THE NUCLEUS & NUCLEOCYTOPLASMIC TRANSPORT

1. Introduction
   a. The nucleus
   b. The nuclear envelope
   c. Transport cargoes

2. The solid phase: the nuclear pore complex (NPC)

3. Signals
   a. Nuclear localization signals (NLSs)
   b. Nuclear export signals (NESs)

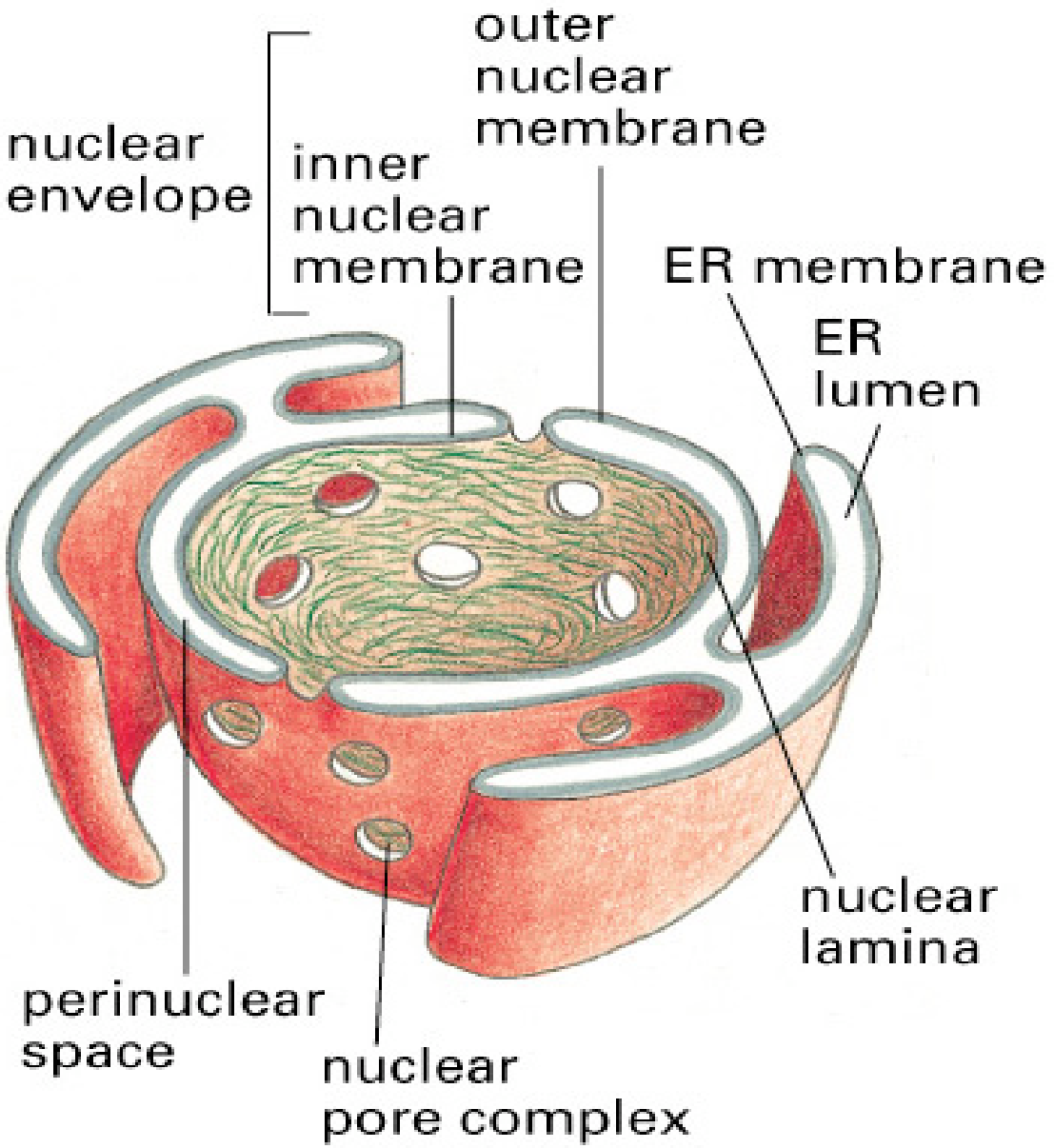
4. The soluble phase: nuclear transport factors
   a. Importins and exportins
   b. The GTPase Ran

5. Mechanism of transport through the NPC

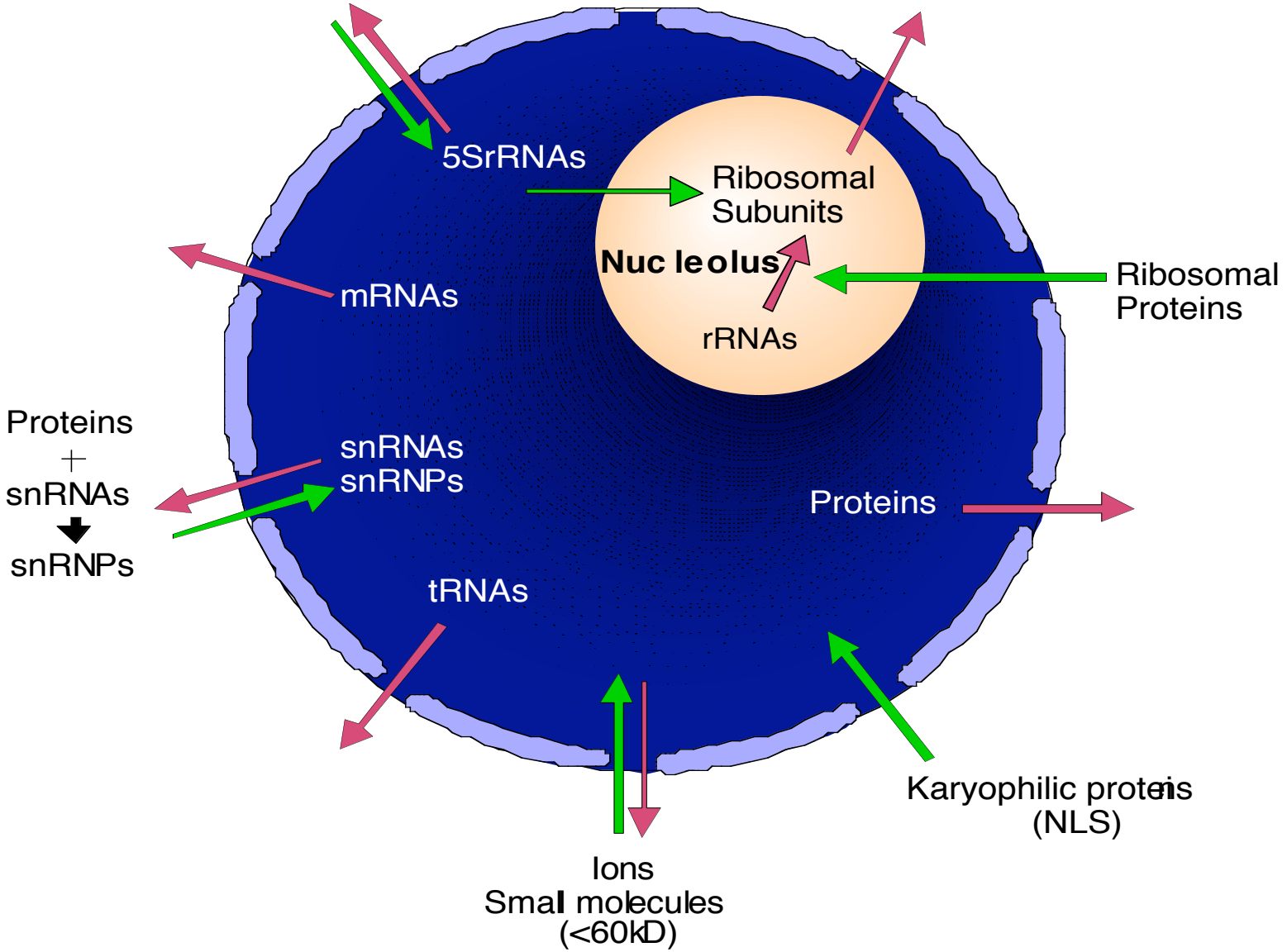
6. Segregation of nuclear components during cell division

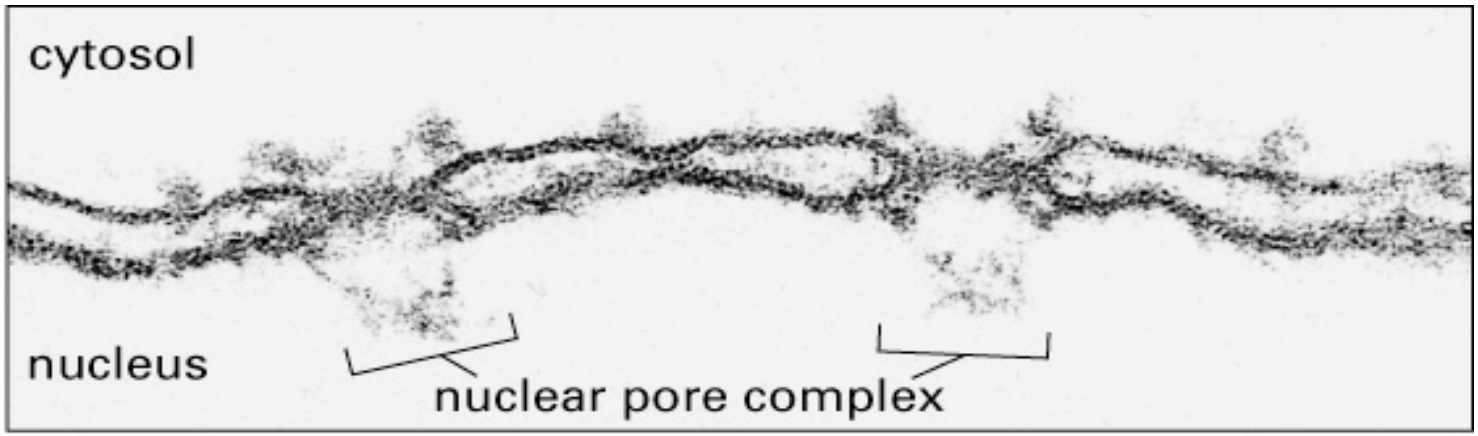
1. Introduction

a. The Nuclear Envelope

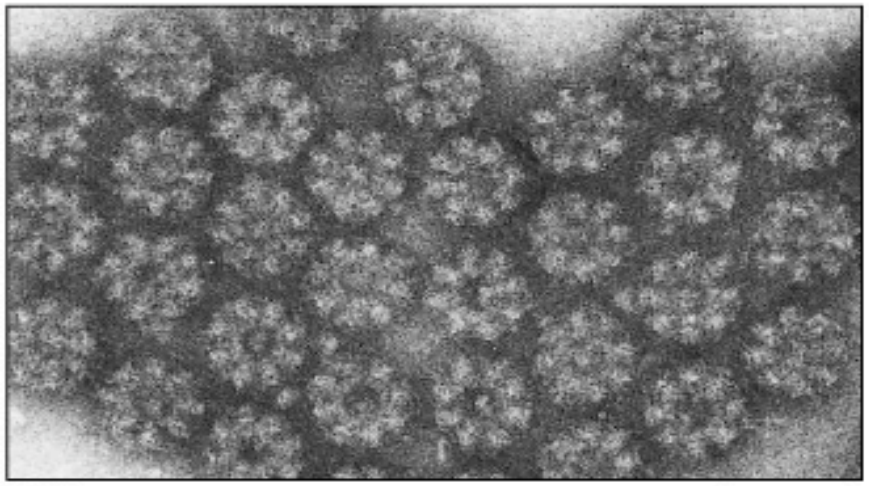


b. Transport Cargoes





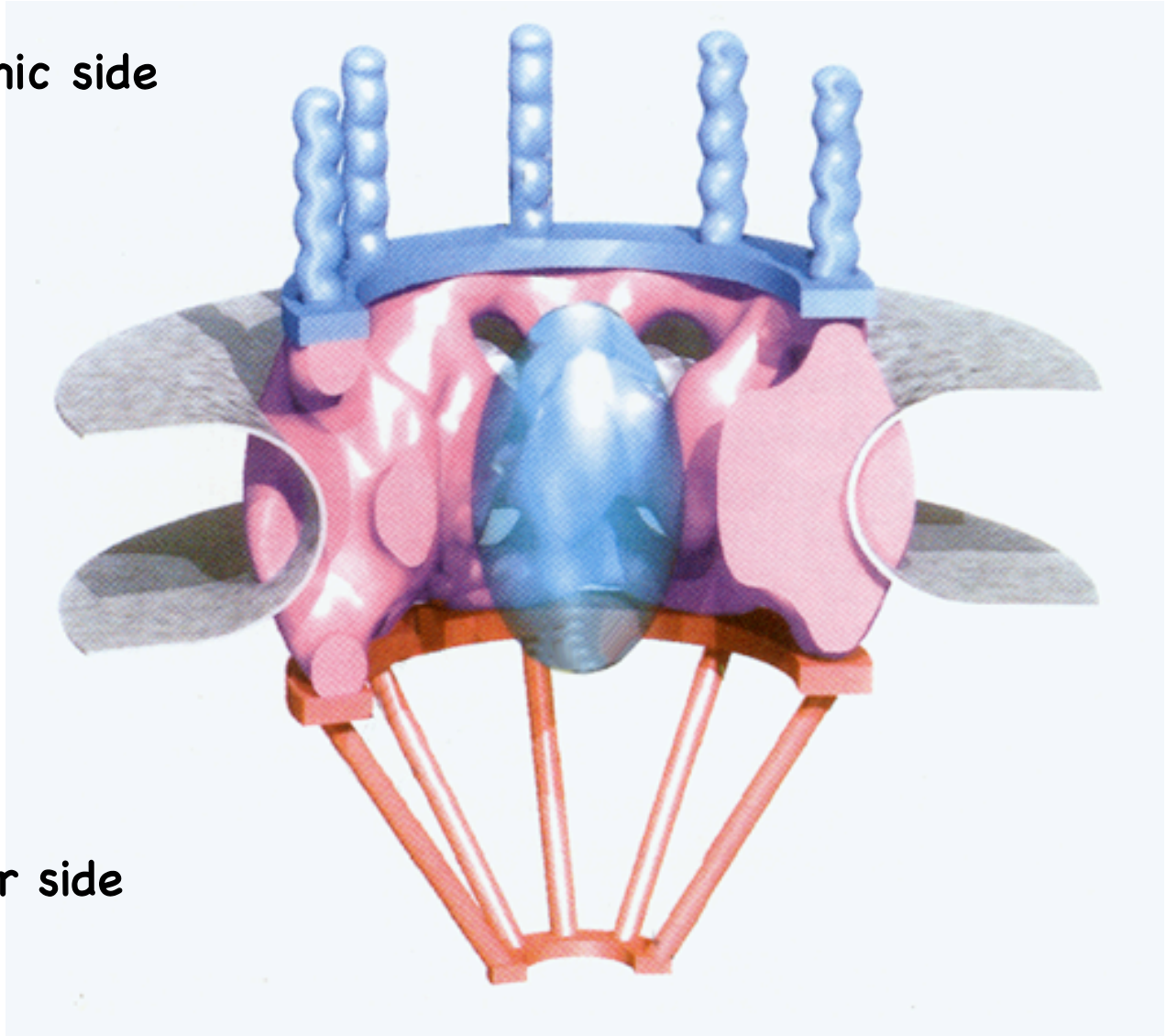
(C)



(D)

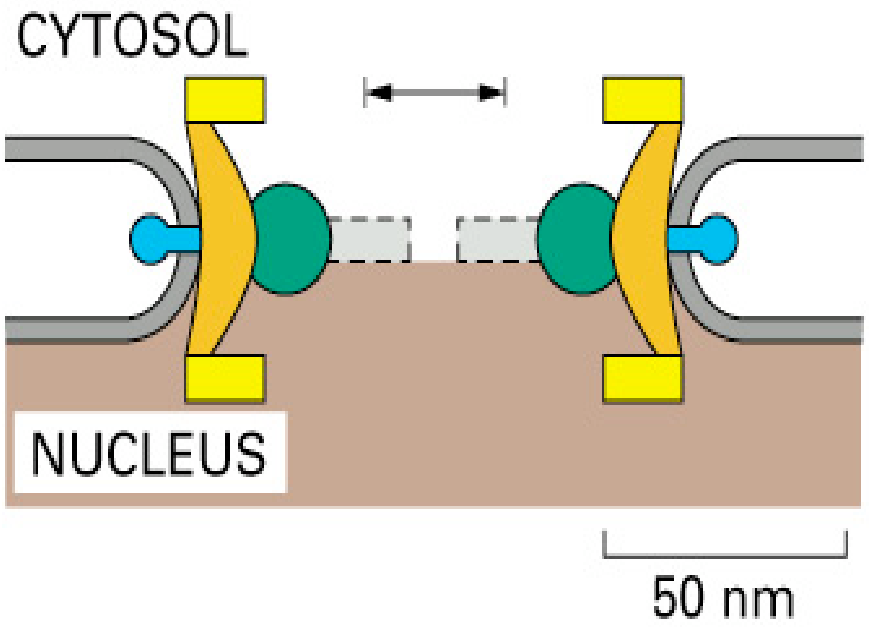
2. The solid phase: the nuclear pore complex (NPC)

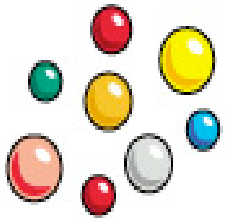
Cytoplasmic side



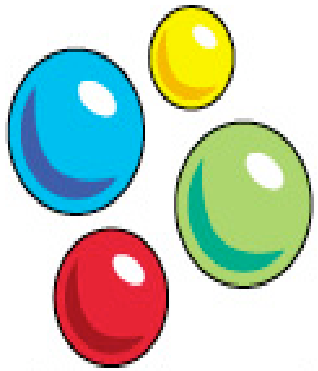
Nuclear side

The nuclear pore has a 9nm wide aqueous diffusion channel





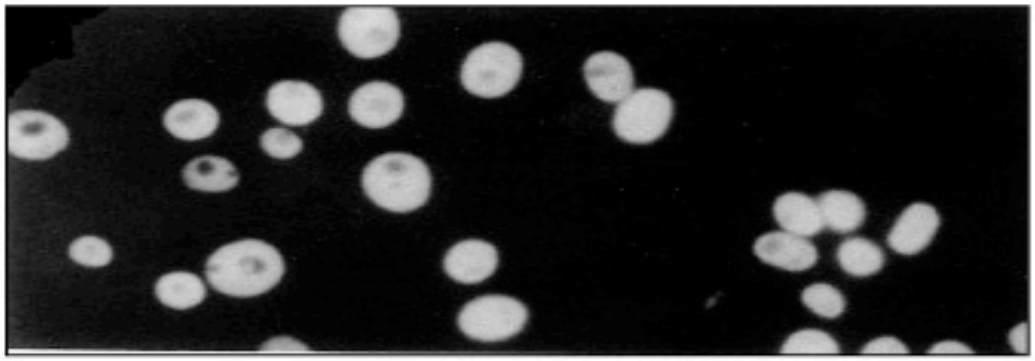
size of proteins that enter nucleus by free diffusion



size of proteins that enter nucleus by active transport

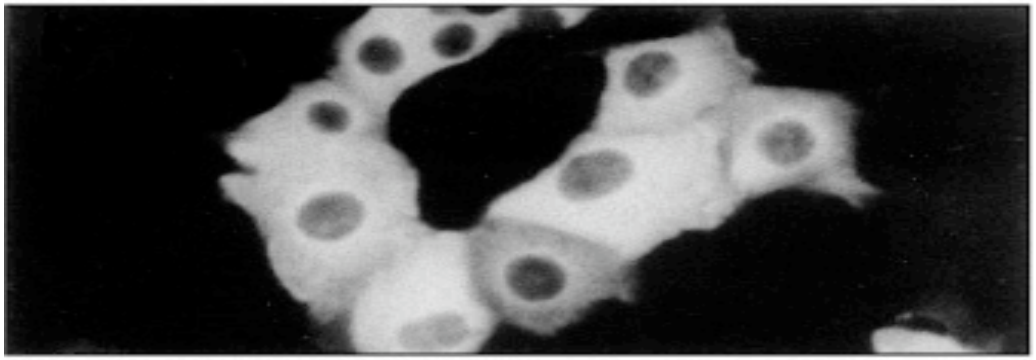
(A) LOCALIZATION OF T-ANTIGEN CONTAINING ITS NORMAL NUCLEAR IMPORT SIGNAL

Pro–Pro–Lys–Lys–Lys–Arg–Lys–Val–

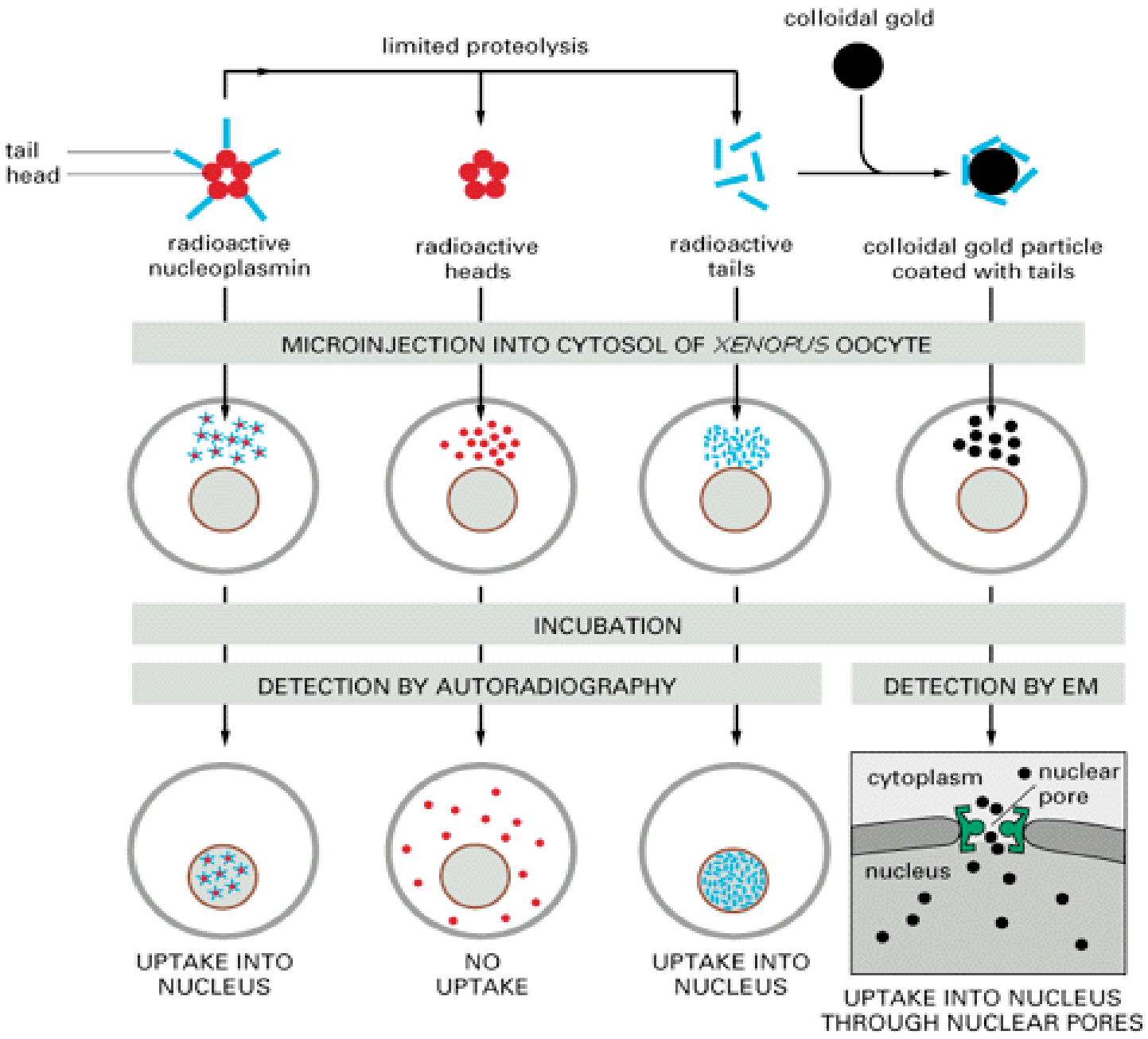


(B) LOCALIZATION OF T-ANTIGEN CONTAINING A MUTATED NUCLEAR IMPORT SIGNAL

Pro–Pro–Lys–Thr–Lys–Arg–Lys–Val–

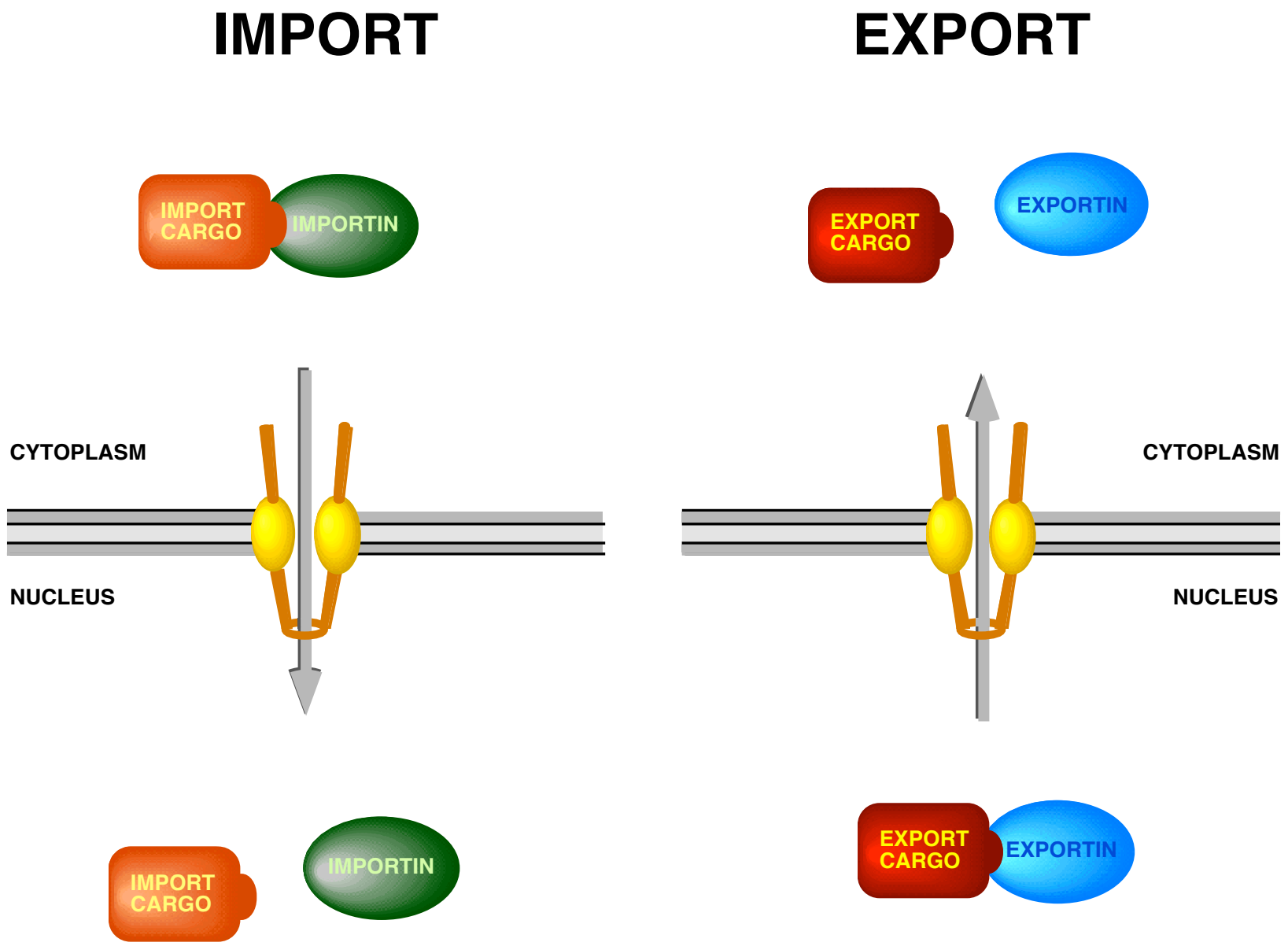


3. Signals: how were they identified?

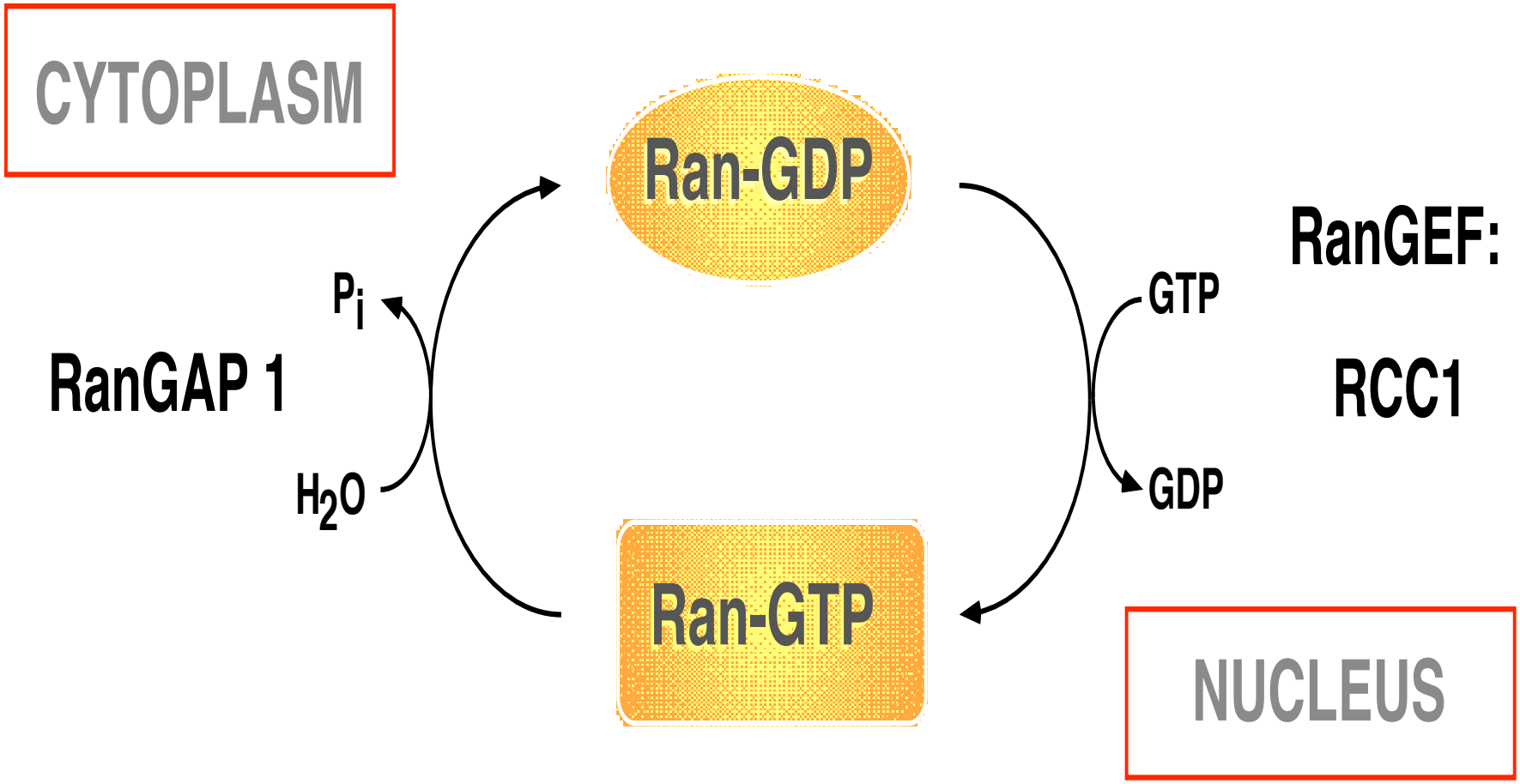


4. The soluble phase

a. Importins and Exportins



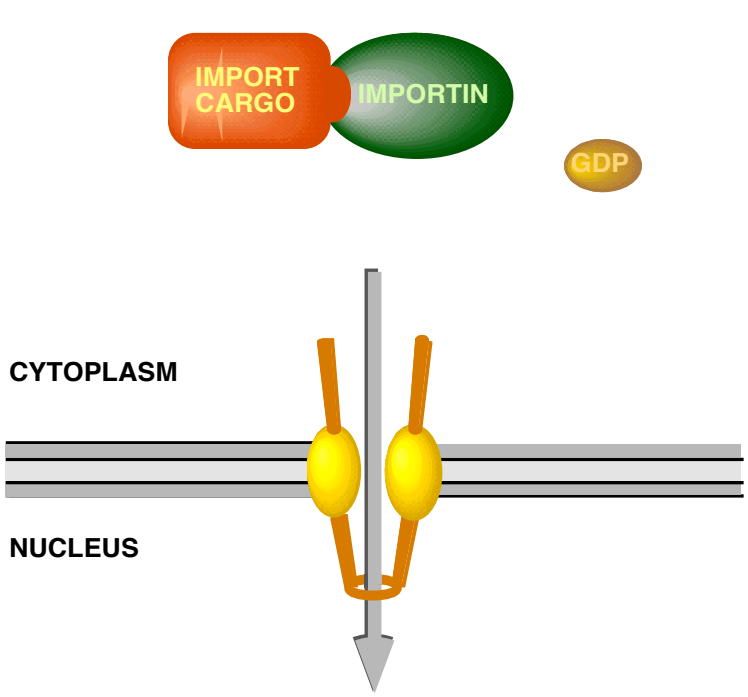
4. The soluble phase b. The GTPase Ran



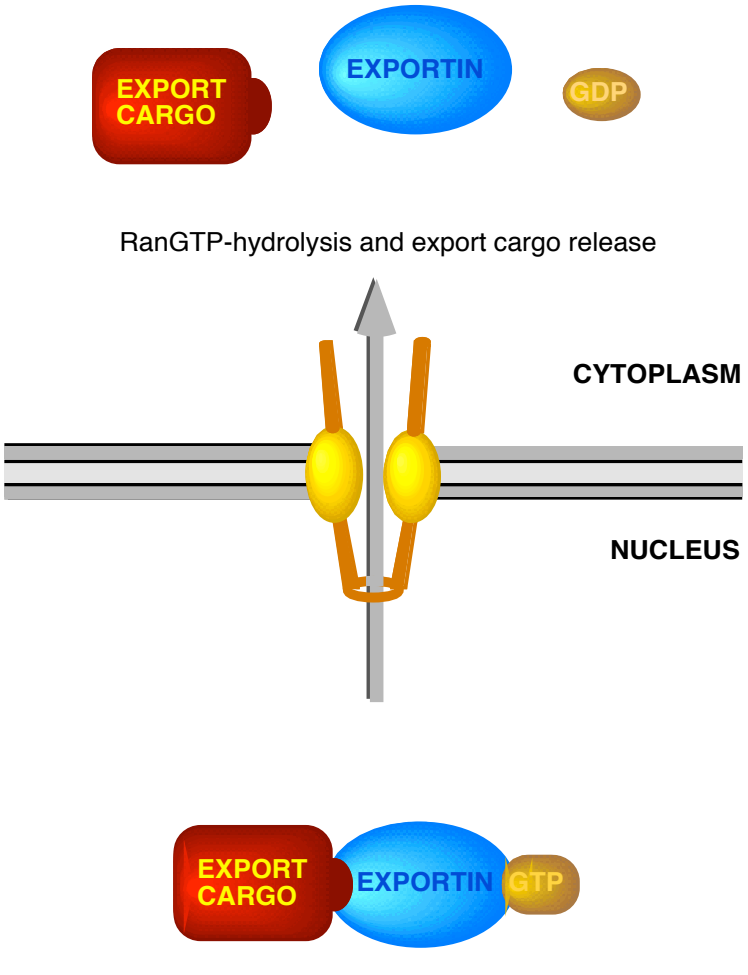
4. The soluble phase

Ran regulates substrate binding and release

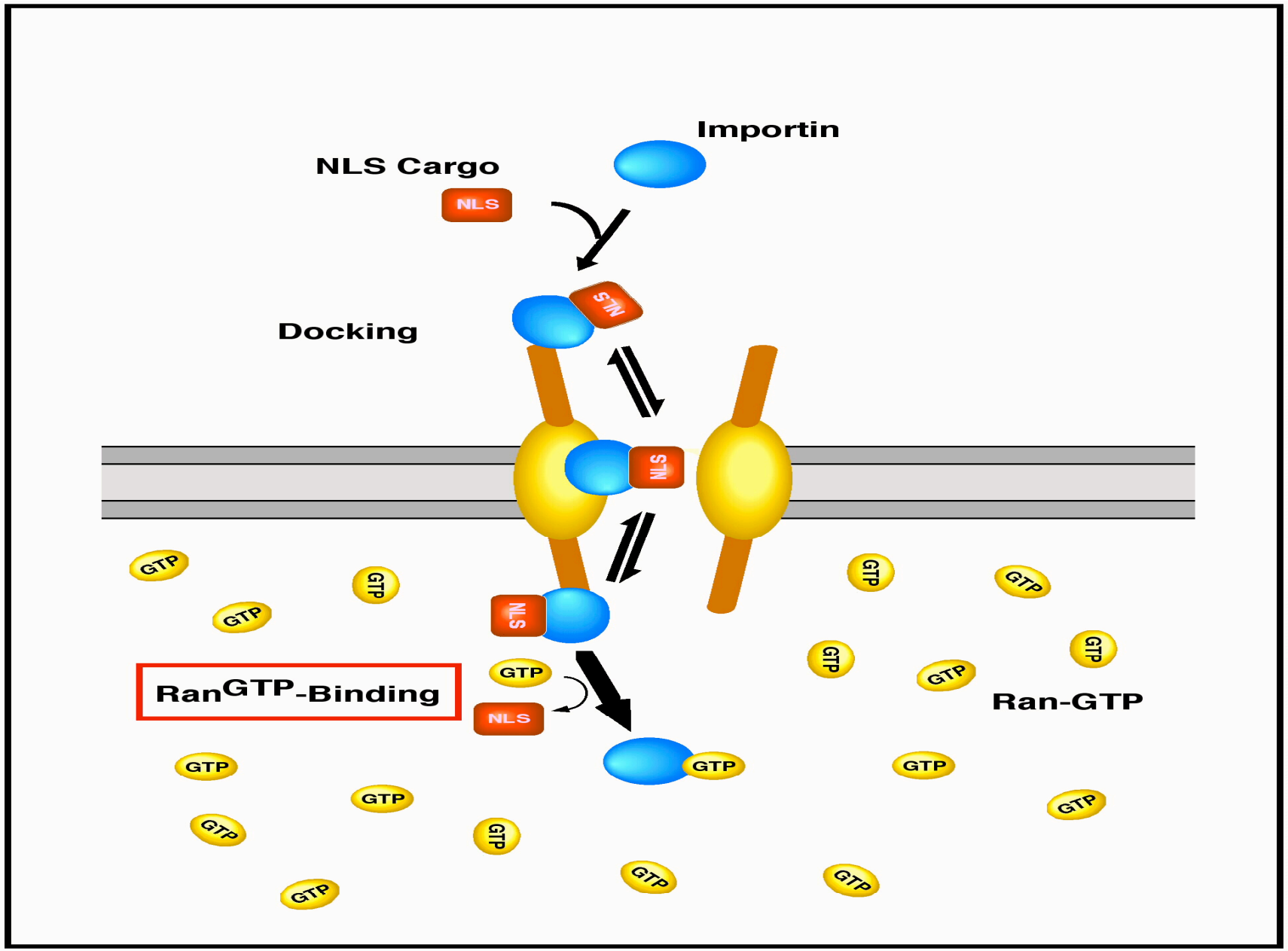
IMPORT

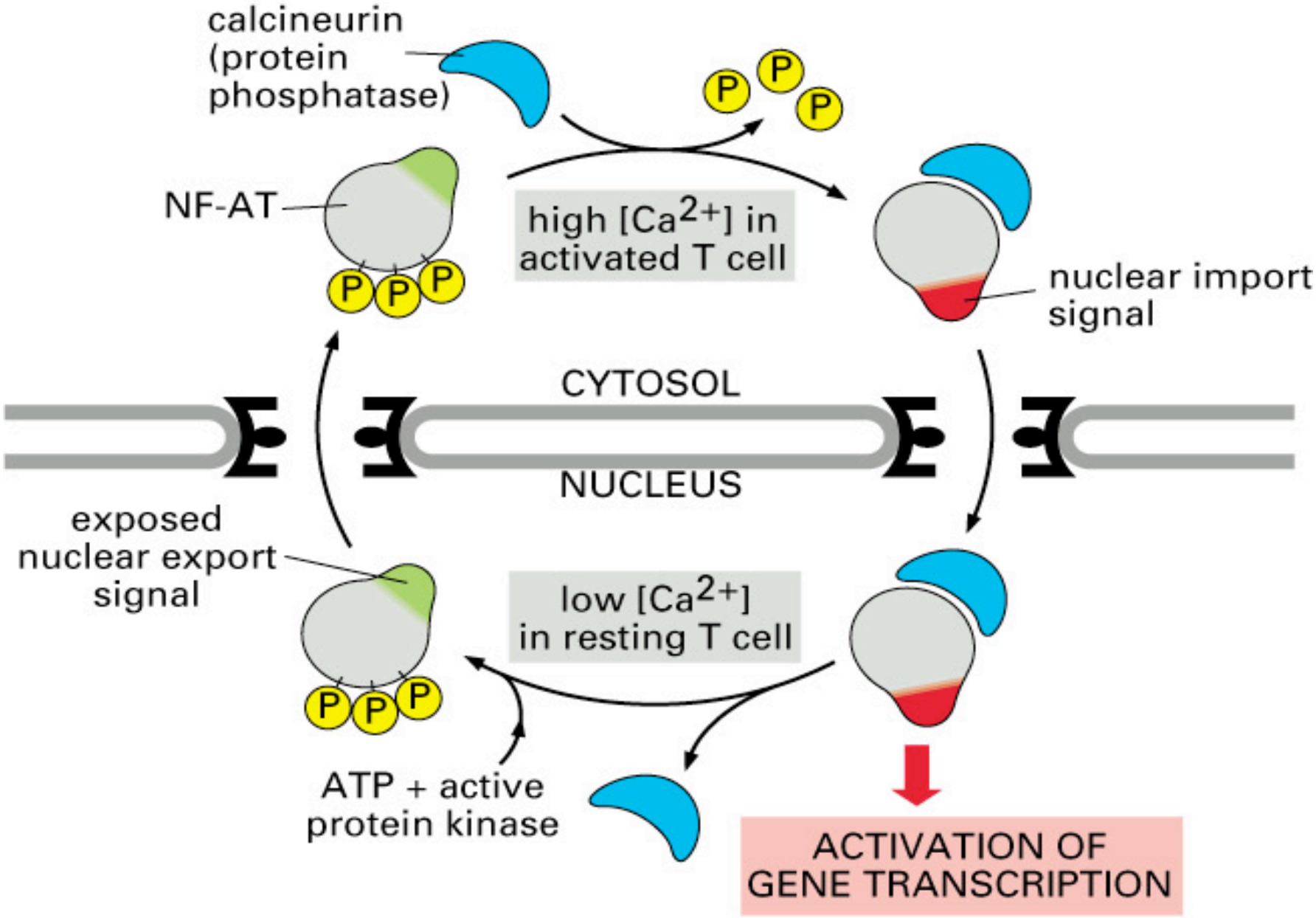


EXPORT



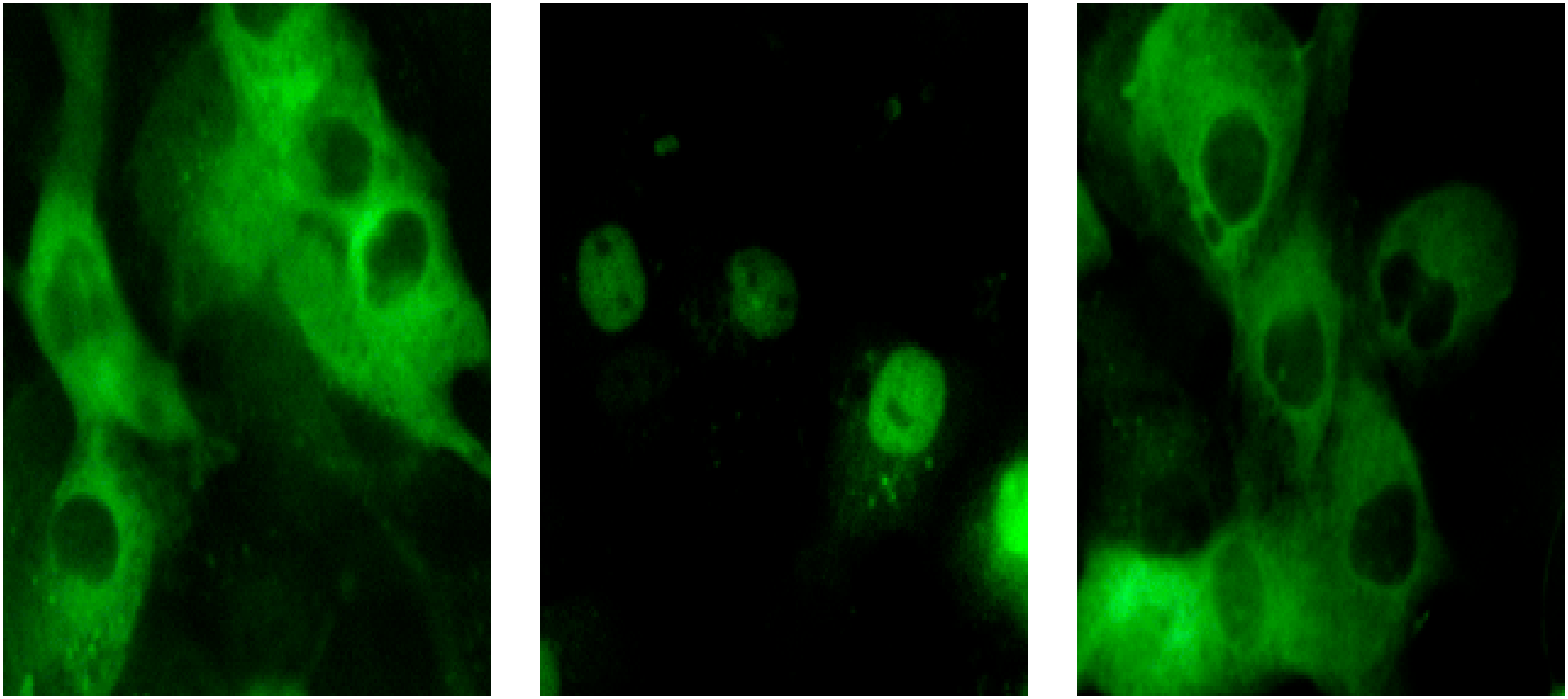
5. A model for translocation through the NPC




calcineurin
(protein
phosphatase)
P
P
P
NF-AT
high [Ca2+] in
activated T cell
nuclear import
signal
P
P
P
CYTOSOL
NUCLEUS
exposed
nuclear export
signal
low [Ca2+]
in resting T cell
P
P
P
ATP + active
protein kinase
ACTIVATION OF
GENE TRANSCRIPTION

Compartmentalisation allows the regulation of gene expression

Example: Reversible nuclear accumulation of NF-AT



SUMMARY

I. Signals target proteins into and out of the nucleus

a. active transport

b. signals are necessary and sufficient

II. Transport occurs through the nuclear pore complex (NPC), a large multi-protein complex

a. NPC has aqueous channel

b. no unfolding of cargoes is required

c. transport is bi-directional

III. Signals are recognized by soluble receptors: Importins and exportins

a. bind specific classes of cargo

b. shuttle between cytoplasm and nucleus

c. interact with nucleoporins

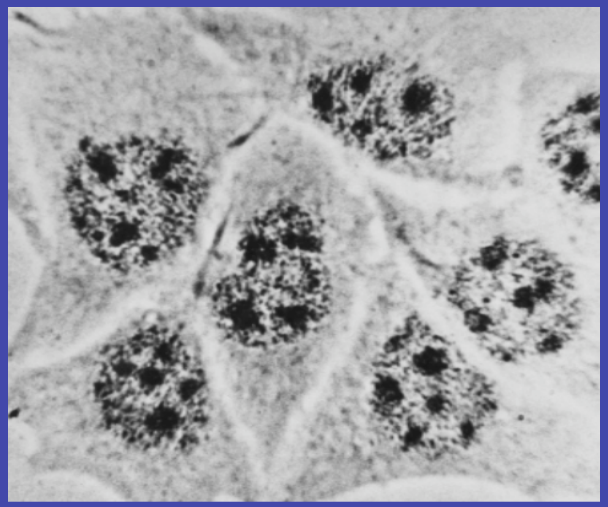
IV. Ran-GTP defines the nucleoplasm and the perichromatin space

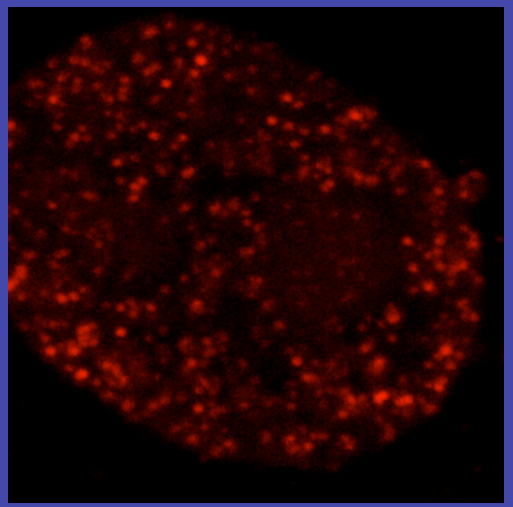
a. Ran-GTP is asymmetrically distributed

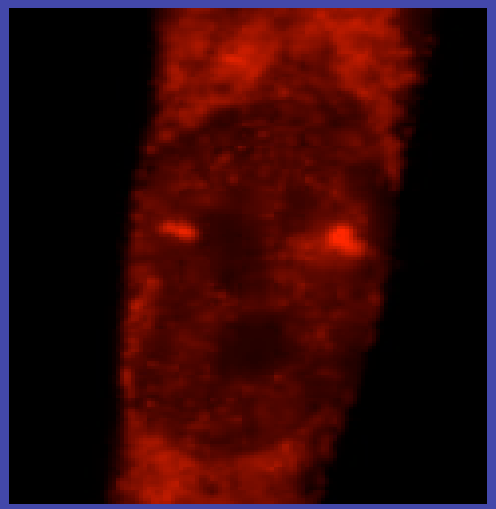
b. Ran regulates cargo binding and release

Visualization of Transcription in Cells or Tissue Sections

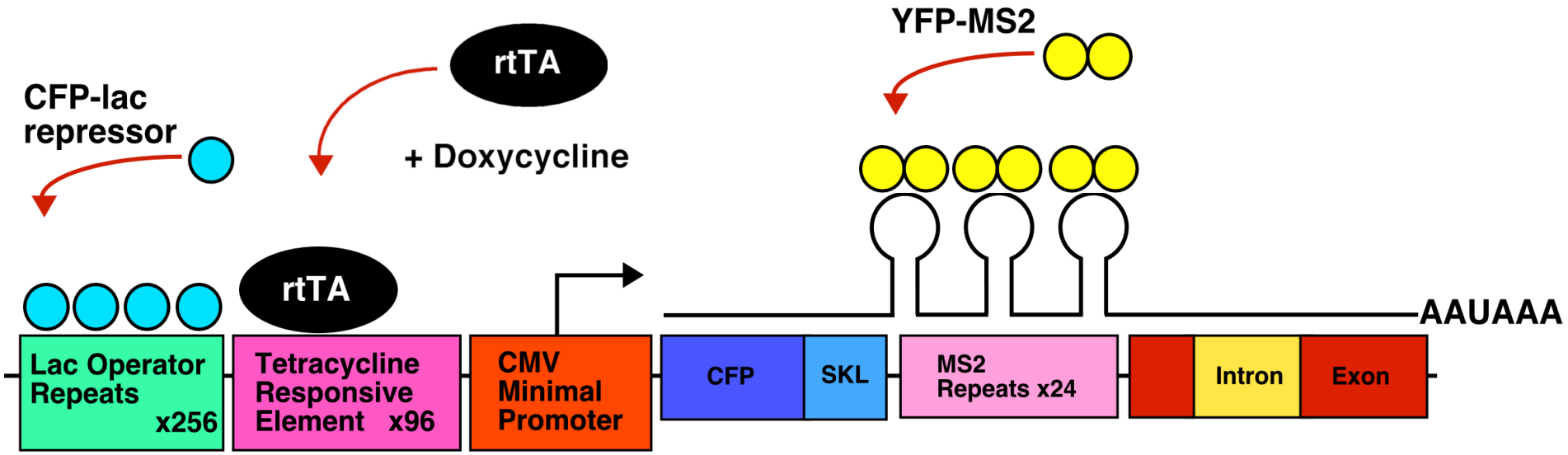
- ^{3}H-Uridine Incorporation
- Br-UTP Incorporation
- Fluorescence In Situ Hybridization





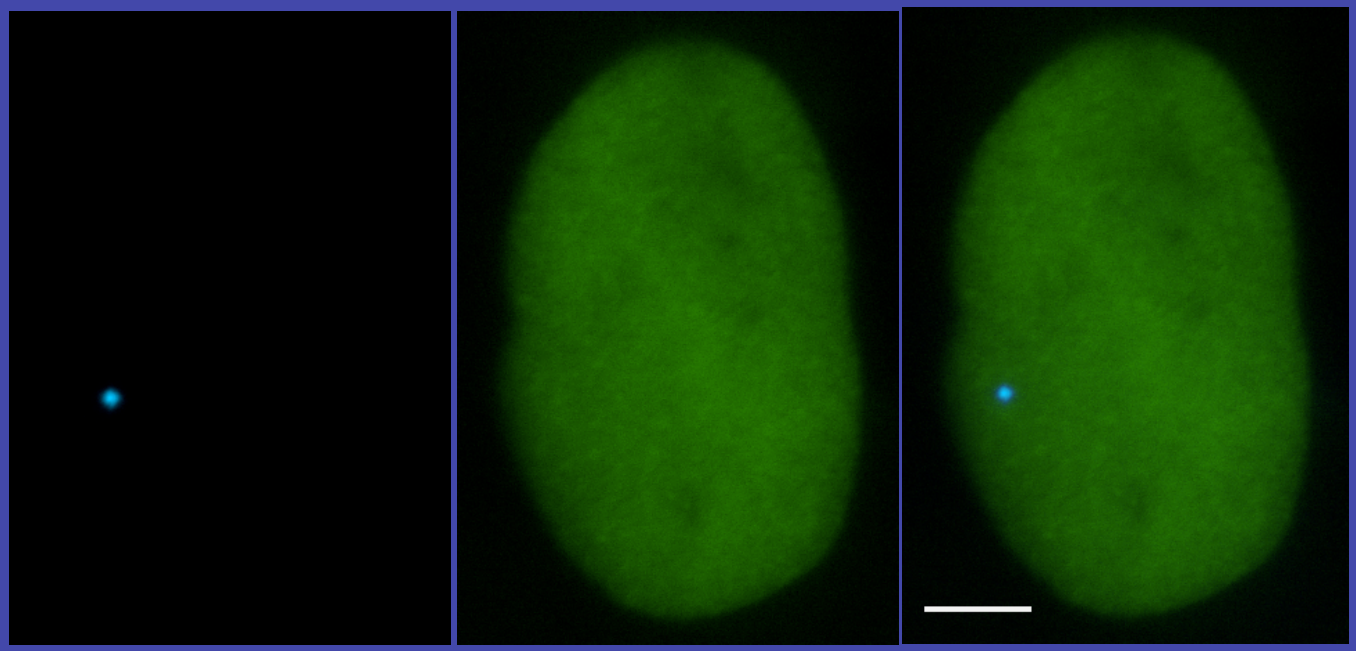


Visualizing Gene Expression in Living Cells



Janicki et al. (2004) Cell 116, 683-698

2.5 hrs. post-transfection (-) Dox



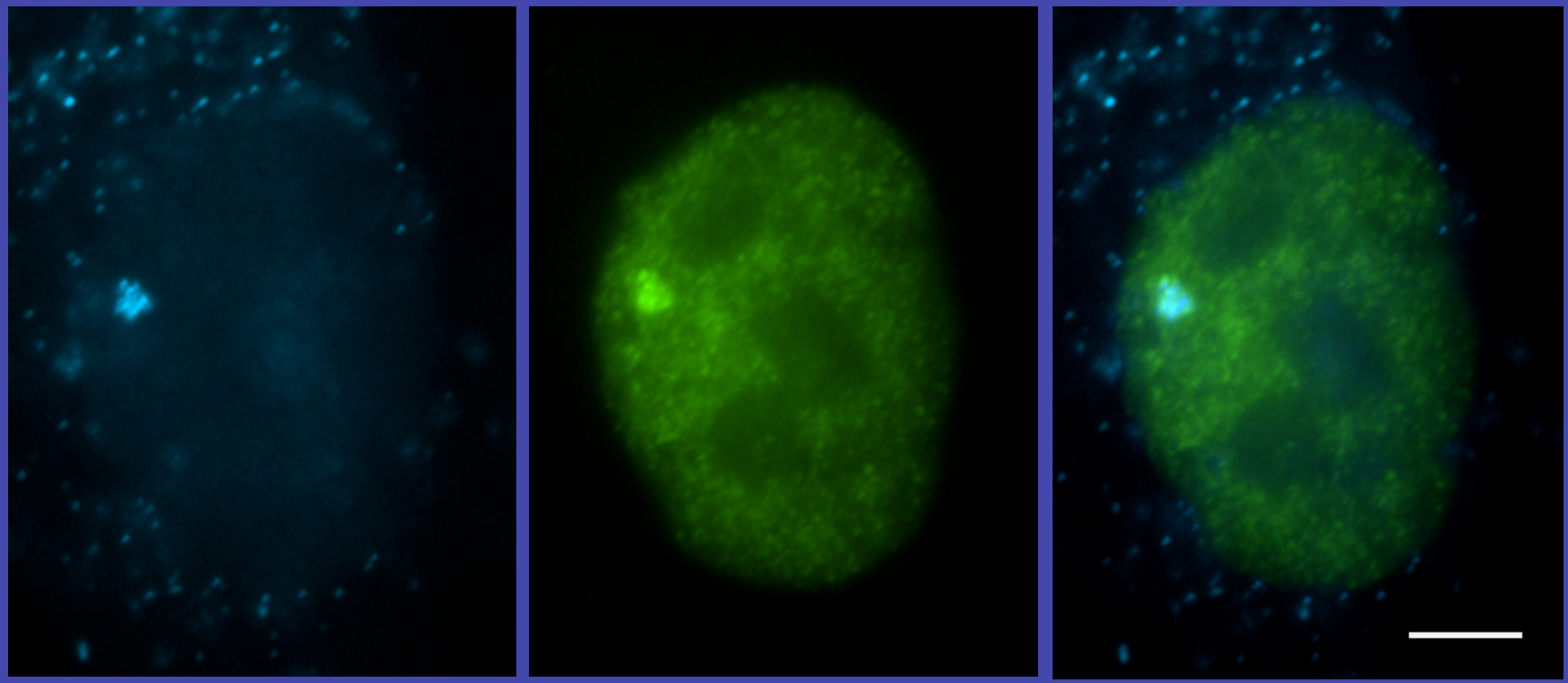
CFP-Lac Repressor

MS2 Binding Protein-YFP

Merge

Janicki et al. (2004) Cell 116, 683-698.

2.5 hrs. post-transfection + 2.5 hrs. after the addition of Dox



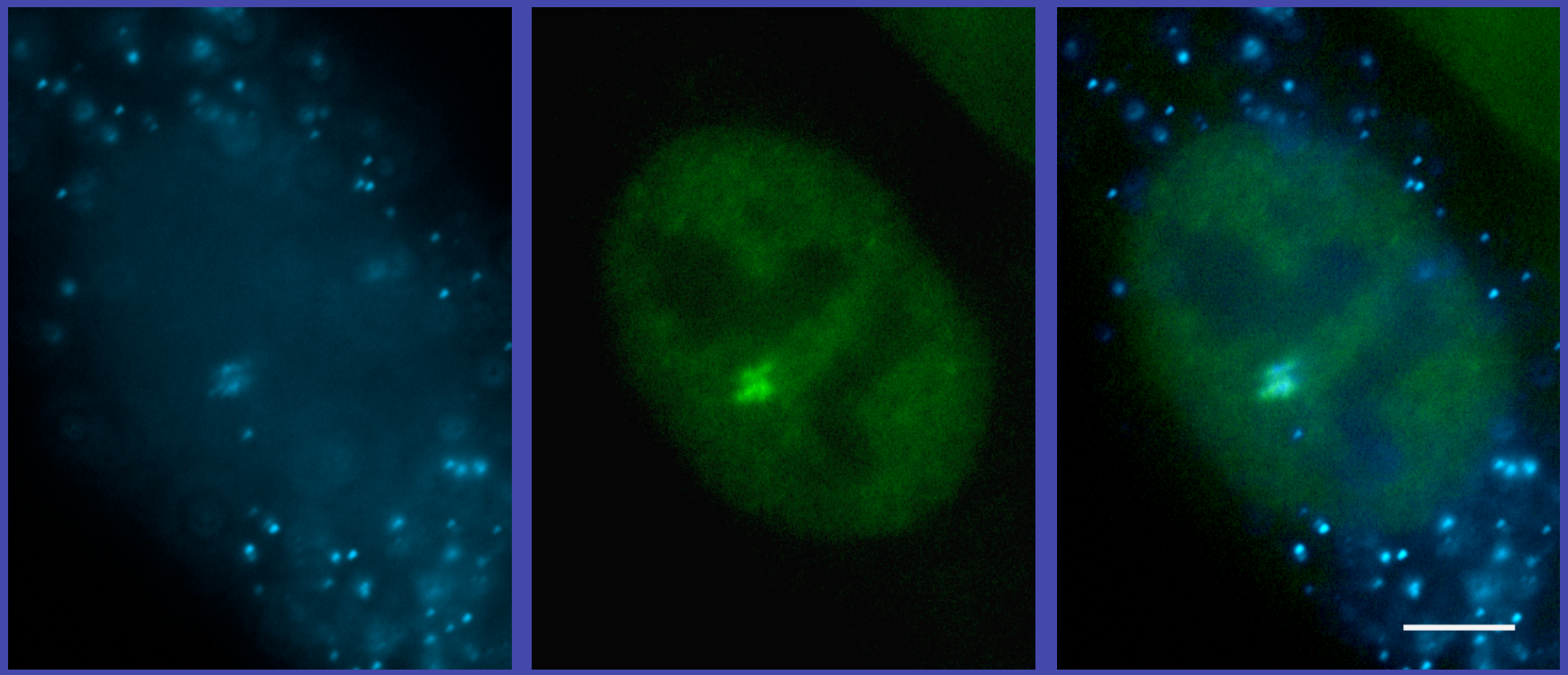
CFP-Lac Repressor
CFP-SKL

MS2 Binding
Protein-YFP

Merge

Janicki et al. (2004) Cell 116, 683-698.

The RNA polymerase II large subunit is recruited to the active locus



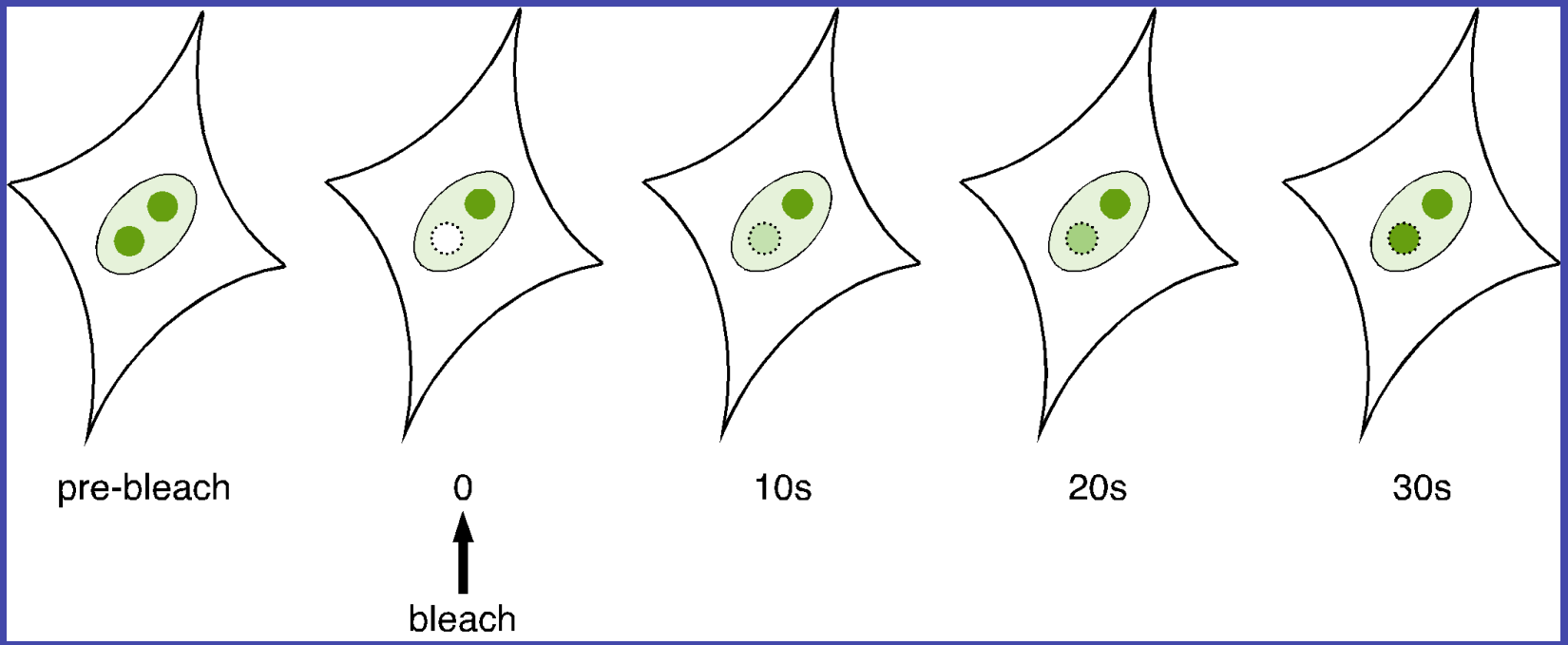
CFP-lac repressor
CFP-SKL

YFP-RNA pol II

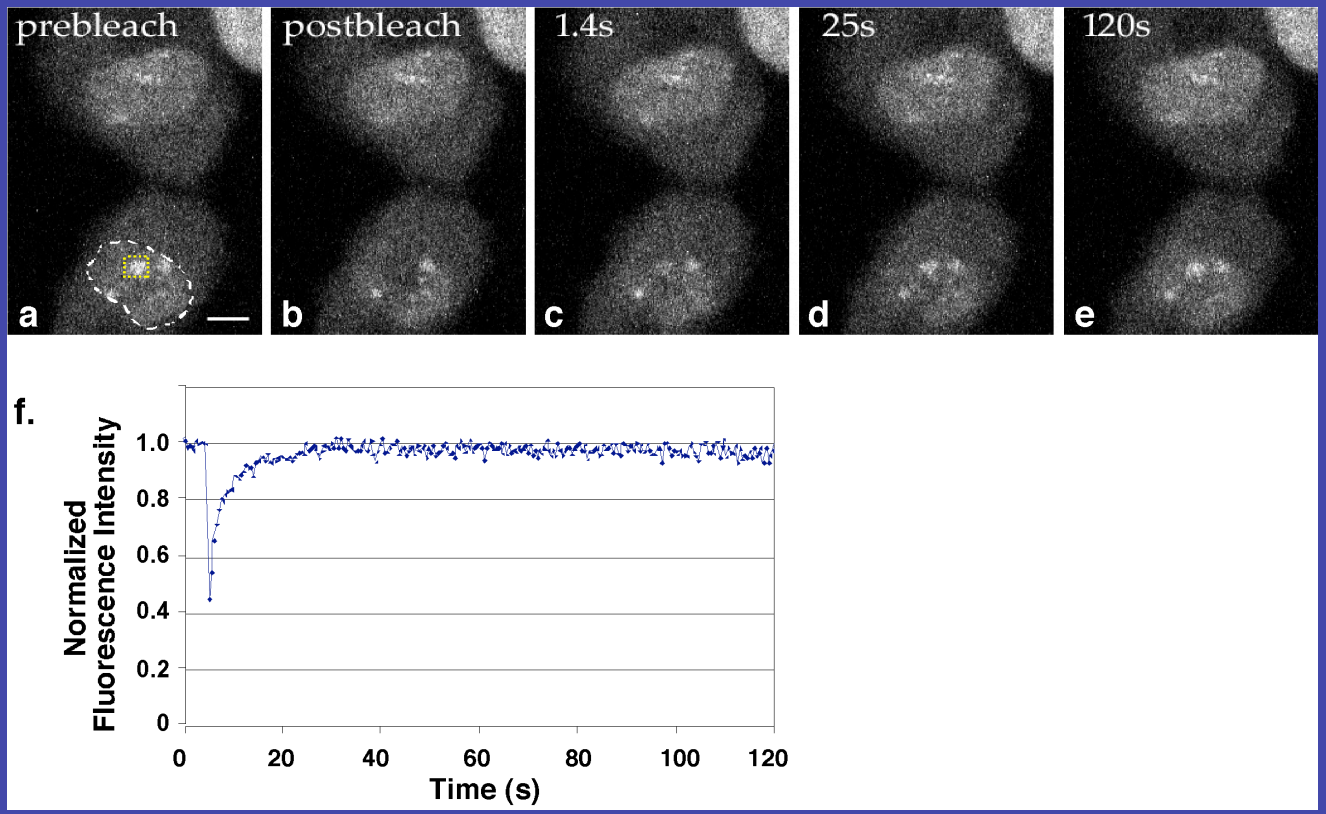
Merge

Janicki et al. (2004) Cell 116, 683-698.

Fluorescence Recovery after photobleaching (FRAP)



FRAP analysis of a pre-mRNA splicing factor in the cell nucleus



SF2/ASF has a half-time of fluorescence recovery of approximately 1.8 (+/- 0.6) seconds.

Bubulya et al. (2004) J. Cell Biol. 167, 51-63.

SUMMARY

I. Single Cell Imaging of Gene Regulation

II. Development of in vivo fluorescent tags

III. New ways to amplify gene loci and detect transcripts

IV. Clever ways of using RNA binding proteins to localize RNA products

V. Using laser to bleach and measure recovery of proteins to a locus

VI. Fluorescence in situ hybridization (FISH) and multi-copy gene arrays to localize specific genes