Analysis of the dynamic property of Tat translocase and the fate of Tat signal peptides

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List of abbreviations

Alb3	Albino 3
amp	Ampicillin
APS	Ammonium peroxodisulphate
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
Bis-acrylamide	N′N′-methylene-bisacrylamide
BN-PAGE	Blue-native polyacrylamide gel electrophoresis
BSA	Bovine serum albumin
CAP	m7G(5′)ppp(5′)G
cDNA	copy (or complementary) DNA
CFoII	Chloroplast Fo ATP synthase subunit II
C-terminal	Carboxyl-terminal
DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoside triphosphate
DTT	1,4-Dithiothreitol
ECL	Enhanced chemiluminescence
E.coli	*Escherichia coli*
EDTA	Ethylenediaminetetra-acetic acid
EGFP	Enhanced green fluorescent protein
ER	Endoplasmic reticulum
Ffh	Fifty-four homologue
FtsY	Filamentous temperature sensitive mutant Y
g	Gram
g	Gravity
GTP	Guanosine triphosphate
GTPase	Guanosine triphosphatase
Hepes	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
HM	Hepes/magnesium buffer
Hsp	Heat shock protein
IgG	Immunoglobulin G
IPTG	Isopropyl-beta-D-thiogalactopyranoside

IVT	*in vitro* translation
kDa	Kilo-Dalton
l	Liter
Leu	Leucine
LHC	Light harvesting complex
LHCP	Light harvesting chlorophyII a/b binding protein
M	Molar
Met	Methionine
mg	Milligram
min	Minute
ml	Millilitre
mM	Millimole per litre
mRNA	Messenger RNA
μg	Microgram
μl	Microlitre
nm	Nanometer
NMR	Nuclear magnetic resonance
N-terminal	Amino-terminal
NTP	Nucleoside triphosphate
OD	Optical density
OEC16	16 kDa oxygen evolving complex protein
OEC23	23 kDa oxygen evolving complex protein
OEC33	33 kDa oxygen evolving complex protein
Oxa-1	Cytochrome oxidase assembly 1
PAA	Polyacrylamide
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PC	Plastocyanin
PCR	Polymerase chain reaction
PE	Phycoerythrin
Pftf	plastid fusion/protein translocation factor
PMSF	Phenylmethylsulfonyl fluoride
PS I	Photosystem I
PS II	photosystem II
PsbW	Photosystem II subunit W
PsbX	Photosystem II subunit X
PsbY	Photosystem II subunit Y
REMPs	Redox enzyme maturation proteins
Rieske	Rieske iron-sulfur protein of the cytochrome complex

RIP	Regulated intramembrane proteolysis
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Rounds per minute
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
SDS	Sodium dodecyl sulphate
Sec	Secretory
SPP	Stromal processing peptidase
SRP	Signal recognition particle
STD	Stroma targeting domain
Tat	Twin arginine translocation
TEMED	N,N,N',N'-tetramethylethylenediamine
Tic	Translocon at the inner chloroplast envelope membrane
TMAO	Trimethylamine N-oxide
Toc	Translocon at the outer chloroplast envelope membrane
TPP	Thylakoidal processing peptidase
Tris	Tris(hydroxymethyl)methylamine
Tween20	Polyoxyethylenesorbitan monolaurate
v/v	Volume/volume
w/v	Weight/volume
°C	Degree Celsius
ΔpH	Proton gradient
$\Delta\psi$	membrane potential

Summary

Translocation of folded proteins across the thylakoid membrane of chloroplasts and the plasma membrane of bacteria distinguishes the Tat pathway from the other protein transport pathways. The work presented in this thesis characterizes the Tat pathway in the following aspects

(1) Evolutionary conservation of the targeting information of the Tat protein transport pathway.
In contrast to plant plastids derived from endosymbiosis of a cyanobacterium, cryptophytes acquire their plastids by engulfing and stably integrating a red algal cell, leading to a eukaryote-eukaryote chimera. The light-harvesting apparatus in cryptophytes is differentially arranged in comparison with that found in the thylakoids of cyanobacteria and red algae. In cryptophytes, the photosynthetic pigments like phycobilin and the relative phycobiliproteins are located on the lumenal rather than the stromal side of the thylakoid membrane. However, how and by which mechanism these phycobiliproteins like phycoerythrin (PE) are sorted is not known.

The transport properties as well as the organelle localization of one such PE protein, PEα, was analyzed in this work. The results show that the PEα subunit is transported into the thylakoid lumen and that the Tat translocase mediates this transport. This analysis, from the evolutionary point of view, strongly suggests that a protein transport pathway corresponding to the Tat pathway of higher plant chloroplasts exists also in cryptophyte plastids and that their targeting information is evolutionary conversed.

(2) Mechanism analysis of the Tat transport process.
Many models have speculated that the Tat translocase is a dynamic and transient translocon as it is formed only in the presence of a Tat transport substrate and the proton gradient across the membrane. To provide experimental evidence for the dynamic properties of the Tat translocon and thus to understand the Tat transport mechanism, a "train-like"protein (16/23-EGFP), in which EGFP (enhanced green fluorescent protein) was attached to the C-terminus of the 16/23 chimeric protein by use of a small peptide linker, has been constructed and analyzed in this work. The results show that the thylakoid transport of this chimeric protein was significantly retarded at indivi-

dual steps giving rise to three transport intermediates. Time course, competition as well as immunoprecipitation experiments were carried out to further characterize these transport intermediates. The results indicate that a single Tat-targeting signal peptide allows the transport of two different mature proteins. Furthermore, the 16/23-EGFP chimera is probably transported in a step-by-step manner. This supports the idea that the Tat translocase could dynamically adapt to different sizes and shapes of the cargo substrates in the course of the transport process.

(3) Analysis of the fate of Tat signal peptides after release by the signal peptidase.

Tat signal peptides play a key role in mediating the Tat transport. After translocation, the signal peptide is cleaved off from the precursor by the signal peptidase. However, what happens to these small signal peptides after signal peptidase cleavage is totally unknown so far.

To analyze the fate of Tat signal peptides, a "tandem-substrate‘" which is composed of two precursors fused in series as well as derivatives thereof have been constructed. The results show that Tat signal peptides are cleaved into subfragments after Tat-transport and processing by signal peptidase. Both events are necessary for the subsequent signal peptide cleavage. Different types of protease inhibitors have been tested for elucidation of the protease involved. It turned out that probably a metalloprotease catalyzes this cleavage. Additionally, the distance between the cleavage site and the C-terminal end of the signal peptide as well as the properties of the signal peptide, like the folding state, have an effect on the cleavage event. These data provide the first analysis of the fate of Tat signal peptides.