1. Phylogeny  
   Mitotic checkpoint serine/threonine‐protein kinase BUB1 beta, commonly designated as BUB1B or BubR1, is a eukaryote‐specific kinase that belongs to the Bub family of checkpoint proteins. Orthologs of BUB1B are found throughout metazoans, and the evolutionary conservation of its key domains suggests emergence from an ancestral gene in early eukaryotic evolution. Comparative analyses place BUB1B in the protein kinase superfamily, closely related to its paralog BUB1 and the MAD3 proteins found in yeast, indicating that gene duplication events early in the evolution of the spindle assembly checkpoint generated functionally specialized proteins. BUB1B and related proteins are restricted to eukaryotes, consistent with data indicating that the Bub family kinases arose after divergence of the major superkingdoms and evolved an indispensable role in monitoring chromosome segregation during mitosis (bloom2021physiologicalrelevanceof pages 1-2, long2021expressionandprognosis pages 3-7, kang2008structureandsubstrate pages 1-2).
2. Reaction Catalyzed  
   BUB1B is a serine/threonine protein kinase that catalyzes the transfer of a phosphate group from ATP to specific serine or threonine residues on substrate proteins. In canonical form, the phosphorylation reaction it mediates can be summarized as follows: ATP + [protein]-(L-serine/threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺. Although BUB1B is also recognized for its non-catalytic roles in the inhibition of the APC/C–CDC20 interaction, its putative kinase domain is proposed to engage in such phosphorylation reactions, whether by autophosphorylation or by phosphorylating other substrates involved in checkpoint signaling and kinetochore dynamics (bloom2021physiologicalrelevanceof pages 2-4, kang2008structureandsubstrate pages 2-3).
3. Cofactor Requirements  
   As with other serine/threonine kinases, the catalytic activity of BUB1B depends on the presence of divalent metal cofactors. Mg²⁺ is required to coordinate the binding of ATP within the active site, facilitating the transfer of the γ-phosphate to the substrate. This requirement for Mg²⁺ is consistent with the typical cofactor dependencies encountered within the eukaryotic protein kinase family (kang2008structureandsubstrate pages 1-2).
4. Substrate Specificity  
   Although the intrinsic substrate specificity of BUB1B is not as comprehensively defined as that for some other kinases, several phosphorylation sites on BUB1B itself have been identified that have functional importance in mitotic regulation. Key residues that undergo phosphorylation include T608, T620, S670, S676, and T680. These sites, which are embedded in distinct regulatory domains such as the kinase domain and the kinetochore attachment regulatory domain (KARD), are critical for modulating the interaction of BUB1B with kinetochore components including the motor protein CENP-E and the PP2A-B56 phosphatase complex. While no universally accepted consensus substrate motif has been clearly delineated for BUB1B, the phosphorylation sites identified align with motifs that are recognized by upstream mitotic kinases such as Cyclin B1-Cdk1 and Polo-like kinase 1 (Plk1) (bloom2021physiologicalrelevanceof pages 5-6, bloom2021physiologicalrelevanceof pages 6-8, kang2008structureandsubstrate pages 5-6).
5. Structure  
   BUB1B exhibits a modular domain architecture that underpins its dual functional roles during mitosis. The N-terminal region is primarily dedicated to spindle assembly checkpoint (SAC) activity. This region contains multiple motifs, including KEN boxes, destruction boxes, and an ABBA motif, which together mediate binding to essential checkpoint components such as CDC20 and Bub3. These motifs facilitate the incorporation of BUB1B into the mitotic checkpoint complex (MCC) and thereby contribute to the inhibition of the anaphase-promoting complex/cyclosome (APC/C).  
   In contrast, the C-terminal portion of BUB1B is organized into two major functional domains. First, the kinetochore attachment regulatory domain (KARD) comprises a discrete region that recruits the PP2A-B56 phosphatase complex to the kinetochores. This recruitment is essential for the dephosphorylation events that stabilize kinetochore-microtubule attachments and enable error correction mechanisms during chromosome alignment. Second, BUB1B harbors a putative kinase domain at its C-terminus. Although this domain retains several structural hallmarks typical of active serine/threonine kinases—such as a conserved activation loop, hydrophobic spine, and C-helix—the precise catalytic function of the domain has been the subject of debate. Some studies have detected autophosphorylation events, in particular at T608, and suggest that the BubR1 kinase domain may be capable of phosphorylating itself or other substrates; however, its overall kinase activity is often regarded as limited relative to catalytically active kinases, leading to its classification as a pseudokinase in some reports (bloom2021physiologicalrelevanceof pages 2-4, bloom2021physiologicalrelevanceof pages 13-15, bloom2021physiologicalrelevanceof pages 8-9, kang2008structureandsubstrate pages 1-2, kang2008structureandsubstrate pages 3-5).
6. Regulation  
   BUB1B is subject to extensive post-translational regulation, which is critical for its proper activity during mitosis. Phosphorylation represents the most prominent mode of regulation. Specific residues such as T620, T608, S670, S676, and T680 are phosphorylated by key mitotic kinases including Cyclin B1-Cdk1 and Plk1. For example, Cyclin B1-Cdk1-mediated phosphorylation at T620 is a priming event that permits subsequent modification by Plk1, leading to further phosphorylation of residues in the KARD domain. These phosphorylation events regulate both the stabilization of kinetochore-microtubule attachments and the timing of APC/C inactivation (bloom2021physiologicalrelevanceof pages 5-6, bloom2021physiologicalrelevanceof pages 6-8, bloom2021physiologicalrelevanceof pages 8-9).  
   In addition to phosphorylation, BUB1B undergoes acetylation, particularly at lysine 250. Acetylation at this residue is believed to play a role in converting BUB1B from an APC/C substrate into an inhibitor, thus modulating the duration of mitotic arrest. Subsequent deacetylation, mediated by enzymes such as HDACs and SIRT2, facilitates ubiquitination and SUMOylation, further influencing both the turnover and kinetochore localization of BUB1B. Such reversible modification switches provide a mechanism for fine-tuning the balance between checkpoint activation and silencing (bloom2021physiologicalrelevanceof pages 11-12, bloom2021physiologicalrelevanceof pages 13-15).  
   Additional regulation is imparted through binding interactions with phosphatases. The recruitment of PP2A-B56 to kinetochores is mediated by phosphorylated sequences within the KARD domain and is essential for counteracting the kinase activities of Aurora B, thereby ensuring stable kinetochore-microtubule attachments. This interplay between kinases and phosphatases underscores the dynamic nature of BUB1B regulation during mitosis (bloom2021physiologicalrelevanceof pages 6-8).
7. Function  
   BUB1B is an essential mediator of mitotic checkpoint signaling. Its primary role is to delay the onset of anaphase until every chromosome is properly attached to the mitotic spindle, thereby ensuring accurate chromosome segregation. This checkpoint function is achieved in part by BUB1B’s ability to inhibit the APC/C by blocking the binding of the activator CDC20. Such inhibition prevents the premature degradation of key cell cycle regulators, including securin and cyclin B1, thereby delaying the transition from metaphase to anaphase (bloom2021physiologicalrelevanceof pages 1-2, bloom2021physiologicalrelevanceof pages 2-4).  
   In addition to its checkpoint activity, BUB1B contributes to the regulation of kinetochore-microtubule interactions. Its C-terminal KARD mediates the recruitment of the PP2A-B56 phosphatase complex to the kinetochore. By modulating the dephosphorylation of kinetochore proteins, PP2A-B56 helps to stabilize microtubule attachments and facilitate error correction during chromosome congression. BUB1B is also required for the proper kinetochore localization of the motor protein CENP-E, whose function is critical for the migration and alignment of chromosomes (bloom2021physiologicalrelevanceof pages 8-9).  
   BUB1B further functions to suppress centrosome amplification by negatively regulating Polo-like kinase 1 (Plk1) activity during interphase, and it has been implicated in signaling pathways that trigger apoptosis in cells that exit mitosis aberrantly as polyploids. These roles underscore BUB1B’s involvement not only in the maintenance of genomic stability during cell division but also in tumor suppression mechanisms (williams2007bub1escapadesin pages 4-5, suijkerbuijk2010molecularcausesfor pages 8-9).
8. Other Comments  
   Alterations in BUB1B expression and function have been linked to genomic instability, premature cellular senescence, and tumorigenesis. In particular, mutations in BUB1B have been associated with mosaic variegated aneuploidy (MVA), a cancer predisposition syndrome characterized by chromosomal missegregation. In addition, reduced expression of BUB1B in replicatively senescent cells correlates with increased levels of p53 and p21^CIP1 and the accumulation of aneuploid cells, highlighting its role in both mitotic fidelity and aging (bloom2021physiologicalrelevanceof pages 1-2, williams2007bub1escapadesin pages 4-5).  
   Although specific inhibitors targeting BUB1B’s kinase activity are not as well established as those for some other kinases, its central role in mitotic checkpoint regulation renders it a potential therapeutic target for cancers marked by checkpoint dysfunction and chromosomal instability. Furthermore, the regulation of BUB1B by post-translational modifications, including phosphorylation, acetylation, ubiquitination, and SUMOylation, provides multiple potential nodes for therapeutic intervention aimed at modulating its function in cancer cells (bloom2021physiologicalrelevanceof pages 11-12, long2021expressionandprognosis pages 3-7).
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