Chapter 1

Introduction

We now know that almost every major biological process is controlled by protein assemblies that comprise two or more proteins that interact with one another to exert their regulatory function (*1*). These assemblies are called protein machineries because the way that they work at a microscopic scale resembles the way that machines work at a macroscopic scale. Underlying these highly controlled activities are the ordered conformational changes that take place in the protein components of the machineries. These conformational changes often lead to molecular events that drive efficient regulation of the biological processes.

One key process in protein biogenesis is the cotranslational protein targeting (*2*). This process transports roughly one-third of proteins in a cell's genome from cytoplasmic space to the membrane compartments. This process, like many others, is controlled by the protein machinery in which the protein components interact with one another. This machinery is composed primarily of the signal recognition particle (SRP) and its receptor (SR). In all cells, the SRP machinery carries out targeting of secretory and membrane proteins to the endoplasmic reticulum membrane in eukaryotic cells or to the plasma membrane in bacteria.

Although the SRP machinery varies widely in size and composition through evolution, the functional core of the SRP machinery is well conserved in a variety of organisms. The center of the SRP is comprised of the universally conserved SRP54 guanosine 5'-triphosphatases (GTPases) in complex with the SRP RNA. The SRP

receptor also contains a highly conserved SR GTPase (SRα) subunit that serves as a connector between the SRP and the cellular membrane. Thus, SRP and SR GTPases together form the center of the SRP machinery and provide exquisite spatial and temporal controls to the protein targeting process.

The SRP-dependent protein targeting process involves a series of highly ordered molecular events (*3*). These events begin when a nascent polypeptide chain destined for the endoplasmic reticulum (ER) or the secretory pathway emerges from a translating ribosome. The signal sequence that specifies its cellular localization is recognized by the SRP. The ribosome•nascent chain complex (RNC), herein denoted as cargo, is then directed to the cellular membrane by the interaction between the SRP and SR. Upon arrival at the membrane, the conformation of the SRP•RNC complex switches from a cargo-loading mode to a cargo-releasing mode in which the RNC is unloaded from the SRP and passed on the protein conducting channel, or the translocon, on the membrane. After the cargo is released, the SRP dissociates from the SR to allow the cargo to be recycled in the next round of protein targeting. Meanwhile, the synthesis of the nascent polypeptide is finished and the nascent protein is either integrated into the membrane or translocated through the membrane to enter its journey to the destined cellular compartment.

SRP and SR GTPases together compose a class of noncanonical GTPases in comparison to the classical GTPases such as Ras, Gα, and EF-Tu (*3*). They do not exhibit significant conformational changes among the apo, guanosine 5'-diphosphate (GDP)-bound and guanosine 5'-triphosphate (GTP)-bound states. Further, these GTPases bind to nucleotides weakly and exchange from GDP to GTP rapidly. However, free SRP and SR

GTPases bound to GTP have a low basal activity to hydrolyze GTP because the key catalytic residues for GTP hydrolysis are not correctly aligned with the bound nucleotide. Instead, GTP binding enables the SRP and SR GTPases to form a thermodynamically stable heterodimeric complex. In this complex, the two GTPases reciprocally activate the GTP hydrolysis activity of one another by two to four orders of magnitude. Following GTP hydrolysis, the GDP-bound SRP-SR complex would lose its affinity and quickly dissociate to regenerate free SRP and SR GTPases for the next cycle.

Since the SRP and SR GTPase are intrinsically capable of multiple rounds of dimerization and GTP hydrolysis, it is of interest to understand how the complex assembly and GTPase activation are controlled so that these GTPase function as molecular switches to regulate the series of molecular events in space and time. The goal of this dissertation is to elucidate the "hidden facts" inside the SRP machinery that control the protein targeting process both efficiently and faithfully. In particular, most efforts have been made to understand how the SRP and SR interact with one another to coordinate the ordered series of molecular events during the protein targeting. Thus, the studies that were carried out in this dissertation focus on the molecular mechanism of the interaction between the SRP and SR GTPases, and on how this interaction responds actively to the cues such as cargos and thus how this interaction helps maintain the efficiency and fidelity of the protein targeting process.

Chapter 2 (*4*) defines the kinetic and thermodynamic framework of the SRP-SR interaction. A transient, GTP-independent early intermediate during the assembly of a stable SRP•SR complex is discovered by a highly sensitive fluorescence assay in real time. This further demonstrates that the SRP•SR complex assembly is a complex process

that involves at least two steps. In the initial step, an early, GTP-independent SRP-SR complex is formed via the fast association between the SRP and SR. In the second step, the GTP-dependent conformational rearrangements precede the formation of a stable complex. The SRP RNA significantly stabilizes the early, GTP-independent intermediate. Further, mutational analyses show that there is a strong correlation between the ability of the mutant SRP RNAs to stabilize the early intermediate and their ability to accelerate the SRP•SR complex formation. Thus, the SRP RNA is proposed to stabilize the transient early intermediate to give it a longer dwell time and therefore a higher probability to rearrange to the stable complex.

Chapter 3 defines the landscape of the transient intermediate during assembly of a stable SRP-SR complex. Direct structural characterization of an transient intermediate ensemble is challenging because the intermediate tends to either dissociate or rearrange to the stable complex. In this work, an ensemble of the SRP-SR early intermediate is generated by stalling the conformational rearrangements to form the stable complex. Thus, the structural properties of the early intermediate can be directly characterized under equilibrium condition. The interaction surface of the early intermediate is both similar to and different from that of the stable complex. Further, a nanosecond timescale experiment reveals a broad conformational distribution of the early intermediate. These conformational states allow the free SRP and SR GTPases to search the optimal routes in the configurational space toward an efficient assembly of the stable complex. Interestingly, the landscape of the early intermediate actively responds to the cargos, suggesting that the early intermediate could potentially serve as a control point to the protein targeting process.

Chapter 4 (*5*) focuses on how SRP machinery ensures the efficiency of the protein targeting reaction by examining the effect of cargos on the SRP-SR interaction. Since the conformational dynamics of the early intermediate actively responds to the cargos, I investigate how the cargos kinetically and thermodynamically modulate a series of discrete conformational rearrangements during the SRP-SR interaction. The cargo for SRP is found to accelerate the SRP-SR complex assembly by over two orders of magnitude, thereby driving rapid and efficient delivery of cargo to the membrane. A series of subsequent rearrangements in the SRP•SR GTPase complex switch the SRP from the cargo-binding mode to the cargo-releasing mode where the cargo can be unloaded during the late stages of protein targeting. Further, the cargo delays GTPase activation in the SRP•SR complex by an order of magnitude. The slower GTP hydrolysis in a RNC•SRP•SR complex creates an important time window that could further improve the efficiency of protein targeting. This work shows that the SRP and SR GTPases constitute a self-sufficient system that provides exquisite spatial and temporal control points to maintain the efficiency of protein targeting.

Chapter 5 answers how SRP machinery maintains the fidelity of the substrate selection in the protein targeting process. The "signal hypothesis" postulates that the signal sequence on a protein allows it to be specifically recognized by targeting factors such as SRP, which mediates the delivery of the protein to the correct cellular compartments. It was generally thought that fidelity arises from the inability of SRP to bind strongly to incorrect cargos. Instead, I show that incorrect cargos are further rejected through a series of fidelity checkpoints during subsequent steps of targeting, including complex formation between the SRP and SR and kinetic proofreading through GTP

hydrolysis. Thus, the SRP pathway achieves high fidelity of substrate selection through the cumulative effect of multiple checkpoints; this principle may be generally applicable to other complex cellular pathways that need to recognize degenerate signals or discriminate between correct and incorrect substrates based on minor differences.

Overall, this thesis establishes the framework of how the SRP machinery achieves an efficient and faithful co-translational protein targeting process. In particular, I show that the function of the SRP machinery is governed by a series of ordered conformational changes during the SRP-SR interaction that culminates in their GTPase activation. These conformational changes respond actively to the cargos so that they could provide discrete control points at which regulation can be exerted on the protein targeting process spatially and temporally. The paradigm provided in this thesis adds to an increasing collection of knowledge on how critical biological processes are regulated by multistate protein machineries.