



Title	Translocation complex facilitating chloroplast protein import at the outer and inner membranes of <i>Passiflora edulis</i>
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Abstract of Thesis

Name (Prianshika Lata Sen)	
Title	Translocation complex facilitating chloroplast protein import at the outer and inner membranes of <i>Passiflora edulis</i> (<i>Passiflora edulis</i> 葉緑体膜において蛋白質輸送に関わる膜透過複合体)
Abstract of Thesis Chloroplast, found in photosynthetic eukaryotes originated from a result of primary endosymbiosis whereby, a eukaryote engulfed a cyanobacterium, more than a billion years ago. Instead of being digested, the engulfed cyanobacterium became more reduced and compartmentalized in the eukaryotic cell. Over the course of millions of years, gene transfers between plastid genome and nucleus occurred resulting in many proteins needed by plastids being nuclear encoded. Almost 3000 of these proteins needed by plastids are nuclear encoded, translated in the cytosol, then imported into chloroplast via the TOC (Translocon at the Outer envelope membrane of Chloroplast) and TIC (Translocon at the Inner envelope membrane of Chloroplast) complexes linked with Ycf2/FtsHi motor complex which provides ATP-driven pulling forces [1]. Extensive studies have been done on TOC-TIC and Import motor complex in <i>Arabidopsis thaliana</i> and <i>Pisum sativum</i> to elucidate the components of the complex. The main components for TOC are Toc75, Toc34 and Toc159. Toc34 and Toc159 are pre-protein receptors having the GTPase domains that bind to transit peptides. Toc75 belonging to the Omp85 family, has N-terminal POTRA domain which is located in the inter-membrane space interacting with TIC components located at the Inter-Membrane Space [2]. The main components for TIC are Tic20, Tic56, Tic100, Tic214 (<i>ycf1</i>) and Tic12[3,4]. Tic20 protein which is the channel protein, has four homologous isoforms in <i>Arabidopsis thaliana</i>, in which Tic20-I and Tic20-IV play a role in the import complex. The core channel protein for the main TIC complex has Tic20-I as an essential component, whereas Tic20-IV is non-essential and found in the alternative TIC system for non-photosynthetic plastids in <i>Arabidopsis</i>. The Ycf2/FtsHi motor complex is a 2MD complex that has ycf2 encoded protein and FtsH-like proteins, which are FtsHi1, FtsHi2, FtsHi4, FtsHi5, and FtsHi12 with NAD-malate dehydrogenase [5]. Four additional components for import motor has been recently identified using cryo-electron microscopy. Recent studies in <i>Chlamydomonas reinhardtii</i> showed that components of TOC, Main TIC complex and Ycf2/FtsHi are well conserved with land plants. Molecular studies conducted thus far shows all components of TOC, main TIC system and the import motor are crucial for chloroplast biogenesis and viability in <i>Arabidopsis</i>. It is also well conserved in the green lineage including green alga and land plants. However some species of land plants shows exception to this, especially for main TIC system and import motor. In the genus <i>Passiflora</i>, recent genomic evidences show loss of functional genes like <i>ycf1</i> and <i>ycf2</i> found in the plastid genome. These two are the largest open reading frame found in the plastid genome that encode two large proteins found in main TIC complex (Tic214) and ATP-driven import motor protein (Ycf2) implying a possible alternate import system. It opens the question to whether they have retained the components of TOC system if they are utilizing alternate TIC and import motor system. It also challenges the question whether the alternate TIC system is composed of the homolog Tic20-IV as identified in <i>Arabidopsis thaliana</i>. In this study, I aimed to elucidate the TOC components of <i>Passiflora edulis</i> as my model plant which belong to the group of plants that has lost function to <i>ycf1</i> and <i>ycf2</i>.	

My data reveals that model plant *Passiflora edulis* has conserved TOC complex while TIC and YCF2/FtsHi complex has been lost. The TOC complex is around 1MD with its two large constituents TOC159 and TOC75 to have well conserved regions and interact with each other in pull-down experiments. TIC20-IV forms a cluster with the rest of the TIC20-IV homologs which utilizes the main TIC system. My research has just scratched the surface on studying dicot plants which are using conserved TOC and alternate TIC and import motor system. More research on other dicot plants would be interesting to do has lost *ycf1* and *ycf2* gene function.

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- Chloroplast protein import in *Passiflora edulis* involves well conserved TOC but is undergoing drastic changes in composition of TIC. Prianshika Lata Sen 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 Prianshika Lata Sen 氏は、葉緑体の外包膜および内包膜に存在する蛋白質輸送装置 TOC トランスロコンおよび TIC トランスロコン複合体、引き込みモータ複合体に関して、幅広く様々な植物のゲノム解析データをデータベースマイニングすることで、非常に稀な例外が存在することを見出した。その一つが生化学材料と用いた双子葉パッションフルーツである。遺伝情報から、他の植物ではマイナーな因子（TIC20-IV）がパッションフルーツではメジャーな役割を果たし、そのためにメジャーな TIC が失われた、という作業仮説に基づいて、このマイナーな TIC を単離同定することを目指して研究を進めてきた。本学位論文の重要な成果は、パッションフルーツにおいては、外包膜の TOC に関しては、植物に広く保存されている TOC が同様に存在し機能しているものの、内包膜のコンポーネントにおいては、他の植物で主要な役割を果たす TIC の中心因子である TIC20-I が欠損し、それに代わって、マイナーな因子である TIC20-IV が働いていることを丁寧な生化学解析により明らかにしていることである。本論文では、非モデル植物の葉緑体包膜という生化学実験の難しい対象に挑戦し、TIC20-IV の存在を実験的に確認して機能的に保存性が高いと思われる蛋白質輸送装置において、マイナーコンポーネントが果たした進化的役割について議論するに至っている。

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に関する植物生化学分野および植物進化学分野での知見を拓げる重要な発見であり、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。