

Characterization of ArfGAP1 and FinGER7/FinGER8 interaction by quantitative yeast two-hybrid analysis

メタデータ	言語: English 出版者: 公開日: 2017-10-05 キーワード: 作成者: Hasina, Akhter メールアドレス: 所属:
URL	http://hdl.handle.net/2297/26882

氏名	HASINA AKHTER
学位の種類	博士(薬学)
学位記番号	博甲第1034号
学位授与の日付	平成20年3月22日
学位授与の要件	課程博士(学位規則第4条第1項)
学位授与の題目	Characterization of ArfGAP1 and FinGER7 /FinGER8 interaction by quantitative yeast two-hybrid analysis (定量的酵母ツーハイブリッド法による ArfGAP1 と FinGER7/ FinGER8 の相互作用の解析)
論文審査委員(主査)	中村 暢宏(自然科学研究科・准教授)
論文審査委員(副査)	中西 義信(医学系研究科・教授), 松永 司(自然科学研究科・教授), 横井 毅(医学系研究科・教授), 山下 克美(自然科学研究科・准教授)

Abstract

By using a quantitative method for yeast two-hybrid analysis, we tried to identify interacting regions of ArfGAP1, a GTPase activating protein for Arf, and FinGER7 and FinGER8, family members of five span transmembrane proteins localizing in the Golgi apparatus and ER. The N-terminal region (1-152) containing a GTPase activation domain, and the C-terminal region (256-406) of ArfGAP1 was dispensable and the central region (153-257) reported to function for sensing membrane curvature and also for targeting to the Golgi apparatus was necessary and enough for the interaction with FinGER7 or FinGER8. A mutation of 211th tryptophan of ArfGAP1 to alanine that disrupts the Golgi targeting and lipid curvature sensing abolished the interaction with FinGER7 or FinGER8 suggesting the role of FinGER7 and FinGER8 for the targeting of ArfGAP1 to the Golgi apparatus. The severe reduction of the interaction was observed by deletion of the N-terminal 31st to 41st residues of FinGER7 showing the importance of this region (31-41) for the interaction. Deletion of any parts of the C-terminal transmembrane segments completely abolished the interaction. Replacement of the cytoplasmic region or the transmembrane region of FinGER7 by FinGER8 did not recreate the original higher-level interaction suggesting that the cytoplasmic region and the transmembrane region cooperate for the interaction with ArfGAP1.

Introduction

The Golgi apparatus is situated at the center of the vesicular transport pathway. It receives secretory and transmembrane proteins from the ER, processes, sorts and sends out to the final destinations. Transport vesicles are produced by COPII, COPI and clathrin coat at the ER, the Golgi apparatus and plasma membrane, respectively. Coat proteins after recruitment on to the membrane by activated Arf1/Sar1, deform flat membranes into spherical buds that finally pinch off the membrane forming transport vesicles. Activation of Arf is mediated by Arf specific guanine nucleotide exchange factor (GEF). Two subfamilies of large Arf-GEFs operate at the Golgi complex, BIGs (Brefeldin inhibited GEFs) and GBF1 (Golgi specific Brefeldin A resistance factor 1). In vesicular transport mediated by COPI coat, the GTPase activity of Arf1 is stimulated by ArfGAP1, a GTPase activating protein, that catalyzes the prerequisite step of uncoating of vesicles. ArfGAPs constitute a large family of proteins containing a highly conserved (from yeast to mammals) catalytic domain of ~130 residues (GTPase activation or GAP domain). A central region of ArfGAP1 (residue 192-231) termed as ALPS domain acts as a lipid-packing sensor and helps anchor ArfGAP1 at the surface of the highly curved membrane, thus allowing GTP hydrolysis on Arf1. Ypt/rabs are known to regulate vesicle budding and/or fusion at the various steps of vesicular transport pathway. In mammals, rab1 regulates ER to Golgi and intra-Golgi protein transport whereas rab6 is involved in transport processes between endosomes and trans-Golgi network (TGN) and Golgi to ER transport. In yeast, Ypt1p is thought to play a key role in the tethering of ER- derived transport vesicles to the Golgi apparatus. Yip1p is an Ypt1p-interacting protein required for transport through the early secretory pathway and Yip1p like FinGER family proteins has been identified in humans which are five-span transmembrane proteins localizing in the ER and Golgi. Eight family members were found in human (FinGER1~FinGER8) and they are all expressed widely in various tissues. It was reported previously that the Golgi apparatus is fragmented by the over expression of FinGER1, 2, 3, 4 and 7

suggesting their function in the maintenance of the Golgi structure and/or the transport between the ER and the Golgi apparatus. Yip1p is a putative yeast orthologue of FinGER4 and FinGER5. Yif1p is that of FinGER3, FinGER7, FinGER8. Yip1p and Yif1p are essential yeast gene products localized in the Golgi apparatus. The loss of function of these proteins causes a block of the ER to Golgi transport and accumulation of ER membranes. These proteins interact with Ypt1p and Ypt31p, suggesting their involvement in vesicle docking and fusion. Supporting this possibility, it was reported that Yip1p and Yif1p were necessary to form vesicles competent for fusion with the Golgi apparatus. On the contrary, it was also reported that Yip1p functions in vesicle budding from the ER but not in the fusion with the Golgi apparatus. Therefore, it remains obscure whether Yip1p and Yif1p function in vesicle budding and/or fusion steps.

Results

To explore the function of FinGER proteins in the secretory pathway, it was tried to identify their interacting partner(s). A quantitative method of yeast two-hybrid analysis was developed and using this approach, the interaction of all the FinGERs with rab1, rab6, GBF1, ArfGAP1 was checked. No significant level of interaction was detected for all those FinGERs with rab1, rab6 and GBF1 (unpublished observation). Interestingly, ArfGAP1 showed reproducible interaction signal with FinGER7 and FinGER8 (Fig 1).

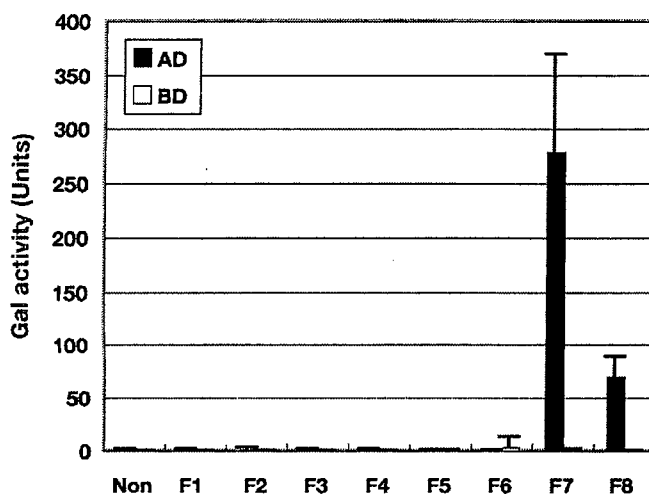


Figure 1 Specific interaction of ArfGAP1 with FinGER7/FinGER8

Quantitative yeast two-hybrid analysis was performed and the mean activities of triplicate assays are shown (bars) with SD (error bars).

In the deletion mapping experiments, the region containing the 153rd to 257th residues found necessary and sufficient for the interaction of ArfGAP1 with FinGER7 or FinGER8 (Fig 2). Since Δ N192- Δ C231 (ALPS domain only), Δ 136 ArfGAP1 (without the GAP domain) did not show interaction with either FinGER7 or FinGER8. Therefore, GAP domain is dispensable and the ALPS region only is not enough for the interaction. ALPS as well as ~ 40 residues upstream of ALPS and 20 residues downstream of ALPS have a pivotal role in this protein-protein interaction. A mutation of 211th tryptophan of ArfGAP1 to alanine that disrupts the Golgi targeting and lipid curvature sensing abolished the interaction with FinGER7 or FinGER8 suggesting the role of FinGER7 and FinGER8 for the targeting of ArfGAP1 to the Golgi apparatus.

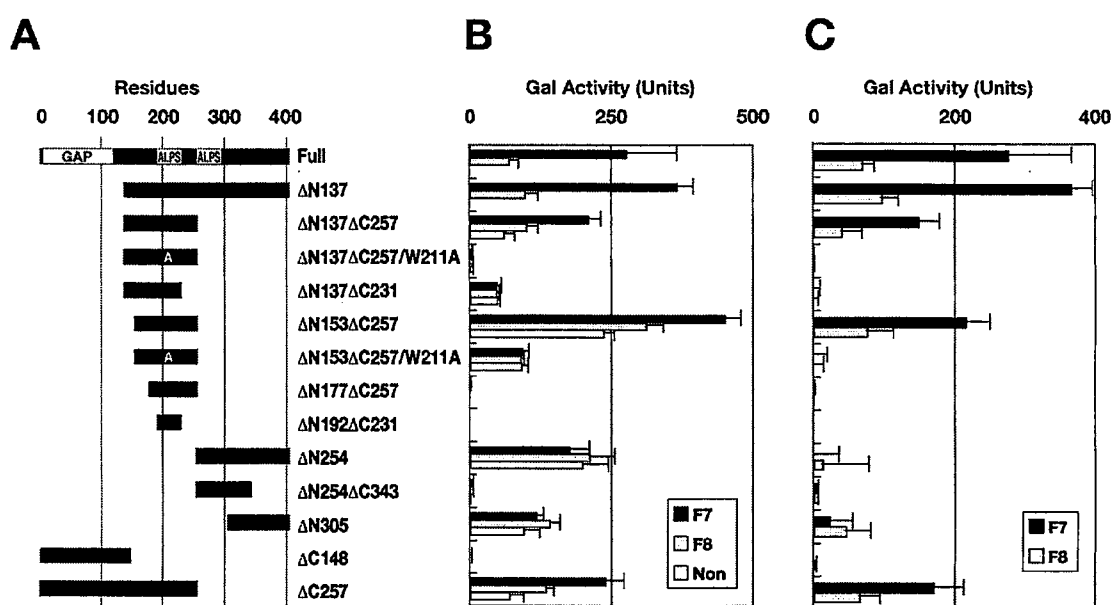


Figure 2 Interaction of ArfGAP1 mutants with FinGER7/FinGER8

(A) Schematic representation of ArfGAP1 mutants. A GTPase activation domain (GAP) and ALPS domains are shown in the top full length ArfGAP1, the W211A mutation is indicated by the letter A. (B) Quantitative yeast two-hybrid analysis was performed and the raw activity is shown. Triplicate assays were performed and the mean activities are shown (bars) with SD (error bars). (C) The results after subtraction of the background activity are shown. The propagation of error (SD) was calculated and is shown (error bars).

The result of deletion analysis of FinGER7 showed severe reduction of the interaction by deletion of the N-terminal 31st to 41st residues implying the importance of this region (31-41) for the interaction with ArfGAP1 (Fig 3). Deletion of any parts of the C-terminal

transmembrane segments completely abolished the interaction suggesting the importance of transmembrane region for the interaction.

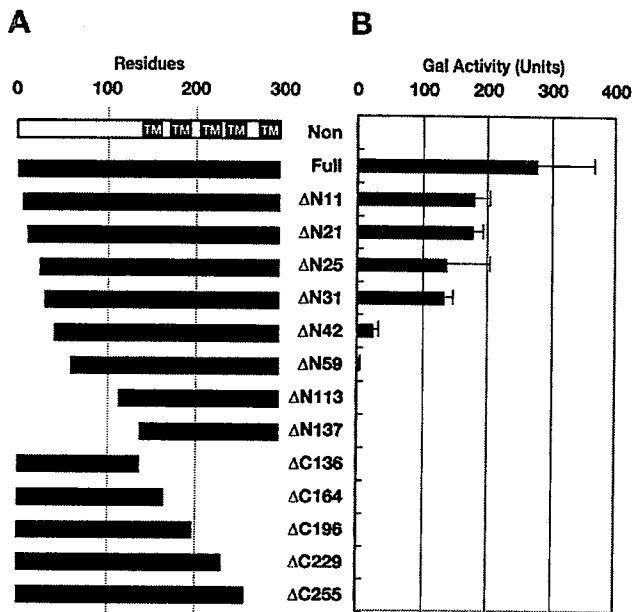


Figure 3 Interaction of FinGER7 deletion mutants with ArfGAP1

(A) Schematic representation of FinGER7 mutants. Putative transmembrane segments are shown in the top full length FinGER7. (B) Quantitative yeast two-hybrid analysis was performed as above. Triplicate assays were performed more than three times and the mean activities are shown (bars) with SD (error bars).

It is possible that deletion of any parts of the transmembrane region could prevent FinGER7 from properly inserting into the membrane, take a proper conformation or topology, or be transported to the correct sub cellular destination for the interaction. Therefore, it was then tried to replace the whole transmembrane domain of FinGER7 with that of other FinGER proteins (FinGER8, FinGER5, FinGER3) but replacement of the putative cytoplasmic region or the transmembrane region of FinGER7 by FinGER8 did not recreate the original higher-level interaction suggesting the co-operation of cytoplasmic and transmembrane domains for the interaction with ArfGAP1 (Fig 4).

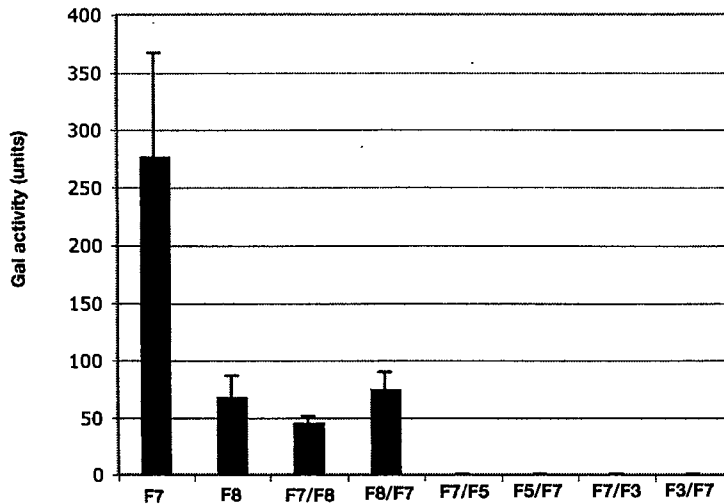


Figure 4 Interaction of chimeric mutants of FinGER7 and FinGER8 or FinGER5 or FinGER3 with ArfGAP1

Putative cytoplasmic (1-111) or transmembrane regions (112-293) of FinGER7 were swapped with homologous regions of FinGER8 (1-126, 127-311) or FinGER5 (1-112, 113-257) or FinGER3 (1-138, 139-350). Quantitative yeast two-hybrid analysis was performed as above. Triplicate assays were performed and the mean activities are shown (bars) with SD (error bars).

Conclusion

It is therefore implicated that FinGER7 and FinGER8 are involved in the COPI vesicle formation or cargo sorting into a COPI vesicle. In this aspect, the fact that FinGER7 interacts with ArfGAP1 at the lipid curvature sensing domain overlapping with the Golgi targeting domain appeals for its significance. It is possible that FinGER7 and FinGER8 control the activity of ArfGAP1 to modulate the activation of Arf1 leading to the stimulation or inhibition of the COPI coat assembly on Golgi membranes.

学位論文審査結果の要旨

本研究は、小胞体- ゴルジ体間の小胞輸送の調節機構を明らかにすることを目的とし、その調節機構に関わる事が示唆されていた小胞体- ゴルジ体間に局在する複数回膜貫通タンパク質のうち、特に FinGER7 と FinGER8 に着目して、これらの分子と相互作用するタンパク質を探索・同定し、さらにその相互作用部位の分子生物学的解析を行ったものである。酵母ツーハイブリッド法を用いて相互作用分子の探索を行ったところ、ArfGAP1 を FinGER7 と FinGER8 の両方に特異的に相互作用する分子として同定することに成功した。ArfGAP1 は、rab/Ypt ファミリーの小型 GTPase の GTP/GDP 交換因子であり、ゴルジ体からの COPI 輸送小胞の形成と輸送小胞へのタンパク質の選択的取り込みに関わる事が報告されている。したがって、本研究から FinGER7 と FinGER8 が ArfGAP1 と相互作用することによって、小胞体- ゴルジ体間の小胞輸送の調節に働くという画期的な可能性が初めて明らかとなった。また、酵母ツーハイブリッド法の手法を改良し定量的に解析する事を可能にし、FinGER7 と FinGER8 が ArfGAP1 と相互作用する部位と特定したことも注目に値する。

以上のことから、本研究の成果は今後の当該研究分野の学術的發展に大きく寄与するものと考えられ、博士（薬学）の授与に値すると判定された。