MOCB 639 Nuclear Transport 2/19/04
Lodish, ch. 11-4; Alberts, Ch. 12
Moore 1998. JBC Ran and Nuclear transport
Rout & Aitchison 2001 JBC. Nuclear pore complex
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**:
5. Identification of essential cytosolic factors: Imp α, Imp β, NTF2, Ran
Nuclear protein: RCCI (GEF)
6. **Principles**
a) Ran-GTPase system
b) Energy
c) Translocation
7. **Diversity of pathways**
Import and Export: proteins and RNAs

Cross section of nucleus

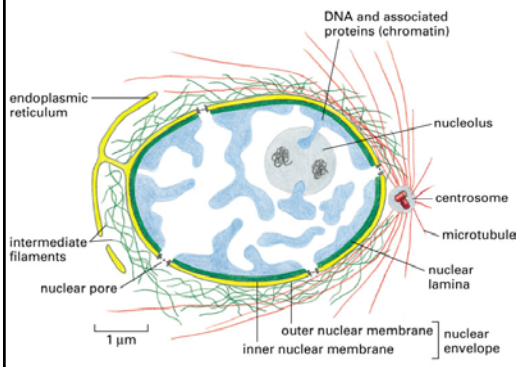


Figure 4–9. Molecular Biology of the Cell, 4th Edition.

Nuc envelope breakdown & reformation during mitosis

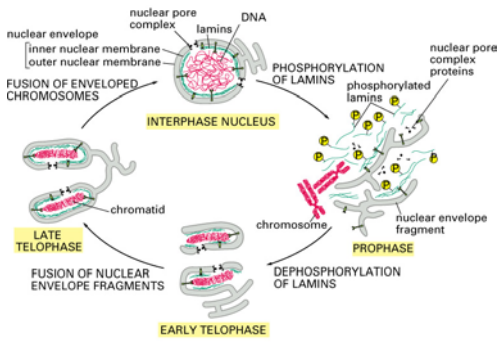
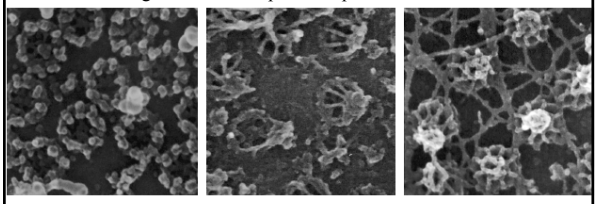


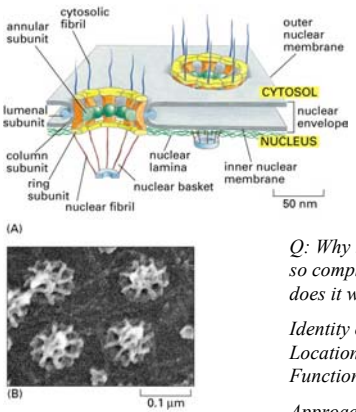
Figure 12–21. Molecular Biology of the Cell, 4th Edition.

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



Cytoplasmic face | Nucleoplasmic face | Nucleoplasmic face after detergent treatment. Note lamin network

Yeast	189 NPC/nuc	66 Mda	~30 proteins
Human (div)	3000-5000	125	~60 proteins
Frog oocyte	500,000	125	50-100

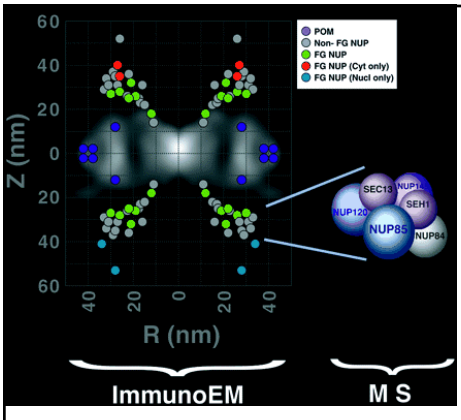


Q: Why is nuc pore so complex? How does it work?

Identity of proteins? Location ? Function?

Approach:??

Figure 12–10 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



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

Long Sec. : Bottom Nuc side. NUP nucleoporins, POM, pore membrane protein

How do molecules move through pore?

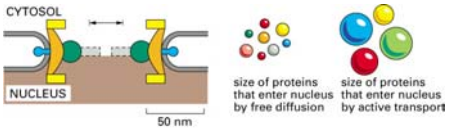


Figure 12–11. Molecular Biology of the Cell, 4th Edition.

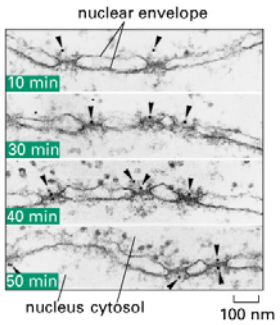
Proteins < 60 kd can diffuse thru 9 nm diam pore.

Pore will allow active transport of particles of 26 nm diam

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

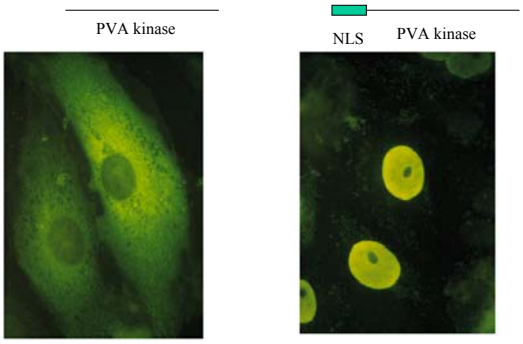


Gold particles coated with a protein-tagged with NLS is microinjected in a cell.

Particle initially at the cytoplasmic face is later found in the nuclear face of the pore.

Figure 12–13. Molecular Biology of the Cell, 4th Edition.

17-35. NLS (nuc localization signal) directs cytosolic protein to the nucleus.



Protein is expressed in transfected cells

Protein detected with immunofluorescence.

NLS = PKKKRKV from SV40 T antigen synthetic peptide alone enters nuc

SV40 large T antigen binds to origin of replication

```
 1  mdkvlnrees lqlmdllgle rsawgniplm
rkaylkkcke fhpdkggdee kmkkmntlyk
61  kmedgvkyah qpdfggfwda teiptygtde
weqwwnafne enlfcseemp ssddeatads
121 qhstppkkkr kvedpkdfps ellsflshav
fsnrtlacfa iyttkekaal lykkimekys
181 vtfisrhnsy nhnilffltp hrhrvsainn
yaqklctfsf lickgvnkey lmysaltrdp
241 fsvieeslpg glkehdfnpe eaeetkqvsw
klvteyamet kcddvllllg mylefqysfe
301 mclkcikkeq pshykyhekh yanaaifads
knqkticqqa vdtvlakkrv dslqltreqm
361 ltnrfndlld rmdimfgstg sadieewmag
vawlhcllpk mdsvvydflk cmvynipkkr
421 ywlfkgpids gkttlaaall elcggkalnv
nlpldrlnfe lgvaidqflv vfedvkgtgg
481 esrdlpsgqg innldnlrdy ldgsvkvnle
kkhlnkrtqi fppgivtmne ysvpktlqar
541 fvkqidfrpk dylkhclers efllekriiq
sgialllmli wyrpvaefaq siqsrivewk
601 erldkefsls vyqkmkfnva mgigvldwlr
nsddddedsq enadknedgg eknmedsghe
661 tgidsqsqgs fqapqssqsv hdhnqpyhic
rgftcfkkpp tpppepet
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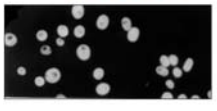
(A) LOCALIZATION OF T-ANTIGEN CONTAINING ITS NORMAL NUCLEAR IMPORT SIGNAL

Pro–Pro–Lys–Lys–Lys–Arg–Lys–Val–



(B) LOCALIZATION OF T-ANTIGEN CONTAINING A MUTATED NUCLEAR IMPORT SIGNAL

Pro–Pro–Lys–Thr–Lys–Arg–Lys–Val–

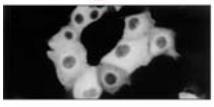
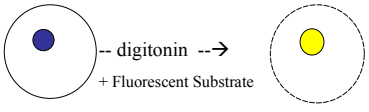


Figure 12–12. Molecular Biology of the Cell, 4th Edition

What cytosolic components are required?
What are the proteins needed? Receptors? Chaperones?
Is energy needed?

Method to study nuclear transport

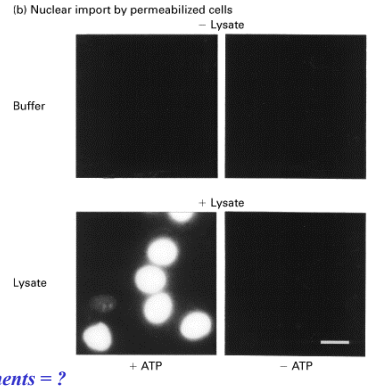
1. In vitro import assay (microscopy) in permeabilized cell
2. Combine with genetics & molecular genetics



Assay:

a. permeabilize cell
b. introduce Fluor substrate
c. monitor uptake with fluorescence microscopy

Fig. 11-36 b. Import requires cytosolic components and ATP



Cytosolic components = ?

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

Related family of import receptors (= trucks)

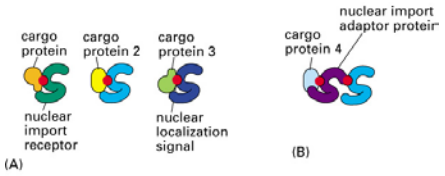


Figure 12–14. Molecular Biology of the Cell, 4th Edition.

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

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?

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

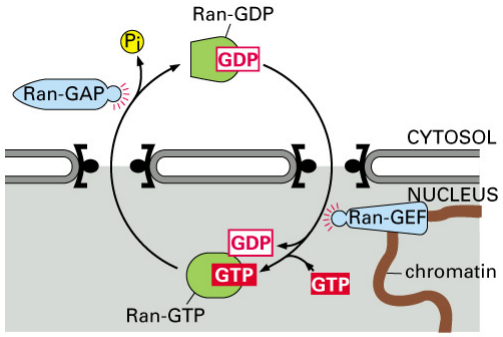


Figure 12–15. Molecular Biology of the Cell, 4th Edition.

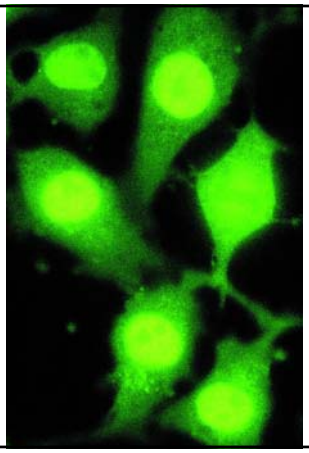
Ran distribution in cell

80% in nucleus of interphase cell

From: Moore 1998, JBC minireview

RAN: Ras-related nuclear protein.

A family of proteins



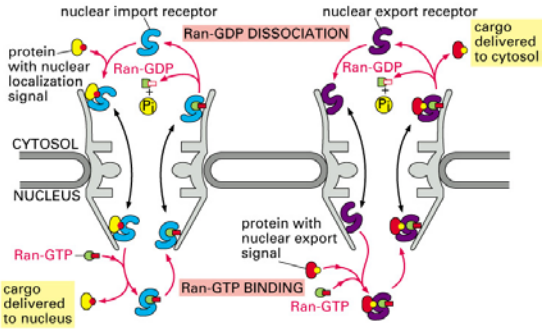


Figure 12–16. Molecular Biology of the Cell, 4th Edition.

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

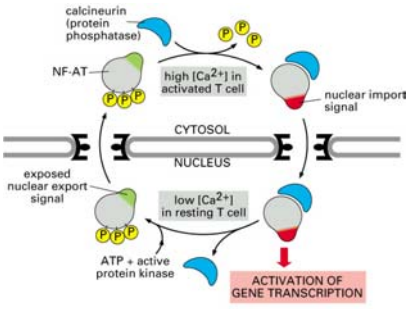


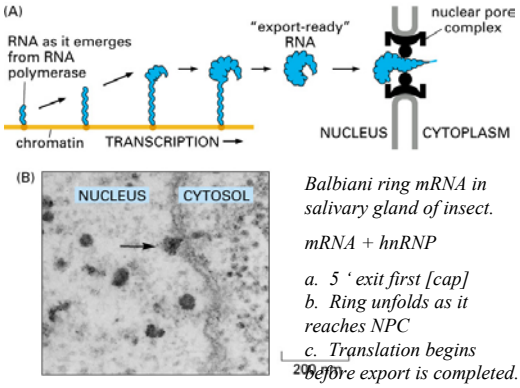
Figure 12–19. Molecular Biology of the Cell, 4th Edition.

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.

Figure 6–39. Molecular Biology of the Cell, 4th Edition.

mRNA exported with other proteins

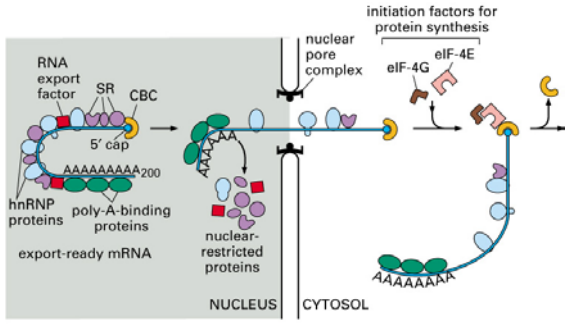
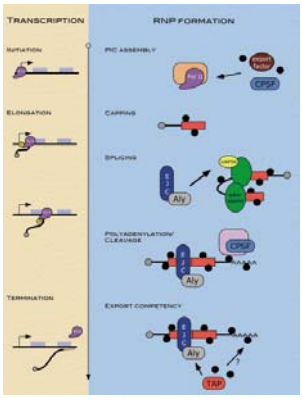


Figure 6–40 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



mRNA export

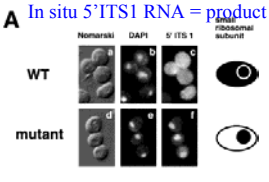
Figure 2. Coordination of Transcription, Pre-mRNA Processing, and mRNA Export in Metazoans(Left) The timeline of transcription begins with preinitiation complex (PIC) formation (tan block) and recruitment of Pol II to the promoter. Soon after promoter escape, the Pol II C-terminal domain is phosphorylated, 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 NXF1), by Aly and perhaps other factors.

unidentified RNA binding proteins (black circles).

Lei & Silver 2002. Dev Cell

Ribosome export in yeast

A In situ 5'ITS1 RNA = product of rRNA processing



B L11-GFP

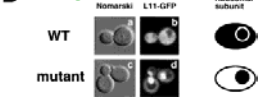


Figure 4. In Vivo Assays for Ribosome Export in *S. cerevisiae*(A) In situ hybridization to 5′ ITS1 RNA denotes localization of the small ribosomal subunit. Wild-type (WT) cells (a–c) display 5′ ITS1 signal throughout the nucleus and cytoplasm (c), while a Ran regulator mutant (d–f) shows accumulation of 5′ ITS1 signal throughout the entire nucleus (f). Both strains are in an *xrn1Δ* 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