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Letter to the Editor

Structural basis for targeting BIG1 to Golgi apparatus through interaction of its DCB domain with Arl1

Dear Editor,

The ADP ribosylation factor (Arf) family small GTPases, consisting of Arf, Arf-like (Arl), and Sar proteins, can recruit a specific set of effectors to the membrane, such as coat complexes and membrane tethers, to modulate vesicular and lipid trafficking (Donaldson and Jackson, 2011). Arf proteins are activated by guanine nucleotide exchange factors (GEFs) that catalyze the switch from the GDP-bound inactive form to the GTP-bound active form. Human GBF/Gea and BIG/Sec7 proteins, including BIG1, BIG2, and GBF1, are a family of Sec7 domain-containing Arf GEFs, which all consist of six conserved domains: an N-terminal dimerization and cyclophilin binding (DCB) domain, a homology upstream of Sec7 (HUS) domain, a Sec7 domain, and three C-terminal homology downstream of Sec7 (HDS 1–3) domains (Bui et al., 2009). The Sec7 domain bears the GEF activity; however, the exact functions of the other domains are still elusive. It is suggested that the conserved modular architecture may participate in the Arf-dependent trafficking pathway, which is necessary for the recruitment of cytosolic coat protein complex I (COPI) vesicle coats to Golgi membranes (Bui et al., 2009). Several lines of evidence have shown that Sec71, the *Drosophila* ortholog of BIG1/2, acts as an effector of Arl1, and could be recruited to the trans-Golgi, leading to the activation of the downstream Arf1 pathway in a cascade manner. This interaction is mapped to the N-terminal region of Sec71 comprising of the DCB domain and part of the HUS domain (Christis and Munro, 2012).

To investigate the molecular basis of the Arl1–BIG1 interaction, we coexpressed the constitutively active Q71L Arl1 mutant (residues 17–181) with different constructs of the N-terminal region of BIG1. Gel filtration, dynamic light scattering, and GST pull-down analyses showed that the DCB domain (residues 1–224) of BIG1 is sufficient to form a stable heterodimer with Arl1-GTP, rather than Arl1-GDP, in high stability and homogeneity (Figure 1A, Supplementary Figures S1A and B, S2, and S3A).

We then determined the crystal structure of Arl1-GTP–DCB at 3.4 Å resolution (Supplementary Table S1). In the structure, each asymmetric unit contains one Arl1 (residues 17–181) bound with a GTP and a Mg^{2+} ion at the active site and one DCB domain (residues 14–49 and 64–221) (Figure 1B). The overall structure of Arl1-GTP in this complex is very similar to that in the Arl1-GTP–golgin-245 GRIP domain complex (PDB code 1UPT) and the Arl1-GTP–Arfaptin2 BAR domain complex (PDB code 4DCN), suggesting that Arl1-GTP adopts nearly identical conformation in binding to different effectors. The DCB domain consists of eight α-helices forming four HEAT (Huntingtin, Elongation factor 3, protein phosphatase 2A, and the yeast kinase TOR1) repeats, and each repeat comprises of a pair of antiparallel α-helices connected by a flexible linker, resembling that in *Thielavia terrestris* Sec7 protein (Richardson et al., 2016) (Supplementary Figure S4A).

The interaction between Arl1-GTP and the DCB domain exhibits an extensive interface, burying 1685 $Å^2$ or 9.4% of the total solvent accessible surface area of the complex. The four HEAT repeats of the DCB domain form a short arc to embrace Arl1; Arl1 interacts with the DCB domain mainly via the switch I, switch II, and interswitch regions against helices α4, α6, and α8 on the concave of the DCB domain (Figure 1B). Structural comparison shows that the GRIP, BAR, and DCB domains all adopt α-helical conformations and interact with the switch and interswitch regions of Arl1; however, as these domains have varied sizes and shapes, their binding modes and orientations with Arl1 are different (Panic et al., 2003; Wu et al., 2004; Nakamura et al., 2012) (Supplementary Figure S4B). The switch I region of Arl1 assumes a loop conformation and interacts mainly with helices α4 and α6 of the DCB domain. Thr44 on switch I forms a hydrogen bond with Tyr109 on helix α4. Ile46 on switch I forms hydrophobic interactions with Ala108 and Tyr109 on helix α4. Ile49 on switch I forms hydrophobic interactions with Ala108 on helix α4 and Thr157 and Thr160 on helix α6 (Figure 1C). Structural comparison of Ar1-GTP in the Arl1-GTP–DCB complex with *Saccharomyces cerevisiae* Arl1-GDP (Amor et al., 2001) indicates that the switch I region of Arl1-GDP forms a β-strand instead of a loop, which appears to cause steric conflict with DCB binding (Supplementary Figure S4C). The switch II region of Arl1 forms an α-helix (α2) and interacts mainly with helix α8 of the DCB domain. Tyr77 and Tyr81 on switch II form a hydrogen bond with Asn203 and Gln200 on helix α8, respectively. There are also numerous hydrophobic interactions among Tyr77, Cys80, and Tyr81 of Arl1 and Thr193, Ala196, Thr199, and Gln200 of the DCB domain (Figure 1D). The interswitch region of Arl1 forms two β-strands (β2 and β3) and makes extensive interactions with helices α4, α6, and α8 of the DCB domain. Phe51 and Val53 from strand β2 and Trp66 from

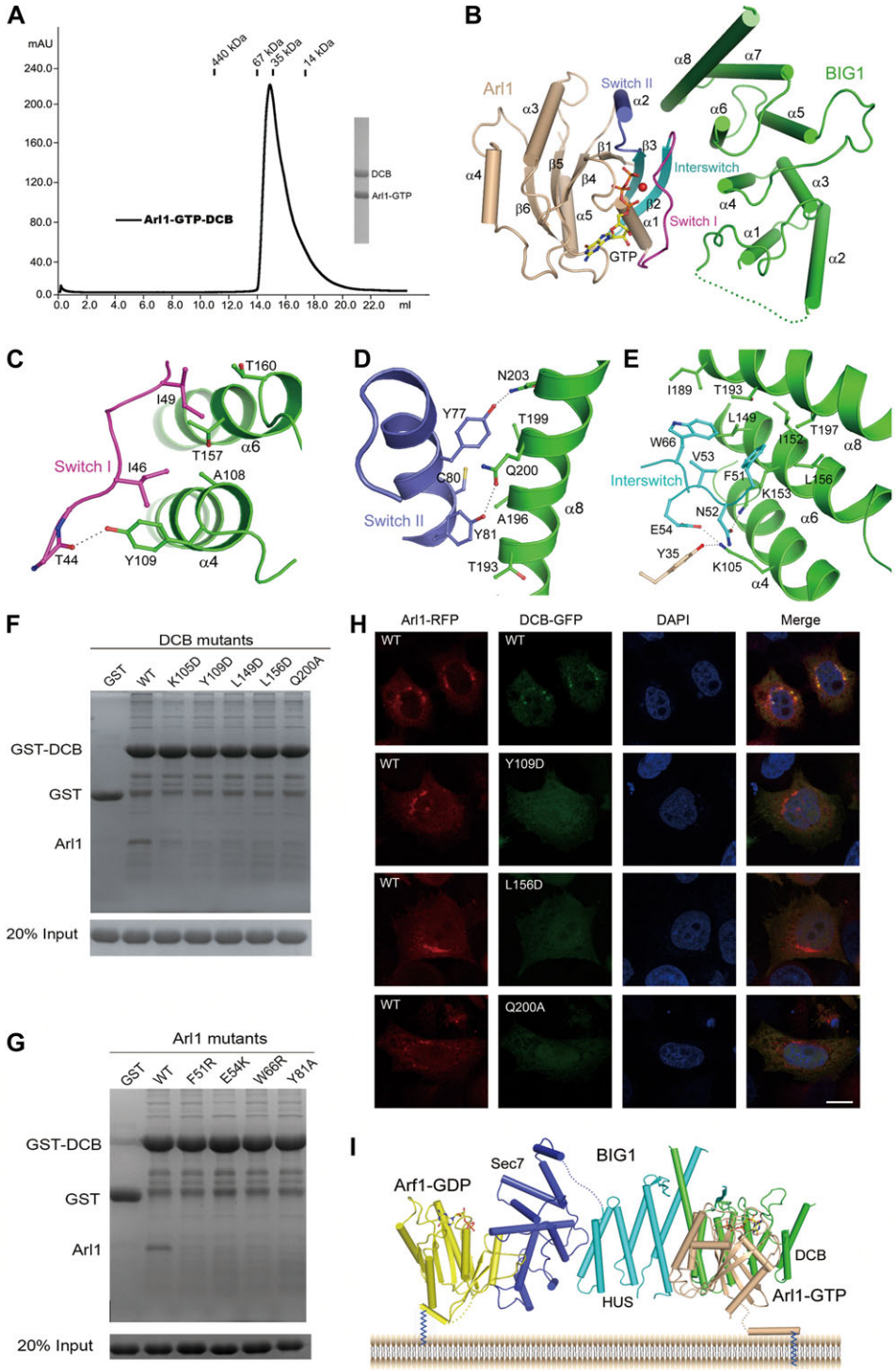

Figure 1 Crystal structure and biochemical characterization of Arl1-GTP–DCB. (**A**) Size-exclusion chromatography of Arl1-GTP–DCB on Superdex 200 10/300 column. The elution peak is analysed by SDS-PAGE and Coomassie staining. (**B**) Overall structure of Arl1-GTP–DCB. The bound GTP and Mg^{2+} are shown with a stick model and a red sphere, respectively. (**C**–**E**) Interactions of the DCB domain with the switch I, switch II, and interswitch regions of Arl1, respectively. The interacting residues are shown with side chains and the hydrophilic interactions are indicated with dotted lines. (**F**) *In vitro* GST pull-down assays between the wild-type and mutant GST-DCB and the wild-type His_6-Arl1. (**G**) *In vitro* GST pull-down assays between the wild-type GST-DCB and the wild-type and mutant His_6-Arl1. (**H**) Confocal fluorescence microscopy images of HeLa cells coexpressing Arl1-RFP and the wild-type DCB-GFP or its mutants. Scale bar, 10 μm. (**I**) The working model for targeting BIG1 to the trans-Golgi via interaction of its DCB domain with Arl1-GTP. The model is constructed based on the structures of the Arl1-GTP–DCB complex, the DCB–HUS domain of *Thielavia terrestris* Sec7, and the Arf1-GDP–Sec7 complex.

strand β3 protrude into a hydrophobic recess formed by Leu149, Ile152, Lys153, and Leu156 from helix α6 and Ile189, Thr193, and Thr197 from helix α8. Additionally, Tyr35 from helix α1 of Arl1 forms a hydrogen bond with Lys105 from helix α4; Glu54 from strand β2 forms a salt bridge with Lys105 from helix α4; and Asn52 from strand β2 forms a hydrogen bond with Lys153 from helix α6 (Figure 1E). Most of the residues at the interface are strictly or highly conserved in Arl1 and BIG1 from different species (Supplementary Figure S5A and B), suggesting that the interactions are very likely conserved in other species.

To confirm the interactions, we performed site-directed mutagenesis and *in vitro* GST pull-down assays on several residues at the interface, including F51R, E54K, W66R, and Y81A on Arl1 and K105D, Y109D, L149D, L156D, and Q200A on the DCB domain. The results showed that all these mutations impair the interaction dramatically (Figure 1F and G). In addition, we analysed the colocalization patterns of wild-type Arl1 with wild-type DCB domain and several DCB mutants. The results showed that the DCB mutants cannot colocalize with the wild-type Arl1 at the trans-Golgi (Figure 1H and Supplementary Figure S6). Moreover, several mutations of the equivalent residues on yeast Sec7 displayed significant growth defects (Richardson et al., 2016). We further investigated the binding of the DCB domain with several available small GTPases (Supplementary Figure S3B). We did not detect direct interaction of the DCB domain with Arl2, Arl3, and Rab1b, probably because the key residue Glu54 of Arl1 is changed to Lys53 in Arl2, Lys54 in Arl3, and Lys46 in Rab1b (Supplementary Figure S5C). On the other hand, we identified Arl4a as a binding partner of the DCB domain. As Arl4a and Arl1 share high sequence similarity in the switch regions and similar trans-Golgi localization (Lin et al., 2011; Christis and Munro, 2012), it is likely that BIG1 is a shared effector recruited to trans-Golgi by Arl1 and Arl4a.

In mammalian cells, knockdown of Arl1 resulted in the displacement of BIG1 from the Golgi (Christis and Munro, 2012), suggesting that the interaction between Arl1-GTP and the DCB domain is essential for targeting BIG1 to Golgi apparatus. Our study reveals the structural basis for the specific interaction of Arl1-GTP with the DCB domain of BIG1, which together with the available biological data allow us to propose a working model for targeting BIG1 to the trans-Golgi (Figure 1I). The membrane localized Arl1-GTP interacts directly with the DCB domain of BIG1 and recruits BIG1 to the trans-Golgi, and hence the Sec7 domain of BIG1 can exert the GEF activity towards and activate the Arf proteins. Accompanied with the activation, the anchoring of the Arf proteins with the membrane via the N-terminal myristoylated helix is further stabilized, and thus the Arf proteins can recruit the effectors such as coat complexes to the trans-Golgi, leading to the activation of the downstream trafficking pathway (Renault et al., 2003; Bui et al., 2009; Christis and Munro, 2012; Richardson et al., 2016).

[Supplementary material is available at Journal of Molecular Cell Biology online. The structure of Arl1-GTP–DCB has been deposited in the RCSB Protein Data Bank (accession code 5J5C). We are grateful to the staff members at BL19U of National Facility for Protein Science in Shanghai for technical support in data collection. This work was supported by grants from the National Natural Science Foundation of China (31230017 and 31370015) and the Chinese Academy of Sciences (Y419S11041).]

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