## Phylogeny

BUB1B (BubR1) and its paralogue BUB1 arose from an early eukaryotic duplication of an ancestral spindle-checkpoint kinase, generating the Bub1/BubR1 sub-family within the CMGC branch of the protein-kinase-like superfamily (Bolanos-Garcia & Blundell, 2011). Orthologues span fungi (S. cerevisiae Mad3, S. pombe Mad3) to insects (D. melanogaster BubR1) and vertebrates (X. laevis, M. musculus, H. sapiens), underscoring a highly conserved spindle-assembly-checkpoint (SAC) role (Chen, 2002; Elowe, 2011). Although the C-terminal lobe retains a Bub1-like bilobal fold, degeneration of the HRD and DFG motifs renders BubR1 a catalytically inactive pseudokinase (Bolanos-Garcia & Blundell, 2011; Breit et al., 2015).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr ⇌ ADP + [protein]-O-phospho-Ser/Thr (canonical Ser/Thr kinase reaction).  
Purified BubR1 binds ATP yet shows no measurable phosphoryl-transfer, confirming catalytic silence (Breit et al., 2015).

## Cofactor Requirements

ATP binding is magnesium-independent; no divalent-cation requirement has been demonstrated in vitro (Breit et al., 2015).

## Substrate Specificity

Comprehensive peptide-library screens failed to define a phosphorylation consensus, consistent with pseudokinase status (Breit et al., 2015; Corno et al., 2023).

## Structure

Domain organisation (human residue numbers approximate):  
• KEN box-1 (7-13) – high-affinity CDC20 binder initiating MCC formation (Krenn, 2014).  
• Triple TPR stack (40-200) – scaffolds MCC interactions (Krenn, 2014).  
• KEN box-2 (300-306) – blocks APC/C substrate entry (Krenn, 2014).  
• GLEBS motif / TPR helix (400-440) – wraps around BUB3 for kinetochore targeting (Bolanos-Garcia & Blundell, 2011).  
• KARD / LxxIxE motif (665-682) – docks PP2A-B56 (Overlack et al., 2015).  
• C-terminal pseudokinase domain (720-1050) – bilobal fold with disrupted catalytic and regulatory spines (Breit et al., 2015).

Three-dimensional data  
Cryo-EM of the human mitotic-checkpoint complex (PDB 6F0I) positions BubR1 with BUB3, MAD2 and CDC20 at the APC/C interface (Banerjee et al., 2022). The PP2A-B56–BubR1 KARD structure (PDB 4OMA) reveals an LxxIxE docking groove (Overlack et al., 2015). AlphaFold modelling corroborates an inactive kinase conformation lacking an intact R-spine (Banerjee et al., 2022).

Key inactive features  
• Truncated / displaced activation segment abolishes the Lys–Glu catalytic salt bridge.  
• HRD and DFG substitutions break the hydrophobic spine (Bolanos-Garcia & Blundell, 2011; Breit et al., 2015).

## Regulation

Post-translational modifications  
Acetylation – Lys250 (PCAF) stabilises BubR1 during prometaphase; deacetylated by HDAC2/3 and SIRT2 (Bloom & North, 2021). Lys668 (CBP) promotes APC/C-mediated ubiquitination; reversed by SIRT2 (UnknownAuthors, 2022).  
SUMOylation – Lys250 conjugation accelerates kinetochore removal, promoting anaphase onset (Yang et al., 2012).  
Phosphorylation – Thr680 (CDK1) and Ser676 (Plk1) within the KARD enhance PP2A-B56 binding; Thr620 (CDK1) creates a Plk1-docking site; unattached kinetochores trigger BubR1 hyperphosphorylation in a Bub1/Mad1-dependent manner (Bloom & North, 2021; Chen, 2002; Corno et al., 2023).  
Ubiquitination – APC/C targets BubR1 for proteasomal degradation in late mitosis (Bloom & North, 2021).

Allosteric interactions  
BUB3 engagement orders the GLEBS helix and is essential for kinetochore localisation and MCC incorporation (Overlack et al., 2015). PP2A-B56 binding via the KARD counteracts Aurora-B to stabilise bi-oriented attachments and facilitates SAC silencing (Overlack et al., 2015).

## Function

Expression – protein accumulates from late G₂, peaks in mitosis and declines with organismal age (Bloom & North, 2021).

Mitotic checkpoint – BUB1–BUB3 bound to Mps1-phosphorylated KNL1 MELT motifs recruits BubR1–BUB3, nucleating MCC assembly; the MCC (BubR1, BUB3, MAD2, CDC20) inhibits APC/C until chromosome bi-orientation is achieved (Banerjee et al., 2022; Krenn, 2014; Overlack et al., 2017).

Kinetochore–microtubule regulation – BubR1 KARD recruits PP2A-B56 to oppose Aurora-B and stabilise attachments; interaction with CENP-E and APC/EB1 further supports chromosome congression (Elowe, 2011; Overlack et al., 2015; Breit et al., 2015).

Signalling network – Upstream regulators include Bub1, Mps1, CDK1 and Plk1; downstream effect is APC/C inhibition to maintain metaphase arrest (Bloom & North, 2021; Banerjee et al., 2022).

## Other Comments

Disease associations – Biallelic germline BUB1B mutations cause mosaic variegated aneuploidy syndrome with childhood cancer predisposition (Bloom & North, 2021; Bolanos-Garcia & Blundell, 2011). Somatic over-expression or mutation correlates with chromosomal instability in several cancers (Bolanos-Garcia & Blundell, 2011). Age-related BubR1 decline promotes tissue degeneration and tumour susceptibility in mice (Bloom & North, 2021). Missense changes at Lys250 or within the pseudokinase domain disrupt stability or MCC assembly, driving aneuploidy and oncogenesis (Bloom & North, 2021; Bolanos-Garcia & Blundell, 2011).

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