Molecular Mechanisms of Membrane Traffic

Edited by

D. James Morré

Department of Medicinal Chemistry
HANS Life Science Research Building
Purdue University
West Lafayette, IN, USA

Kathryn E. Howell

C & S Biology
University of Colorado
School of Medicine
Denver, CO, USA

John J. M. Bergeron

Department of Anatomy and Cell Biology
McGill University
Montreal PQ H3A 2B2, Canada

Springer-Verlag
Berlin Heidelberg New York London Paris Tokyo
Hong Kong Barcelona Budapest
Published in cooperation with NATO Scientific Affairs Division

CONTENTS

IMPORT INTO MITOCHONDRIA, PEROXISOMES, CHLOROPLASTS AND VACUOLES

TRAFFIC WITHIN AND FROM THE GOLGI COMPLEX

THE ENDOCYTIC PATHWAY AND SIGNALS

LYSOSOMES AND DEGRADATION

MOLECULES IDENTIFIED IMPORTANT IN MEMBRANE TRAFFIC

Isolation And Characterization Of A Functionally Active Protein Translocation Apparatus From Chloroplast Envelopes.

K. Waegemann, J. Soll
Botanisches Institut, Universität Kiel, Olshausenstraße 40,
D-2300 Kiel, Germany

Chloroplast structure and function depends vitally on the import of nuclear coded and cytosolically synthesized polypeptide constituents (de Boer and Weisbeek 1991). Proteins of the outer and inner envelope from chloroplasts collaborate to form an import machinery which is responsible for the specific recognition of chloroplast destined precursor proteins and their translocation through the two membrane barrier. Outer envelope membrane vesicles are purified from pea chloroplasts in a right side-out orientation, i.e. like in the intact organelle (Waegemann et al. 1992). Precursor proteins are bound to the membrane vesicles in an ATP, receptor and transitpeptide dependent manner (Waegemann and Soll 1991, Soll and Waegemann 1992). The translocation process of a precursor proceeds via distinct steps which can be detected in vitro as translocation intermediates, named deg 1-4 in chloroplasts (Fig 1). The outer envelope localized import apparatus yields deg 1 and 2 while deg 3 and 4 are translocation intermediates which occur in connection with the inner envelope import machinery (Fig 1).

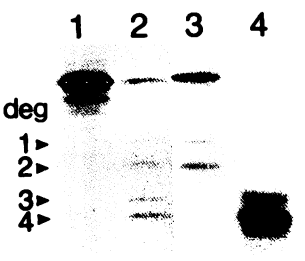

Fig 1) Localisation of pSSu translocation intermediates. pSSu binds to intact chloroplasts in the presence of 100 μM ATP (lane 1). Protease treatment results in the occurence of deg 1-4 (lane 2). Envelope membranes isolated from these plastids show that deg 3 and 4 are localized in the inner envelope (lane 4). Outer envelopes incubated with pSSu as above yield deg 1 and 2 after protease treatment (lane 3).

NATO ASI Series, Vol. H 74
Molecular Mechanisms of Membrane Traffic
Edited by D. J. Morré, K. E. Howell, and J. J. M. Bergeron

Using a solubilisation protocol of outer envelope membranes as outlined in Fig 2A (Kiebler et al. 1990) a protein complex could be enriched by sucrose density centrifugation which contained bound precursor protein (import complex I) (Fig 2B). Detection of this import complex I depended on the presence of ATP, a transit sequence and protease sensitive components, e.g. receptor molecules during the incubation of the membrane vesicle with the precursor (Waegemann and Soll 1991). The protein composition of import complex I is distinctively different from total outer envelope membrane proteins and shows an enrichment of a number of proteins as judged by gel electrophoresis followed by silver staining (Fig 2C). Using immunological technics we have identified four outer envelope proteins (OEP) of 86, 70, 75, and 34 kDa (Fig 2D). The 70 kDa polypeptide crossreacts with an antiserum against heatshock protein 70 (hsp 70). Furthermore it is possible to immunoprecipitate the precursor protein by antibodies against hsp 70 (Waegemann and Soll 1991) indicating a close interaction between these two components. Envelope localized hsc 70 might thus act as a chaperone to facilitate the translocation of a precursor protein by interacting with the transport competent, i.e. partially folded, conformation of the polypeptide chain (Marshall et al. 1990, von Heijne and Nishikawa 1991, Waegemann and Soll 1991). The role and function of other import complex I constituents remains to be established.

After solubilisation of outer envelope membranes and centrifugation through sucrose-gradients the fractions which contained import complex I were pooled and subsequently incubated with precursor protein (see Fig. 2A, right panel). This isolated membrane complex recognized specifically the precursor polypeptide in relation to the mature form and interacted with it in an ATP dependent manner. Following protease treatment translocation intermediates deg 1 and 2, identical to those described above (Fig 1) for the organellar system were detected. Transit peptide dependent interaction of the isolated import complex with the precursor points to the

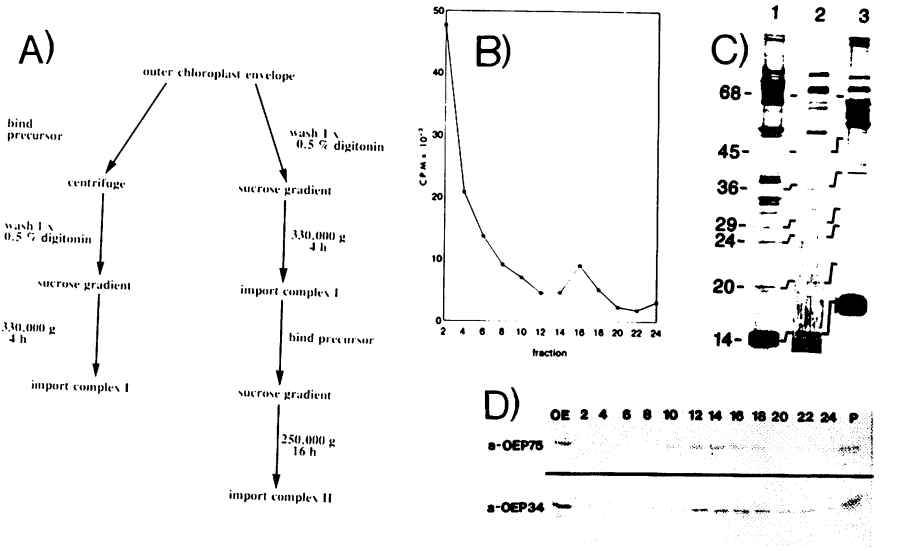

Fig 2) Characterization of the protein import complex from chloroplast outer envelopes. A) Solubilisation and isolation protocol of import complex I and II. B) Distribution of radioactivity in the sucrose density gradient as determined by liquid scintillation counting (line drawing). C) Polypeptide composition of outer chloroplast envelope (lane 1), complex I (lane 2) and complex II (lane 3). D) Immunoblot analysis of fractions obtained from a sucrose density gradient to enrich complex I using antisera against OEP 75 and OEP 34. Numbers on top indicate fraction numbers.

presence of a receptor in this complex. Protease sensitive components in import complex II are OEP 86 (Fig 3) and OEP 34 (see Fig 2C, D), however, direct evidence for their function as receptor is still missing. We were unable to detect a putative receptor of 30 kDa molecular (Pain et al. 1988, Schnell et al. 1990) in import complex II and conclude from this and from other results that the receptor for chloroplastic protein import is not yet conclusively identified (Flügge et al. 1991). The presence of hsc 70 in import complex II indicates again the involvement of this protein in the translocation mechanism (Fig 3). The protein composition of import complex I and II is similar (Fig 2C). To

our knowledge the results described above demonstrate for the first time that it is possible to isolate a protein translocation apparatus as a functional active unit from chloroplasts. This will enable us to study the mechanism of protein translocation in greater detail.

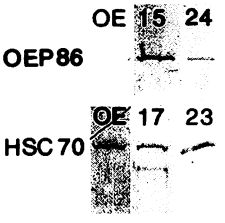

Fig 3) OEP 86 and a hsc 70 homologue are present in import complex II. Outer envelope membranes (OE) and samples of the sucrose density gradient (figures indicate fraction numbers) were tested using the respective antiserum.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft.

References

de Boer, A D & Weisbeek, P J (1992) Biochim Biophys Acta, in press.

Flügge, U -I , Weber, A , Fischer, K , Lottspeich, F , Eckerskorn, Ch , Waegemann, K & Soll, J (1991) Nature 353, 364-367.

Kiebler, M , Pfaller, R , Söllner, T , Griffiths, G , Hartmann, H , Pfanner, N & Neupert, W (1990) Nature 348, 610-616.

Marshall, J S , DeRocher, A E , Keegstra, K & Vierling, E (1990) Proc Natl Acad Sci USA 87, 374-378.

Pain, D , Kanwar, Y S & Blobel, G (1988) Nature 331, 232-237.

Schnell, D J , Blobel, G & Pain, D (1990) J Cell Biol 111, 1825-1838.

Soll, J & Waegemann, K (1992) Plant J. 2, 253-256.

v.Heijne, G & Nishikawa, K (1991) FEBS Lett 278, 1-3.

Waegemann, K & Soll, J (1991) Plant J 1, 149-158.

Waegemann, K , Eichacker, S & Soll, J (1992) Planta 187, 89-94.