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Prokaryotic cell wall synthesis is a dynamic process that is continuously coordinated with cell growth and division, but the mechanisms by which this process is regulated remain obscure. We define a phosphosignaling pathway in *Mycobacterium tuberculosis (Mtb)* that controls peptidoglycan (PG) biosynthesis in response to PG substructures. The eukaryotic-like Ser/Thr protein kinase (STPK) PknB initiates the signal, phosphorylating the putative flippase for PG precursors, FlpA. The phosphorylation site borders a novel, deeply diverged pseudokinase domain that adopts the kinase fold and forms a back-to-back, N-lobe dimer characteristic of bacterial receptor STPKs. Biochemical and structural studies show that the pseudokinase doman fails to bind ATP, lacks an ATP binding site, and has lost substrate-binding and catalytic motifs. Importantly, the phosphorylated FlpA pseudokinase binds a Forkhead Associated (FHA) domain protein, FhaA, in vitro and in vivo. The crystal structure of the pseudokinase: FHA domain complex reveals unanticipated three-dimensional contacts that augment current models of FHA domain recognition. Knocking down the essential FlpA flippase and FhaA regulator in vivo using a regulated proteolysis tag reveals that these proteins are crucial for cell-wall integrity, cell growth and normal cell morphology. Overall, our studies define a pseudokinase and FHA-domain interaction that mediates an essential bacterial STPK signaling network.

**Keywords: Pseudokinase, FHA domain, kinase signalling**

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**Structural basis for vitamin $B_{12}$ uptake**

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Vitamin $B_{12}$ is a bacterial organic compound and an essential coenzyme in mammals, which take it up from the diet. This occurs by the combined action of the gastric intrinsic factor (IF) and the ileal endocytic cubam receptor formed by the 460 kDa protein cubilin and the 45 kDa transmembrane protein amnionless. Loss of function of any of these proteins ultimately leads to vitamin $B_{12}$ deficiency in man. We have determined the crystal structure of the complex between IF-$B_{12}$ and the cubilin IF-$B_{12}$-binding-region ($CUB_{5-8}$) at 3.3 Å resolution [1]. The structure provides insight into how several CUB (for 'complement C1r/C1s, Uegf, Bmp1') domains collectively function as modular ligand-binding regions, and how two distant CUB domains embrace vitamin $B_{12}$ by binding the two IF domains in a $Ca^{2+}$-dependent manner. This dual-point model provides a probable explanation of how vitamin $B_{12}$ indirectly induces ligand-receptor coupling. In addition, the comparison of $Ca^{2+}$-binding CUB domains and the low-density lipoprotein (LDL) receptor-type A modules suggests that the electrostatic pairing of a basic ligand arginine/lysine residue with $Ca^{2+}$-coordinating acidic aspartates/glutamates is a common theme of $Ca^{2+}$-dependent ligand-receptor interactions.

[1] C.B.F. Andersen, M. Madsen, T. Storm, S.K. Moestrup, G.R. Andersen, *Nature* **2010**, *464*, 445-448.

**Keywords: vitamin, calcium, receptor**

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**Structural basis of cargo recognition by the myosin-X MyTH4-FERM domain**

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A large number of unconventional myosins appeared early in eukaryotic evolution and these play vital roles in diverse cellular processes including intracellular transport, organization of F-actin, mitotic spindle regulation and gene transcription. Myosins consist of three distinct regions, a head, neck and tail. The heads contain actin-based motor domains that display homology among different myosins and these structures have been extensively studied. In contrast to the head, the tails display diversity by combination of a variety of functional domains that mediate cargo recognition, which determine the individual cellular functions of myosins. However, little is known about the tail domain structures and their specific cargo recognition.

Myosin-X is an unconventional myosin implicated in elongation of filopodia, which function as tentacles that explore and interact with cell surroundings to determine the direction of cell movement and to establish cell adhesion such as in the case of synapses. The manner by which myosin-X discriminates between cargos for transportation to the tip and how these cargos contribute to filopodial processes at the tip remains unknown. Myosin-X contains myosin tail homology 4 (MyTH4) and 4.1 and ezrin/radixin/moesin (FERM) domains for cargo recognition.

One of the most exciting processes involving myosin-X relates to the axon path-finding of neurons, which is essential for proper wiring in the brain. During neural development, axons are navigated by extracellular guidance cues such as those provided by netrins. Deleted in colorectal cancer (DCC) and neogenin are membrane proteins that function as netrin receptors. Myosin-X recognizes these receptors as cargos and redistributes to the cell periphery or to the tips of neurites, where growth cones dynamically develop filopodia.

In addition to mediating the biological function of selective cargo transportation on actin cables, myosin-X directly interacts with microtubules and plays a key role in spindle assembly during meiosis to ensure faithful delivery of replicated chromosomes to daughter cells following cell division. This surprising myosin-X function is mediated by a direct interaction between microtubules and the MyTH4-FERM cassette. However, the manner by which myosin-X recognizes microtubules has remained unclear.

Here, we report on a series of structural and biochemical/biophysical studies concerning DCC recognition by the myosin-X MyTH4-FERM cassette. We reveal the presence of a VHS-like fold within the MyTH4 domain. Our 1.9 Å-resolution structure clarifies details of an unexpected binding mode of DCC to the myosin-X FERM domain which is distinct from those found in the FERM domain of radixin that links membrane protein/plasma membrane and actin cytoskeletons. We also show that the cassette binds the C-terminal acidic tails of tubulins and that this binding is obstructed by DCC binding. Our results reveal the structural mechanism that underlies cargo recognition by the cassette and provide the molecular basis for further structural and functional investigations of biologically and medically important myosin-X, as well as of the related unconventional myosins containing MyTH4-FERM cassette.

**Keywords: biomacromolecule, molecular_recognition, X-ray_diffraction**