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Review

The chloroplast protein import system: From algae to trees ☆

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ABSTRACT

Chloroplasts are essential organelles in the cells of plants and algae. The functions of these specialized plastids are largely dependent on the ~3000 proteins residing in the organelle. Although chloroplasts are capable of a limited amount of semiautonomous protein synthesis – their genomes encode ~100 proteins – they must import more than 95% of their proteins after synthesis in the cytosol. Imported proteins generally possess an N-terminal extension termed a transit peptide. The importing translocons are made up of two complexes in the outer and inner envelope membranes, the so-called Toc and Tic machineries, respectively. The Toc complex contains two precursor receptors, Toc159 and Toc34, a protein channel, Toc75, and a peripheral component, Toc64/OEP64. The Tic complex consists of as many as eight components, namely Tic22, Tic110, Tic40, Tic20, Tic21 Tic62, Tic55 and Tic32. This general Toc/Tic import pathway, worked out largely in pea chloroplasts, appears to operate in chloroplasts in all green plants, albeit with significant modifications. Sub-complexes of the Toc and Tic machineries are proposed to exist to satisfy different substrate-, tissue-, cell- and developmental requirements. In this review, we summarize our understanding of the functions of Toc and Tic components, comparing these components of the import machinery in green algae through trees. We emphasize recent findings that point to growing complexities of chloroplast protein import process, and use the evolutionary relationships between proteins of different species in an attempt to define the essential core translocon components and those more likely to be responsible for regulation. This article is part of a Special Issue entitled: Protein Import and Quality Control in Mitochondria and Plastids.

1. Introduction

The chloroplast is the major organelle in plant and algal cells responsible for photosynthesis. It is also the factory in which many other essential biosynthetic reactions occur, including synthesis of amino acids, fatty acids and terpenes. In plants, the chloroplast is only one of a number of plastids that include proplastids, etioplasts, chromplasts, leucoplasts, amyloplasts, elaioplasts, proteinoplast or aleuronoplasts and gerontoplasts. These plastids display different morphologies, perform specialized functions and store various biochemical compounds during plant development. Under certain conditions, plastid types can interconvert. Chloroplasts possess three membrane systems: the outer envelope, the inner envelope and thylakoid membranes. These in turn enclose three aqueous compartments: the intermembrane space, stroma and thylakoid lumen. The structural and functional complexities of this organelle require the concerted action of some 3000 different proteins [1].

Chloroplasts originated from endosymbiotic cyanobacteria, with the original symbiotic event estimated to have occurred approximately 1.5 billion years ago [2]. In order to achieve a mutually beneficial endosymbiosis, two major events happened. First, the endosymbiont transferred the bulk of its genes to the host genome, and second, it developed protein import systems to translocate proteins from the host cytoplasm back into the endosymbiont. Two sources were used by the endosymbiont to construct the protein transport machinery; one native to the host cell and the other native to the prokaryotic endosymbiont [3–11]. Eventually, two interacting protein conducting complexes were established in the envelope membranes; termed Toc (for Translocon at Outer envelope membrane of Chloroplast) and Tic (for Translocon at Inner envelope membrane of Chloroplast) [12]. Components originating with the prokaryotic endosymbiont include Toc75, Tic22, Tic21, Tic20, Tic55, Tic32 and Tic62, while those originating with the eukaryotic host are Toc159, Toc34, Tic110, Tic40 and Toc64/OEP64 (Table 1, and also see [3–7]).

The genome of extant chloroplasts encodes only ~100 proteins. Accordingly, more than 95% of chloroplast proteins are nuclear encoded, synthesized in the cytoplasm as precursor proteins and post-translationally imported into the plastids. Most chloroplast precursor proteins have a cleavable N-terminal extension, the transit peptide, to direct them into chloroplasts and to target them to their final destinations within plastids. The transit peptides are not conserved at the primary structural level. However, they share some common structural and physical features, such as being rich in serine, possessing a low abundance of acidic amino acids and having lengths ranging from 20 to >100 residuals [13–16]. Precursors are

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Table 1
Toc and Tic components from green algae to trees.

Component	Gene in organism					Transit peptide and Domain	Proposed function	Origin
	C .reinhardtii	*P. patens*	*A. thaliana*	*P. sativum*	*P. trichocarpa*			
Toc159	Cre17.g707500.t1.1 (8.3e–27) Cre17.g734300.t1.2 (9.5e–25)	Pp1s123_61V6.1 (0) Pp1s123_63V6.1 (0) Pp1s136_62V6.1 (1.6e–180) Pp1s136_59V6.1 (3.8e–175)	At4g02510 (Toc159; 0) At2g16640 (Toc132; 0) At3g16620 (Toc120; 1.2e–178) At5g20300 (Toc90; 4.9e–118) At4g15810 (Toc100; 2.8e–22)	**AAF75761**	POPTR_0004s17740.1 (0) POPTR_0008s22130.1 (0) POPTR_0008s22140.1 (0) POPTR_0009s13370.1 (0) POPTR_0009s13380.1 (6.8e–179) POPTR_0010s01800.1 (2.5e–136) POPTR_0006s06230.1 (1.1e–131) POPTR_0010s01820.1 (7.5e–123) POPTR_0025s00620.1 (1.5e–120)	Acidic, GTPase , Membrane domain	Precursor recognition; Translocation driving motor	Eukaryotic
Toc34	Cre06.g252200.t1.1 (2.5e–37)	Pp1s72_197V6.1 (6.1e–95) Pp1s54_12V6.2 (2.3e–92) Pp1s6_441V6.1 (5.7e–65)	At5g05000 (Toc33, 1.3e–121) At1g02280 (Toc34, 2.2e–103)	**Q41009**	POPTR_0002s18420.1 (9.3e–102) POPTR_0014s10500.1 (3.7e–75)	GTPase , 1 TMH[a]	Precursor recognition	Eukaryotic
Toc75	Cre03.g175200.t1.1 (3.7e–85)	Pp1s2_62V6.1 (0) Pp1s23_111V6.1 (0) Pp1s317_51V6.1 (0) Pp1s44_230V6.1 (2.6e–112)	At3g46740 (Toc75–III, 0) At4g09080 (Truncated, 2.6e–139) At1g35860 (Pseudogene, 1.9e–128)	**Q43715**	POPTR_0001s12500.1 (0) POPTR_0003s15670.1 (0)	N–terminal cleavable bipartite transit peptide, POTRA repeats, β–barrel	Channel, Precursor recognition	Cyanobac-terial
OEP80	Cre02.g122700.t1.1 (1.1e-24)	Pp1s1_532V6.1 (2.6e–33)	At5g19620 (OEP80, Toc75–V,7.0e–29)	P0C891 (partial sequence)	POPTR_0001s05640.1 (1.8e–28) POPTR_0003s20390.1 (7e–28)	POTRA repeats, β–barrel	Chloroplast biogenesis	Cyanobac-terial
Toc64/ OEP64	Cre18.g747150.t1.2 (AMI2, 6.7e-47)	Pp1s167_52V6.2 (9.1e–165) Pp1s42_259V6.1 (1.6e–161) Pp1s301_54V6.1 (9.7e–153)	AT3G17970 (0) AT5G09420 (MtOM64, 7e–154) AT1G08980 (AMI1, 1.2e–93)	Q9MUK	POPTR_0012s04440.1 (0) POPTR_0015s04580.1 (0) POPTR_0001s21240.1 (7.3e–153)	TMH, TPR, Amidase region	Receptor	Eukaryotic
Tic22	Cre14.g625750.t1.1 (4.5e–21)	Pp1s282_30V6.1 (1.4e–58) Pp1s81_171V6.1 (2.7e–37)	At4g33350 (2.0e–91) At3g23710 (9.1e–37)	**Q9ZST9**	POPTR_0002s12830.1 (9.6e–96) POPTR_0014s02990.1 (6.4e–92) POPTR_0002s23710.1 (3.5e–41)	N–terminal transit peptide	Scaffold between Toc and Tic	Cyanobac-terial
	None	Pp1s68_153V6.1 (1.7e–59)	**At5g62650 (Tic22–like)**	N/A[b]	POPTR_0017s00970.1 (6.4e–179) POPTR_0004s07060.1 (1.1e–156)	Predicted N–terminal transit peptide	N/A	N/A
Tic20	Cre08.g379650.t1.1 (7.5e–10) Cre04.g225050.t1.2 (2.2e–8) Cre01.g039150.t1.1 (2.7e–8)	Pp1s54_101V6.1 (1.5e–61) Pp1s197_89V6.1 (7.8e–61) Pp1s5_404V6.1 (2.6e–7)	At1g04940 (Tic20–I, 3.6e–82) At4g03320 (Tic20–IV, 5.6e–32) At2g47840 (Tic20–II, 1.1e–7) At5g55710 (Tic20–V, 6.2e–6)	**Q9ZST8**	POPTR_0002s03320.1 (1.0e–83) POPTR_0005s25300.1 (1.2e–83) POPTR_0019s13630.1 (3.1e–37) POPTR_0004s20320.1 (3.5e–5) POPTR_0013s08250.1 (9.27e–4)	N–terminal transit peptide, 4 TMHs	Channel	Cyanobac-terial
Tic21	Cre10.g454771.t1.1 (1.4e–27) Cre22.g763750.t1.2 (5.1e–13) Cre01.g039450.t1.2 Cre01.g062500.t1.1	Pp1s54_55V6.1 (1.7e–68) Pp1s58_244V6.1 (1.3e–66) Pp1s257_95V6.1 (6.4e–37) Pp1s113_77V6.1 (10e–29)	At2g15290/PIC1 (1.5e–78)	**ABG00264**	POPTR_0001s30780.1 (7e–88) POPTR_0009s09920.1 (2.3e–85)	N–terminal transit peptide, 4 TMHs	Channel, assembly factor of importing complex, iron transport	Cyanobac-terial
Tic110	Cre10.g452450.t1.1 (9.1e–46)	Pp1s26_23V6.1 (0) Pp1s509_22V6.2 (0)	At1g06950 (0)	**O24303**	POPTR_0013s15040.1 (0) POPTR_0019s14760.1 (0)	N–terminal transit peptide, 2 TMHs (or 6TMHs)	Channel; Precursor and chaperone docking	Eukaryotic
Tic40	Cre12.g508000.t1.1 (7.7e–40)	Pp1s159_2V6.1 (4.0e–73) Pp1s110_13V6.1 (2.5e–72)	At5g16620 (2.8e–100)	**Q8GT66**	POPTR_0004s08560.1 (7.4e–117) POPTR_0017s02900.1 (1.7e–115)	N–terminal transit peptide, Ser/Pro–rich, 1 TMH, TPR, Hip/Hop/Sti1	Co-chaperone for import motor(s)	Eukaryotic
Tic55	None	Pp1s153_103V6.1 (1.6e–156) Pp1s11_89V6.1 (1.4e–152)	At2g24820 (0)	**O49931**	POPTR_0018s02890.1 (0) POPTR_0006s28310.1 (0)	N–terminal transit peptide, Rieske [2Fe–2S] cluster, Mononuclear iron–binding site, CxxC motif,	Redox sensor and regulator	Cyanobac-terial

(*continued on next page*)