Protein Folding and Chaperones II

3387-Pos Board B115

Driving Forces for Protein Secretion Across the Bacterial Outer Membrane

Igor Drobnak, Patricia L. Clark.
University of Notre Dame, Notre Dame, IN, USA.

Autotransporters are a large and diverse class of monomeric virulence proteins secreted from Gram-negative bacterial pathogens. Secretion across the outer membrane is facilitated by the C-terminal translocator domain that creates a pore in the outer membrane, through which the N-terminal passenger (the functional, extracellular part of the protein) exits the cell. However, there is no ATP in the periplasm nor an ion gradient across the outer membrane. It is therefore not clear where the free energy comes from to drive the transport of the passenger through the pore. Using a computational model of secretion kinetics, we show that the free energy of passenger folding could be used as a driving force for secretion, provided that the passenger does not fold prematurely in the periplasm. We have tested this model experimentally by reversibly stalling secretion and probing the periplasmic conformation of the stalled protein. Our results show that the passenger remains unfolded in the periplasm, confirming that the major requirement for coupling folding to secretion is met. In addition, preliminary data indicates that mutations that disrupt folding also prevent efficient secretion. Further work is in progress to experimentally characterize the interplay between autotransporter passenger folding and secretion.

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Identification of Protein-Protein Interactions Between the TatB and TatC Subunits of the Twin-Arginine Translocase System and the Redox Enzyme Maturation Protein Chaperones

Lalita Kuzniatsova, Raymond J. Turner.
University of Calgary, Calgary, AB, Canada.

The Twin-arginine translocation pathway (Tat) serves for targeting and translocation of fully folded proteins across cytoplasmic membrane in bacterial and plant chloroplast thylakoid membranes. TatA, TatB, and TatC are the core components of the Escherichia coli Tat system, where the TatB and TatC subunits are considered to form a receptor complex for Tat-bound proteins. TatB protein is composed of a transmembrane helix and extramembrane part facing the cytoplasmic side of the cell, the structure of TatC revealed six transmembrane helixes and four extramembrane loops. Redox Enzyme Maturation Proteins (REMPs) are the system specific chaperons, which play significant roles in respiratory enzyme maturation, including targeting to the Tat translocase system. We applied a bacterial two-hybrid technique to determine whether REMPs interact with the extramembrane loops of the TatBC recognition module. Individual loops were cloned and fused with one of the two catalytic domains of adenylate cyclase from Bordetella pertussis. The constructs were co-expressed in Escherichia coli where peptides interaction leads to functional complementation between the two adenylate cyclase catalytic domains resulting in increased cAMP levels and induction of beta-galactosidase expression. Our study demonstrated that the DmsD chaperone interacts with the loops 1, 2 and 4 of TatC and with the proposed unfolded domain of TatB. Other E. coli chaperones homologous to DmsD- YcdY and NarJ - also show an interaction with the TatB domain. This suggests that there may be a handoff mechanism of Tat substrates to the translocase via the REMP.

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Determining the Sorting Pathways of Multi-Spanning Inner Membrane Proteins of the Mitochondria In Vivo

Kwangjin Park, Hyun Kim.
Seoul National University, Seoul, Korea, Republic of.

Most mitochondrial inner membrane (MIM) proteins in *Saccharomyces cerevisiae* are encoded in the nucleus and are fully synthesized as precursors before they are targeted to the mitochondria. The translocase of inner mitochondrial membrane (TIM22 or TIM23) mediates the translocation and integration of the precursors including multi-spanning MIM proteins. However, the exact insertion mechanism of multi-spanning MIM proteins is not well characterized owing to lack of robust tools. The recently established Mgm1 fusion approach, which takes advantage of the rhomboid cleavage region in the C-terminal domain of Mgm1p, has been useful in elucidating the sorting pathways of single-spanning MIM proteins. In this study, we tested whether the Mgm1 fusion approach can be applied to multi-spanning membrane proteins using Mdl1p. It is an ABC transporter whose membrane insertion pathway is already known. Our data shows that only the first two transmembrane (TM) domains are integrated into the lipid bilayer and the subsequent four domains are translocated to the matrix. Having validated the approach, we determined the insertion pattern of Mdl2p, a homolog of Mdl1p. Unlike Mdl1p, only the last two TM segments are translocated to the matrix. Altogether, our results suggest that the Mgm1 fusion assay can be used as a useful tool to determine the membrane insertion pathways of not only single-spanning MIM proteins, but also of multi-spanning membrane proteins.

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Tracking Folding Events During the Cell-Free Expression of Bacteriorhodopsin into Nanodiscs by Seiras

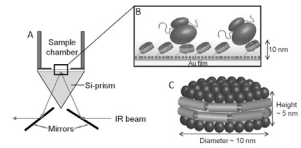
Axel Baumann[1], Kenichi Ataka[2], Silke Kerruth[2], Jörg Fitter[1,3], Joachim Heberle[2], Georg Bueldt[1], Ramona Schlesinger[2].
[1]Research Centre Jülich GmbH, Jülich, Germany, [2]Free University Berlin, Berlin, Germany, [3]RWTH Aachen University, Aachen, Germany.

Cell-free expression systems offer a valuable approach to study protein folding in situ.

However, studies on such molecular processes over time are difficult to perform because of the disturbing crowded cellular conditions.

Applying Surface Enhanced Infrared Absorption Spectroscopy (SEIRAS, Fig. A) can circumvent many of the difficulties. In particular, SEIRAS is exclusively sensitive to processes occurring on the surface. Hence, it is possible to monitor a folding process of a membrane protein by creating a membrane support (here: nanodiscs; Fig. C) on such a surface, in which the target protein can incorporate. Meanwhile, other processes of the cell-free expression system from the bulk will not be visible.

We immobilized nanodiscs to a gold surface via His-tag Ni-NTA linkage. By introducing a cell-free transcription/translation protein expression system, the synthesis of bacteriorhodopsin (bR), a seven-helical trans-membrane retinal protein, was initialized (Fig. B). During the reaction infrared spectra were collected in snap-shot mode. Thus, it was possible to monitor events of the translational process in situ. Here, we present secondary structural data as strong evidence for bR folding and incorporation into nanodiscs.



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Role of Environment in Protein Folding

Sai Janani Ganesan[1], Joel Schneider[2], Robert Blumenthal[2], Silvina Matysiak[1].
[1]University of Maryland, College Park, College Park, MD, USA, [2]National Cancer Institute, Frederick, MD, USA.

It has long been known that the spontaneous folding process of a polypeptide chain is governed both by its sequence and environment. Consequently, the use of coarse graining to explore larger biological systems and longer time scales has become increasingly popular. We recently developed a coarse grained protein model with induced structural polarization, by adding oppositely charged dummies inside backbone coarse grained beads. The dummies represent a dipole, which can be influenced by the surrounding environment. The presence of polarization in the backbone beads, enabled us to explore the role of sequence patterning in protein folding, and we achieved alpha/beta content de novo without any added biases. In this work, we look more closely at the relationship between environment, folding and sequence. We also explore the role of lipids as triggers in the folding process. Anticancer peptides and varying membrane compositions of phosphatidylserine and phosphatidylcholine are considered as a model system for this study.

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Investigating the Residues that are Responsible for PH Dependent Activity of the ATPase Domain of DnaK

Gizem Dinler Doganay, Umut Gunsel, Bulent Balta.
Istanbul Technical University, Istanbul, Turkey.

Hsp70 proteins have essential roles in cells such as de novo folding of newly synthesized proteins, refolding or ubiquitination of denatured proteins, protein tarfficking and translocation through membranes. DnaK, Escherichia coli homolog of Hsp70 molecular chaperone, is comprised an N-terminal ATPase domain (NBD), a C-terminal substrate-binding domain (SBD) and a partially conserved hydrophobic linker that connects the domains. Substrate-binding affinities on SBD are driven by ATP-ADP conversion cycles in NBD. Allosteric communication between the two domains is provided by the conserved 389VLLL392 sequence on the linker region. Previous studies done using truncated DnaK(1-392) construct, containing the 389VLLL392 sequence, showed a pH-dependent enhanced ATPase activity, similar to the substrate-stimulated activity of the full-length protein; whereas construct lacking this sequence, DnaK(1-388) showed an activity resembling to the unstimulated-form of