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Is chloroplast import of photosynthesis proteins facilitated by an actin-TOC-TIC-VIPP1 complex?

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Actin filaments are major components of the cytoskeleton that interact with chloroplast envelope membranes to allow chloroplast positioning and movement, stromule mobility and gravitropism perception. We recently reported that Toc159, a component of the TOC complex of the chloroplast protein import apparatus, interacts directly with actin. The interaction of Toc159 and actin was identified by co-immunoprecipitation and co-sedimentation experiments with detergent-solubilised pea chloroplast envelope membranes. In addition, many of the components of the TOC-TIC protein import apparatus and VIPP1 (vesicle-inducing protein in plastids 1) were identified by mass spectroscopy in the material co-immunoprecipitated with antibodies to actin. Toc159 is the receptor for the import of photosynthesis proteins and VIPP1 is involved in thylakoid membrane formation by inducing vesicle formation from the chloroplast inner envelope membrane, suggesting we may have identified an actin-TOC-TIC-VIPP1 complex that may provide a means of channeling cytosolic preproteins to the thylakoid membrane. The interaction of Toc159 with actin may facilitate exchange between the putative soluble and membrane forms of Toc159 and promote the interaction of cytosolic preproteins with the TOC complex.

Key words: actin, chloroplast, protein import, TOC complex, TIC complex, VIPP1

Abbreviations: SPP, stromal processing peptidase; TIC, translocon of the inner envelope membrane of chloroplasts; TOC, translocon of the outer envelope membrane of chloroplasts; VIPP1, vesicle-inducing protein in plastids 1

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Actin is a ubiquitous protein of eukaryotic cells and a major component of the cytoskeleton as microfilaments. In plant cells, plastids are closely associated with actin microfilaments.[1,2] A direct interaction of plastids with the actin cytoskeleton has been postulated to anchor chloroplasts at appropriate intracellular positions,[3] to support chloroplast light-intensity-dependent movement,[4] to facilitate plastid stromule (stroma-filled tubule) mobility[5,6] and to participate in gravity perception.[7] The known proteins implicated in plastid-actin interaction are CHUP1 (chloroplast unusual positioning 1), a protein exclusively targeted to the chloroplast outer envelope membrane that is essential for chloroplast anchorage to the plasma membrane,[8] and myosin XI proteins that play a role in stromule movement[9] and in gravitropism.[10,11] Recently, we found that Toc159 also interacts with actin.[12]

Toc159 is a component of the TOC complex, which is part of the chloroplast protein translocation apparatus. This apparatus consists of two membrane protein complexes that associate to allow translocation of nucleus-encoded proteins from the cytoplasm to the interior stromal compartment (reviewed in ref. 13). The translocon at the outer envelope membrane of chloroplasts (TOC complex) mediates the initial recognition of preproteins and their translocation across the outer membrane.[14] The translocon at the inner envelope membrane of chloroplasts (TIC complex) physically associates with the TOC complex and provides the membrane translocation channel for the inner membrane. In addition, the TOC and TIC complexes interact with a set of molecular chaperones (ClpC and Hsp70), which assist the transfer of imported proteins[15-17] (**Fig. 1**).

The interaction between actin and Toc159 was identified by co-immunoprecipitation and co-sedimentation

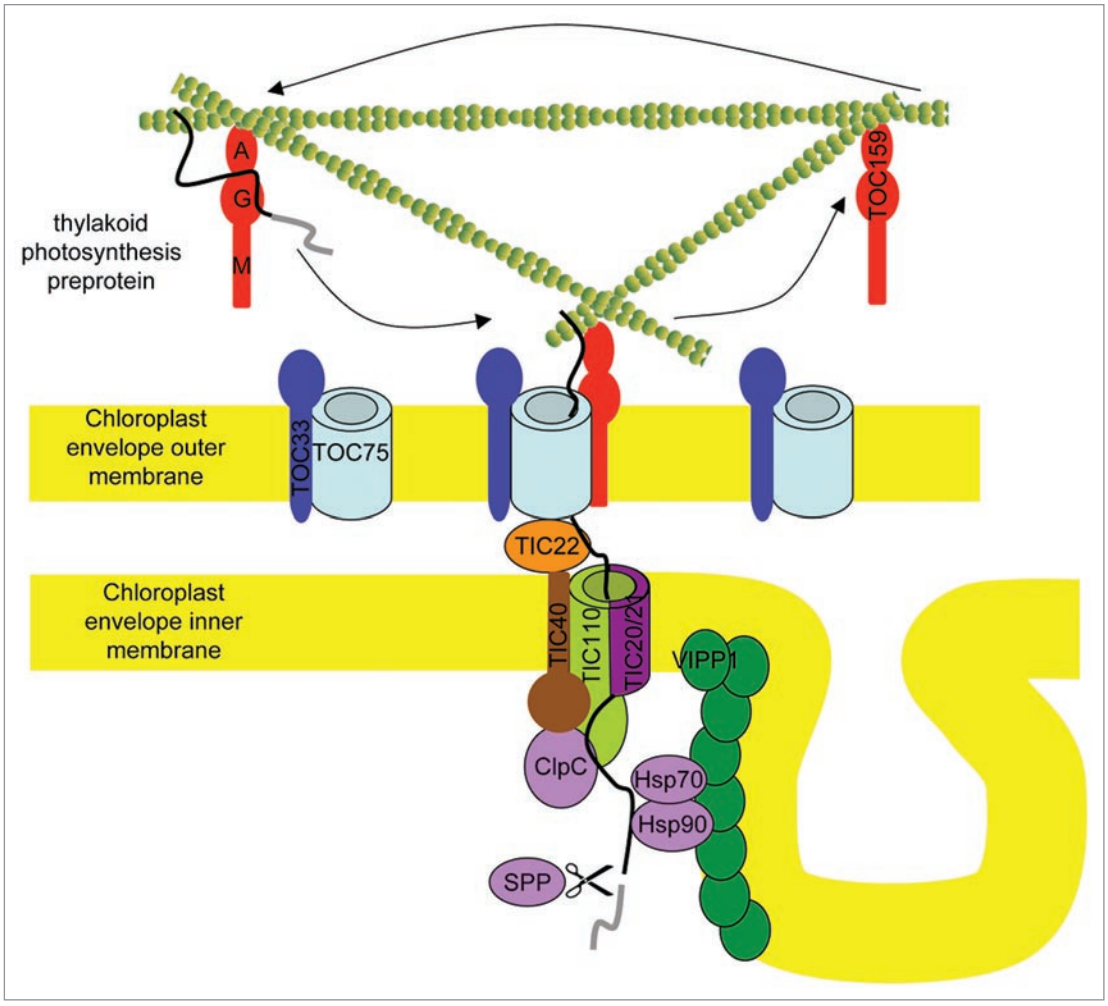

Figure 1. Schematic diagram of Toc159-actin interactions and the import of photosynthesis proteins. Toc159, linked to actin by its A-domain, recruits a newly synthesized photosynthesis preprotein by its G-domain. Actin filaments facilitate Toc159 movement to the chloroplast outer envelope membrane for integration into the TOC complex. The core TOC complex is formed by Toc159, Toc34 and Toc75. Tic22 acts to facilitate the passage of preproteins across the intermembrane space and interacts with the TIC complex. The core TIC complex is composed of Tic110, Tic20 and/or Tic21, and Tic40. The Tic110 protein recruits stromal molecular chaperone ClpC. On arrival in the stroma, the transit peptide is cleaved by SPP, and other chaperones (Hsp90 or Hsp70) may assist in the folding. VIPP1 interacts with the chaperones and polymerises, inducing chloroplast inner envelope membrane budding, leading to thylakoid formation.

experiments with detergent-solubilised pea chloroplast envelope membranes, and confirmed with Toc159 expressed in *Escherichia coli*. In addition, many other components of the TOC-TIC protein import apparatus were co-immunoprecipitated by antibodies to actin and co-sedimented with added F-actin filaments.[12] Using mass spectrometry, we identified the principal components of the TOC complex (Toc159, Toc75 and Toc34) and three accepted components of the TIC core complex (Tic110, Tic40 and ClpC). The presence of Tic20/21 and Tic22 could not be examined because they migrate in the same position on SDS-PAGE as the light chains of antibody molecules but, since they are involved in linking the TOC and TIC complexes,[6] they may also be part of the complex with actin.

The identification of the region of Toc159 that interacts with actin is an important feature to help establish whether any of the other Toc159 isoforms (such as Toc132 and Toc120) are likely to interact with actin. Toc159 family proteins are composed of three different domains: the A (acidic) domain, the G (GTPase) domain and the M (membrane) domain.[18] The interaction of Toc159 with actin appears most likely to be through the A-domain; the G-domain did not co-sediment with actin filaments[12] and the M-domain is embedded in the chloroplast envelope outer membrane and therefore is unlikely to be accessible to actin. Toc132 and Toc120 have shorter A-domains than Toc159 and this may affect their ability to bind actin. Although all the Toc159 isoforms are implicated in chloroplast protein import, Toc132 and Toc120 are involved in the import of chloroplast housekeeping proteins and Toc159 is specialized for the import of photosynthesis proteins.[18] For import of photosynthesis proteins, two models have been proposed for preprotein recognition by the TOC complex: the 'targeting model' where the newly synthesized

preprotein is first bound by a free cytosolic form of Toc159, and the 'motor model' where the transit peptide is first phosphorylated and then bound to Toc34 associated with the other TOC subunits in the outer envelope membrane.[13] In support of the first model, Toc159 has been reported to exist in both cytosolic and membrane-bound forms[19,20] and the soluble form of Toc159 is able to bind preproteins.[20,21] Toc159 is proposed to be the major point of contact for preproteins during the early stages of protein import through its A-domain.[22] The interaction of Toc159 with actin might provide a means to favor exchange between the putative soluble and membrane forms of Toc159 and potentially facilitate chloroplast photosynthesis protein import (**Fig. 1**).

Several features of this model require additional experimental evidence. The involvement of a soluble form of Toc159 is highly controversial,[13] and evidence for a physiological role in vivo is required. Experimental evidence for a facilitating role of the actin cytoskeleton in chloroplast protein import is also required. Does the presence of a basket of actin filaments surrounding the chloroplasts[2] provide a means of concentrating cytosolic Toc159 in the vicinity of the chloroplasts? Or do actin filaments provide a trackway for movement of Toc159 to or from chloroplasts? Myosin, the motor protein for movement along actin filaments, was not detected in the co-immunoprecipitated complex, but this does not necessarily rule out its involvement.

VIPP1 was also identified in the complex with actin. VIPP1 is involved in thylakoid membrane formation by vesicle formation from the chloroplast inner envelope membrane[23] and the quantity of thylakoid membrane proteins is closely correlated to the amount of VIPP1 in chloroplasts.[24] VIPP1 is also known to interact with Hsp70 and Hsp90 chaperones[25-27] and these chaperones may associate with the stromal face of the TIC complex to support protein folding.[15] This raises the possibility that an actin-TOC-TIC-VIPP1 complex may facilitate thylakoid formation by channeling the import of thylakoid-located photosynthesis proteins through the chloroplast envelope membrane into vesicles directed to the thylakoid membrane (**Fig. 1**).

Our study of actin-binding proteins in the chloroplast envelope membrane may have provided an initial glimpse at previously unrecognized mechanisms facilitating the import of photosynthesis proteins by chloroplasts. The formation of an actin-TOC-TIC-VIPP1 complex may provide a means of channeling cytosolic preproteins to the thylakoid membrane.

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