

Minireview

Protein translocation across the thylakoid membrane – a tale of two mechanisms

Colin Robinson^a, Ralf Bernd Klösgen^b, Reinhold G. Herrmann^b and Jamie B. Shackleton^a

^a*Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK* and ^b*Botanisches Institut der Ludwig-Maximilians-Universität, Menzinger Strasse 67, 8000 München 19, Germany*

Received 7 April 1993

In vitro reconstitution assays have been used in recent years to probe the mechanisms by which a variety of cytosolically synthesised proteins are transported across the thylakoid membrane within higher plant chloroplasts. The emerging data suggest that two distinct mechanisms operate. Translocation of a subset of luminal proteins, namely the 23 kDa and 16 kDa proteins of the oxygen-evolving complex, and of the CFo2 protein (an integral membrane protein), requires only the presence of the thylakoidal ΔpH . In contrast, two other luminal proteins, the 33 kDa oxygen-evolving complex protein and plastocyanin, require also the presence of ATP and at least one stromal factor for efficient transport into isolated thylakoids to take place.

Chloroplast; Energetics; Protein transport; Thylakoid protein

1. INTRODUCTION

It has been clear for a long time that chloroplast biogenesis requires a considerable amount of protein trafficking, and extensive efforts have been made to understand the underlying translocation mechanisms. Such studies have relied heavily on *in vitro* transport assays, originally developed in 1978 [1,2] in which precursors of chloroplast proteins are synthesised in a cell-free translation system and incubated with isolated, intact chloroplasts. Using this type of assay, significant progress has been made in attempts to understand how proteins are transported into higher plant chloroplasts (reviewed in [3]). However, a number of photosynthetic proteins are transported across both the envelope and thylakoid membranes, and the intact-chloroplast import assay is of limited use for analysing the later stages of the biogenesis of these luminal proteins. More recently, an *in vitro* assay has been developed in which isolated thylakoids efficiently import a variety of proteins, and the purpose of this article is to describe how this assay has been used to investigate the mechanisms by which these proteins are translocated across the thylakoid membrane.

2. BIOGENESIS OF THYLAKOID LUMEN PROTEINS

The thylakoid lumen contains relatively few well-

studied proteins, but the biogenesis of these proteins has attracted considerable interest in view of the complex import pathway involved. The best-studied luminal proteins are plastocyanin, a small, soluble electron carrier, and the 33, 23 and 16 kDa proteins (33K, 23K and 16K) of the photosynthetic oxygen-evolving complex. All of these proteins are synthesised in the cytosol as larger precursors and transported to the thylakoid lumen by a two-step mechanism. The pre-proteins are initially imported into the stroma and processed to intermediate forms by a stromal processing peptidase, after which the intermediates are transported across the thylakoid membrane and processed to the mature size by a second, thylakoidal processing peptidase [4–6]. The transit peptides of these proteins contain two signals in tandem. The first ‘envelope transit’ signal directs transport into the stroma, and is structurally and functionally equivalent to the transit peptides of imported stromal proteins; the second ‘thylakoid transfer’ signal directs translocation across the thylakoid membrane. The structures of the latter signals is of particular interest because they are strikingly similar in several key respects to ‘signal’ peptides which mediate protein export in bacteria. Indeed, expression of luminal protein precursors in *E. coli* can lead to efficient export into the periplasmic space [7,8]. Furthermore, the processing peptidases which remove the two types of peptide display remarkably similar reaction specificities [9]. These findings suggest that the thylakoidal protein import system may be derived from an ancestral translocation system in the cyanobacterial progenitors of higher plant chloroplasts.

Correspondence address: C. Robinson, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK.

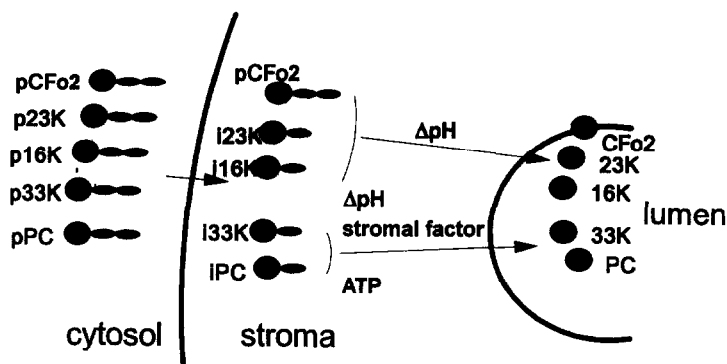


Fig. 1. Import pathways for cytosolically-synthesised thylakoid proteins. Precursors (p) of 33K, 23K, 16K, CFo2 and plastocyanin (PC) are synthesised in the cytosol and transported into the stroma, where each precursor, with the probable exception of CFo2, is processed to an intermediate form (denoted i). Further translocation of i23K and i16K across the thylakoid membrane, and of pCFo2 into the membrane, is accomplished by a mechanism which appears only to require the thylakoidal ΔpH . In contrast, transport of i33K and iPC into the lumen requires the activity of at least one stromal factor and the presence of ATP. Complete maturation of the proteins is carried out by a thylakoidal processing peptidase.

3. DEVELOPMENT OF AN ASSAY FOR THE IMPORT OF PROTEINS BY ISOLATED THYLAKOIDS

Although precursors of thylakoid lumen proteins are efficiently imported by isolated intact chloroplasts, it is impossible to analyse in detail the later stages of the import pathway (especially translocation across the thylakoid membrane) using this assay system. In many cases, the stromal intermediates can hardly be detected because import into the chloroplast is followed rapidly by transport across the thylakoid membrane. In view of this problem, attempts have been made to persuade isolated thylakoids to import lumenal proteins. Mould et al. [10] showed that isolated pea thylakoids are capable of efficiently importing 23K and 33K in a light-driven import assay; further analyses [11] showed that the effect of light is to generate a ΔpH which is essential for efficient translocation across the thylakoid membrane. Using the same import assay, efficient, ΔpH -driven import of 16K was also demonstrated [12]. Cline et al. [13] used similar assay conditions to demonstrate import of 23K and 16K by pea thylakoids, and in addition showed that import of these proteins into thylakoids does not require ATP.

4. TWO TYPES OF TRANSLOCATION MECHANISM

Previous work [10–13] has shown that 23K and 16K have simple requirements for transport across the thylakoid membrane: translocation is driven solely by the thylakoidal ΔpH , and neither stromal factors nor ATP are required. Interestingly, cleavage to the intermediate form by the stromal peptidase is also unnecessary; both proteins can be efficiently imported by thylakoids as the full precursor form. In contrast, import of 33K into thylakoids was found to require the presence of stromal

extract in addition to a ΔpH [10]. At first, it was thought that this may reflect a requirement for stromal processing activity, in order to generate the intermediate form which was presumed to be the natural substrate for the thylakoidal protein transport machinery. However, we have since generated an artificial stromal intermediate which is no longer recognised by the stromal peptidase, and import of this intermediate by thylakoids still requires the presence of stromal extract.

The active factor is a heat-sensitive macromolecule, presumably a protein, and such a factor has also been found to be required for the import of spinach pre-plastocyanin by thylakoids (Hazell, Cai, Herrmann, Klosgen and Robinson, manuscript submitted). In addition, import of 33K and plastocyanin also requires the presence of NTPs, since apyrase treatment of the wheat germ translation mixture and stromal extract to remove ATP leads to a complete inhibition of import.

Additional work has centred on the biogenesis of another thylakoid protein, the CFo2 component of the ATP synthase. Unlike the proteins described above, this protein is an integral thylakoid membrane protein, but it is nevertheless synthesised in the cytosol with a bipartite transit signal similar to those of plastocyanin and the oxygen-evolving complex proteins. This protein is imported into isolated thylakoids by a mechanism similar to those utilised by 23K and 16K; import requires a ΔpH but not ATP or stromal extract (Michl, Hulford, Robinson, Herrmann and Klosgen, manuscript in preparation). Interestingly, there is some evidence that, in this case, the transit peptide does not contain an intermediate processing site, suggesting that the cleavage site was either never present, or was lost during the course of evolution.

5. CONCLUSIONS

On the basis of the studies described above, it appears

that proteins are translocated across the thylakoid membrane by two distinct mechanisms. A subset of proteins, including 23K, 16K and CFo2, are transported by a mechanism which, in comparison with other protein translocation systems, is extremely simple: translocation is driven solely by the thylakoidal ΔpH , and there is no necessity for stromal factors or ATP hydrolysis. Other proteins, including 33K and plastocyanin, have more complex transport requirements which include the presence of a stromal factor(s) and ATP. These findings, which are summarised in Fig. 1, could be explained by either of two scenarios. One possibility is that there are (at least) two distinct translocation systems in the thylakoid membrane, each responsible for the transport of a discrete set of proteins. If this is the case, it is difficult to predict how the various luminal proteins are distinguished, because there are no clear differences in the thylakoid transfer signals of the proteins described above. However, since only a few luminal proteins have been studied in detail, it is nevertheless possible that the transfer signals of 23K, 16K and CFo2 have specific features in common which are recognised by one translocation system, and that the transfer signals of 33K and plastocyanin contain targeting signals which are recognised by a different system. An alternative possibility is that these proteins are transported by a single translocator in the thylakoid membrane, and that the differing requirements for stromal factors and ATP reflect differing characteristics of the mature proteins. For example, one possibility is that the stromal factor plays an unfolding or antifolding role, and is

required to prevent 33K and plastocyanin from folding into import-incompetent forms. In contrast 23K, 16K and CFo2 might be naturally less tightly folded, and thereby able to bypass any such requirement. The role of ATP in the transport of 33K and plastocyanin is currently unclear, but one obvious possibility under consideration is that it is required for the activity of the stromal factor.

REFERENCES

- 1 Highfield, P.E. and Ellis, R.J. (1978) *Nature* 271, 420–424
- 2 Chua, N.-H. and Schmidt, G.W. (1978) *Proc. Natl. Acad. Sci. USA* 75, 6110–6114
- 3 Smeekens, S., Weisbeek, P. and Robinson, C. (1990) *Trends Biochem. Sci.* 15, 73–76
- 4 Hageman, J., Robinson, C., Smeekens, S. and Weisbeek, P. (1986) *Nature* 324, 567–569
- 5 James, H.E., Bartling, D., Musgrove, J.E., Kirwin, P.M., Herrmann, R.G. and Robinson, C. (1989) *J. Biol. Chem.* 264, 19573–19576
- 6 Ko, K. and Cashmore, A.R. (1989) *EMBO J.* 8, 3187–3194
- 7 Seidler, A. and Michel, H. (1990) *EMBO J.* 9, 1743–1748
- 8 Meadows, J.W. and Robinson, C. (1991) *Plant Mol. Biol.* 17, 1241–1243
- 9 Halpin, C., Elderfield, P.D., James, H.E., Zimmermann, R., Dunbar, B. and Robinson, C. (1989) *EMBO J.* 8, 3917–3921
- 10 Mould, R.M., Shackleton, J.B. and Robinson, C. (1991) *J. Biol. Chem.* 266, 17286–17289
- 11 Mould, R.M. and Robinson, C. (1991) *J. Biol. Chem.* 266, 12189–12193
- 12 Klosgen, R.B., Brock, I.W., Herrmann, R.G. and Robinson, C. (1992) *Plant Mol. Biol.* 18, 1031–1034
- 13 Cline, K., Ettinger, W.F. and Theg, S.M. (1992) *J. Biol. Chem.* 267, 2688–2696