



Title	YlmG1 is localized exclusively to the chloroplast envelope membrane and is involved in preprotein translocation in Arabidopsis thaliana
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Abstract of Thesis

Name (Mengyi Li)	
Title	YlmG1 is localized exclusively to the chloroplast envelope membrane and is involved in preprotein translocation in <i>Arabidopsis thaliana</i> (シロイヌナズナYlmG1は葉緑体包膜に局在し前駆体蛋白質の膜透過に関与する)
Abstract of Thesis Most chloroplast proteins are nucleus-encoded, synthesized in the cytosol as a larger precursor protein called preprotein and are translocated across the outer and inner envelope membranes successively through TOC and TIC translocons, respectively [1], with the aid of the inner envelope membrane-localized ATP-dependent pulling motor, the Ycf2/FtsHi complex [2]. TOC and TIC constituents have been extensively characterized by means of biochemistry, cell biology, and molecular genetics as well as structural biology. Green lineages including <i>Chlorophyta</i> and <i>Embryophyta</i>, plants, possess well conserved TOC and TIC constituents as well as Ycf2/FtsHi motor complex constituents [1-3]. CryoEM structures of TIC from <i>Chlamydomonas reinhardtii</i> and from <i>Arabidopsis thaliana</i> revealed that Tic20 bears four transmembrane helices and forms an inner envelope membrane-embedded part of TIC with other two essential components, namely Tic12 and Tic214 (Ycf1), which contain one and six transmembrane helices, respectively. Tic214 forms a large intermembrane space domain of TIC, which is intertwined with two additional essential proteins, Tic56 and Tic100 [1,3]. While <i>Chlamydomonas</i> TIC and <i>Arabidopsis</i> TIC shows overall structural similarities, there are several remarkable differences: i) <i>Chlamydomonas</i> TIC forms a stable supercomplex with TOC in which a small part of TIC214 is integrated into the Toc75/Toc159 mixed barrel, whereas <i>Arabidopsis</i> TIC does not; ii) <i>Chlamydomonas</i> TIC contains another essential two-transmembrane helices-containing YlmG which binds to a transmembrane segment of Tic20 located opposite to the Tic214/Tic12-interacting face of Tic20 [4]. In <i>Arabidopsis</i>, YlmG1-1 (At3g07430; hereafter referred as YlmG1) which is the closest homolog of <i>Chlamydomonas</i> YlmG, was shown to be an indispensable protein required for chloroplast biogenesis [5]. On the other hand, another study demonstrated that YlmG1 was exclusively localized on the thylakoid membrane and was involved in nucleoid distribution [6]. Thus far, involvement of YlmG1 in chloroplast protein import in plants has not been investigated. In this report I revisited suborganellar location of YlmG1 in <i>Arabidopsis</i> chloroplasts and analyzed its molecular interaction with TIC as well as with translocating preproteins. Our biochemical data demonstrated that, in <i>Arabidopsis</i>, YlmG1 protein is exclusively localized in the envelope membrane of chloroplast. While YlmG1 is not a stably associated component of TIC and exists most likely as a monomeric form but is recruited to the preprotein translocation intermediate complexes together with other TIC components. Since YlmG1 was shown to be absolutely required for chloroplast biogenesis as well as for plant viability [5], this protein is assumed to play an important and indispensable role in preprotein translocation across the inner envelope membrane	

of chloroplast. This is a clear discrepancy with the previous report that YlmG1 is exclusively localized on the thylakoid membrane and play a role in nucleoid distribution [6]. The reason for the latter can be explained if the knock-down of *YLMG1* in *Arabidopsis* affect an import efficiency of certain protein involved in nucleoid distribution. A possible reason for the former might be related to a considerable difficulty I experienced to obtain strong enough antibody that can specifically recognize the endogeneous YlmG1 protein. To overcome this, in my study, I tried and selected several antisera of different sources, which commonly recognized the same envelope-localized protein, confirming the exclusive localization of YlmG1 in the chloroplast envelope membrane with confidence.

1. Kikuchi, S., Bédard, J., Hirano, M., Hirabayashi, Y., Oishi, M., Imai, M., Takase, M., Ide, T. and Nakai, M., 2013. Uncovering the protein translocon at the chloroplast inner envelope membrane. *Science*, **339** (6119), pp.571-574.
 2. Kikuchi, S., Asakura, Y., Imai, M., Nakahira, Y., Kotani, Y., Hashiguchi, Y., Nakai, Y., Takafuji, K., Bédard, J., Hirabayashi-Ishioka, Y. Mori, H., Shiina, T. and Nakai, M. (2018) A Ycf2-FtsHi heteromeric AAA-ATPase complex is required for chloroplast protein import. *Plant Cell*, **30**, 2677-2703.
 3. Zhao, X., Higa, T., and Nakai, M. (2022) Tic12, a 12-kDa essential component of the translocon at the inner envelope membrane of chloroplasts in *Arabidopsis*. *Plant Cell* **34**, 4569-4582.
 4. Liu, H., Li, A., Rochaix, J.D., and Liu, Z. (2023) Architecture of chloroplast TOC-TIC translocon supercomplex. *Nature* **615**, 349-357.
 5. Chen, H., Li, S., Li, L., Hu, H., and Zhao, J. (2018) *Arabidopsis* *EMB1990* Encoding a Plastid-Targeted YlmG Protein Is Required for Chloroplast Biogenesis and Embryo Development. *Front. Plant Sci.* **9**, 181.
 6. Kabeya, Y., Nakanishi, H., Suzuki, K., et al. (2010) The YlmG protein has a conserved function related to the distribution of nucleoids in chloroplasts and cyanobacteria. *BMC Plant Biol.* **10**, 57.
- 1 YlmG1 is localized exclusively to the chloroplast envelope membrane and is involved in preprotein translocation in *Arabidopsis thaliana*. Mengyi Li, Xueyang Zhao, and Masato Nakai (*Submitted*)

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

申請者 Mengyi Li 氏は、別グループから報告された葉緑体内包膜に存在する蛋白質輸送装置 TIC トランスロコンの 2 つのクライオ電顕構造において、緑藻クラミドモナスの TIC には YlmG が結合した状態で見られたのに対し、後で発表された植物シロイヌナズナの TIC には YlmG は見られていない事に着目し、植物シロイヌナズナの YlmG に関して研究を進めてきた。YlmG は小さな疎水性の高い蛋白質であり、特異抗体の取得に大変苦労しているが、最終的に抗体を得て生化学解析を実施している。その結果、YlmG が包膜に局在する蛋白質であること、TIC に安定して結合性している因子ではないが、葉緑体蛋白質を輸送する過程で輸送装置にリクルートされてくる因子であることを発見している。申請者はミトコンドリアの TIM 複合体ではたらく Mgr2 とのアナロジーから、シロイヌナズナの YlmG が TIC 複合体のゲートキーパーとして働いているという作動モデル提唱するに至っており、生命が生み出した蛋白質膜透過装置全般に関する作動原理やその進化に洞察を与えるものとなっている。

以上の研究成果は、葉緑体包膜に存在する蛋白質輸送装置の構造機能連関に関して、クライオ電子顕微鏡構造解析では明らかにすることのできていない基質輸送中の TIC トランスロコンを生化学解析したものであり、高く評価される。よって本論文は博士（理学）の学位論文として十分価値あるものと認める。