Vesicular traffic during exocytosis is regulated by Rab GTPase, Sec4p in yeast. Sec2p is a guanine nucleotide exchange factor (GEF) for Sec4p, and the N-terminal 160 residues of Sec2p are sufficient for the GEF activity. Since this region of Sec2p shows no sequence similarity to any other GEFs with known structures, the GEF mechanism by Sec2p has remained unknown. To elucidate this nucleotide exchange mechanism by X-ray crystallography, we crystallized three constructs of the native Sec2p (Sec21-160p, Sec218-160p, and Sec231-160p) and three constructs of the selenomethionine (SeMet)-labeled Sec2p [Sec231-160p, Sec231-160p (M115L), and Sec231-160p (M115L, K121M, T142M)]. These six crystals diffracted to 8.8, 4.8, 2.6, 4.0, 3.3, and 3.0 Å resolutions, respectively. The data set of the SeMet-labeled Sec231-160p (M115L, K121M, T142M) crystal was processed for SAD phasing, producing an interpretable map after density improvement. The atomic model of the Sec2p GEF domain was refined to an Rfree value of 28.9%. Unexpectedly, the Sec2p GEF domain consists of a homodimeric, parallel coiled coil that extends over 180 Å. Pull-down and guanine nucleotide exchange assay using a series of deletion and point mutants of Sec2p unveiled the catalytic residues for its GEF activity and the Sec4p binding site.

Keywords: vesicle membrane fusion, GTP-binding proteins, membrane trafficking

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**Structure and inhibition of Arf GTPases**

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Transport of proteins and membranes sustains all aspects of cellular life. It is therefore associated to major cellular processes such as signalling, morphology and division and it is also sensitive to subversion by pathogens. Small GTP-binding proteins (GTPases) of Arf families are major player in cellular traffic, where they tag and organize membranes for specific trafficking events. They are activated by a family of guanine nucleotide exchange factors (GEFs) that carry a catalytic domain (the Sec7 domain), which stimulates the exchange of the tightly bound GDP nucleotide for GTP. Structural studies have elucidated the exchange mechanism of the Sec7 domain, yet its exquisite ability to discriminate between closely related Arf isoforms remains unexplained. Combining X-ray crystallography, NMR and the use of small molecular weight inhibitors, we identify structural dynamics as a previously overlooked aspect of Arf GTPases functions.

Keywords: GTPase, guanine nucleotide exchange factor, traffic

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**Crystal structure of E.coli MacA reveals the assembly of the tripartite bacterial efflux pump**

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Periplasmic membrane fusion proteins (MFPs), including MacA and AcrA, are an essential component of multidrug efflux pumps in Gram-negative bacteria. They play a crucial role in bridging the outer membrane porin ToIC and two distinctive types of inner membrane transporters. MacA and AcrA form the MacA-MacB-ToIC and AcrA-AcrB-ToIC efflux pumps, respectively, in Escherichia coli. Although the crystal structures of two MFPs have been reported, the functional form and the mechanistic role of MFPs are only vaguely understood. Here, we show that MacA forms a funnel-like hexameric assembly with a central channel whose diameter is similar to that of ToIC and a conical mouth that appears to accommodate the periplasmic end of MacA. In accordance with the results of biochemical experiments, we propose a structural model for how MFP induces the opening of the central channel of ToIC in the periplasmic space of Gram-negative bacteria. Based on the complementing available structures and information, realistic models for the tripartite multidrug efflux transporters are proposed.