## Phylogeny

• Orthologs reported in Saccharomyces cerevisiae, Schizosaccharomyces pombe and Drosophila melanogaster underline conservation from fungi to insects (Bolanos-Garcia, 2009)  
• A vertebrate duplication generated paralog BUBR1; BUB1 retained catalytic competence whereas BUBR1 became a pseudokinase (Suijkerbuijk, 2012)  
• BUB1 and BUBR1 constitute the BUB sub-family placed in the “Other” group of the serine/threonine kinome (Suijkerbuijk, 2012)

## Reaction Catalyzed

ATP + protein-Ser/Thr-OH ⇄ ADP + protein-Ser/Thr-O-PO₃²⁻ (Kang, 2008)

## Cofactor Requirements

Catalysis requires Mg²⁺, as shown by Mg²⁺–nucleotide coordination in crystal structures and biochemical assays (Breit, 2015)

## Substrate Specificity

• Consensus motif ψ(x)₅T/S, with ψ denoting an aliphatic residue and small upstream aliphatic side chains enhancing recognition (Breit, 2015)  
• Histone H2A-Thr120 matches this motif and is a validated in-vivo substrate (Asghar, 2015)

## Structure

• Residues 1–179 form a triple TPR array that docks onto Blinkin/KNL1 for kinetochore targeting; crystal structure at 1.74 Å (PDB 3ESL) (Bolanos-Garcia, 2009)  
• A central GLEBS motif (~240–280) binds Bub3, enabling kinetochore recruitment (Bolanos-Garcia, 2011)  
• C-terminal kinase domain (residues 735–1085) exhibits canonical bilobal fold; inactive (PDB 4R8Q) and active (PDB 4QPM) states reveal P+1-loop rearrangement after Ser969 autophosphorylation (Breit, 2015)  
• Gatekeeper Gly866 enlarges the ATP pocket, allowing binding of bulky adenine analogues (Kang, 2008)  
• An N-terminal extension wraps the N-lobe and functions as an intrinsic “mini-cyclin” stabilising the activation segment (Kang, 2008)  
• Regulatory spine is pre-aligned in both conformations, facilitating rapid activation once the P+1 blockade is lifted (Breit, 2015)

## Regulation

• Autophosphorylation on Ser969 within the P+1 loop relieves substrate occlusion and activates the kinase (Breit, 2015)  
• Autophosphorylation at Thr589 modulates kinetochore residence and local substrate phosphorylation (Asghar, 2015)  
• Mps1-dependent phosphorylation of the CD1 region promotes Mad1 recruitment to unattached kinetochores (Kim, 2021)  
• CDK1 and Plk1 introduce additional phosphorylations in the central segment that fine-tune checkpoint timing (Bolanos-Garcia, 2011)  
• PP1 and PP2A-B56 dephosphorylate Bub1/Bub3, opposing kinetochore binding (Kim, 2021)  
• Two N-terminal KEN boxes target Bub1 for APC/C-Cdh1-mediated proteolysis in G1 (Bolanos-Garcia, 2011)

## Function

• Initiates spindle-assembly checkpoint signalling and sustains mitotic arrest after spindle perturbation (Hoffmann, 2006)  
• Required for kinetochore localisation of BUBR1, BUB3, MAD2, CENPE, CENPF and PLK1 (Bolanos-Garcia, 2011)  
• Phosphorylates CDC20-Ser153, reinforcing APC/C inhibition (Bolanos-Garcia, 2011)  
• Phosphorylates histone H2A-Thr120, creating the SGO1 docking site for cohesion protection (Asghar, 2015)  
• Promotes centromeric enrichment of Aurora B and other chromosomal passenger complex components (Kim, 2021)  
• Bub1:Bub3 forms a heterotetramer with BubR1:Bub3 on KNL1 phospho-MELT repeats to assemble the checkpoint platform (Breit, 2015)

## Inhibitors

• 2OH-BNPP1 is an ATP-competitive inhibitor exploiting the expanded gatekeeper pocket; IC₅₀ ≈ 0.25 µM (Kang, 2008)  
• Pharicin A, a diterpenoid, inhibits Bub1 and induces mitotic arrest in tumour cells (Bolanos-Garcia, 2011)  
• BAY-320 and BAY-524 inhibit Bub1 and potentiate microtubule poisons in aneuploid cancer models (Kim, 2021)

## Other Comments

• Bub1 loss causes chromosome mis-segregation and is lethal in Drosophila, underscoring essentiality (Bolanos-Garcia, 2009)  
• Tumour-derived mutations clustering near the kinase domain destabilise the protein and weaken checkpoint fidelity (Bolanos-Garcia, 2011)