZAK-dependent ribotoxic signalling (vind2020ribosomalstresssurveillancethree pages 5-6).

## Other Comments

• Off-target inhibition of ZAK by RAF inhibitors (vemurafenib, dabrafenib, sorafenib) diminishes UV-induced apoptosis and contributes to cutaneous squamous-cell carcinoma in mouse models (mathea2016structureofthe pages 3-6).  
• Germline SAM-domain mutation F368C causes congenital split-hand/foot malformation via constitutive pathway activation and reduced protein stability (vind2020zakαrecognizesstalled pages 11-13).  
• Homozygous MAP3K20 loss results in a myofibrillar myopathy with FLNC and BAG3 accumulation in patients and Zak-deficient mice (stonadge2023myofibrillarmyopathyhallmarks pages 1-1).  
• Zak knockout mice display atrophy of slow-twitch fibres and impaired p38/JNK activation after muscle contraction (nordgaard2022zakβisactivated pages 8-10).

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