

LECTURE 2

The nuclear envelope and nucleocytoplasmic traffic

Textbook reading:

Lodish - pp. 426-436; 510-517 (see Lecture 1).

PART A: THE NUCLEAR ENVELOPE, LAMINA, AND NUCLEAR PORES

(See outline for Lecture #1 for more detailed sections I – III)

I. Overall structure of the nuclear envelope.

ONM and INM, nuclear lamina, and nuclear pore complexes (NPCs)

II. The nuclear lamina.

Composed lamins, which belong to the intermediate filament superfamily:

1) Lamins A and C:

- Are solubilized in mitosis;
- Developmentally regulated expression
- Lamin C is alternatively spliced isoform of lamin A that lacks the CaaX box.
- CaaX box of lamin A removed by proteolysis.

2) Lamin B, ubiquitous. Remains associated with N.E. throughout the cell cycle.

Modifications: Isoprenylation, carboxyl methylation, and phosphorylation.

Assembly of the lamina:

- 1) Formation of coiled-coil dimers;
- 2) Polar longitudinal association of dimers;
- 3) Lateral association of polymers into filaments;
- 4) Paracrystalline fibers with ~25nm axial repeats.

Integral membrane proteins of INM mediate interactions with lamina and with chromosomes.

III. Nuclear envelope disassembly and reassembly

Stages in disassembly of the nucleus in mitosis:

- Chromosome condensation;
- Lamina depolymerization (mediated by phosphorylation of lamins);
- Envelope vesicularization

Stages in reassembly of nuclei:

- Binding of membrane vesicles to condensed chromosomes;
- Membrane fusion;

- Chromatin decondensation and envelope growth;
- Nuclear transport of proteins

IV. Nuclear pore complexes.

Allow passive diffusion of small solutes, as well as mediated, selective transport of macromolecules. All exchange of molecules between the nucleus and the cytoplasm believed to take place through NPCs.

General structural features:

- Octagonal symmetry
 - ca. 125MDa
 - Up to 100 different (nucleoporins) in vertebrates.
- There are ca. 3,000 - 5,000 NPC's in a proliferating human cell (by contrast, there are ca. 190 NPCs per nucleus in yeast, and $\sim 5 \times 10^7$ in a mature *Xenopus* oocyte).

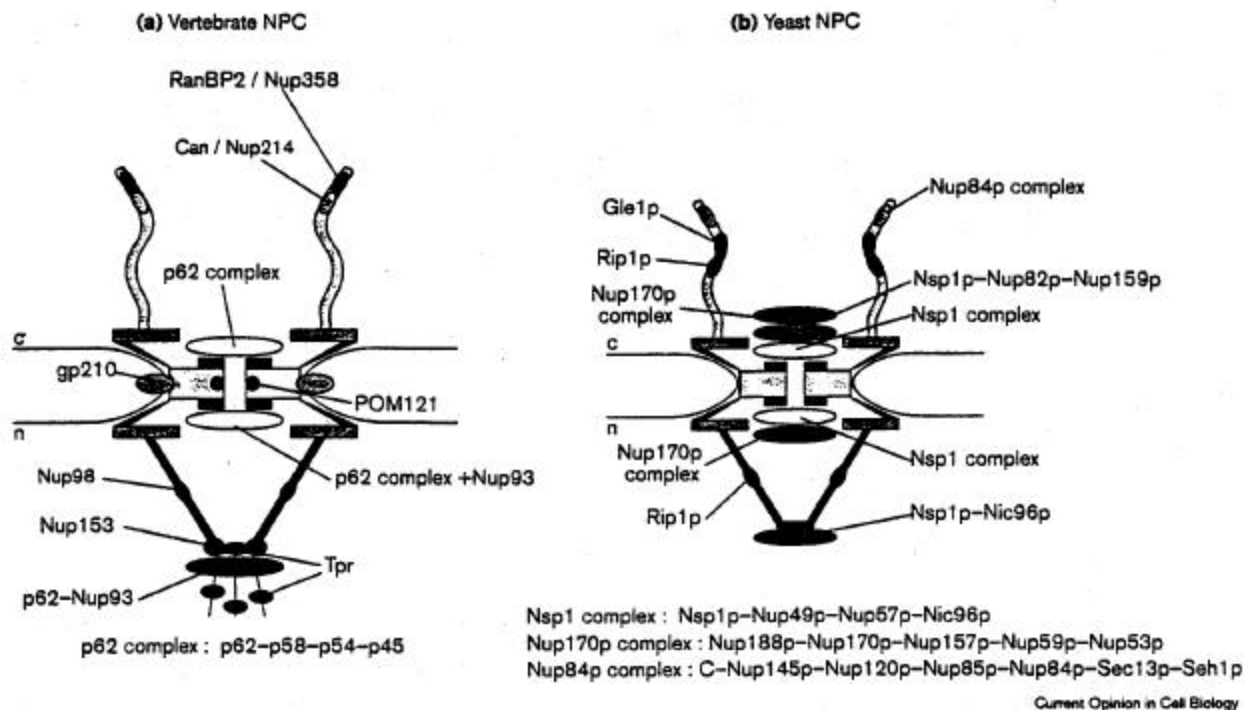


Figure 1. Overall structure of NPCs in vertebrates and in yeast:

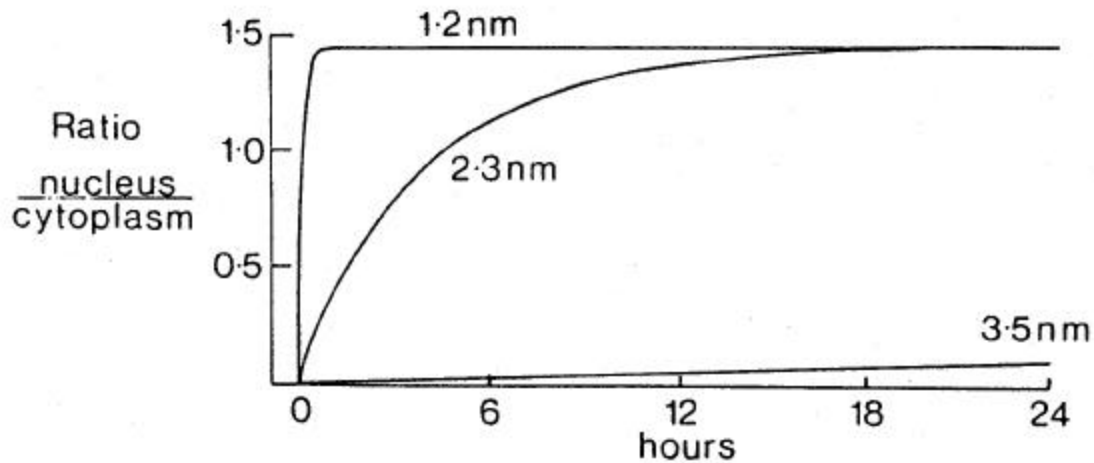
PART B: NUCLEAR IMPORT

Review article: E.A. Nigg (1997) Nucleocytoplasmic transport: signals, mechanism and regulation. *Nature* **386**:779-787.

(For a more detailed review, see: Görlich, D., and U. Kutay (1999) Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Dev. Biol.* **15**:607-660)

I. Passive diffusion of solutes between the nucleus and the cytoplasm.

Diffusion size limit for passage of solutes between nucleus and cytoplasm is ca. 9nm, corresponding to a globular protein of 45-68kDa (see Fig. 2). However, passive



diffusion for proteins even within this limit is exceedingly slow.

Figure 2. Entry of tritiated dextrans of known radius into *Rana pipiens* nuclei after microinjection into the cytoplasm, as a function of time (Redrawn from Paine et al., 1975).

I. Evidence for mediated nuclear import

- The size limit for passive diffusion across the NPC is ca. 9Å (90nm; ~ 45,000-60,000kDa for globular proteins). This does not explain how large proteins such as nucleoplasmin (pentamer of ca. 165,000Da) and other macromolecules enter into and accumulate efficiently in the nucleus.
- Nucleoplasmin pentamer accumulates in the nucleus of oocytes after microinjection into the cytoplasm. The 'core' fragment, generated by limited proteolysis, cannot enter the nucleus. Other proteolytic products that retain 'tail' fragments enter efficiently (Figure 3).

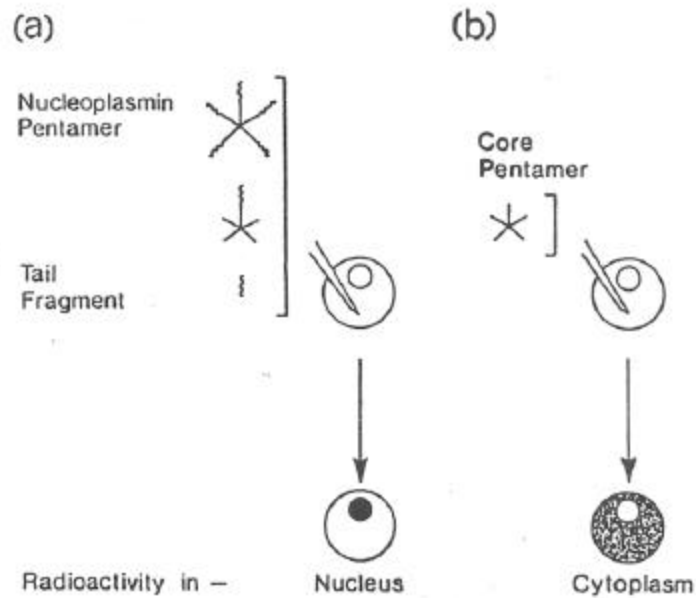


Figure 2 Diagram illustrating the transport of nucleoplasmin molecules into the *Xenopus* oocyte nucleus. (a) The intact nucleoplasmin pentamer, a pentamer with a single intact subunit, and the isolated "tail" fragment can all accumulate in the nucleus after microinjection into the cytoplasm. (b) The nucleoplasmin "core" molecule cannot enter the nucleus after microinjection into the cytoplasm.

II. Nucleocytoplasmic transport occurs through nuclear pores.

- Nucleoplasmin-coated gold particles are transported into the nucleus through nuclear pores, as visualized by E.M. (Feldherr and co-workers).
- Initial attachment appears to occur at cytoplasmic filaments of the NPC.
- Upper size limit for mediated transport is ca. 260Å (26nm).

III. Basic-type Nuclear Location Sequence (NLS)

- Single amino acid change in SV40 T antigen prevents its nuclear localization
- NLS is **necessary** for nuclear localization of SV40 T antigen (mutation and deletion analysis)
- NLS of SV40 T antigen is **sufficient** for nuclear localization: can direct otherwise cytoplasmic proteins to the nucleus; NLS-coated gold particles, or BSA chemically coupled to NLS, also accumulate in the nucleus when injected into the cytoplasm.

Sequence of the SV40 NLS, characterized by a preponderance of basic (Lys and Arg) amino acids:

PKKKRKV

Bipartite NLS in nucleoplasmin:

KRPAATKKAGQAKKKK

- Bold (Lys and Arg) amino acids essential, but others in the ca. 10a.a. spacer tolerant to mutations.

- While these prototype NLSs are the most prevalent, many other classes of nuclear location signals have been identified (e.g. M9 in hnRNP A1; TMG + Sm proteins in snRNP complexes).

Some salient characteristics of NLS-mediated nuclear protein import:

- NLS function is independent of its location in a protein, so long as it is accessible for interaction with the appropriate factors;

- NLS can mediate import of heterologous proteins into the nucleus, even when synthetic NLS peptides are coupled chemically to proteins.

- NLS is not cleaved after nuclear import. This is important, for example, for proper nuclear location of pre-existing proteins at the end of mitosis, as well as for nuclear return of nucleocytoplasmic shuttling proteins..

- Nuclear protein import does not require unfolding/conformational changes of the substrate for translocation.