Lecture 4

Protein Translocation & Nucleocytoplasmic Transport

Reference paper: Tran and Wente, Cell 125, 1041-1053, 2006

2/6/2013
On binding an ER signal sequence (which acts as a start transfer signal), the translocator opens its pore, allowing the transfer of the polypeptide chain across the lipid bilayer as a loop. After the protein has been completely translocated, the pore closes, but the translocator now opens laterally within the lipid bilayer, allowing the hydrophobic signal sequence to diffuse into the bilayer, where it is rapidly degraded. * In this figure, the ribosomes have been omitted for clarity.
How a Single-Pass Transmembrane Protein with a Cleaved ER Signal Sequence Is Integrated into the ER Membrane

In this protein, the co-translational translocation process is initiated by an N-terminal ER signal sequence (red) that functions as a start-transfer signal, as in Figure 12–45. In addition to this start-transfer sequence, however, the protein also contains a stop-transfer sequence (orange). When the stop-transfer sequence enters the translocator and interacts with a binding site, the translocator changes its conformation and discharges the protein laterally into the lipid bilayer.

* In this figure, the ribosomes have been omitted for clarity.
An internal ER signal sequence (also binds to the SRP) that functions as start transfer signal can bind to the translocator in one of two different ways, leading to a membrane protein that has either its C-terminus (pathway A) or its N-terminus (pathway B) in the ER lumen.

Proteins are directed into either pathway by features in the polypeptide chain flanking the internal start transfer sequence.

* In this figure, the ribosomes have been omitted for clarity.
Integration of a Double-Pass Membrane Protein with an Internal Signal Sequence into the ER Membrane

In this protein, an internal ER signal sequence acts as a start-transfer signal (as in Figure 12–47) and initiates the transfer of the C-terminal part of the protein. At some point after a stop-transfer sequence has entered the translocator, the translocator discharges the sequence laterally into the membrane. * In this figure, the ribosomes have been omitted for clarity.
The Insertion of the Multipass Membrane Protein Rhodopsin into the ER Membrane

(A) A hydropathy (also called hydrophobicity) plot identifies seven short hydrophobic regions in rhodopsin.

(B) The hydrophobic region nearest the N-terminus serves as a start-transfer sequence that causes the preceding N-terminal portion of the protein to pass across the ER membrane. Subsequent hydrophobic sequences function in alternation as start-transfer and stop-transfer sequences.

(C) The final integrated rhodopsin has its N-terminus located in the ER lumen and its C-terminus located in the cytosol. The blue hexagons represent covalently attached oligosaccharides. Arrows indicate the paired start and stop signals inserted into the translocator.
(A) The CIC chloride channel, which consists of two subunits, has a complex arrangement of helices. (B) The EmrE protein has two identical subunits with completely inverted topology. (C) The topology of aquaporin 1 changes before and after insertion into the membrane. The red arrows highlight the direction of helix rearrangements. Several helices must either insert into the membrane or flip orientations. (D) Topology rearrangements in lactose permease with varying lipid composition. The helices that undergo topology rearrangements in the distinct lipid environments are circled in the dashed red box.
Almost as soon as a polypeptide chain enters the **ER lumen**, it is glycosylated on **target Asn** (Asn-X-Ser/Thr, X is not Pro) amino acids. The precursor oligosaccharide is transferred to the Asn as an intact unit in a reaction catalyzed by a membrane-bound oligosaccharyl transferase enzyme. As with signal peptidase, one copy of this enzyme is associated with each protein translocator in the ER membrane.

Figure 12-51
The ER-membrane-bound chaperone protein calnexin binds to incompletely folded proteins containing one terminal glucose on N-linked oligosaccharides, trapping the protein in the ER. Removal of the terminal glucose by a glucosidase releases the protein from calnexin. A glucosyl transferase is the crucial enzyme that determines whether the protein is folded properly or not: if the protein is still incompletely folded, the enzyme transfers a new glucose from UDP-glucose to the N-linked oligosaccharide, renewing the protein’s affinity for calnexin and retaining it in the ER. The cycle repeats until the protein has folded completely.
Improperly Folded Proteins Are Exported from the ER and Degraded in the Cytosol

Misfolded soluble proteins in the ER lumen are translocated back into the cytosol, where they are deglycosylated, ubiquitylated, and degraded in proteasomes.
Some Membrane Proteins Acquire a Covalently Attached Glycosylphosphatidylinositol (GPI) Anchor

Immediately after the completion of protein synthesis, the precursor protein remains anchored in the ER membrane by a hydrophobic C-terminal sequence (15–20 aa). Within less than a minute, an enzyme in the ER cuts the protein free from its membrane bound C-terminus and simultaneously attaches the new C-terminus to an amino group on a preassembled GPI intermediate. The sugar chain contains an inositol attached to the lipid from which the GPI anchor derives its name. It is followed by a glucosamine and three mannoses. The terminal mannose links to a phospho-ethanolamine that provides the amino group to attach the protein. **The signal that specifies this modification is contained within the hydrophobic C-terminal sequence and a few amino acids adjacent to it on the lumenal side of the ER Membrane.**
The Production of Mitochondrial and Chloroplast proteins by Two Separate Genetic Systems

Most of the proteins in these organelles are encoded by the nucleus and must be imported from the cytosol.
The Organization of the Human Mitochondrial genome

The genome contains 2rRNA genes, 22 tRNA genes, and 13 protein-coding genes. The DNAs of many other animal mitochondrial genomes have also been completely sequenced. Most of these animal mitochondrial DNAs encode precisely the same genes as humans, with the gene order being identical for animals that range from fish to mammals.

Figure 14-60
Protein Transport into Mitochondria Matrix

Mitochondria proteins have a signal peptide. Most proteins are too large to fit through pore and are unfolded by hsp70. Mitochondria proteins have specialized membrane proteins to assist the translocation. The signal peptide binds receptor on outer membrane. The receptor/protein complex diffuses until it reaches a contact site. When a mitochondria matrix protein is translocated into matrix by passing through two membranes, the signal peptide is cleaved.
Transport into the Inner Mitochondrial Membrane and Intermembrane Space Occurs via Several Routes

Routes for (A) inner membrane protein containing a stop-transfer sequence (hydrophobic); (B) inner membrane proteins containing a hydrophobic signal sequence; (C) intermembrane space protein; (D) metabolite transporters in the Inner membrane.
Mitochondrial precursor proteins remain *unfolded* in the cytosol through interactions with other proteins (e.g. *chaperone proteins* of the *Hsp70 family*).

*The isolated mitochondria were used in this experiment.*

Proteins transiently span the inner and outer mitochondrial membranes during their translocation into the matrix space.
Transport of Molecules Between the Nucleus and the Cytosol - Nucleocytoplastic Transport
Topologically equivalent spaces are shown in red. In principle, cycles of membrane budding and fusion permit the lumen of any of these organelles to communicate with any other and with the cell exterior by means of transport vesicles. Blue arrows indicate the extensive outbound and inbound vesicular traffic. Some organelles, most notably mitochondria and (in plant cells) plastids, do not take part in this communication and are isolated from the traffic between organelles shown here.
The double membrane envelope is penetrated by pores in which nuclear pore complexes (NPCs) are positioned and is continuous with the ER. The ribosomes that are normally bound to the cytosolic surface of the ER membrane and outer nuclear membrane are not shown. The nuclear lamina is a fibrous meshwork underlying the inner membrane.

Using field emission scanning electron microscope Elena Kiseleva, *Nature Cell Biol.* 6, 497, 2004
The Arrangement of NPCs in the Nuclear Envelope

Figure 12-9

(A) A small region of the nuclear envelope (cross section).
(B) A scanning electron micrograph of the nuclear side of the nuclear envelope of an oocyte.
(C) An electron micrograph showing a side view of two NPCs (brackets). The inner and outer nuclear membranes are continuous at the edges of the pore.
(D) An electron micrograph showing face-on views of negatively stained NPCs.
The Nuclear Pore Complex

(a) Cut-away view of the structure of the NPC from Dictyostelium determined by cryo-ET (electron tomogram). The central plug or transporter is transparently rendered.

(b) Schematic diagram of nucleoporin localization within the metazoan NPC. Biochemically characterized subcomplexes are boxed. Note that this two-dimensional representation is simplified and not exhaustive.

Nucleoporins (Nups) Are Building Blocks of the NPCs

- **Yeast:** ~60 MD, ~30 Nups, **Vertebrate:** ~125 MD, ~30 Nup
- **Symmetry:** 8-fold rotational symmetry and 2-fold pseudo-mirror symmetry
- **Nups form subcomplexes**
- **FG-Nups and non-FG-Nups** (*FG: Phe-Gly repeats*)

D’Angelo and Hetzer *Trends in Cell Biology* 2008
Nuclear Pore Complex (NPC) Assembly and the Dynamics of Nucleoporins Are Correlated with their Proposed Functions within the NPC

A cross-section of an NPC is shown, with the central region magnified in the right three panels. The order of Nup assembly (late to early) into NPCs following mitosis and the relative Nup NPC residence time or shuttling activity (stable to transient) are illustrated. The predicted function (structural to transport) for each Nup or subcomplex is also shown. Common colors between the three figures in each structure indicate correlations in assembly, dynamics, and function. Those that are early in assembly and stable in residence time are likely structural in function. In contrast, those that are late in assembly and transient in residence time are likely directly involved in transport.

Tran and Wente, *Cell* 125, 1041-1053, 2006
A side view of an NPC. Unstructured regions of the proteins lining the central pore form a tangled meshwork, which blocks the passive diffusion of large macromolecules. During active transport, however, even particles up to 39 nm in diameter can pass through NPCs.
Many different classes of molecules and macromolecules are transported through NPCs. Small, uncharged molecules (<100 Da) can diffuse through the membrane of the nuclear envelope.

When gold particles of various sizes are injected into cells, those smaller than 9 nm in diameter can pass through NPCs by passive diffusion.
Immunofluorescence micrographs showing the cell location of SV40 virus T-antigen containing or lacking a short sequence that serves as a nuclear localization signal.

(A) The normal T-antigen protein contains the lysine-rich sequence indicated and is imported to its site of action in the nucleus, as indicated by immunofluorescence staining with antibodies against the T-antigen.

(B) T-antigen with an altered nuclear localization signal (a threonine replacing a lysine) remains in the cytosol.
This series of EMs shows *colloidal gold spheres* (arrowheads) coated with peptides containing nuclear localization signals entering the nucleus through NPCs.

Gold particles were injected into living cells, which then were fixed and prepared for electron microscopy at various times after injection.

**(A)** Gold particles are first seen in proximity to the cytosolic fibrils of the NPCs.

**(B, C)** They migrate to the center of the NPCs, where they are first seen exclusively on the cytosolic face.

**(D)** They then appear on the nuclear face.

These gold particles have much larger diameters than the diffusion channel in the NPC and are imported by active transport.
Nuclear transport of baculovirus: Revealing the nuclear pore complex passage

EM of NPC cross-sections from Xenopus oocytes microinjected with baculovirus AcMNPV capsid and incubated at room temperature for 3.5 h. Capsids of 250–300 nm in length are seen traversing the NPCs. Capsids appear fully intact in its native conformation while crossing the NPC. Note the capsid in the middle panel appear shorter due to the variability in the length of these capsids. Bar, 100 nm. n, nucleus; c, cytoplasm.

EM of Xenopus oocytes microinjected with WGA-gold into either the cytoplasm (A) or nucleus (B), incubated at room temperature for 2 h, followed by cytoplasmic injection of baculovirus AcMNPV capsids and further incubated for 8 h.

When NPCs were inhibited by WGA-gold particles, capsids remained interacting with the NPC cytoplasmic filaments 8 h post micro-injection. No capsids were found inside the nucleus when NPCs were inhibited with WGA. Bar, 200 nm. n, nucleus; c, cytoplasm. Black arrows point to capsids and white arrowheads point to WGA-gold particles at NPCs.

(A) Many nuclear import receptors bind both to NPC proteins and to a nuclear localization signal (NLS) on the cargo proteins they transport. Cargo proteins 1, 2, and 3 in this example contain different NLSs, and therefore each binds to different nuclear import receptors.

(B) Cargo protein 4 requires an adaptor protein to bind to its nuclear import receptor. The adaptors are structurally related to nuclear import receptors and recognize NLSs on cargo proteins. They also contain an NLS that binds them to an import receptor.
## Proteins in Kapβ Receptor Family

<table>
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<th>Homologs</th>
<th>Substrates</th>
<th>NLS/NES</th>
<th>References</th>
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A Model Explaining How GTP Hydrolysis by Ran in the Cytosol Provides Directionality to Nuclear Transport

Movement through the NPC of loaded nuclear transport receptors occurs along the FG-repeats displayed by certain NPC proteins. The differential localization of Ran-GTP in the nucleus and Ran-GDP in the cytosol provides directionality (red arrows) to both nuclear import (left) and nuclear export (right).

Ran-GAP stimulates the hydrolysis of GTP to produce Ran-GDP on the cytosolic side of the NPC.

Ran-GDP is imported into the nucleus by its own import receptor, which is specific for the GDP-bound conformation of Ran. The Ran-GDP receptor is structurally unrelated to the main family of nuclear transport receptors. However, it also binds to FG-repeats in NPC proteins and hops across the NPC.
Xylourgidis and Fornerod, *Dev. Cell* 2009
The compartmentalization of Ran-GDP and Ran-GTP. Localization of Ran-GDP in the cytosol and Ran-GTP in the nucleus results from the localization of two Ran regulatory proteins: Ran GTPase-activating protein (Ran-GAP) is located in the cytosol and Ran guanine nucleotide exchange factor (Ran-GEF) binds to chromatin and is therefore located in the nucleus.

* Ran-REF is also call RCC1 (regulator of chromosome condensation 1).
(A) The structure of a nuclear transport receptor with bound Ran-GTP. The receptor contains repeated α-helical motifs that stack on top of each other, forming a flexible springlike structure, which can adopt multiple conformations in response to the binding of cargo proteins and Ran-GTP. *Cargo proteins and Ran-GTP bind to different regions of the coiled spring. The Ran-GTP partly covers a conserved loop (red) of the receptor, which, in the Ran-free state, is thought to be important for cargo binding. (B) The cycle of loading in the cytosol and unloading in the nucleus of a nuclear import receptor.
The nuclear factor of activated T cells (NF-AT) is a gene regulatory protein that, in the resting T cell, is found in the cytosol in a phosphorylated state. When T cells are activated by foreign antigen, the intracellular Ca\(^{2+}\) concentration increases. In high Ca\(^{2+}\), the protein phosphatase calcineurin binds to NF-AT and dephosphorylates it. The dephosphorylation exposes nuclear import signals and blocks a nuclear export signal. The complex of NF-AT and calcineurin is then imported into the nucleus, where NF-AT activates the transcription of numerous genes required for T cell activation. The response shuts off when Ca\(^{2+}\) levels decrease, releasing NF-AT from calcineurin. Rephosphorylation of NF-AT inactivates the nuclear import signals and re-exposes the nuclear export signal, causing NF-AT to relocate to the cytosol.

Some of the most potent immunosuppressive drugs, including cyclosporin A and FK506, inhibit the ability of calcineurin to dephosphorylate NF-AT and thereby block the nuclear accumulation of NF-AT and T cell activation.
Multiple Models for the Mechanism of Nuclear Transport

The contacts between Kaps and Nups via FG repeats are the key to understanding how translocation through the nuclear pore occurs.

The interaction between FG repeats prevent most proteins from translocating through the NPC. Proteins that contain binding sites for FG repeats can disrupt those interactions and partition through the NPC.