## Phylogeny

Haspin orthologs occur across the eukaryotic kingdom, including mammals (Mus musculus Gsg2), birds, amphibians (Xenopus laevis), insects (Drosophila CG8878), nematodes (Caenorhabditis elegans gene expansion), fungi (Saccharomyces cerevisiae Alk1/Alk2) and plants (Arabidopsis thaliana AtHaspin) (Higgins, 2001; Kurihara et al., 2011). Kinome surveys place these enzymes in a stand-alone “Haspin family” within the Other-Protein-Kinase group, distinct from classical eukaryotic protein-kinase subfamilies (Higgins, 2001; Eswaran et al., 2009).

## Reaction Catalyzed

ATP + histone H3 (Thr-3) ⇌ ADP + histone H3 (O-phospho-Thr-3) (Eswaran et al., 2009).

## Cofactor Requirements

Catalytic turnover requires divalent cations; Mg²⁺ supports maximal activity, whereas Mn²⁺ or Ca²⁺ can substitute in vitro (Eswaran et al., 2009).

## Substrate Specificity

The preferred motif is Ala(P-2)-Arg(P-1)-Thr(0)-Lys(P + 1); acidic residues at any position are disfavoured (Maiolica et al., 2014). Phosphorylation efficiency decreases as Lys-4 adjacent to Thr-3 becomes progressively methylated (Eswaran et al., 2009).

## Structure

• Residues 1–≈470 form an intrinsically disordered N-terminal segment that contains a basic autoinhibitory element (HBIS) (Amoussou et al., 2018).  
• Residues ≈471–798 comprise a bilobed catalytic domain (Eswaran et al., 2009).

Distinctive 3-D features include:  
– An activation segment remodelled into helix α\_AS with a DYT motif replacing the canonical DFG, and a divergent APE tail (Amoussou et al., 2018).  
– Helix ulH plus β-hairpin and β7–β8 inserts that immobilise helix C and the P-loop, locking the kinase in an active conformation independent of activation-loop phosphorylation (Eswaran et al., 2009; Higgins, 2010).  
– A metal-binding site at the catalytic-loop/helix F interface that stabilises the hydrophobic spine (Eswaran et al., 2009).

Representative crystal structures: PDB 2VUW, 3DLZ, 3IQ7, 5V6O (Eswaran et al., 2009; Lavogina et al., 2016).

## Regulation

Autoinhibition by the HBIS operates during interphase; CDK1 priming followed by PLK1-mediated multisite phosphorylation of HBIS at G2/M relieves this inhibition (Amoussou et al., 2018). H3-Thr-3 phosphorylation (H3T3ph) recruits Aurora B, which in turn further activates Haspin, establishing positive feedback (Amoussou et al., 2018). Dephosphorylation by the PP1γ–Repo-man complex removes H3T3ph during anaphase (Amoussou et al., 2018). More than 30 mitotic phosphosites accumulate in the disordered N-terminus without altering intrinsic catalytic rate (Higgins, 2010). Lys-4 methylation or acetylation on histone H3 modulates substrate recognition (Maiolica et al., 2014).

## Function

Expression/localisation: high expression in haploid germ cells and detectable levels in proliferating somatic tissues (Higgins, 2003; Amoussou et al., 2018). During mitosis the kinase localises to condensed chromosomes (prophase–metaphase), centrosomes post-nuclear-envelope breakdown, spindle microtubules (metaphase) and the midbody (telophase) (Amoussou et al., 2018; Dai & Higgins, 2005).

Mitotic roles: H3T3ph provides the centromeric docking site for the chromosomal passenger complex (Aurora B, Survivin, INCENP), thereby protecting cohesin, promoting chromosome congression and sustaining spindle-assembly-checkpoint signalling (Higgins, 2010; Dai & Higgins, 2005). Haspin depletion causes prometaphase arrest with cohesion loss, whereas over-expression delays early mitosis (Dai & Higgins, 2005; Higgins, 2010).

Signalling partners: upstream kinases CDK1 and PLK1 prime/activate Haspin; downstream effectors include Aurora B, Survivin, INCENP, Sgo1 and PP1γ (Amoussou et al., 2018; Higgins, 2010).

## Inhibitors

Potent ATP-competitive inhibitors include 5-iodotubercidin (IC₅₀ ≈ 5–9 nM), CHR-6494 (IC₅₀ ≈ 2 nM), ARC-3354 (K\_d ≈ 0.42 nM) and LDN-192960 (IC₅₀ ≈ 10 nM). Bisubstrate imidazo[1,2-b]pyridazine complexes have been structurally characterised (Amoussou et al., 2018; Cuny et al., 2012; Lavogina et al., 2016).

## Other Comments

The human GSG2 gene maps to chromosome 17p13.2/13.3, a region frequently deleted in tumours (Dai & Higgins, 2005). Pharmacological inhibition of Haspin suppresses tumour-cell proliferation in vitro and in xenograft models (Amoussou et al., 2018). Mutation His651Ala abolishes catalytic activity and is widely used as a kinase-dead control (Eswaran et al., 2009).

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