## Phylogeny

• Orthologs are documented in mammals (Mus musculus Gsg2), birds, amphibians (Xenopus laevis), insects (Drosophila CG8878), nematodes (Caenorhabditis elegans multi-gene expansion), fungi (Saccharomyces cerevisiae Alk1/Alk2), and plants (Arabidopsis thaliana AtHaspin) (higgins2001haspin‐likeproteinsa pages 1-2, kurihara2011identificationandcharacterization pages 1-2).  
• Kinome analyses place the enzyme in the stand-alone “Haspin family” within the Other-Protein-Kinase (OPK) group, clearly separated from classical ePK subfamilies (higgins2001haspin‐likeproteinsa pages 7-8, eswaran2009structureandfunctional pages 1-2).

## Reaction Catalyzed

ATP + histone H3 (threonine-3) ⇌ ADP + histone H3 (O-phospho-threonine-3) (eswaran2009structureandfunctional pages 1-2).

## Cofactor Requirements

Catalytic turnover requires divalent cations; Mg²⁺ supports maximal activity, with Mn²⁺ or Ca²⁺ acting as permissible substitutes in vitro (eswaran2009structureandfunctional pages 3-4).

## Substrate Specificity

• Preferred consensus motif: Ala(P-2)-Arg(P-1)-Thr(0)-Lys(P + 1); acidic residues in any position are disfavoured (maiolica2014modulationofthe pages 11-12).  
• Phosphorylation efficiency declines with increasing Lys-4 methylation adjacent to Thr-3 (eswaran2009structureandfunctional pages 3-4).

## Structure

Domain organisation  
• Residues 1–≈470: intrinsically disordered N-terminal region containing a basic inhibitory segment (HBIS) (amoussou2018haspinapromising pages 2-3).  
• Residues ≈471–798: bilobed catalytic kinase domain (eswaran2009structureandfunctional pages 1-2).

Three-dimensional features  
• Activation segment is re-configured into helix αAS; canonical DFG and APE motifs are replaced by a DYT motif and divergent tail (amoussou2018haspinapromising pages 2-3).  
• Helix ulH, a β-hairpin insert and a β7–β8 insertion immobilise helix C and the P-loop, locking the enzyme in an active conformation without activation-loop phosphorylation (eswaran2009structureandfunctional pages 1-2, higgins2010haspinanewly pages 16-17).  
• A metal-binding site at the catalytic-loop/helix F interface stabilises the hydrophobic spine (eswaran2009structureandfunctional pages 3-4).  
Representative crystal structures: PDB 2VUW, 3DLZ, 3IQ7, 5V6O (eswaran2009structureandfunctional pages 1-2, lavogina2016cocrystalstructuresof pages 7-7).

## Regulation

• Autoinhibition by HBIS during interphase; CDK1 priming followed by PLK1 multisite phosphorylation of HBIS at G2/M releases inhibition (amoussou2018haspinapromising pages 2-3).  
• Positive feedback: H3T3ph recruits Aurora B, which further phosphorylates and enhances the kinase (amoussou2018haspinapromising pages 1-2).  
• Termination: PP1γ-Repo-man complex removes H3T3ph during anaphase (amoussou2018haspinapromising pages 1-2).  
• >30 mitotic phosphosites accumulate in the disordered N-terminus without altering intrinsic catalytic rate (higgins2010haspinanewly pages 8-9).  
• Substrate recognition is modulated by Lys-4 methylation or acetylation on histone H3 (maiolica2014modulationofthe pages 12-14).

## Function

Expression and localisation  
• Highly expressed in haploid germ cells; detectable in proliferating somatic tissues (higgins2003structurefunctionand pages 1-2, amoussou2018haspinapromising pages 1-2).  
• Localises to condensed chromosomes from prophase to metaphase, centrosomes after nuclear-envelope breakdown, spindle microtubules at metaphase, and the midbody during telophase (amoussou2018haspinapromising pages 2-3, dai2005haspinamitotic pages 2-3).

Mitotic roles  
• H3T3ph forms the centromeric docking platform for the chromosomal passenger complex (Aurora B, Survivin, INCENP), preserving cohesin, ensuring chromosome congression and maintaining spindle-assembly checkpoint signalling (higgins2010haspinanewly pages 4-5, dai2005haspinamitotic pages 2-3).  
• Depletion causes prometaphase arrest and cohesion loss; over-expression delays early mitosis (dai2005haspinamitotic pages 1-2, higgins2010haspinanewly pages 4-5).

Upstream and downstream partners  
• Upstream kinases: CDK1, PLK1.  
• Downstream effectors: Aurora B, Survivin, INCENP, Sgo1, PP1γ (amoussou2018haspinapromising pages 1-2, higgins2010haspinanewly pages 4-5).

## Inhibitors

• 5-Iodotubercidin, IC₅₀ ≈ 5–9 nM (amoussou2018haspinapromising pages 5-6).  
• CHR-6494, IC₅₀ ≈ 2 nM (amoussou2018haspinapromising pages 5-6).  
• ARC-3354, K\_d ≈ 0.42 nM (amoussou2018haspinapromising pages 5-6).  
• LDN-192960, IC₅₀ ≈ 10 nM (cuny2012structure–activityrelationshipstudy pages 1-2).  
• Co-crystal structures with bisubstrate imidazo[1,2-b]pyridazines define active-site engagement (lavogina2016cocrystalstructuresof pages 7-7).

## Other Comments

• GSG2 maps to 17p13.2/13.3, a chromosomal region frequently lost in human tumours (dai2005haspinamitotic pages 2-3).  
• Pharmacological inhibition suppresses tumour cell proliferation in vitro and in xenograft models (amoussou2018haspinapromising pages 1-2).  
• His651Ala mutation abolishes catalytic activity and is utilised as a kinase-dead control in mechanistic studies (eswaran2009structureandfunctional pages 1-2).

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