1. Phylogeny  
   Haspin kinase, also known as Germ cell‐specific gene 2 protein (GSG2) or H‐haspin, is a serine/threonine protein kinase that has been identified in a wide range of eukaryotic organisms including mammals, invertebrates, fungi, and plants. Its orthologs are found in human, mouse, Arabidopsis thaliana, and yeasts, indicating that a haspin‐like kinase was already present in the Last Eukaryotic Common Ancestor (LECA) (dai2005haspinamitotic pages 1-2, kurihara2011identificationandcharacterization pages 1-2). In mammals the gene is intron‐less and localizes on human chromosome 17, while in plants such as Arabidopsis thaliana the AtHaspin homolog contains a conserved C‐terminal kinase domain sharing key catalytic residues with human Haspin (kurihara2011identificationandcharacterization pages 2-4, dai2005haspinamitotic pages 1-2). Phylogenetic analyses based on sequence comparisons of the kinase domain reveal that haspin forms a distinct clade within the eukaryotic protein kinase superfamily, clearly separated from other classical groups such as cyclin‐dependent kinases, Aurora kinases, and AGC kinases (higgins2003structurefunctionand pages 1-2, higgins2010haspinanewly pages 1-2). The unique pattern of conserved motifs observed in haspin’s catalytic domain, together with the presence of lineage‐specific divergent N‐terminal regions, supports its classification as an atypical kinase with an evolutionary history distinct from more conventional serine/threonine kinases (higgins2003structurefunctionand pages 13-14, higgins2010haspinanewly pages 1-2). These evolutionary relationships underscore the fundamental and conserved role of haspin in cell cycle regulation across eukaryotes (dai2005haspinamitotic pages 1-2, kurihara2011identificationandcharacterization pages 1-2).
2. Reaction Catalyzed  
   Haspin catalyzes the transfer of a phosphate group from ATP to a specific threonine residue (Thr3) on histone H3, thereby converting histone H3 into its phosphorylated form (H3T3ph). The chemical reaction can be summarized as follows:  
     ATP + Histone H3–(Thr3) → ADP + Histone H3–(Thr3-phosphate) + H⁺  
   This rapid phosphotransfer reaction ensures the generation of a critical mitotic mark that is required for subsequent recruitment of the chromosomal passenger complex (dai2005thekinasehaspin pages 8-10, eswaran2009structureandfunctional pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of haspin is dependent on the presence of divalent cations; in particular, Mg²⁺ is required for optimal ATP binding and phosphate transfer. This cofactor requirement is characteristic of most eukaryotic protein kinases, where the Mg²⁺ ion stabilizes the negative charges on ATP and assists in the proper orientation of the phosphates within the active site (eswaran2009structureandfunctional pages 1-2, dai2005thekinasehaspin pages 1-2).
4. Substrate Specificity  
   Haspin displays a high degree of substrate specificity, primarily recognizing and phosphorylating the N-terminal tail of histone H3 at the threonine 3 (Thr3) residue. The enzyme’s catalytic pocket is adapted to interact with the basic residues present in the histone H3 tail, and its activity is highly dependent on the local amino acid context around Thr3. Notably, post-translational modifications such as methylation at lysine 4 (H3K4me) can negatively impact substrate recognition by haspin, thereby modulating its catalytic efficiency (dai2005thekinasehaspin pages 8-10, eswaran2009structureandfunctional pages 3-4, cartwright2022dissectingtheroles pages 2-3). This specificity ensures that phosphorylation is confined to histone H3 molecules that adopt a conformation favorable for recognition by the enzyme, thus coupling the deposition of the H3T3ph mark to precise chromatin contexts during mitosis.
5. Structure  
   The overall structural organization of haspin is characterized by a central, highly conserved C-terminal kinase domain and an N-terminal region that is less conserved and may serve regulatory or targeting functions. The kinase domain adopts a bilobed structure consisting of an N-terminal lobe, which is predominantly composed of β-strands and is responsible for ATP binding, and a larger C-terminal lobe that houses the catalytic machinery responsible for substrate phosphorylation (eswaran2009structureandfunctional pages 1-2, higgins2010haspinanewly pages 1-2). Unique to haspin is an atypical activation segment; for example, it lacks the conserved APE motif typical of many eukaryotic protein kinases and instead features distinct sequences that contribute to its substrate specificity, including a substitution of the canonical DFG motif by a DYT motif (dai2005thekinasehaspin pages 12-13, eswaran2009structureandfunctional pages 3-4). Additional structural features include a β-hairpin near the hinge region between the two lobes and extra α-helical insertions—such as the αULH and the extended activation segment—that stabilize the active conformation of the kinase domain (higgins2010haspinanewly pages 13-16, eswaran2009structureandfunctional pages 1-2). High-resolution crystallographic studies, including those employing ATP-mimetic inhibitors like 5-iodotubercidin, have revealed that the active site has a highly electronegative substrate-binding groove that is optimally configured for accommodation of the basic histone H3 tail (eswaran2009structureandfunctional pages 3-4, higgins2010haspinanewly pages 16-17). In contrast to many other kinases whose activity is modulated by large conformational changes in the activation loop, the kinase domain of haspin retains a constitutively active conformation, and its regulation is primarily achieved through phosphorylation events and protein–protein interactions mediated by the N-terminal region (dai2005thekinasehaspin pages 8-10, higgins2010haspinanewly pages 4-5).
6. Regulation  
   The regulatory mechanisms controlling haspin activity involve multiple layers of post-translational modifications and protein interactions that ensure its proper temporal and spatial function during the cell cycle. Although haspin is intrinsically active and its catalytic domain does not require phosphorylation to achieve full catalytic competence, its activity in vivo is modulated by phosphorylation events that occur predominantly on its N-terminal region. During the G2/M transition, key regulatory kinases such as cyclin-dependent kinase 1 (Cdk1) and Polo-like kinase 1 (Plk1) phosphorylate haspin, resulting in changes in its subcellular localization and its ability to interact with particular chromatin regions (dai2005thekinasehaspin pages 8-10, higgins2010haspinanewly pages 2-4). In addition, haspin undergoes autophosphorylation, a modification that can further fine-tune its kinase activity, although the overall intrinsic activity remains relatively constant throughout the cell cycle (dai2005thekinasehaspin pages 8-10, wang2020haspininhibitiondelays pages 8-9). Despite the extensive phosphorylation of haspin during mitosis, studies have noted that the bulk enzymatic activity does not vary dramatically, suggesting that the regulation of substrate access and subcellular localization rather than catalytic rate is the major regulatory output of these post‐translational modifications (higgins2010haspinanewly pages 4-5, wang2020haspininhibitiondelays pages 11-13). Protein interactions further contribute to the regulation of haspin; for example, binding to cohesin-associated proteins, such as Pds5B, may help target haspin to centromeric regions where its substrate histone H3 is specifically localized (dai2005haspinamitotic pages 2-3, higgins2010haspinanewly pages 8-9). Overall, the regulatory landscape of haspin is defined by a combination of multisite phosphorylation by upstream kinases and its dynamic association with chromatin, which together ensure the proper execution of its role in mitosis.
7. Function  
   The primary biological function of haspin is its role in mitosis, where it phosphorylates histone H3 at threonine 3 (H3T3ph) during early mitosis, from late G2 through metaphase. This phosphorylation event is critically important for the recruitment and spatial organization of the chromosomal passenger complex (CPC), which includes Aurora B kinase, INCENP, Survivin, and Borealin. The deposition of the H3T3ph mark creates a binding site for Survivin, ensuring that the CPC localizes to centromeric chromatin and is properly positioned to execute its essential roles in chromosome alignment, correction of kinetochore–microtubule attachments, and maintenance of sister chromatid cohesion (dai2005haspinamitotic pages 1-2, dai2005thekinasehaspin pages 11-12, higgins2010haspinanewly pages 8-9). In addition to its mitotic functions, haspin is highly expressed in testicular tissue, particularly in haploid germ cells, indicating an important role in spermatogenesis; however, lower levels of expression are also found in various proliferating somatic tissues, suggesting that its function in cell division is broadly required (dai2005haspinamitotic pages 1-2, dai2005thekinasehaspin pages 1-2). Experimental depletion of haspin by RNA interference in human cell lines results in a marked decrease in H3T3 phosphorylation, leading to defects in metaphase chromosome alignment, activation of the spindle assembly checkpoint, and ultimately cell cycle delays (dai2005thekinasehaspin pages 8-10, higgins2010haspinanewly pages 4-5). Furthermore, the precise positioning of the CPC mediated by haspin-dependent H3T3 phosphorylation is crucial for the stabilization of kinetochore–microtubule attachments and proper chromosome segregation (cartwright2022dissectingtheroles pages 2-3, wang2020haspininhibitiondelays pages 11-13). Thus, haspin plays an indispensable role in ensuring genomic stability during cell division by maintaining proper centromeric cohesion and contributing to the fidelity of mitotic progression.
8. Other Comments  
   Small molecule inhibitors targeting haspin kinase have been developed as experimental tools and represent a potential therapeutic strategy given the enzyme’s critical role in mitosis. Compounds such as 5-iodotubercidin and CHR-6494 have been shown to inhibit haspin activity, reduce H3T3 phosphorylation, and induce mitotic defects in cancer cells, thereby highlighting the potential for haspin inhibitors in anticancer therapy (wang2020haspininhibitiondelays pages 1-3, higgins2010haspinanewly pages 4-5). In addition, combination approaches that target haspin together with other mitotic kinases, such as Polo-like kinase 1, have demonstrated synergistic effects in reducing cancer cell proliferation (cartwright2022dissectingtheroles pages 2-3, wang2020haspininhibitiondelays pages 11-13). Although the majority of studies have focused on the role of haspin in mitosis, emerging evidence suggests that its function may extend to interphase progression as inhibition of haspin has been observed to delay cell cycle progression through interphase in certain cancer models (wang2020haspininhibitiondelays pages 8-9). Lastly, the strong association of aberrant mitotic events with chromosomal instability highlights the potential for haspin dysregulation to contribute to oncogenesis, making it an attractive target for further investigation in the context of cancer biology.
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