View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Caltech Theses and Dissertations

POST-TRANSLATIONAL MEMBRANE PROTEIN TARGETING BY THE CHLOROPLAST SIGNAL RECOGNITION PARTICLE

Thesis by

Peera Jaru-Ampornpan

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2011

(Defended April 29, 2011)

© 2011

Peera Jaru-Ampornpan

All Rights Reserved

ACKNOWLEDGEMENT

First, I would like to thank my thesis advisor, Shu-ou Shan, for guidance during my graduate work. Shu-ou teaches by example of her rigor and depth in research, as well as her commendable work ethic. She sets high standards for her students, and I am fortunate to have an opportunity to try to meet those and learn many valuable lessons along the way. I am also grateful to my thesis committee, Professors Doug Rees, David Chan and Bil Clemons, for the insightful discussions about my projects we have enjoyed over the years.

I am privileged to have had opportunities to work with the brilliant scientists, who are also wonderful friends, of the Shan lab: Sowmya Chandrasekar, Xin Zhang, Rumana Rashid, Vinh Lam, Thang Nguyen, Kuang Shen, Ishu Saraogi, Aileen Ariosa, David Akopian, Dawei Zhang, Nathan Pierce, and Mike Rome. Special thanks to Sowmya and Thang who have weathered the Monday meetings together with me as Team Chloroplast for the last three years and have helped me proofread parts of this dissertation. Xin has taught me many useful skills, scientific and otherwise, and I thank him for those lessons and his friendship.

Collaboration has been an important part of my research experience at Caltech. I thank Zhenfeng Liu for his invaluable advice and discussion on LHCP and experiments, Peigen Cao for his help in AFM imaging, Alasdair McDowell for his help in TEM imaging, Alex Nisthal and his Robot for the LHCP and cpSRP43 scanning mutagenesis project, and Troy Walton for his help in the multi-angel light scattering trials. I have enjoyed working with two talented SURF students, Tony Jia and Jack Wang, and wish them the best for their future endeavors.

Many supporting staff members have helped smooth my Caltech education experience and become my good friends. I thank Margot Hoyt for being the world's best secretary and beyond; Alison Ross for all the administrative issues regarding the BMB option; Santiago Laparra for many liters of bacterial culture media; Tom Dunn, Jerry Becker, and Scott Smith for promptly fixing various instruments; Joe Drew, Ron Cohen, and Steve Gould of Chemistry purchasing who dealt with many odd purchasing requests for me; and Leslie Fox and Jill Brooks for keeping me sane with their exercise classes. In addition, we have had help from the high-school volunteers, Kevin Kim, Catherine Chan, Grace Qi, Joe Tung, and Irene Chen. I thank them for their hard work and the way their youth has brightened the lab, and wish them the best for their future endeavors.

I could not have done this without personal support from many friends and family. I thank my parents and my sister for being the coolest family I know. I thank all my friends, especially the Thai Scholar network, for the wonderful friendship they have shared with me. Last, I thank my best friend, Knot Pipatsrisawat, for the amazing journey we have together enjoyed.

ABSTRACT

Post-translational transport of membrane proteins poses enormous challenges to the cells. The transport factors must accurately select and deliver the cargos to the appropriate target membranes. In addition, they have to provide chaperone for their hydrophobic cargos. To understand capacity and limitation of a post-translational transport factor, we studied one of the most efficient membrane protein transport pathways, the delivery of light-harvesting chlorophyll-binding (LHC) proteins to the thylakoid membrane. This targeting reaction is mediated by the chloroplast Signal Recognition Particle (cpSRP) and its receptor. Although the core SRP GTPases are close homologues of those in cytosolic SRP pathways, the unique features of cpSRP that might reflect its adaptation to the challenges in post-translational targeting include (i) the lack of the otherwise universally conserved SRP RNA, and (ii) the exclusive presence of a novel protein, cpSRP43. In the first part of this thesis, we define the thermodynamic and kinetic framework for the GTPase cycles of cpSRP and its receptor and uncover the molecular bases that enable their intrinsically fast interactions, such that they can bypass an SRP RNA, an essential accelerator for the cytosolic SRP–receptor interaction. The second part of the thesis is devoted to characterization of the chaperone function of cpSRP43. We show that cpSRP43 specifically and effectively prevents and reverses the aggregation of its cargo, LHC proteins. We further investigate the molecular mechanism of this novel disaggregase activity, using a combination of biochemical and structural approaches. In summary, this dissertation aims to understand how cpSRP and its receptor adapt to their unique requirements in efficiently transporting a family of highly abundant membrane proteins.

TABLE OF CONTENTS

Summary

Compartmentalization offers cells the efficiency and the flexibility to simultaneously carry out vastly different chemical reactions in contained environments. Maintaining specific contents of cellular compartments requires precise control of protein transport from the cytosolic space of the cells, where most proteins are made, to the various organelles. Especially challenging is the transport of membrane proteins, which often are hydrophobic and hence are prone to aggregation during production and transport in aqueous environment. Therefore, cells devote their resources into membrane protein transport to ensure proper protein localization and prevent protein aggregation. Many pathways exist to transport different groups of membrane proteins with great efficiency and accuracy.

A major targeting pathway for membrane proteins is the Signal Recognition Particle (SRP) pathway. SRP handles about one-third of all the cellular proteins and targets secretory and membrane proteins to the endoplasmic reticulum membrane in eukaryotic cells or the plasma membrane in prokaryotic cells (*1*, *2*). Although the size and the composition of the SRP machineries vary in different species, they share the core components. These include the SRP GTPases in the SRP and the SRP receptor (SR) and the SRP RNA that forms a complex with the SRP GTPase (*1*). The cytosolic SRP recognizes its cargos, the translating ribosomes, by binding to the signal sequences (*3*). Because of its co-translational nature of targeting, cytosolic SRP minimizes the exposure of hydrophobic regions of its membrane protein substrates. The first transmembrane

helix is taken as a signal sequence and is sequestered by direct binding to SRP. The rest of the protein is still either inside the ribosome tunnel or is not yet translated during the delivery by SRP (*2*, *4*). Through its interactions with SR, SRP brings its cargo to the target membrane. These interactions involve an extensive series of conformational rearrangements that are modulated by unique environmental cues provided by the cargos and the membrane (*5*, *6*, *7*). Thereby, protein targeting by SRP achieves exquisite spatial and temporal regulations.

Recent discovery of an SRP-mediated targeting pathway in chloroplasts has brought some surprises (*8*, *9*). As opposed to the cytosolic SRP that strictly mediates co-translational targeting, chloroplast SRP (cpSRP) post-translationally delivers its cargos, the nuclear-encoded light-harvesting chlorophyll a/b-binding (LHC) family of proteins, to the thylakoid membrane (*8*). The core SRP GTPases in the cpSRP pathway are highly homologous to those from the cytosolic SRP pathway, sharing over 60% sequence similarity (*10*, *11*). Unexpectedly, however, the otherwise universally conserved SRP RNA has not been found in the cpSRP pathway. Instead, cpSRP is composed of two protein subunits: cpSRP54, which is an SRP GTPase that interacts with the reciprocal chloroplast SR GTPase (*10*), and cpSRP43, which is a novel protein unique to chloroplasts and functions in cargo recognition (*12*, *13*, *14*). Similar to cytosolic SRP, cpSRP brings its cargo to the thylakoid membrane via its interaction with the SR GTPase (*15*). In the thylakoid membrane, the LHC proteins bind photosynthetic pigments and form light-harvesting complexes that allow the photosynthetic reaction centers to capture light efficiently (*16*, *17*). Present in all green plants, LHC proteins are arguably the most abundant membrane protein on earth (*18*). Due to the abundance and the essentiality of

the LHC proteins, the transport system that handles them must be highly effective. Moreover, the post-translational nature of targeting further complicates the situation, adding a requirement for a potent chaperone to prevent the aggregation of the hydrophobic LHC proteins during targeting. cpSRP must therefore adapt itself to accommodate these specialized needs. We believe that these "surprises", the drastic deviations from the cytosolic SRP pathways, reflect such adaptations. This dissertation aims to understand the molecular bases of these adaptations that enable cpSRP to meet the challenges in LHC proteins transport.

First, the otherwise universally conserved SRP RNA is absent from cpSRP. In the cystosolic SRP systems, the SRP-SR interaction is extremely slow and involves multiple discrete conformational changes (*5*, *19*). One of the important roles of the SRP RNA is to accelerate complex formation between the SRP and the SR GTPases by 400-fold, bringing their interaction rate to a physiological range for protein targeting (*20*, *21*). How, then, might this seemingly crucial molecule be left out in the cpSRP system? More specifically, how do cpSRP GTPases that are highly homologous to the slowly-interacting cytosolic SRP GTPases manage the high-load protein targeting without the interaction accelerator? The first part of this dissertation aims to answer these questions.

Chapter 1 shows that the cpSRP pathway bypasses the SRP RNA but still sustains the fast rate of SRP-SR association required for efficient targeting. Indeed, the rate of SRP-SR complex assembly for the chloroplast proteins is 400-fold faster than that of the *E. coli* SRP proteins, matching the interaction rate of the bacterial proteins in the presence of the SRP RNA. The intrinsically fast complex assembly stems partly from the pre-organization of the free cpSRP receptor into a conformation that is conducive for

complex formation. This is not the case for the cytosolic SRP receptor, which undergoes this conformational change only upon binding to SRP (*22*). Therefore, an energy barrier in complex formation is readily reduced for cpSRP-cpSR interaction.

Chapter 2 reveals the molecular bases of this fast interaction. Cross-complementation study using the GTPases from both the chloroplast and *E. coli* pathways confirms the pre-organization of the cpSRP receptor and uncovers the stimulatory role of the cargo-binding "M" domain of cpSRP. The M-domain of *E. coli* SRP, which is the binding site for both the SRP RNA and the signal sequence, does not significantly stimulate the interactions of the cytosolic GTPases unless the SRP RNA is bound to it (*23*). On the contrary, the M-domain of cpSRP, which is the binding site for cargo-binding cpSRP43, raises the interaction rate by 50–100-fold. Therefore, the M-domain of cpSRP subsumes the function of the SRP RNA and eliminates the need for an external regulator for the chloroplast SRP-SR interaction.

Second, cpSRP43 is found exclusively in chloroplast SRPs and has no known homologues in other eukaryotic SRPs. *Chaos*, the cpSRP43-null mutant, has yellow leaves and sustains growth defects, implicating its vital role in LHC biogenesis (*24*). Later biochemical work detected its direct binding to the conserved 18-amino acid motif (called L18) on LHC proteins (*12*, *13*, *14*). Therefore, cpSRP43 is a cargo recognition module specific for cpSRP. Since the nature of post-translational targeting requires a potent chaperone for the membrane protein substrates, it is inviting to speculate that cpSRP43 assumes this chaperone function. The second part of this dissertation explores this idea and is devoted to characterizing the chaperone aspect of cpSRP43.

Chapter 3 discusses the role of cpSRP43 as a potent specific chaperone for the cargo. cpSRP43 can effectively prevent LHC proteins from aggregating in aqueous environment using the specific binding interactions that it establishes with its substrate. Despite its small size, cpSRP43 is an elongated molecule that can provide potential binding surfaces for LHC proteins. Serendipitously, we discovered that cpSRP43 also has a specific disaggregase activity toward the aggregates formed by its substrate protein. Using no external energy input, cpSRP43 can actively reverse aggregation of LHC proteins with speed and efficiency that rival the ATPase-based disaggregases. This is an exciting discovery because, to our knowledge, cpSRP43 represents a rare example of a class of novel disaggregases that utilize binding energy to dissolve insoluble protein aggregates.

Chapters 4 and 5 explore cpSRP43–LHC protein aggregates as a model system for studying a novel mechanism for protein disaggregation. Chapter 4 focuses on the biochemical and biophysical characterization of the aggregates formed by LHC proteins in an attempt to understand the basic nature of the aggregates handled by cpSRP43. We found that these aggregates are disc-shaped micelles with a ~ 12 nm diameter, and they are thermodynamically and kinetically stable. Chapter 5 extends the mechanistic study of the cpSRP43-mediated disaggregation reaction and outlines the molecular requirements of the LHC protein aggregates for efficient disaggregation by cpSRP43. The disaggregation reaction can be dissected into at least two steps: initial binding to the aggregate and subsequent re-solubilization. For the first step, the aggregate must present the cpSRP43 recognition element, L18, on its surface. For the subsequent re-solubilization step, the overall binding energy provided by cpSRP43 must be enough to

overcome the internal packing interactions of the aggregates. Although future work is required, these studies allow us to start probing the capabilities and the limitations of this new disaggregase activity.

In summary, this dissertation aims to explain the "adaptations" made to satisfy special needs in the cpSRP pathway. At the center, the cytosolic and the chloroplast SRP pathways share highly homologous SRP GTPases. However, each system has evolved distinct mechanisms to enable them to fulfill their functions. The cytosolic SRP must select its substrates from a vast pool of translating ribosomes; the pathway has built-in multiple potential regulatory points, making use of the extensive conformational rearrangements of the SRP GTPases (*7*). The indispensable SRP RNA plays a regulatory role and serves as the molecular link between the correct cargos and the GTPases (*25*). On the other hand, the chloroplast SRP pathway, handling one conserved family of proteins, opts for efficiency by bypassing some conformational rearrangements and foregoing external regulators. To cope with the unique problem of protein aggregation during post-translational targeting, cpSRP43 has been evolved as an add-on cargo-binding module for cpSRP to provide effective chaperone during LHC transport. The cytosolic SRP and cpSRP represent only two examples of the numerous membrane protein targeting pathways. Other pathways, such as the GET pathway that delivers tail-anchored proteins (*26*, *27*) or the bacterial SecB–SecA pathway that delivers bacterial outer membrane proteins (*28*), certainly have different requirements and have evolved distinct mechanisms to handle their own substrates. Lessons learned from this dissertation, as well as from numerous comparative studies, emphasize the versatility of nature in its capability to cater to different biological needs.

References:

1. Keenan, R. J., Freymann, D. M., Stroud, R. M., and Walter, P. (2001) The signal recognition particle, *Annu. Rev. Biochem. 70*, 755–775.
2. Walter, P., and Johnson, A. E. (1994) Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane, *Ann. Rev. Cell Biol. 10*, 87–119.
3. Walter, P., and Blobel, G. (1981) Translocation of proteins across the endoplasmic reticulum III. Signal recognition protein (SRP) causes signal sequence-dependent and site-specific arrest of chain elongation that is released by microsomal membranes, *J. Cell Biol. 91*, 557–561.
4. Zheng. N, and Gierasch, L. M. (1996) Signal sequences: the same yet different, *Cell 86*, 849–852.
5. Shan, S., Stroud, R., and Walter, P. (2004) Mechanism of association and reciprocal activation of two GTPases, *PLoS Biology 2*, e320.
6. Zhang, X., Schaffitzel, C., Ban, N., and Shan, S. (2009) Multiple conformational switches in a GTPase complex control co-translational protein targeting, *Proc. Natl. Acad. Sci. USA 106*, 1754–1759.
7. Zhang, X., Rashid, R., Wang, K., and Shan, S. (2010) Sequential checkpoints govern substrate selection during cotranslational protein targeting., *Science 328*, 757–760.
8. Schuenemann, D., Gupta, S., Persello-Cartieaux, F., Klimyuk, V. I., Jones, J. D. G., Nussaume, L., and Hoffman, N. E. (1998) A novel signal recognition particle targets light-harvesting proteins to the thylakoid membranes, *Proc. Natl. Acad. Sci. USA 95*, 10312–10316.
9. Schuenemann, D. (2004) Structure and function of the chloroplast signal recognition particle *Curr. Genet. 44*, 295–304.
10. Li, X., Henry, R., Yuan, J., Cline, K., and Hoffman, N.E. (1995) A chloroplast homologue of the signal recognition particle subunit SRP54 is involved in the posttranslational integration of a protein into thylakoid membranes, *Proc. Natl. Acad. Sci. USA 92*, 3789–3793.
11. Tu, C.-J., Schuenemann, D., and Hoffman, N. E. (1999) Chloroplast FtsY, Chloroplast Signal Recognition Particle, and GTP are required to reconstitute the soluble phase of light-harvesting chlorophyll protein transport into thylakoid membranes, *J. Biol. Chem. 274*, 27219–27224.
12. Delille, J., Peterson, E. C., Johnson, T., Morre, M., Kight, A., and Henry, R. (2000) A novel precursor recognition element facilitates posttranslational binding to the signal recognition particle in chloroplasts, *Proc. Natl. Acad. Sci. 97*, 1926–1931.
13. Tu, C. J., Peterson, E. C., Henry, R., and Hoffman, N. E. (2000) The L18 domain of light-harvesting chlorophyll proteins binds to chloroplast signal recognition particle 43, *J. Biol. Chem. 275*, 13187–13190.

14. Stengel, K. F., Holdermann, I., Cain, P., Robinson, C., Wild, K., and Sinning, I. (2008) Structural basis for specific substrate recognition by the chloroplast signal recognition particle protein cpSRP43, *Science 321*, 253–256.
15. Jaru-Ampornpan, P., Chandrasekar, S., Shan, S. (2007) Efficient interaction between two GTPases allows the chloroplast SRP pathway to bypass the requirement for an SRP RNA, *Mol Biol Cell. 18*, 2636–2645.
16. Peter, G. F., and Thornber, J.P. (1991) Biochemical composition and organization of higher plant photosystem II light-harvesting pigment-proteins, *J. Biol. Chem. 266*, 16745–16754.
17. Liu, Z., Yan, H., Wang, K., Kuang, T., Zhang, J., Gui, L., An, X., and Chang, W. (2004) Crystal structure of spinach major light-harvesting complex at 2.72 Å resolution *Nature 428*, 287–292.
18. Jansson, S. (1999) A guide to the Lhc genes and their relatives in arabidopsis, *Trends Plant Sci. 4*, 236–240.
19. Zhang, X., Kung, S., and Shan, S. (2008) Demonstration of a multistep mechanism for assembly of the SRP- SRP receptor complex: implications for the catalytic role of SRP RNA, *J Mol Biol. 381(3)*, 581–593.
20. Peluso, P., Herschlag, D., Nock, S., Freymann, D. M., Johnson, A. E., and Walter, P. (2000) Role of 4.5S RNA in assembly of the bacterial signal recognition particle with its receptor, *Science 288*, 1640–1643.
21. Peluso, P., Shan, S., Nock, S., Herschlag, D., and Walter, P. (2001) Role of SRP RNA in the GTPase cycles of Ffh and FtsY, *Biochemistry 40*, 15224–15233.
22. Shan, S., and Walter, P. (2003) Induced Nucleotide Specificity in a GTPase, *Proc. Natl. Acad. Sci. U.S.A. 100*, 4480–4485.
23. Bradshaw, N., and Walter, P. (2007) The signal recognition particle (SRP) RNA links conformational changes in the SRP to protein targeting., *Mol Biol Cell 18*, 2728–2734.
24. Klimyuk, V. I., Persello-Cartieaux, F., Havaux, M., Contard-David, P., Schuenemann, D., Meiherhoff, K., Gouet, P., Jones, J.D.G., Hoffman, N.E., and Nussaume, L. (1999) A chromodomain protein encoded by the arabidopsis CAO gene is a plant-specific component of the chloroplast signal recognition particle pathway that is involved in LHCP targeting, *The Plant Cell 11*, 87–99.
25. Bradshaw, N., Neher, S.B., Booth, D.S., and Walter, P. (2009) Signal sequences activate the catalytic switch of SRP RNA, *Science 323*, 127–130.
26. Stefanovic, S., and Hegde, R.S. (2007) Identification of a targeting factor for posttranslational membrane protein insertion into the ER, *Cell 128*, 1147–1159.
27. Schuldiner, M., Metz, J., Schmid, V., Denic, V., Rakwalska, M., Schmitt, H.D., Schwappach, B., and Weissman, J.S. (2008) The GET complex mediates insertion of tail-anchored proteins into the ER membrane, *Cell 134*, 634–645.
28. Randall, L. L., and Hardy, S.J.S. (2002) SecB, one small chaperone in the complex milieu of the cell, *Cell. Mol. Life Sci. 59*, 1617–1623.