Mini Review

Proteins Heading for the Chloroplast

Hong, Choo Bong

(Genetic Engineering Center, K.I.S.T.)

엽록체로 향하는 단백질

洪 周 奉

(韓國科學技術研究院 遺傳工學센터)

ABSTRACT

The chloroplast has been the prime light-energy harvesting organelle on earth. It also carries out several key metabolic processes, such as lipid synthesis and nitrogen metabolism. Even though the chloroplast has its own genome, its coding capacity can afford only dozens of proteins, and most of the proteins functioning in the chloroplast are imported from the cytosol where nuclear encoded chlorplast genes are synthesized on free cytosokic ribosomes. Precursor proteins synthesized on cytosolic ribosomes have transit peptides at the amino termini of the proteins, and the transit peptide is sufficient to transfer chloroplast proteins from the cytosol into the chloroplast. When comparing amino acid sequences duduced from the nucleotide sequences of the clones of the chloroplast proteins, high homologies can be found among the transit peptides of proteins with the same function. Overall amino acid compositions of the transit peptides show amphiphilic characters of the transit peptides, and the amphiphilicity indicates that three dimensional structure of the transit peptide is responsible for the translocation of the chloroplast proteins.

I. Chloroplast transit peptides

Chloroplast, the small organelle whose size is about 5 μm in length, has been attracting a lot of attentions due to its function in photosynthesis. Without chloroplasts that immense light energy coming from the sun can not be utilized as the major energy to keep the life organism on earth going. In addition to assimilating carbohydrates, chloroplast is the location where nitrogen metabolism and lipid biosynthesis occur.

There have been evidences indicating that chloroplast was originated from cyanobacteria. Antibiotic specificities and structural characters of chloroplast are bacteria's rather than eukaryotes'. In this line of thought, cyanobacteria somehow became a part of eukaryotic cell, and a sort of symbiotic relationship between the cell and the cyanobacteria had been maintained which resulted in the photosynthetic eukaryotes.

Even though many features of the individually living cyanobacteria have been lost during the evolution of higher plants, chloroplast still has its own genome, presides several key metabolic processes of plant cells and divides to increase its number which occurs quite independtly from cell division. The size of average bacterial chromosome is about 2×10^{-9} D, and the size of chloroplast genome is about 9×10^{-8} D. Considering all the processes occuring in the chloroplast and chloroplast genome's capacity to code for proteins, it is obvious that genes for the many proteins functioning in

the chloroplast are imported.

Many proteins in the chloroplast are coded on the nuclear chromosome and synthesized on cytosolic ribosomes as large precursors. The synthesized large precursors are translocated across the chloroplast envelope and in some cases across the thylakoid membrane. During this process transit peptides do the functions of translocation and are cleaved off enzymatically resulting in active proteins at the proper location inside the chloroplast. Assays using the transformation systems of plants and *in vitro* analysis confirmed that transit peptides are sufficient to transport chloroplast proteins across the chloroplast envelope into chloroplast and sometimes into the lumenal space of the thylakoids. There have been several excellent reviews regarding this subject (Keegstra and Olsen, 1989; Keegstra, 1989; Dean *et al.*, 1989).

2. General features of transit peptides

Even though there are many chloroplast proteins whose amino acid sequences are analyzed, it is only the *Chlamydomonas reinhardtii* ribulose bisphosphate carboxylase/oxygenase small subunbit (rbcS) whose transit peptide amino acids are actually sequenced (Schmidt *et al.*, 1979). All other amino acid sequences of the transit peptides are from nucleic acid sequencing of the cloned genes coded on the chromosome. Thus most of the available sequences are those of the abundant proteins whose genes are relatively easy to clone.

Transit peptides of the chloroplast stromal proteins. Most intensively studies chloroplast stromal protein is rbsS protein whose genes are coded on the nuclear chromosome as multiple gene families (Dean *et al.*, 1989). Comparisons of the transit peptide amino acid sequences of rbsS among different species revealed striking similarties except a region with large variabilities among plants. This region locates at the end of the transit peptide far from the main body of the rbcS (up to −57 amino acids from the amino terminus of the matured rbcS) and has a length of about twenty amino acids. Usually in monocots this region, is largely deleted (Xie and Wu, 1988; Lebrun *et al.*, 1987).

Other transit peptides for the stromal proteins whose gene clones have been sequenced are; acetolactate synthase (Mazur *et al.*, 1987), acyl carrier proteins (Ross *et al.*, 1987; Safford *et al., 1988). EPSP synthase* (Gasser *et al.*, 1988; Klee *et al.*, 1987), ferredoxin (Smeekens *et al.*, 1985; Dobers *et al.*, 1987), glutamine synthetase (Lightfoot *et al.*, 1988; Tingey *et al.*, 1988), glyceraldehyde-3P-dehydrogenase (Quigley *et al.*, 1988; Shih *et al.*, 1986), heat shock protein (Vierling *et al.*, 1988), nitrate reductase (Back *et al.*, 1988), hosphoribulokinase (Roelser and Ogren, 1988), pyruvate Pi dikinase (Matsuoka *et al.*, 1988), rubisco activase (Werneke *et al.*, 1988), and UDPGlc:starch glucosyl transferase (Klosgen *et al.*, 1986; Rohde *et al.*, 1988).

Sizes of these transit peptides vary from 32 amino acids for nitrate reductase to 76 amino acids for EPSP synthase, and there are no apparent aminoacid sequence homologies among transit peptides of the stromal proteins with different functions.

Transit peptides of thylakoid membrane proteins. Transit peptides of chlorophyll a/b binding proteins (CAB) have been most extensively studied. Depends on the sites of CAB functioning, CAB can be classified into two groups; PSI-type prpteins and PSII-type proteins. Aminoacid sequence comparisons of these transit peptides reveal two groups in each type of protein. Transit peptides in each group share amino acid sequence homolgies, but transit peptides of different groups show clear discrepancies, but transit peptides of different groups show clear discrepancies. Lengths of the transit peptides are usually about 40 amino acids long (Cashmore, 1984; Castresana *et al.*, 1987; Dunsmuir, 1985; Hoffman *et al.*, 1987; Karlin-Neumann, 1985; Kohorn *et al.*, 1986; Lamppa *et al.*, 1985; Leutwiler *et al.*, 1987; Matsuoka *et al.*, 1987; Pichersky *et al.*, 1985; Pichersky *et al.*, 1987; Pichersky *et al.*, 1988; Smeekens *et al.*, 1986; Stayton *et al.*, 1987; Sullivan *et al.*, 1989; Timko *et al.*, 1985).

CF1-delta subunit (Hermans *et al.*, 1988), ferredoxin/NADP reudctase (Jansen *et al.*, 1988; Newman and *Gray, 1988) PS I-subunit II (Hoffman et al., 1988;* Lagoutte, 1988), PS II-10kD protein (Eckes *et al.*, 1986; Lautner *et al.*, 1988) and Rieske FeS (Steppuhn *et al.*, 1987) are the proteins whose genes have been cloned and transit peptide amino acid sequences are characterized. Highly conserved aminoacid sequences can be found from the transit peptides of the proteins with the same function as the case of the stromal proteins.

Transit peptides of thylakoid lumen proteins. To locate in the thylakoid lumen of chloroplast, precursor proteins must cross two membranes; one is chloroplast envelope and the other is thylakoid membrane. Thylakoidal lumen proteins so far characterized have

longer transit peptides with two composite peptides. Transit peptide far from the main body of the protein functions when the protein crosses the chloroplast envelope and has the overall features of stroma and thylakoid membrane precursor proteins. Transit peptide close to the main body of the protein is very hydrophobic and similar to the bacterial signal peptide (Weisbeek *et al.*, 1987). As the cases for stromal and thylakoidal membrane proteins, amino acid sequence homologies can be found from the transit peptides of the proteins with the same function.

Proteins which are part of the oxygen evolving complex (Jansen *et al.*, 1987; Mayfield *et al.*, 1987; Tyagi *et al.*, 1987) and plastocyanin (Rother *et al.*, 1986; Smeekens *et al.*, 1985; Vorst *et al.*, 1988) have been characterized for their transit peptides.

What is the function of transit peptides in translocating chloroplast proteins? Although it is possible that specific receptor proteins reside in the chloroplast membrane and work in translocating chloroplast proteins across the membrane, there is no supporting evidence for the receptor mediated translocation of the chloroplast proteins at this point.

General features of the transit peptides except the second domain of the transit peptide of lumenal proteins are rich in the hydroxylated amino acids, serine and threonine, and small hydrophobic amino acids, alanine and valine. This pattern of amino acid distribution supports the model that essential feature of the transit peptide is its amphiphilicity. Amphiphilic transit peptides could interact directly with the lipid bilayer of the chloroplast envelope and cause alterations of the membrane. This could be the basic mechanism to transport the proteins across the chloroplast envelope (Keegstra, 1989).

3. Finishing remarks

All the chloroplast proteins synthesized on the cytosolic ribosomes so far characterized have trnasit peptides, and there seems to be no exceptions that will be found. Size of the chloroplast genome is much smaller compare to the bacterial chromosome, even though it has many features of independently living cyanobacteria. Thus chloroplast should import a full spectrum of proteins to support its function in photosynthesis, nitrogen metabolism, lipid biosynthesis, transporting in and out various compounds (including proteases necessary to process precursor proteins to matured forms of proteins) and self-replication. Unveiling transport machinery of the chloroplast will give answers to many questions: How chloroplast can be build and maintained? How chloroplast was located in plant cell? One of the important transport machineries seems to be tightly related to the transit peptides coming from the informations residing in the nucleus.

REFERENCES

Back, E., W. Burkhart, M. Moyer, L. Privallen and Rothstein. 1988. *Mol. Gen. Genet.* **212:** 20-26.

Cashmore, A.R. 1984. *Proc. Natl. Acad. Sci.* USA. **81:** 2960-2964.

Castresana, C., R. Staneloni, V.S. Malik and A.R. Cashmore. 1987. *Plant Mol. Biol.* **10:** 117-126.

Dean, C., P. van den Elzen, S. Tamaki, M. Black and P. Dunsmuir. 1987. *Mol. Gen. Genet.* **206:** 465-474.

Dean, C., E. Pichersky and P. Dunsmuir. 1989. *Ann. Rev. Plant Physiol. & Mol. Biol.* **40:** 415-439.

Dobres, M.S., R.C. Elliott, J.C. Watson and W.F. Thompson. 1987. *Plant Mol. Biol.* **81:** 53-59.

Dunsmuir, P. 1985. *Nucl. Acids Res.* **13:** 2503-2518.

Eckes, P., S. Rosahl, J. Schell, L. Willmitzer. 1986. *Mol. Gen. Genet.* **205:** 14-22.

Gasser, C.S., S.A. Winter, C.M. Hironaka and D.M. Shah. 1988. *J. Biol. Chem.* **263:** 4280-4289.

Hoffman, N.E., E. Pichersky, V.S. Malik, C. Castresana and K. Ko. 1987. *Proc. Natl. Acad. Sci.* USA **84:** 8844-8848.

Hoffman, N.E., E. Pichersky, V.S. Malik, K. Ko and A.R. Cashmore. 1988. *Plant Mol. Biol.* **10:** 435-445.

Jansen, T., C. Rother, J. Steppuhn, H. Reinke and K. Bayreuther. 1987. *FEBS Lett.* **216:** 234-240.

Karlin-Neumann, G.A. and E.M. Tobin. 1986. *EMBO J.* **51:** 9-13.

Keegstra, K. 1989. *Cell* **56:** 247-253.

Keegstra, K., L. Olsen and S. Theg. 1989. *Ann. Rev. Plant Physiol. & Mol. Biol.*. **40:** 276-501.

Klee, H.J., Y.M. Muskopf and C.S. Gasser. 1987. *Mol. Gen. Genet.* **210:** 437-442.

Klosgen, R.B., A. Gierl, Z. Schwarz-Sommer and H. Saedler. 1986. *Mol. Gen. Genet.* **203:** 237-244.

Kohorn, B.D., E. Harel, P.R. Chitnis, J.P. Thornber and E.M. Tobin. 1986. *J. Cell Biol.* **102:** 972-981.

Lagouttle, B. 1988. *FEBS Lett.* **232:** 275-280.

Lamppa, G.K., G. Morelli and N.H. Chua. 1985. *Mol. Cell. Biol.* **5:** 1370-1378.

Lautner, A., R. Klein, U. Ljungberg, H. Reilander and D. Bartling. 1988. *J. Biol. Chem.* **263:** 10077-10081.

Lebrun, M., G. Waksman and G. Freyssinet. 1986. *Nucl. Acids Res.*, **15:** 4360.

Leutwiler, L.S., E.M. Meyerowitz and E.M. Tobin. 1986. *Nucl. Acids Res.* **10:** 4051-4064.

Lightfoot, D.A., N.K. Green and J.V. Cullimore. 1988. *Plant Mol. Biol.,* **11:** 191-202.

Matsuoka, M., Y. Kano-Murakami and N. Yamamoto. 1987. *Nucl. Acids Res.* **15:** 6302.

Matsuoka, M., Y. Ozeki, N. Yamamoto, H. Hirano, Y. Kano-Murakami and Y. Tanaka. 1988. *J. Biol. Chem.,* **263:** 11080-11083.

Mayfield, S.P., M. Rahire, G. Frank, H. Zuber and J.D. Rochaix. 1987. *Proc. Natl. Acad. Sci.* USA **84:** 749-753.

Mazur, B.J., C.F. Chui and J.K. Smith. 1987. *Plant Physiol.* **85:** 1110-1117.

Newman, B.J. and J.C. Gray. 1988. *Plant Mol. Biol.* **10:** 511-520.

Pichersky, E., R. Bernatzky, S.D. Tanksley, R.B. Breidenbach and A.P. Kausch. 1985. *Gene* **40:** 247-258.

Pichersky, E., N.E. Hoffman, R. Bernatzky, B. Piechulla and S.D. Tanksley. 1987. *Plant Mol. Biol.* **9:** 205-216.

Pichersky, E., N.E. Hoffman, V.S. Malik, R. Bernatzky and S.D. Tanksley. 1987. *Plant Mol. Biol.,* **9:** 109-120.

Pichersky, E., S.D. Tanksley, B. Piechulla, M.M. Stayton and P. Dunsmuir. 1988. *Plant Mol. Biol.* **11:** 69-71.

Quigley, F., W.F. Martin and R. Cerff. 1988. *Proc. Natl. Acad. Sci.* USA **85:** 2672-2675.

Roesler, K.R. and W.L. Ogren. 1988. *Nucl. Acids Res.* **16:** 7192.

Rohde, W., D. Becker and F. Salamini. 1988. *Nucl. Acids Res.,* **16:** 7185-7186.

Rose, R.E., C.E. DeJesus, S.L. Moylan, N.P. Ridge and D.E. Scherer. 1987. *Nucl. Acids Res.* **15:** 7197.

Rother, C., T. Jansen, A. Tyagi, J. Tittgen and R.G. Herrmann. 1986. *Curr. Genet.* **11:** 171-176.

Safford, R., J.H.C. Windust, C. Lucas, J. DeSilva and C.M. James. 1988. *Eur. J. Biochem.* **174:** 287-295.

Schmidt, G.W., A. Devillers-Thiery, H. Desruisseaux, G. Blobel and N.H. Chua. 1979. *J. Cell Biol.* **83:** 615-622.

Shih, M.C.,G. Lazar and H. Goodman, 1986. *Cell* **47:** 73-80.

Smeekens, S., M. de Groot, J. van Binsbergen and P. Weisbeek. 1985. *Nature* **317:** 456-458.

Smeekens, S., J. van Oosten, M. de Groot and P. Weisbeek. 1986. *Plant Mol. Biol.,* **7:** 433-440.

Stayton, M.M., M. Black, J. Bedbrook, and P. Dunsmuir. 1986. *Nucl. Acids Res.* **14:** 9781-9796.

Stayton, M.M., P. Brosio and P. Dunsmuir. 1987. *Plant Mol. Biol.* **10:** 127-137.

Steppuhn, J., C. Rother, J. Hermans, T. Jansen and J. Salinkow. 1987. *Mol. Gen. Genet.* **210:** 171-177.

Timko, M.P., A.P. Kausch, J.M. Hand, A.R. Cashmore and L. Herrera-Estrella. 1985. *In, Molecular Biology of the Photosynthetic Apparatus.* K.E. Steinback, S. Bonitz, C.J. Arntzen, and L. Bogorad(eds.), pp. 381-396. Cold Spring Harbor Lab.

Tingey, S.T., F.Y. Tsai, J.W. Edwards, E.L. Walker and G.M. Coruzzi. 1988. *J. Biol. Chem.* **263:** 9651-9657.

Tyagi, A., J. Hermans, J. Steppuhn, C. Jansson and F. Vater. 1987. *Mol. Gen. Genet.* **207:** 288-293.

Vierling, E., R.T. Nagao, A.E. DeRocher and L.M. Harris. 1988. *EMBO J.,* **7:** 575-581.

Vorst, O., R. Oosterhoff-Teertstra, P. Vankan, S. Smeekens and P. Weisbeek. 1988. *Gene* 65: 56-69.

Weisbeek, P., J. Hageman, S. Smeekens, D. de Boer and F. Cremers. 1987. *In. Plant Molecular Biology,* D. von Wettstein. N.H. Chua, pp. 77-91, Plenum. New York/London.

Werneke, J.M., R.E. Zielinski and W.L. Ogren. 1988. *Proc. Natl. Acd. Sci. USA* **85:** 787-791.

Xie, Y., and R. Wu. 1988. *Nucl. Acids Res.* **16:** 7749.

(Received March 10, 1990)