Quantitative Analysis of Chloroplast Protein Targeting Pathways

Molecular Cell Biology Seminar

MOAC 1st Year PhD Student Michael Li

EPSRC Life Sciences Programme
Molecular Organisation and Assembly in Cells
The University of Warwick

Spring 2006

Outline of Presentation

Function and Layout of Chloroplasts

Chloroplasts are organelles responsible for photosynthesis. Protein targeting is needed to allow proteins to fulfil their function in the correct compartment.

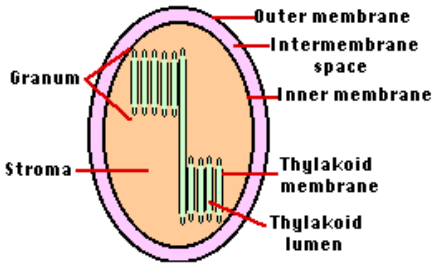


Figure: Schematic diagram of a chloroplast (Copyright ©2006 John W. Kimball)

Function and Layout of Chloroplasts

Chloroplasts are organelles responsible for photosynthesis. Protein targeting is needed to allow proteins to fulfil their function in the correct compartment.

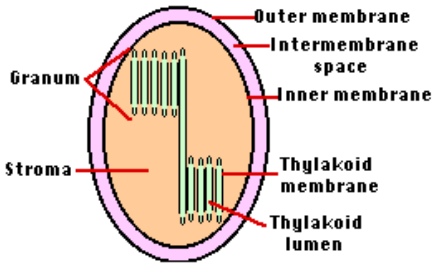


Figure: Schematic diagram of a chloroplast (Copyright ©2006 John W. Kimball)

- Small 70S ribosomes synthesize proteins encoded on small circular DNA within the chloroplasts (in common with prokaryotes)

Function and Layout of Chloroplasts

Chloroplasts are organelles responsible for photosynthesis. Protein targeting is needed to allow proteins to fulfil their function in the correct compartment.

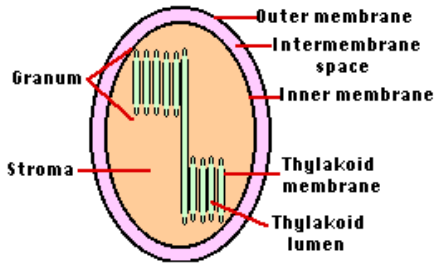


Figure: Schematic diagram of a chloroplast (Copyright ©2006 John W. Kimball)

- Small 70S ribosomes synthesize proteins encoded on small circular DNA within the chloroplasts (in common with prokaryotes)
- Larger 80S cytosolic ribosomes synthesize proteins encoded in the nuclear DNA of the plant cell

Protein Targeting

Some cytosolically-synthesized proteins perform their function in either the chloroplast stroma, or the thylakoid lumen

- An example is the 23 kDa subunit of the oxygen evolving complex

Protein Targeting

Some cytosolically-synthesized proteins perform their function in either the chloroplast stroma, or the thylakoid lumen

- An example is the 23 kDa subunit of the oxygen evolving complex
- A short sequence of amino acids on the N-terminal allows this targeting and sorting to take place

Protein Targeting

Some cytosolically-synthesized proteins perform their function in either the chloroplast stroma, or the thylakoid lumen

- An example is the 23 kDa subunit of the oxygen evolving complex
- A short sequence of amino acids on the N-terminal allows this targeting and sorting to take place
- Nuclear-encoded proteins may have a two-part transit peptide with the first half targeting the stroma, and the second half allowing entry to the thylakoid lumen

DmsA is an example of a protein targeted to the twin-arginine translocation pathway in *E. coli*

DmsA is an example of a protein targeted to the twin-arginine translocation pathway in *E. coli*

- Dimethyl sulphoxide (DMSO) reductase of *E. coli* is involved in anaerobic respiration

DmsA is an example of a protein targeted to the twin-arginine translocation pathway in *E. coli*

- Dimethyl sulphoxide (DMSO) reductase of *E. coli* is involved in anaerobic respiration
- DmsA is a subunit of DMSO reductase and has a protein targeting sequence causing it to be exported to the *E. coli* periplasm; this seqence can also be joined to GFP

DmsA is an example of a protein targeted to the twin-arginine translocation pathway in *E. coli*

- Dimethyl sulphoxide (DMSO) reductase of *E. coli* is involved in anaerobic respiration
- DmsA is a subunit of DMSO reductase and has a protein targeting sequence causing it to be exported to the *E. coli* periplasm; this seqence can also be joined to GFP

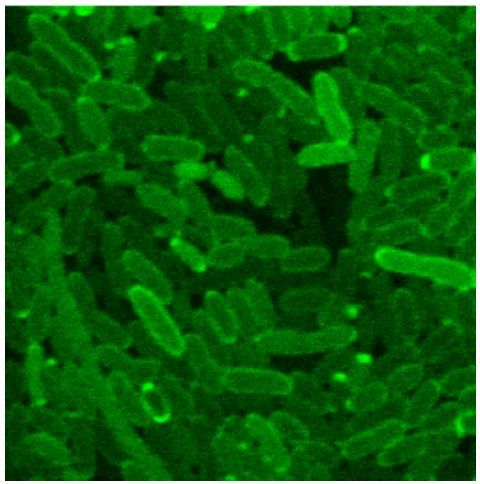


Figure: *E. coli* exporting DmsA-GFP to the periplasm

Multi-disciplinary theme of the research

MOAC, and my study here, is supported by the Engineering and Physical Sciences Research Council[1] and my work is split between Biology and Mathematics

[1] the EPSRC also funds Mathematics

Multi-disciplinary theme of the research

MOAC, and my study here, is supported by the Engineering and Physical Sciences Research Council[1] and my work is split between Biology and Mathematics

- The goal is to bring in expertise from the Engineering and Physical Sciences in a relevant and appropriate way

[1] the EPSRC also funds Mathematics

Multi-disciplinary theme of the research

MOAC, and my study here, is supported by the Engineering and Physical Sciences Research Council[1] and my work is split between Biology and Mathematics

- The goal is to bring in expertise from the Engineering and Physical Sciences in a relevant and appropriate way
- The first step is to use data in a quantitative way (eg estimate how quickly proteins move between compartments)

[1] the EPSRC also funds Mathematics

What will be done

The plan is to use procedures such as silver staining, radiolabelling, and fluorescence confocal imaging in a quantitative way

- Estimations of the amount of protein in cellular compartments at different times will allow comparisons to non-spatial models

What will be done

The plan is to use procedures such as silver staining, radiolabelling, and fluorescence confocal imaging in a quantitative way

- Estimations of the amount of protein in cellular compartments at different times will allow comparisons to non-spatial models
- Fluorescence imaging will allow us to visualize the distribution of components involved in protein targeting, and direct the development of spatial models

Numerical Partial Differential Equations

Partial Differential Equations tell us how a value changes in certain directions, or changes with time, or both.

- We can have values at a certain point and time:

Numerical Partial Differential Equations

Partial Differential Equations tell us how a value changes in certain directions, or changes with time, or both.

- We can have values at a certain point and time:

$$u = u(x, y, z, t)$$

Numerical Partial Differential Equations

Partial Differential Equations tell us how a value changes in certain directions, or changes with time, or both.

- We can have values at a certain point and time:

$$u = u(x, y, z, t)$$

- We can consider the rate of change of the value (as time varies)

Numerical Partial Differential Equations

Partial Differential Equations tell us how a value changes in certain directions, or changes with time, or both.

- We can have values at a certain point and time:

$$u = u(x, y, z, t)$$

- We can consider the rate of change of the value (as time varies)

$$\partial_t u$$

Numerical Partial Differential Equations

Partial Differential Equations tell us how a value changes in certain directions, or changes with time, or both.

- We can have values at a certain point and time:

$$u = u(x, y, z, t)$$

- We can consider the rate of change of the value (as time varies)

$$\partial_t u$$

- We can also consider the rate of change, of the rate of change of the value (as time varies)

Numerical Partial Differential Equations

Partial Differential Equations tell us how a value changes in certain directions, or changes with time, or both.

- We can have values at a certain point and time:

$$u = u(x, y, z, t)$$

- We can consider the rate of change of the value (as time varies)

$$\partial_t u$$

- We can also consider the rate of change, of the rate of change of the value (as time varies)

$$\partial_t^2 u = \partial_t(\partial_t u)$$

Finite Differences and Finite Elements

Approximating by diffusion $\partial_t u = D(\partial_x^2 u + \partial_y^2 u + \partial_z^2 u)$ would be easy except for the complex geometry

Finite Differences and Finite Elements

Approximating by diffusion $\partial_t u = D(\partial_x^2 u + \partial_y^2 u + \partial_z^2 u)$ would be easy except for the complex geometry

- This can be handled in a point-by-point manner with the rates of change replaced by subtractions (finite differences)

Finite Differences and Finite Elements

Approximating by diffusion $\partial_t u = D(\partial_x^2 u + \partial_y^2 u + \partial_z^2 u)$ would be easy except for the complex geometry

- This can be handled in a point-by-point manner with the rates of change replaced by subtractions (finite differences)
- Or we can divide up the domain into elements and proceed in a region-by-region approximation with simple functions to build up the full picture

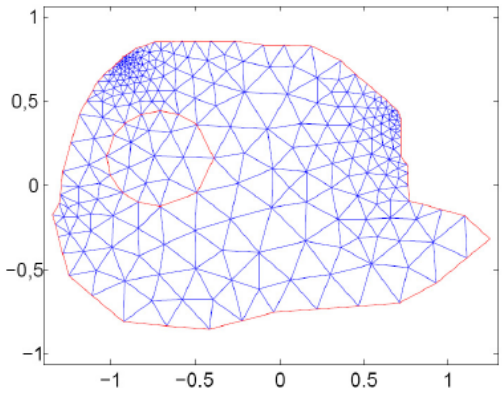


Figure: Finite element mesh (Muller)

How it will be done

The main task is to relate spatial and non-spatial data.

How it will be done

The main task is to relate spatial and non-spatial data.

- This project will aim to reconcile data from different experimental techniques to give a more careful description of chloroplast protein targeting pathways

How it will be done

The main task is to relate spatial and non-spatial data.

- This project will aim to reconcile data from different experimental techniques to give a more careful description of chloroplast protein targeting pathways
- Mathematics and Partial Differential Equations will allowing for a quantitative analysis

Chloroplast Import

- A bipartite presequence will allow targeting of GFP from the medium, to the stroma, and to the thylakoid lumen

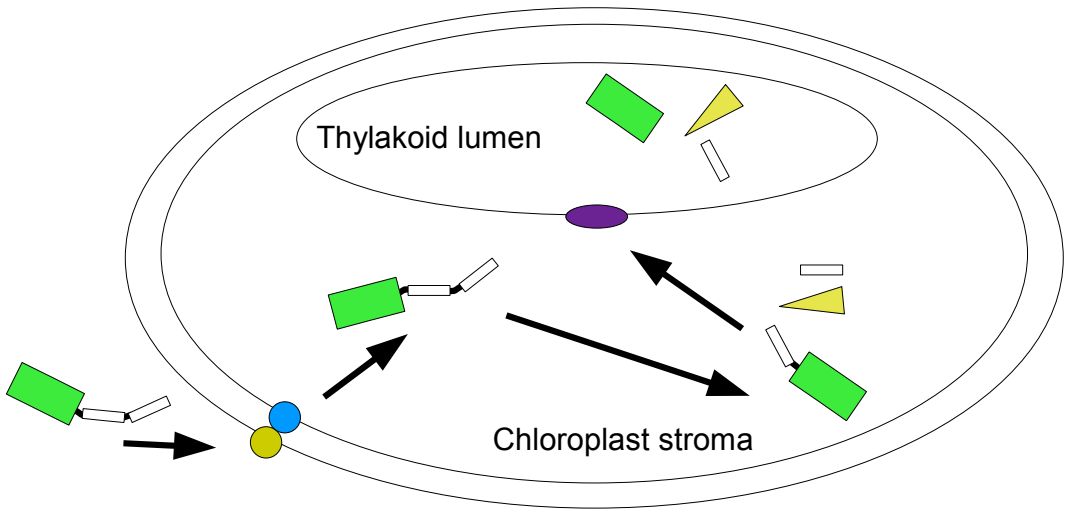
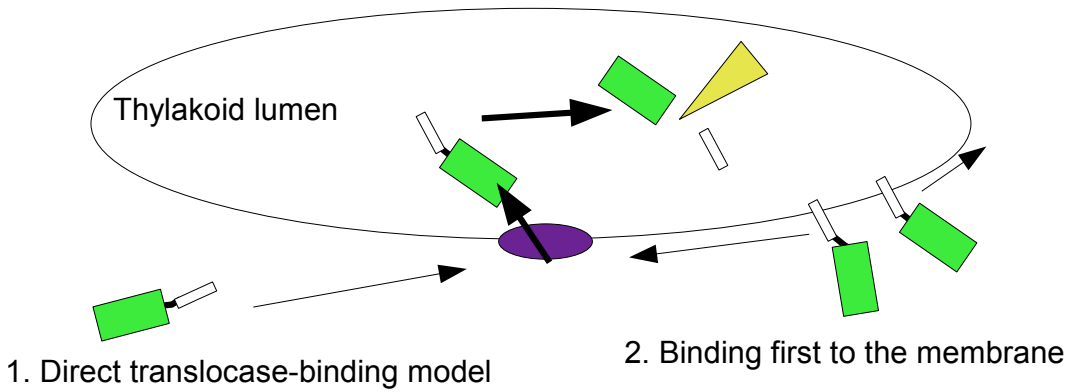


Figure: Entry to the stroma is at the translocons at inner+outer membrane (we do not study directly), and entry to the lumen is at the Tat translocase; various signals are cleaved along the way.

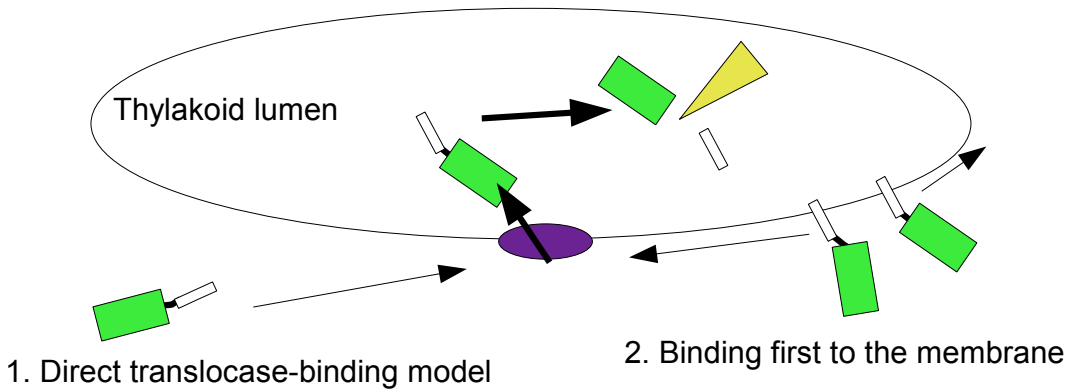
Thylakoid Import

- There are two models for import into the thylakoid



Thylakoid Import

- There are two models for import into the thylakoid



- The idea is to look for accumulation of fluorescence in a quantitative way, and to look for clues about how the targeting process proceeds

Substrate for Thylakoid Import

- Yellow fluorescent protein (YFP) with a DmsA targeting sequence will be purified[2] by affinity chromatography for import into the pea thylakoid lumen

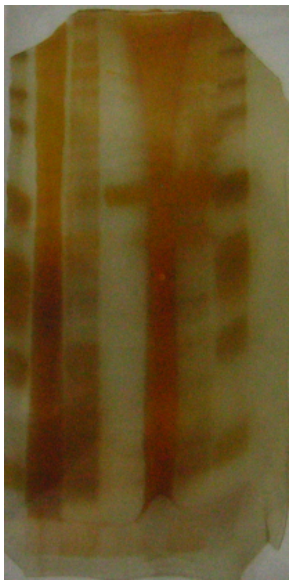


Figure: Silver staining of DmsA-YFP - marker, flow through, wash, elution, top layer of sample, top layer of elution after freezing

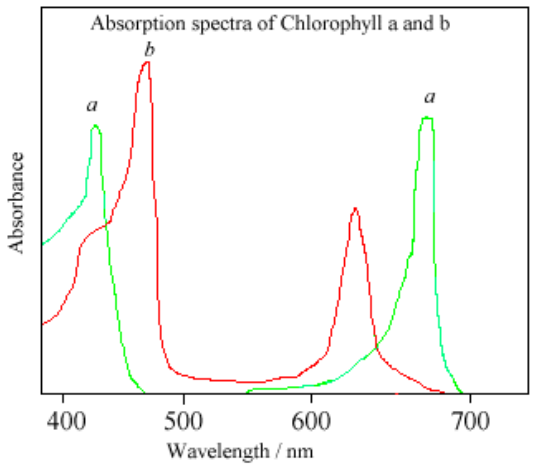
[2] lots of work done on this by James Barnett and others

Purifying and Fixing Thylakoids

- Work is being done to purify thylakoids and to find a way to restrict their movement sufficiently for imaging

Purifying and Fixing Thylakoids

- Work is being done to purify thylakoids and to find a way to restrict their movement sufficiently for imaging
- Chlorophyll in the thylakoid membrane can be visualised by confocal microscopy as it absorbs incident light and fluoresces in the orange-red region



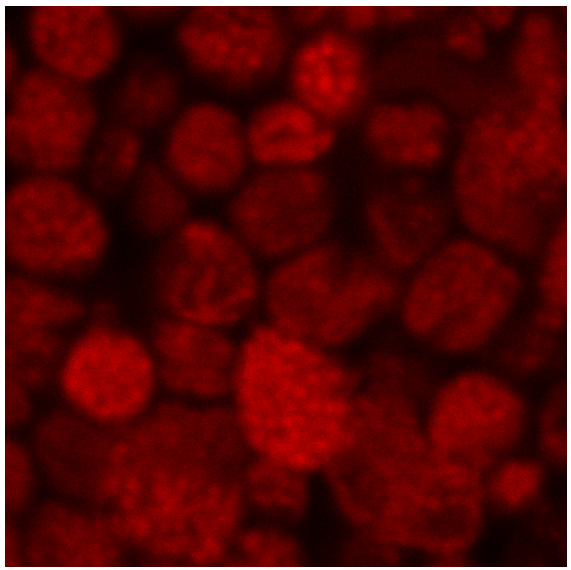


Figure: Absorption spectra of chlorophyll a/b (©Paul May), and autofluorescence of pea thylakoids (line average 8, 63× Oil Immersion Lens, ∼19.8 μm x 19.8 μm)

How it will be done

- Chloroplasts and thylakoids can be isolated by centrifugation for *in vitro* protein imports

How it will be done

- Chloroplasts and thylakoids can be isolated by centrifugation for *in vitro* protein imports
- Cloning in *E. coli* allows the manufacture of fluorescent proteins targeting the thylakoids for fluorescence confocal microscopy of the targeting process

How it will be done

- Chloroplasts and thylakoids can be isolated by centrifugation for *in vitro* protein imports
- Cloning in *E. coli* allows the manufacture of fluorescent proteins targeting the thylakoids for fluorescence confocal microscopy of the targeting process
- Fluorescence imaging only gives an indication of the localisation of the protein, so we check with SDS-PAGE and one of

How it will be done

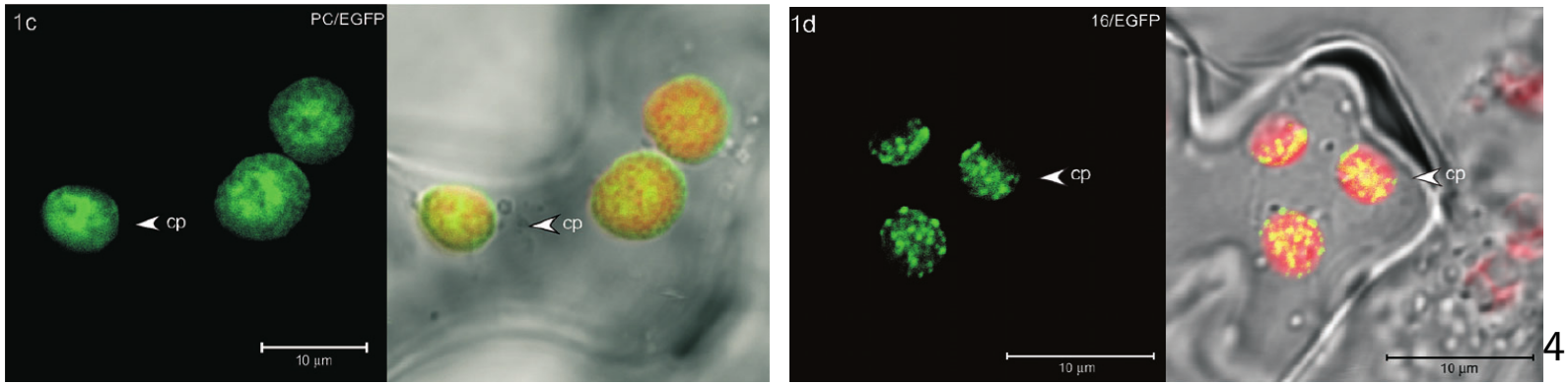
- Chloroplasts and thylakoids can be isolated by centrifugation for *in vitro* protein imports
- Cloning in *E. coli* allows the manufacture of fluorescent proteins targeting the thylakoids for fluorescence confocal microscopy of the targeting process
- Fluorescence imaging only gives an indication of the localisation of the protein, so we check with SDS-PAGE and one of
  - Immuno-blotting with antibodies to GFP

How it will be done

- Chloroplasts and thylakoids can be isolated by centrifugation for *in vitro* protein imports
- Cloning in *E. coli* allows the manufacture of fluorescent proteins targeting the thylakoids for fluorescence confocal microscopy of the targeting process
- Fluorescence imaging only gives an indication of the localisation of the protein, so we check with SDS-PAGE and one of
  - Immuno-blotting with antibodies to GFP
  - Silver staining of proteins in a compartment

Other Labs: Qualitative work on *Arabidopis thaliana*

- Work elsewhere has shown that imaging[3] has a good chance of success



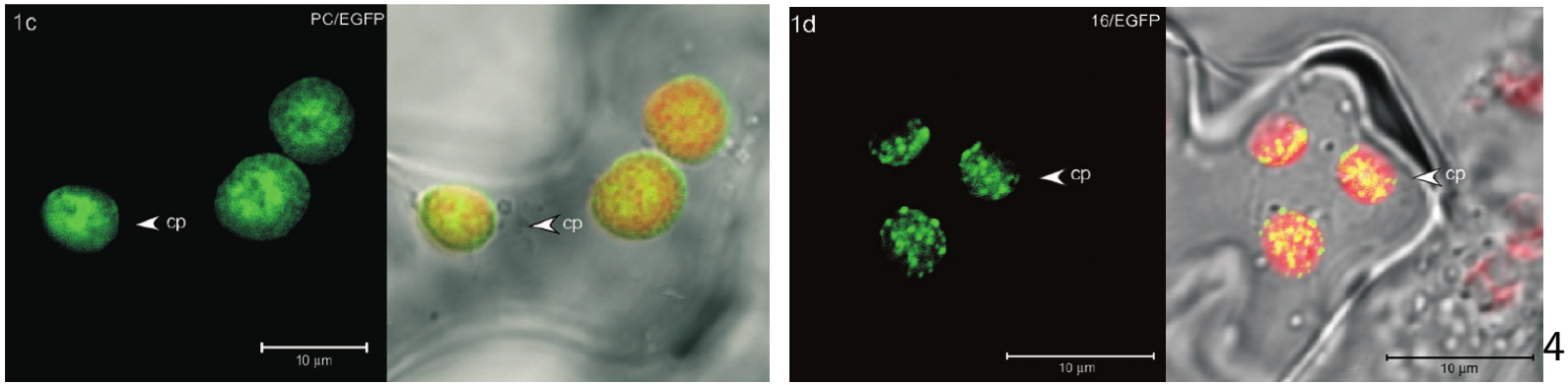
4

[3] In vivo transport of folded EGFP by the ΔpH/TAT-dependent pathway in chloroplasts of Arabidopsis thaliana, Marques et al. 2004

[4] Marques et al. cp marks chloroplasts; PC is plastocyanin transit peptide; 16 is the Tat signal of the 16 kDa subunit of oxygen evolving complex

Other Labs: Qualitative work on *Arabidopis thaliana*

- Work elsewhere has shown that imaging[3] has a good chance of success
- The work is to understand the data better and to check that various ways of processing the data (eg to remove autofluorescence) are valid



[4]

[3] In vivo transport of folded EGFP by the ΔpH/TAT-dependent pathway in chloroplasts of Arabidopsis thaliana, Marques et al. 2004

[4] Marques et al. cp marks chloroplasts; PC is plastocyanin transit peptide; 16 is the Tat signal of the 16 kDa subunit of oxygen evolving complex

Take Home Message

- Chromosomal proteins can be synthesized in the plant cytosol and targeted to different parts of the chloroplasts

[5] for example, the finite element method

Take Home Message

- Chromosomal proteins can be synthesized in the plant cytosol and targeted to different parts of the chloroplasts
- Chloroplast and thylakoid imports will be performed to allow observation of the protein targeting process amd differentiation of schematic models

[5] for example, the finite element method

Take Home Message

- Chromosomal proteins can be synthesized in the plant cytosol and targeted to different parts of the chloroplasts
- Chloroplast and thylakoid imports will be performed to allow observation of the protein targeting process amd differentiation of schematic models
- Computational approaches[5] to partial differential equations can be adapted to describe how the protein targeting process takes place in the complex geometry of the internal membranes

[5] for example, the finite element method

The Presentation Has Ended

Thank you!

Appendices

Total number of frames: 19