Outline:
1. Introduction to nucleus and nuclear envelope
2. Nuclear pore complex: structure
3. Types of nuclear passage: 2 way traffic
   a) passive
   b) signal-mediated: selective transport triggered by specific transport signals
4. Method/approach:
   a) Identification of essential cytosolic factors: Imp α, Imp β, NTF2, Ran
   b) Nuclear protein: RCC1 (GEF)
5. Principles
   a) Ran-GTPase system
   b) Energy
   c) Translocation
6. Diversity of pathways
   a) Import and Export: proteins and RNAs

Q: Why is nuc pore so complex? How does it work?
   a) Identity of proteins?
   b) Location?
   c) Function?
   d) Approach:

Increasing resolution maps of the NPC substructure. Immunoelectron microscopy (ImmunoeM) has begun to map the position of the nucleoporins within the NPC, whereas mass spectrometry (MS) is one of the new techniques being used to map the direct interactions between individual nucleoporins.

From Rout & Aitchison 2001. JBC

Cross section of nucleus

Nuc envelope breakdown & reformation during mitosis

11-28a. Scanning E.M. Nuclear pore complex

<table>
<thead>
<tr>
<th></th>
<th>Yeast</th>
<th>Human (div)</th>
<th>Frog oocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPC/nuc</td>
<td>189</td>
<td>3000-5000</td>
<td>500,000</td>
</tr>
<tr>
<td>Mda</td>
<td>~66</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Protein</td>
<td>~30</td>
<td>~60</td>
<td>50-100</td>
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Proteins < 60 kd can diffuse through a 9 nm diameter pore. Pore will allow active transport of particles of 26 nm diameter.

**Approaches**

1. Nuc import of microinjected protein into oocyte
2. Nuc import in permeabilized cell
3. Yeast mutants lacking an importin or exportin
4. RNAi of transport factors in multicellular organism or cell culture.

**Visualize gold particles coated with NLS**

Gold particles coated with a protein-tagged with NLS is microinjected into a cell. Particle initially at the cytoplasmic face is later found in the nuclear face of the pore.

**Figure 17-35: NLS (nuclear localization signal) directs cytosolic protein to the nucleus.**

Pore is expressed in transfected cells. Protein detected with immuno-fluorescence.

**SV40 large T antigen binds to origin of replication**

```plaintext
SV40 large T antigen binds to origin of replication
1 mdhvrsnes lgamllgig reagynpple
2 skppkklnke fhpdpdpm kekkntylh
3 kmewqyakh qygypgd wtekipytug
4 vwynenaw awrocawmp sadkadmafuds
5 qhstppkkkr kvedpkdfps ellsflshav
6 fsnrtlacfa iyttkekaal lykkimekys
7 vtfisrhnsy nhnilffltp hrhrvsain
8 weqwwnafne enlfcseemp ssddeatads
9 qhstppkkkr kvedpkdfps ellsflshav
10 vtfisrhnsy nhnilffltp hrhrvsain
```

**Method to study nuclear transport**

1. In vitro import assay (microscopy) in permeabilized cell
2. Combine with genetics & molecular genetics

**What cytosolic components are required?**

- What are the proteins needed? Receptors? Chaperones?
- Is energy needed?

**Assay:**

a. permeabilize cell
b. introduce Fluor substrate
c. monitor uptake with fluorescence microscopy
Fig. 11-36 b. Import requires cytosolic components and ATP

![Image of import process](image)

**Cytosolic components = ?**

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**Import Receptors: soluble protein that bind cargo-NLS and nucleoporins**

Related family of import receptors (≈ trucks)

![Diagram of import receptors](image)

More different cargo e.g. mRNA, nuclear proteins, than importins. How is specificity determined?

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**Export from the nucleus** depends on

1. NES- nuclear export signal
2. Export receptor proteins (exportins) are soluble proteins
   - recognize cargo-NES
   - bind cargo in nucleus
   - release them in the cytosol

What determines directionality?

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**Ran GTP gradient is maintained by asymmetric distribution of GAP and GEF.**

![Diagram of Ran GTP gradient](image)

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**Ran distribution in cell**

80% in nucleus of interphase cell

From: Moore 1998, JBC minireview

RAN: Ras-related nuclear protein. A family of proteins

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[Ca]_dependent dePhosphorylation of Transcription factor can determine its import or export

![Diagram](Ca-dependent dePhosphorylation of Transcription factor)

RNA transport

- mRNA
- tRNA
- Ribosome export

Balbiani ring mRNA in salivary gland of insect.

mRNA + hnRNP

- a. 5' exit first [cap]
- b. Ring unfolds as it reaches NPC
- c. Translation begins before export is completed.

mRNA exported with other proteins

Figure 2. Coordination of Transcription, Pre-mRNA Processing, and mRNA Export in Metazoans

- Left: The timeline of transcription begins with Pol II-promoter complex (PIC) formation (tan block) and recruitment of Pol II to the promoter. Soon after promoter escape, Pol II C-terminal domain is phosphorylated, and the nascent transcript is capped. As elongation continues, Pol II undergoes cycles of phosphorylation and dephosphorylation and further recruits processing factors. Pol II terminates and is released. During elongation, the splicing machinery may deposit Aly (also known as REF) and the exon junction complex on the pre-mRNA, and Aly recruitment may be mediated by UAP56. Furthermore, CPSF and the 3' end processing machinery (pink block) produce the mature 3' end of the transcript. Export competency is achieved through recruitment of export factors, such as TAP (also known as NPL1), by Aly and perhaps other factors.
- Right: Unidentified RNA binding proteins (black circles).

Lei & Silver 2002. Dev Cell

Ribosome export in yeast

In situ 5'ITS1 RNA = product of rRNA processing

![Figure 4](In Vivo Assays for Ribosome Export in S. cerevisiae)

(A) In situ hybridization to 5'ITS1 RNA denotes localization of the small ribosomal subunit. Wild-type (WT) cells (a–c) display 5'ITS1 signal throughout the nucleus and cytoplasm (c), while a Ran regulator mutant (d–f) shows accumulation of 5'ITS1 signal throughout the entire nucleus (f). Both strains are in an xrn1∆ background. Nomarski optics (a and d) and DAPI fluorescence (b and e) are shown.

(B) Rpl11b-GFP localization in live cells denotes localization of the large ribosomal subunit. WT cells (a and b) display L11-GFP signal throughout the nucleus and cytoplasm (b), while a ribosome assembly mutant (c and d) shows strong nuclear accumulation of the reporter (d). Nomarski optics are shown (a and c).

Lei & Silver 2002. Dev Cell