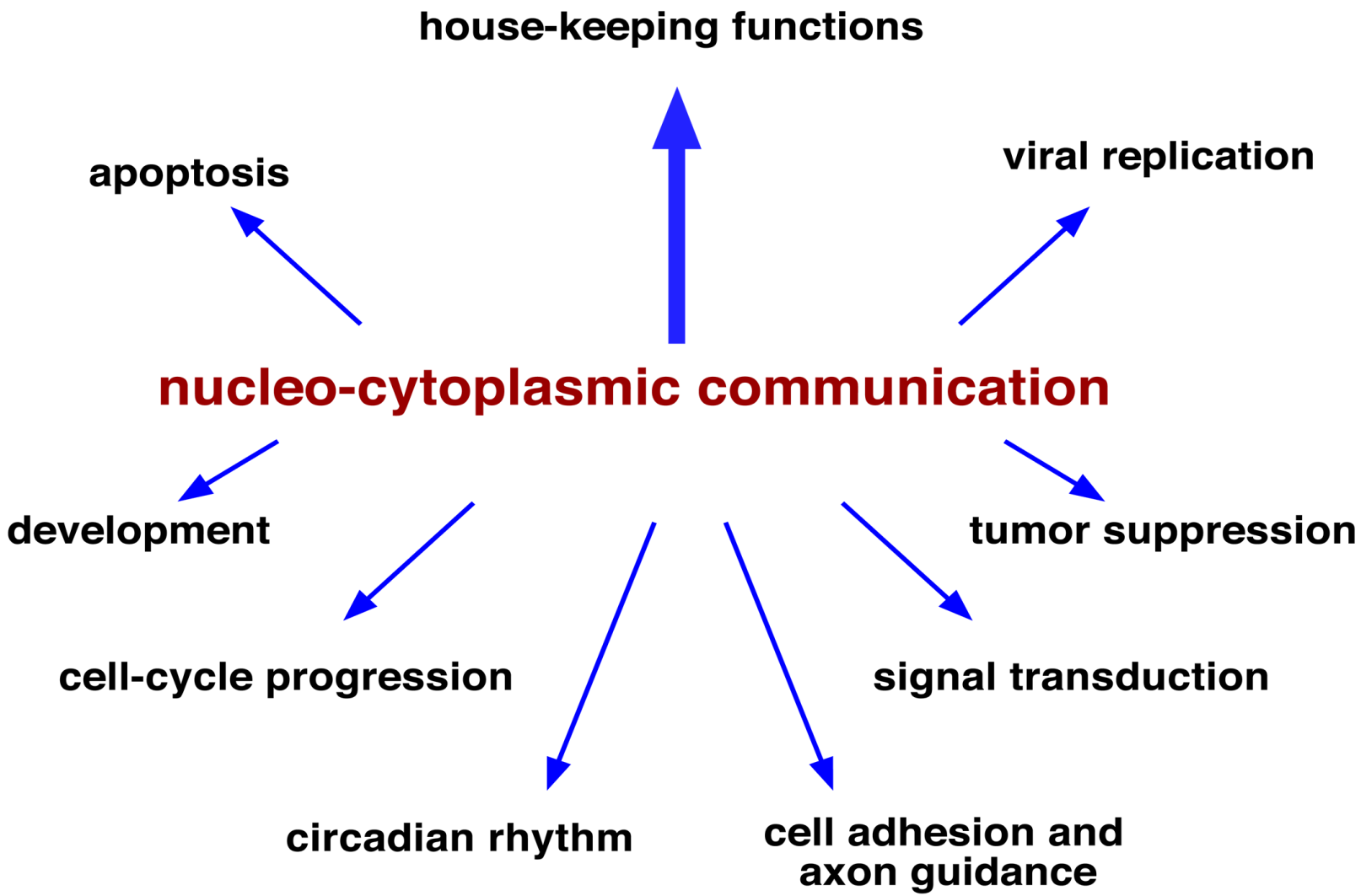
Regulation of nucleo-cytoplasmic transport



Eukaryotic cells control many biological processes by regulating transport into and out of the nucleus.

- cell cycle progession and proliferation
  (CDC25, p27Kip1, p53, APC, cyclinB, survivin...)

- signal transduction pathways
  (MAPK, NF-ATs, NF-kB, glucocorticoid receptor…)

- response to environmental stimuli in yeast (Pho4, Yap1,...)

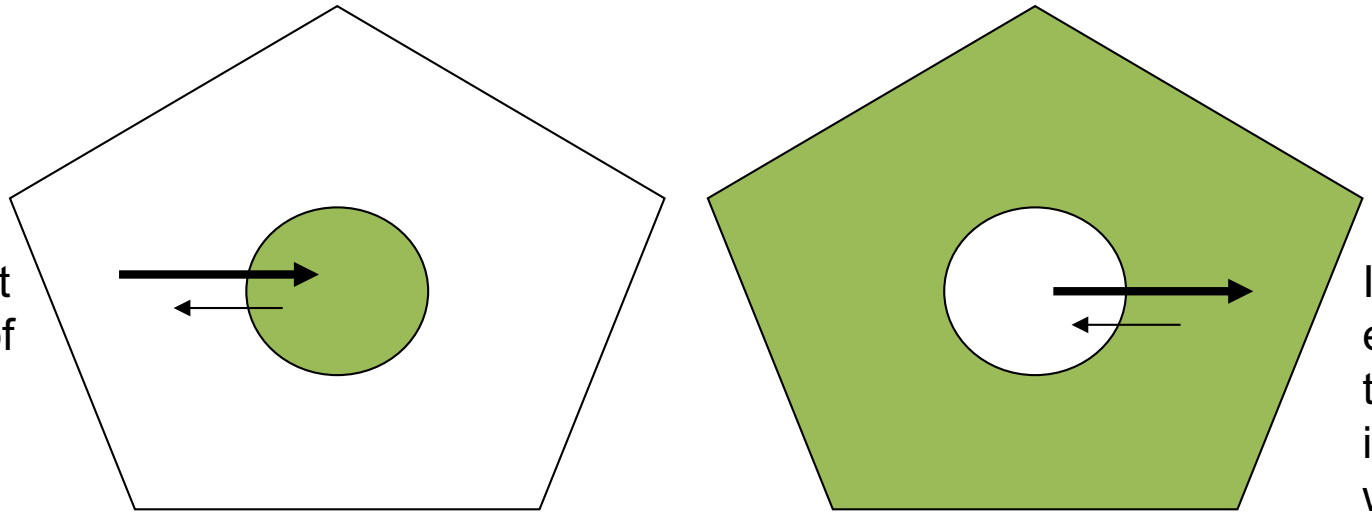
- circadian clock (Period, Timeless)

- organogenesis (SRY)
- muscle differentiation (HDAC4,5)
- bone morphogenesis (Smad1)
- development of nervous, muscular, immune and vascular sytems (NF-ATs)

How can changes in steady-state localization be accomplished?

1. Steady-state localisation of a protein is often determined by its relative rates of nuclear import and export.

If the rate of import exceeds the rate of export, the protein will be localised to the nucleus.



If the rate of export is greater than the rate of import, the protein will be localised to the cytoplasm.

A change in the rate of either import or export can lead to a shift in the steady-state localisation of a protein.

2. Retention in the one or the other compartment plays an equally important role and changes in retention may affect loaclization.

Means to regulate nucleo-cytoplasmic transport

1. Regulation of cargo-transport receptor complex formation (e.g. phosphorylation of cargo)

2. Regulation of cytoplasmic or nuclear anchoring/ retention (also often by phosphorylation)

3. Regulation of the soluble transport machinery

4. Regulation of the NPC

Phosphorylation in regulated transport

only for illustration - dont learn them

- activation/inactivation of NLS or NES signals
  - NLS activation — e.g. Dorsal, SV40 T-ag
  - NLS inactivation — e.g. NF-AT, Pho4p
  - NES activation — e.g. Pho4p
  - NES inactivation — e.g. cyclin B

- complex formation/dissociation
  - dimerization (e.g. MAPK, Period and Timeless)
  - degradation (e.g. NF-kB and IkB, Period and Timeless)
  - binding or release from anchoring partners (e.g. from 14-3-3 proteins)

1. Regulation of cargo-receptor complex formation

we dont need to learn them all - memorize some of them to give them as examples for certain principles for the exam

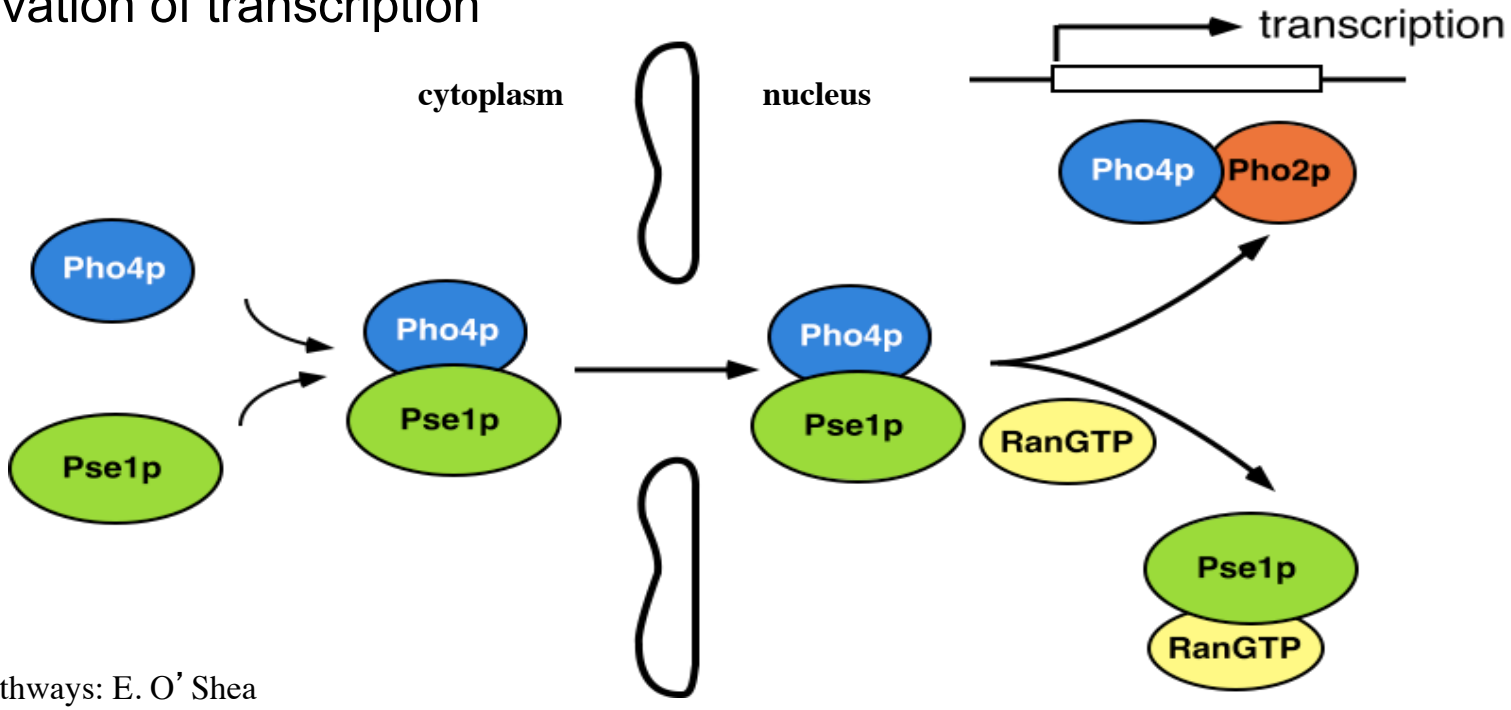
- 1.1. Phosphorylation of cargo
  - 1.1.1. Pho4 (phosphate starvation)
  - 1.1.2. NF-AT
- 1.2. Complex formation
  - 1.2.1. MAPK
  - 1.2.2. NF-κB
  - 1.2.3. Glucocorticoid receptor
- 1.3. Conformational change in cargo
  - yAP1 (response oxidative stress)

Pho4p and the response to phosphate starvation

Transcriptional activator Pho4 is needed for expression of genes under phosphate starvation in yeast.

Low phosphate:

- Pho4p is imported into the nucleus by the importin Pse1p
- Binding to transcription factor Pho2p
- Activation of transcription

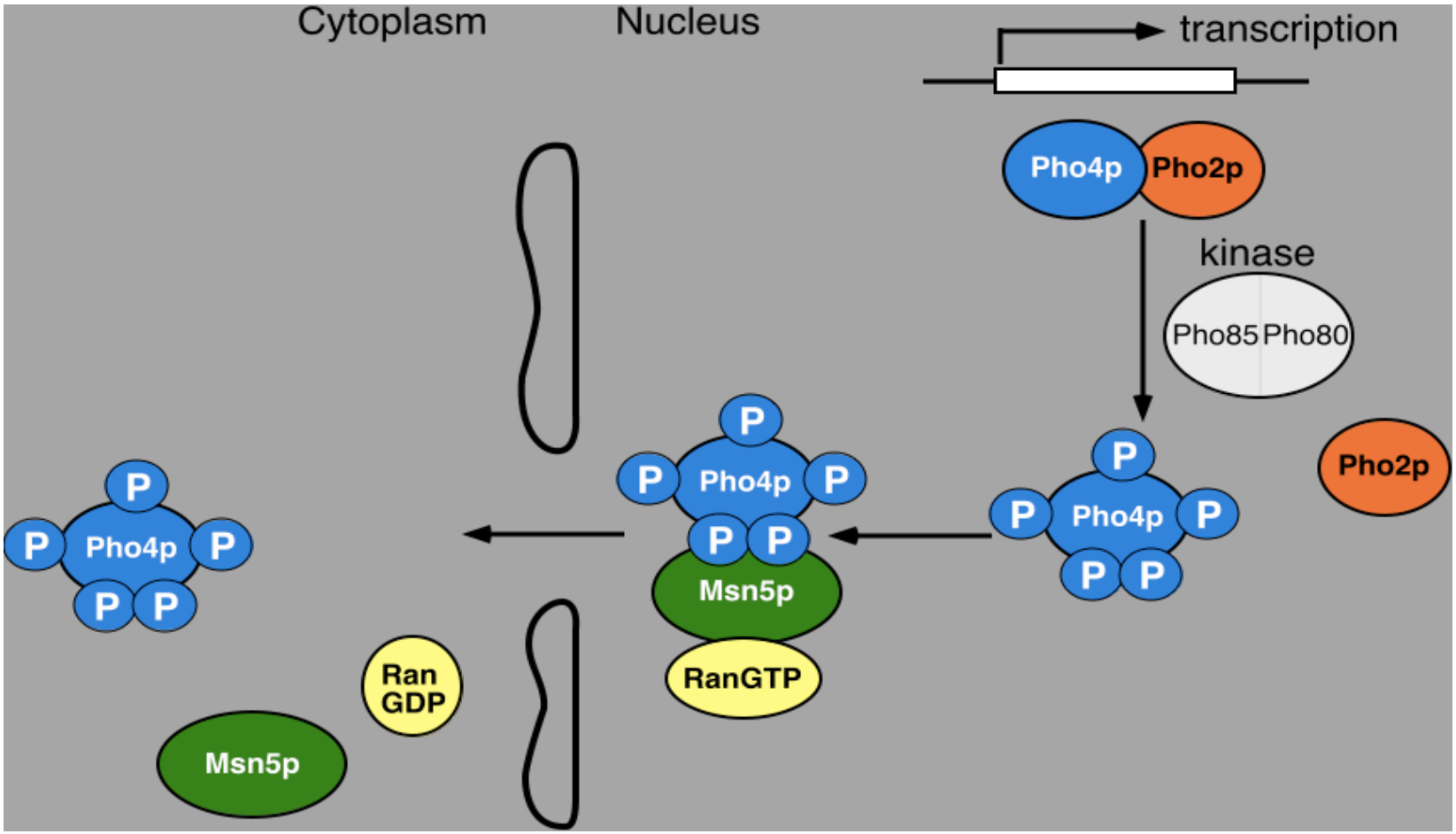


Pho4p import and export pathways: E. O'Shea

Nuclear localisation of Pho4 is regulated by phosphorylation

High phosphate:

- Pho4p is phosphorylated in the nucleus by a kinase complex on five Ser residues
- phosphorylation triggers binding to the exportin Msn5p
- phosphorylated Pho4p is exported to the cytoplasm

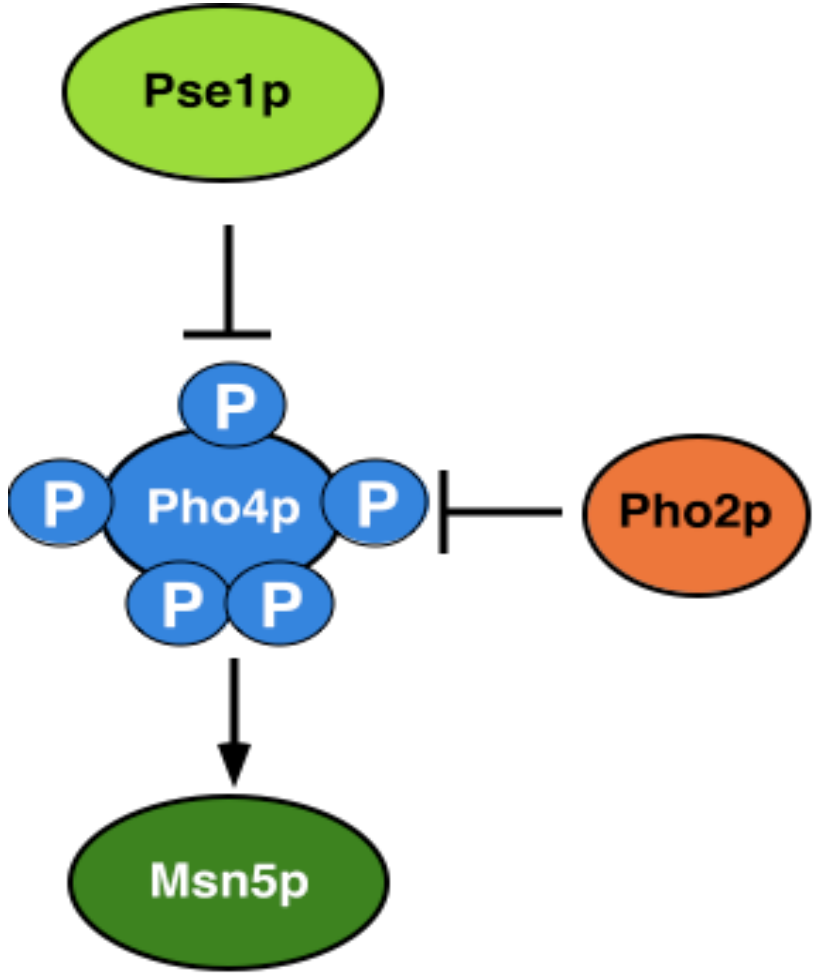


Phosphorylation inactivates Pho4 by three mechanisms

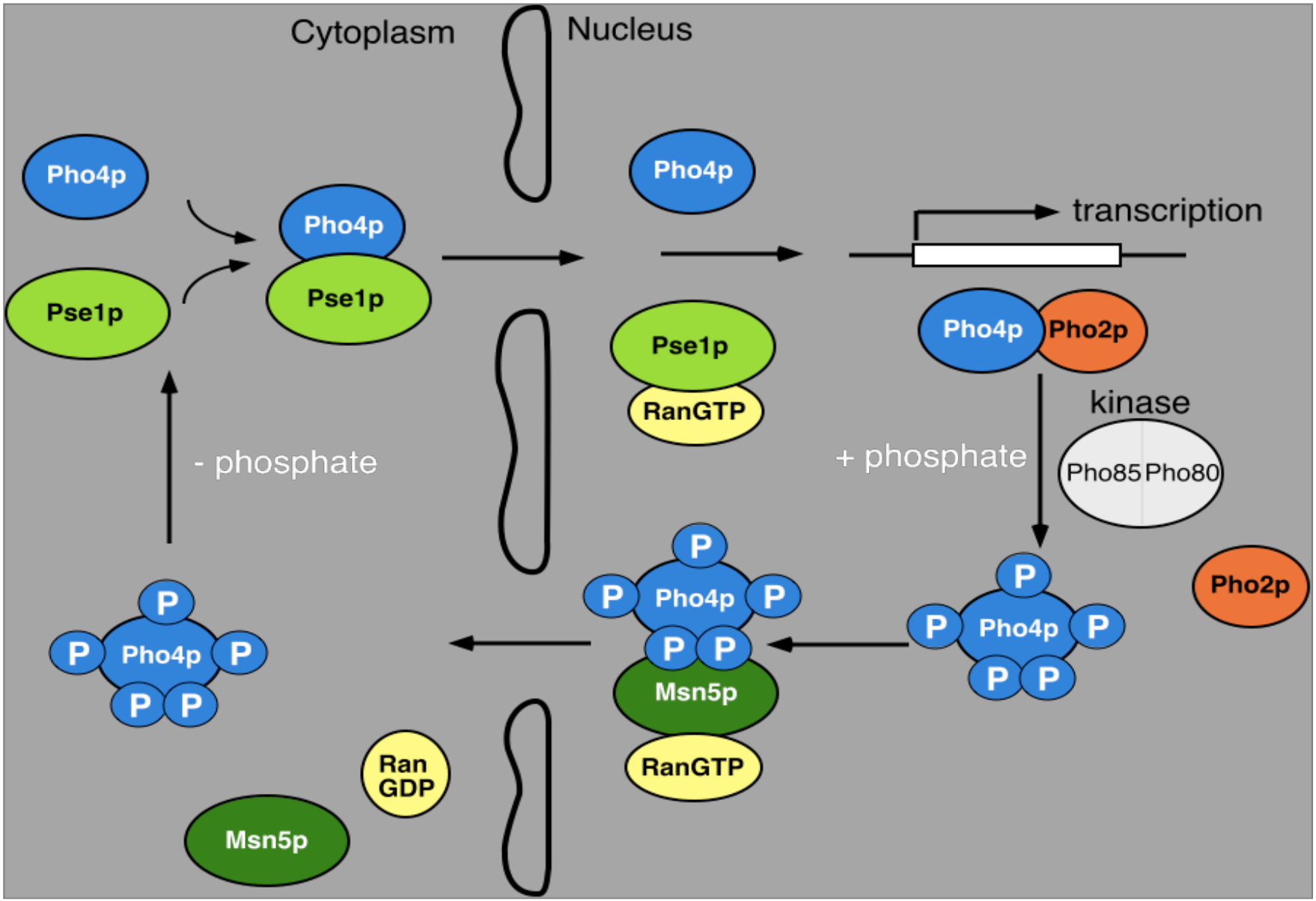
➔ preventing its reimport

➔ dissociating it from Pho2

➔ promoting its export by Msn5

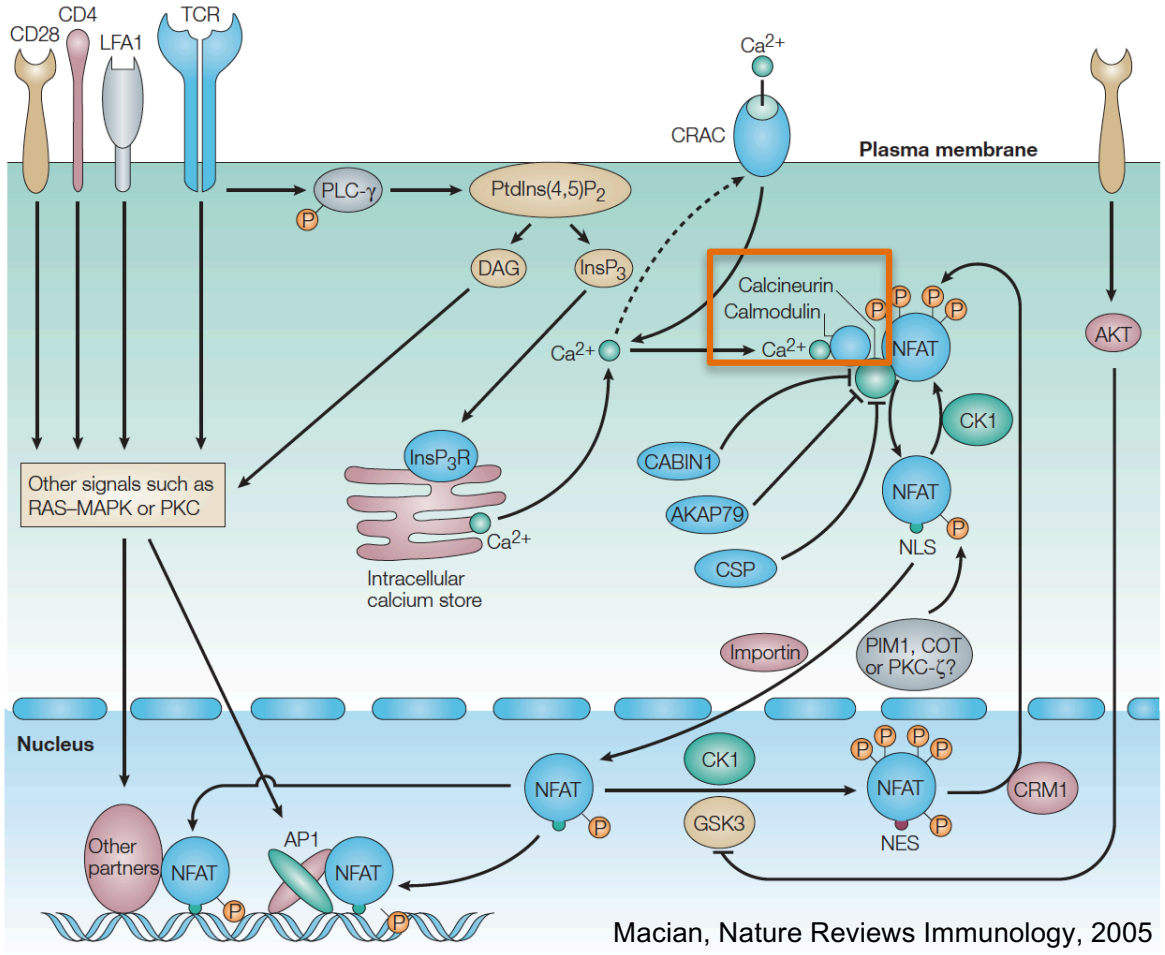


The Pho4 transport cycle



Nuclear factor of activated T cells (NF-AT)

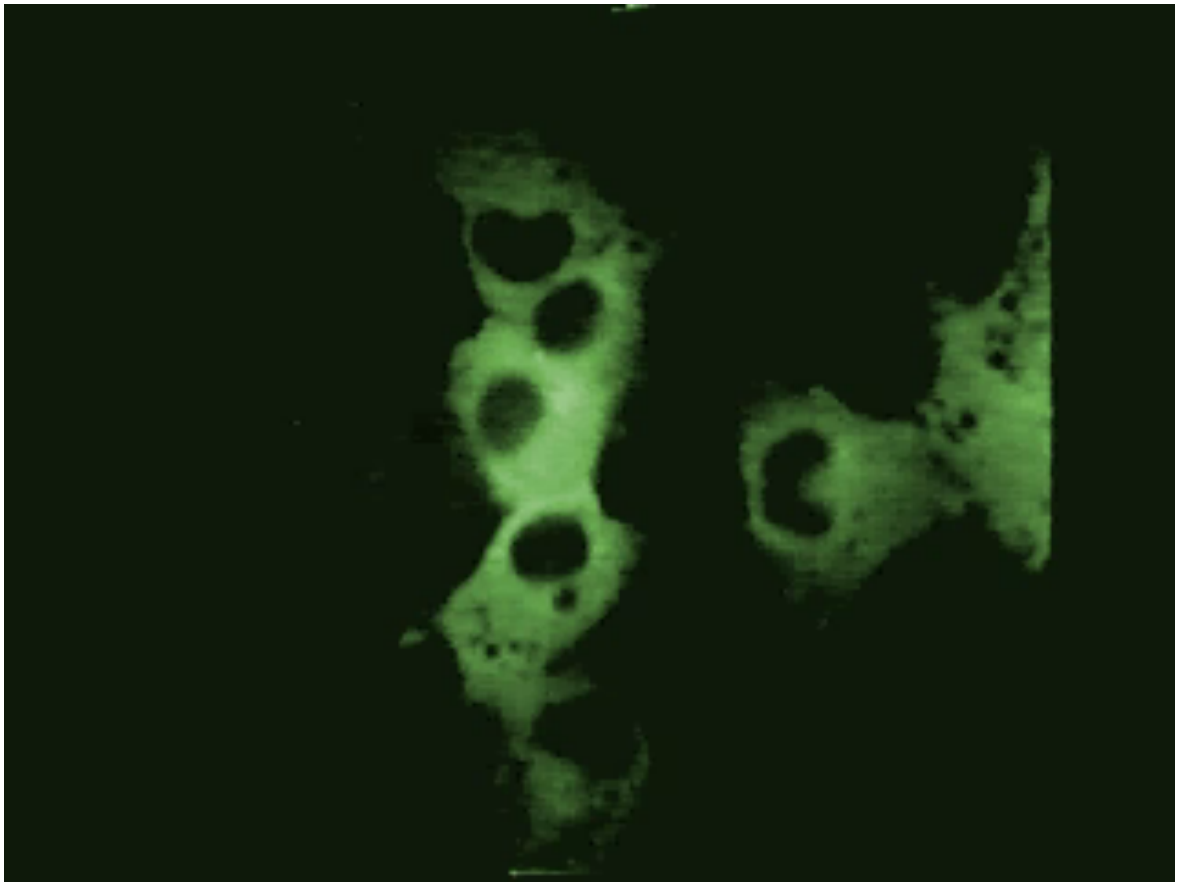
- first discovered in lymphocytes, expressed in most cells of the immune system
- plays a pivotal role in the transcription of cytokine genes and other genes critical for the immune response
- NF-AT also regulates cell differentiation and development



Macian, Nature Reviews Immunology, 2005

activity of NF-AT proteins is tightly regulated by the calcium/calmodulin-dependent phosphatase **calcineurin** on the level of nucleo-cytoplasmic transport

Nuclear factor of activated T cells (NF-AT)

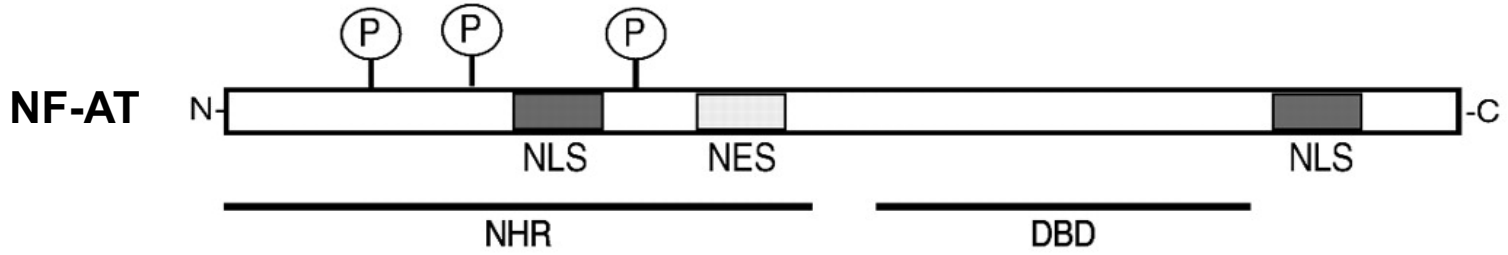


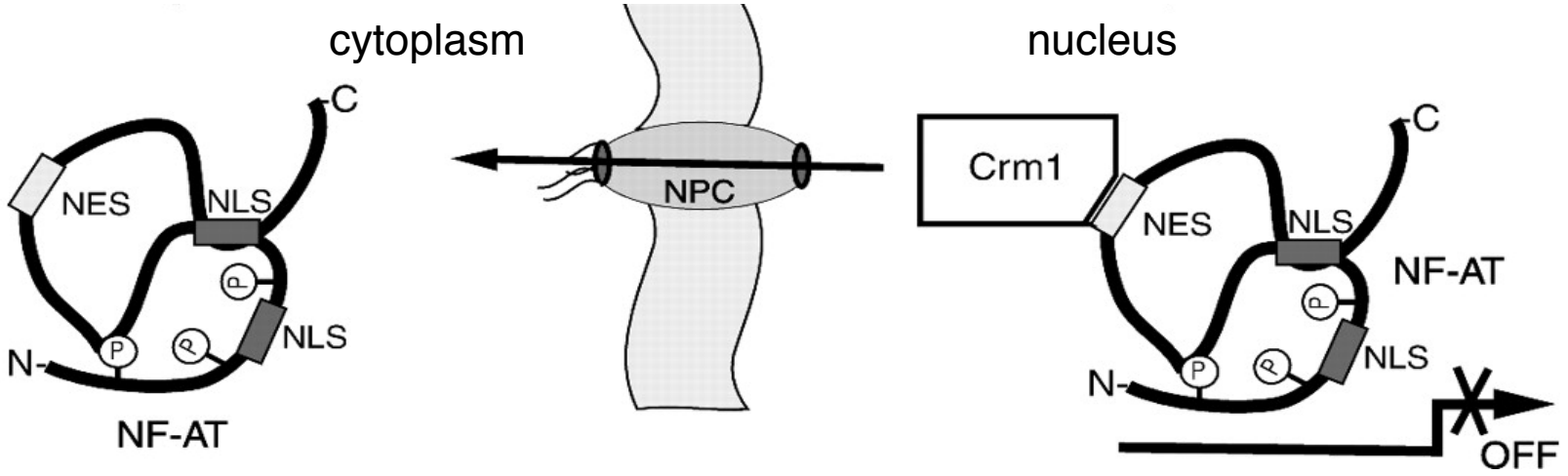
Movie of NF-AT shuttling: Alberts

NF-AT is kept out of the nucleus by CRM1-mediated export

unstimulating conditions:

- NF-AT is phosphorylated and exported to the cytoplasm by virtue of a leucine-rich NES recognised by the exportin CRM1
- phosphorylation decreases the rate of nuclear import





Dephosphorylation triggers import of NF-AT

stimulation of cells (e.g. stimulation of T-cell receptor):

1. elevation of cytoplasmic Ca^{2+} concentration

2. activation of calcineurin; dephosphorylation of NF-AT and NES masking

3. nuclear import and acivation of TK

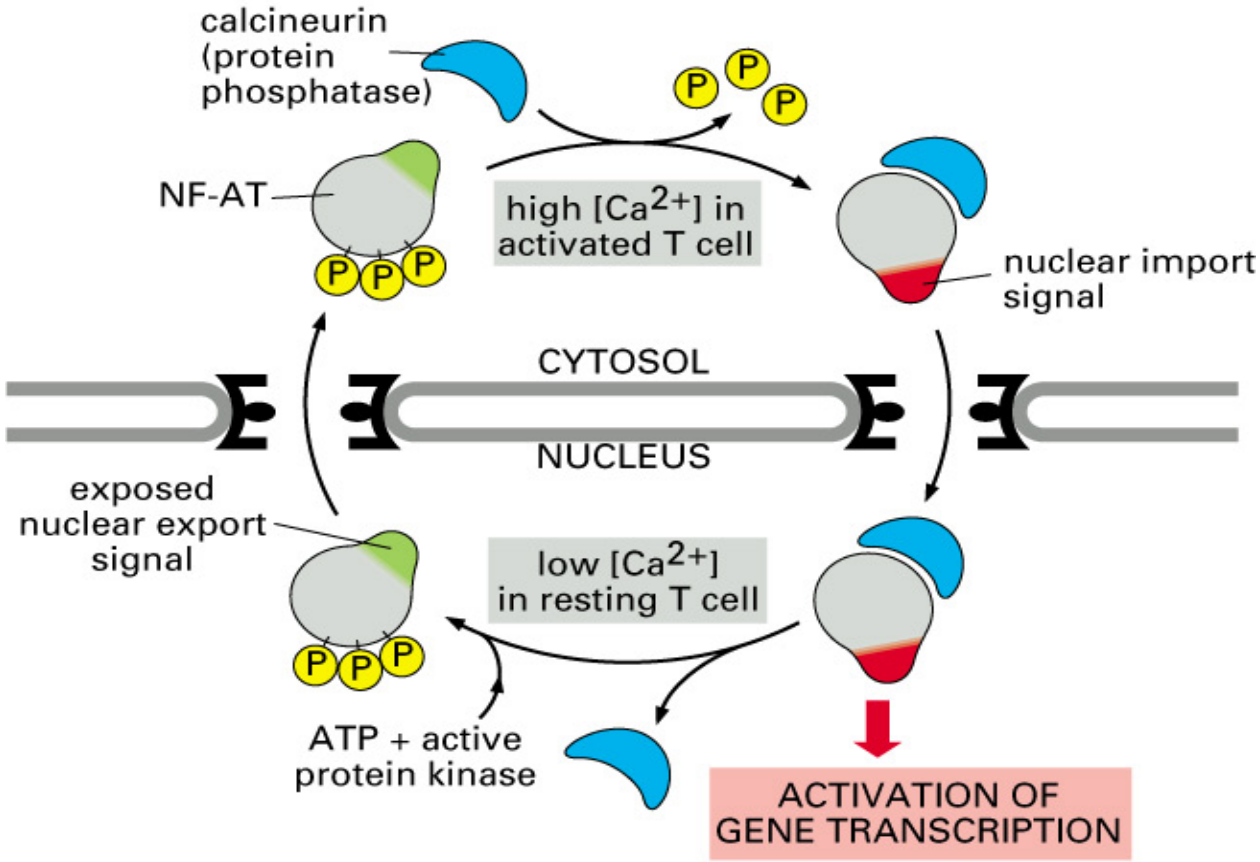
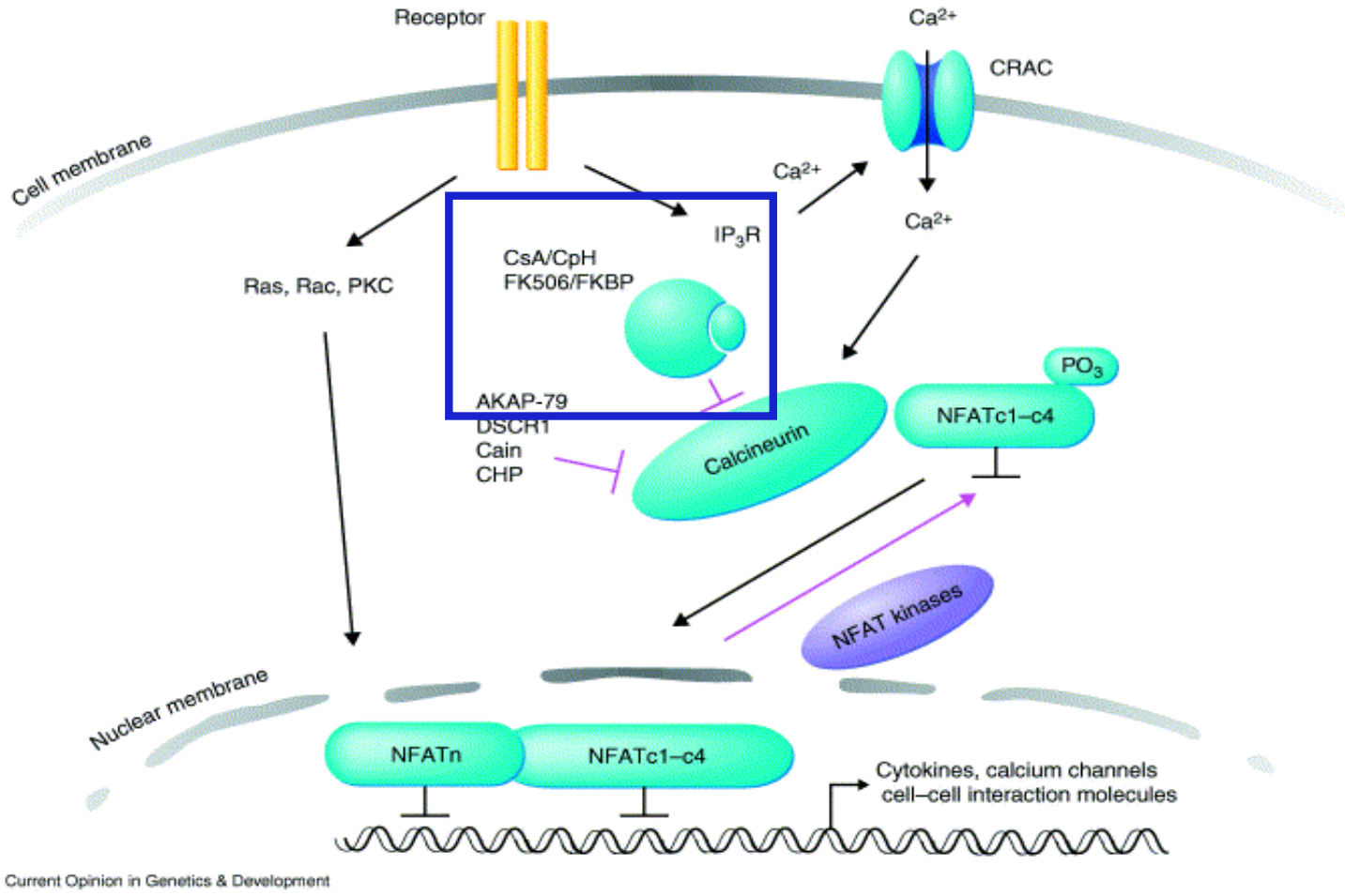


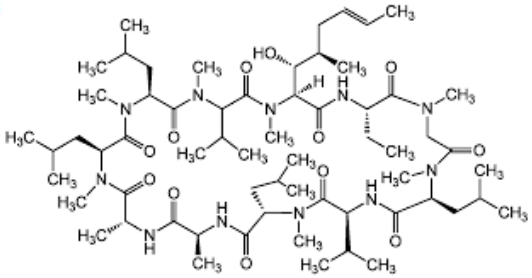
Figure 12–19. Molecular Biology of the Cell, 4th Edition.

Immunosuppressive drugs prevent nuclear accumulation of NF-AT

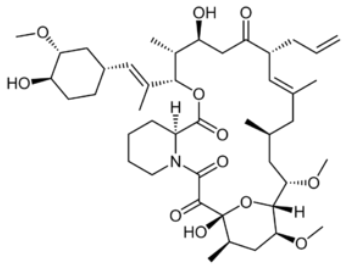
major pathway in our immune response



Cyclosporin A



FK506



cyclosporin A, FK506: inhibit calcineurin

around 200 genes aare regulated by NFAT depending on cell type - there are also 5 isoforms of NFAT

1.2. Complex Formation of Cargo

With itself

With another protein

With an RNA

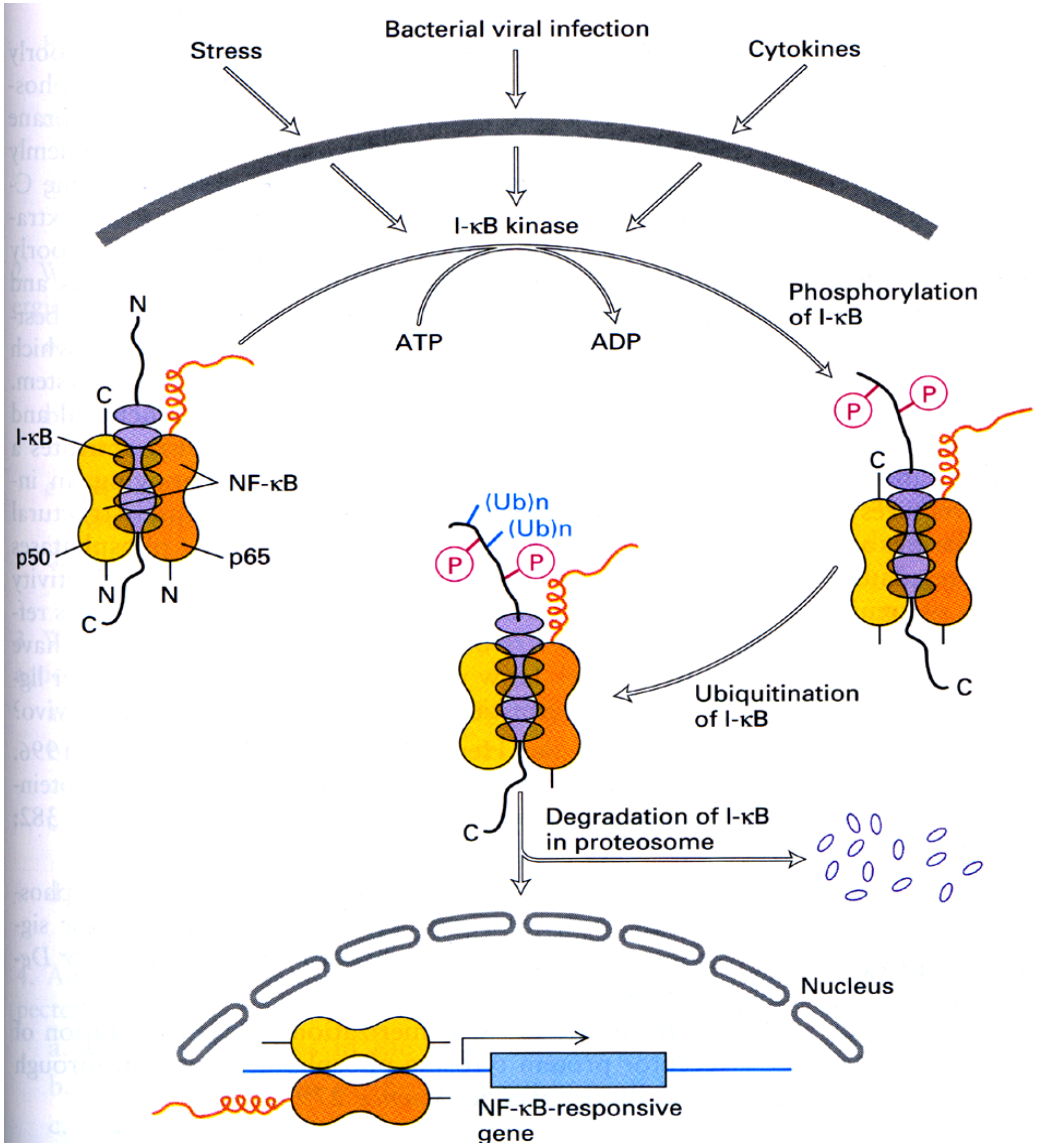
Nuclear import of NF-kB

kappa B

- heterodimeric transcription factor composed of the **RelA (p65)** and the **p50** subunits
- induces transcription of a large number of genes involved in the immune, inflammatory and apoptotic responses.

- activated by numerous stimuli associated with stress, injury or infection:

  - cytokines (interleukin 1b)
  - TNFa
  - lipopolysaccharides
  - dsRNA (via PKR)
  - pro-apoptotic and necrotic stimuli such as oxygen radicals, uv- and g-radiation

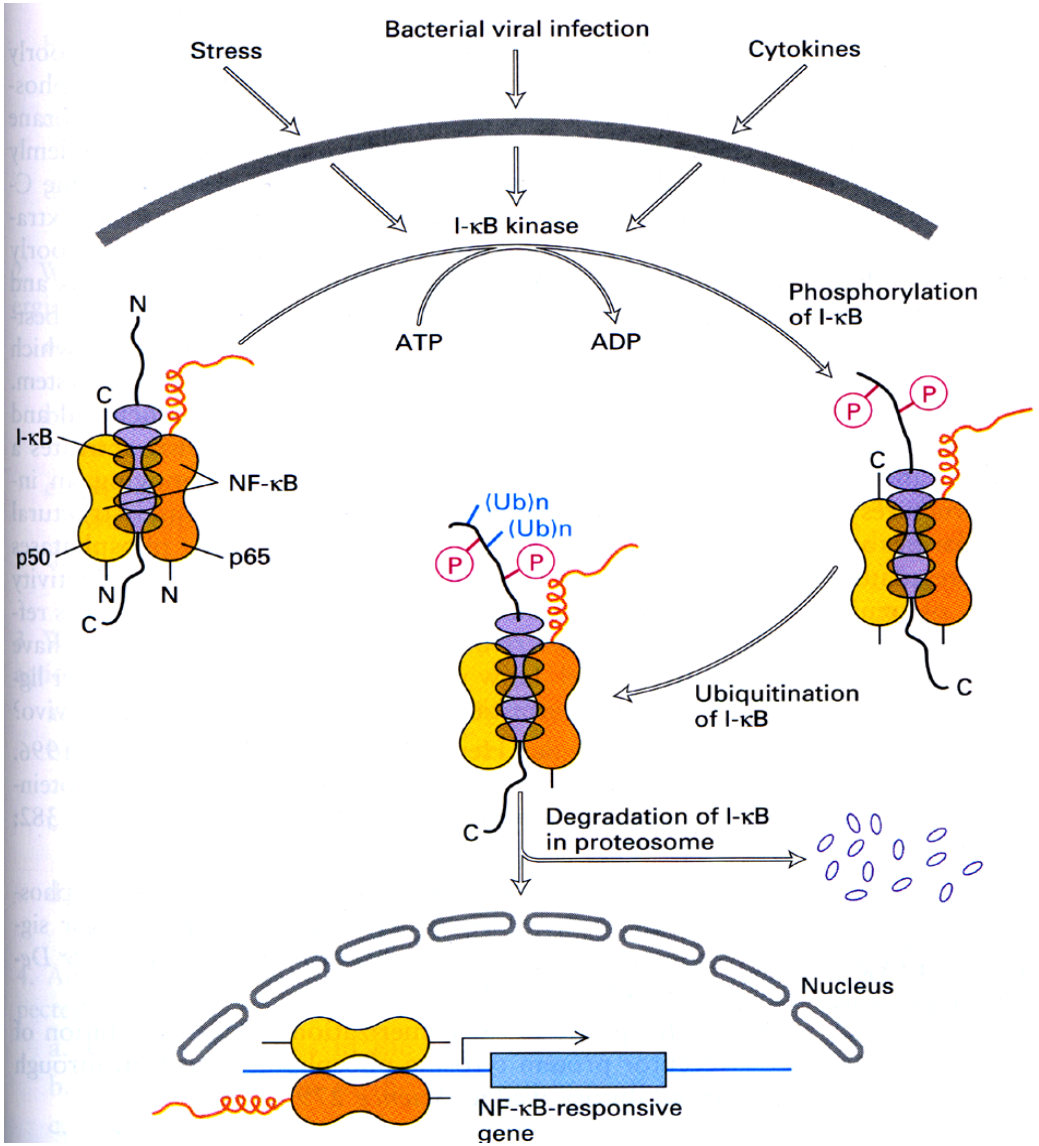


Nuclear import of NF-kB

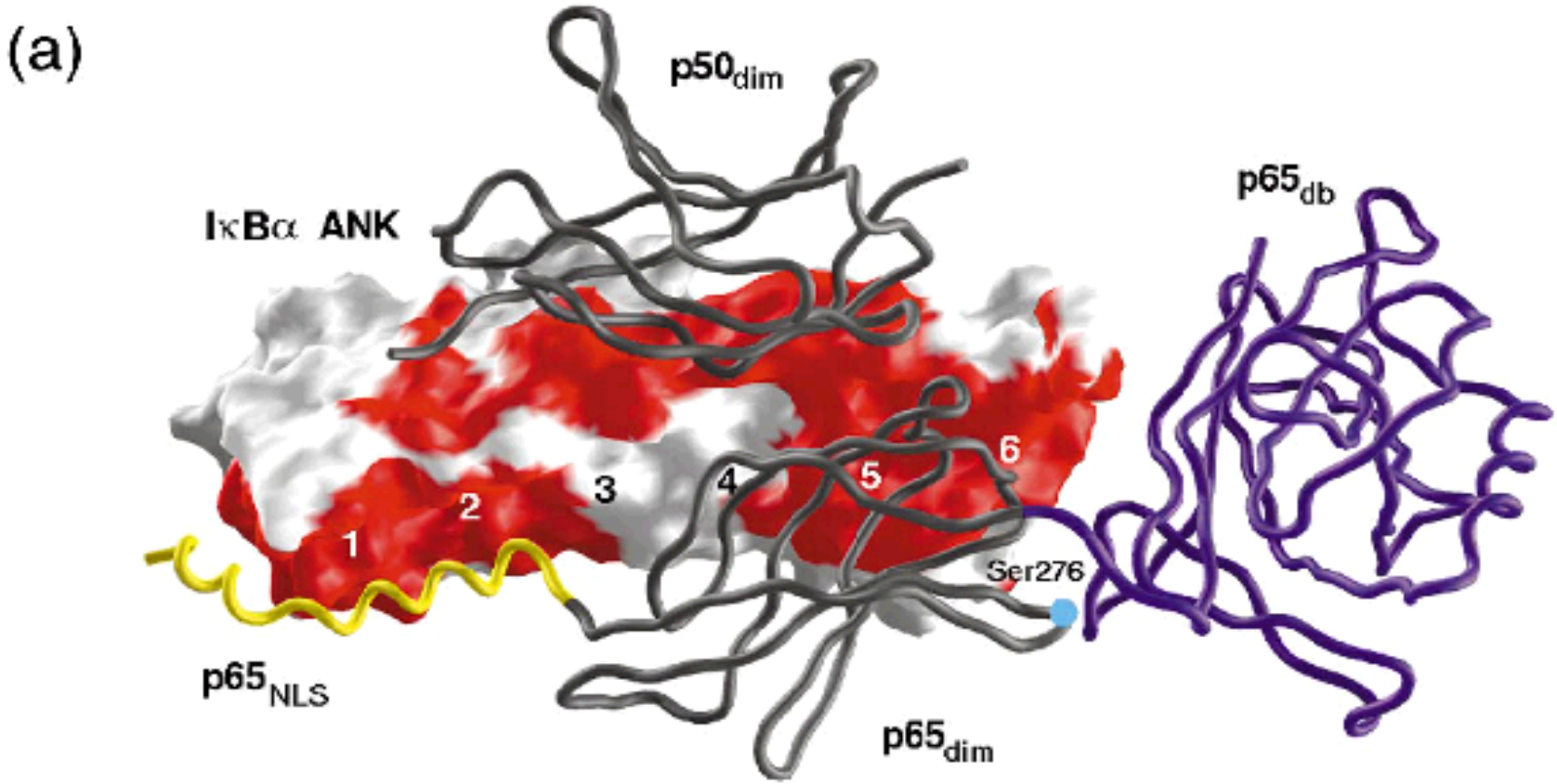
In the cytoplasm, NF-kB is kept inactive by association with IkB:

Different environmental stimuli activate a kinase **(IKK)** that phosphorylates IkB on two serine residues and targets it for degradation.

Degradation of IkB allows for the rapid translocation of NF-kB into the nucleus and the onset of a transcriptional response.

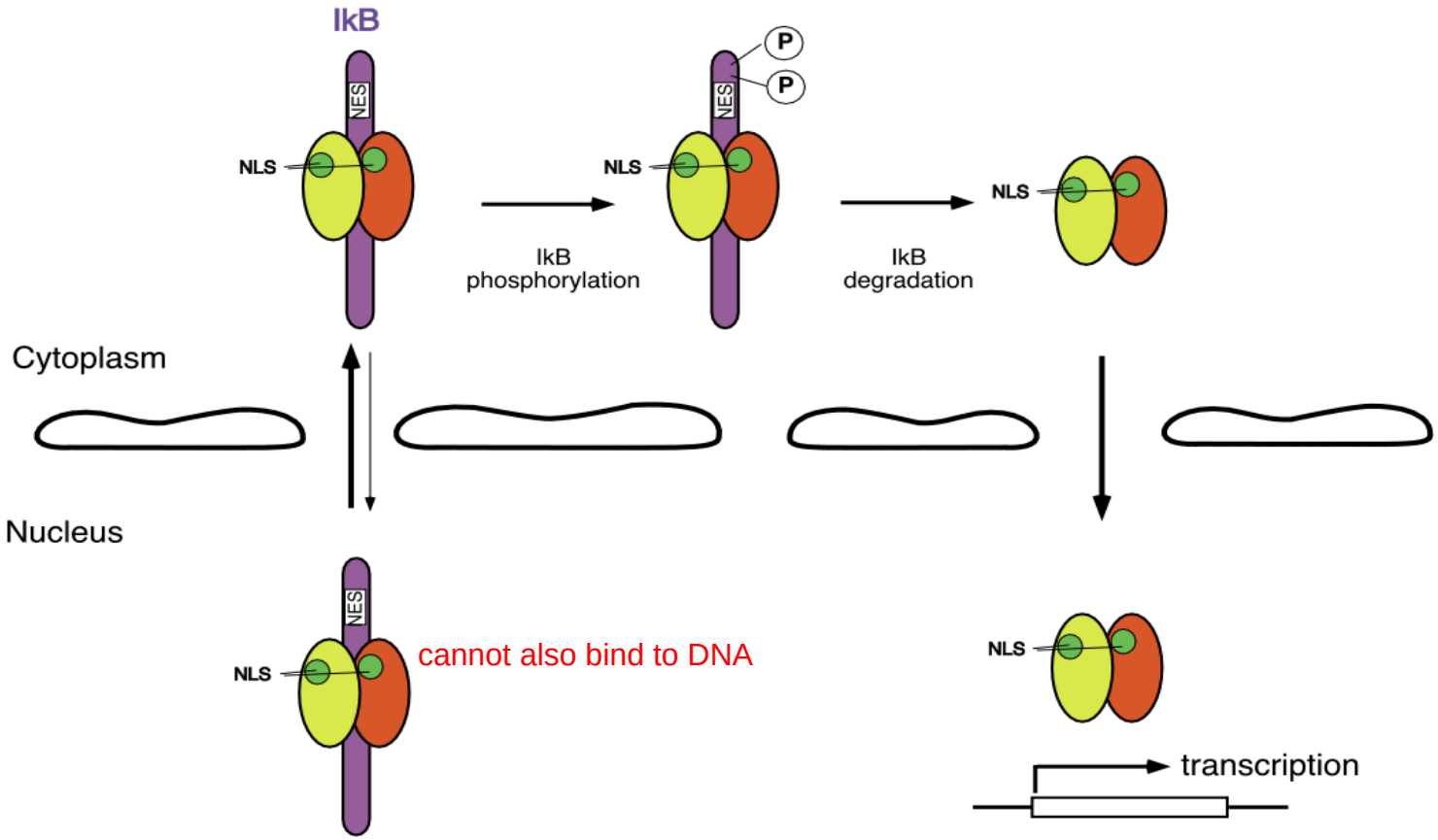


Structure of the IkB/NF-kB complex



➔ **the NLS is not accessible in the IkB/NF-kB compex**

IkB contains an NES sequence that helps to restrict NF-kB to the cytoplasm



IkB inactivates NF-kB by:

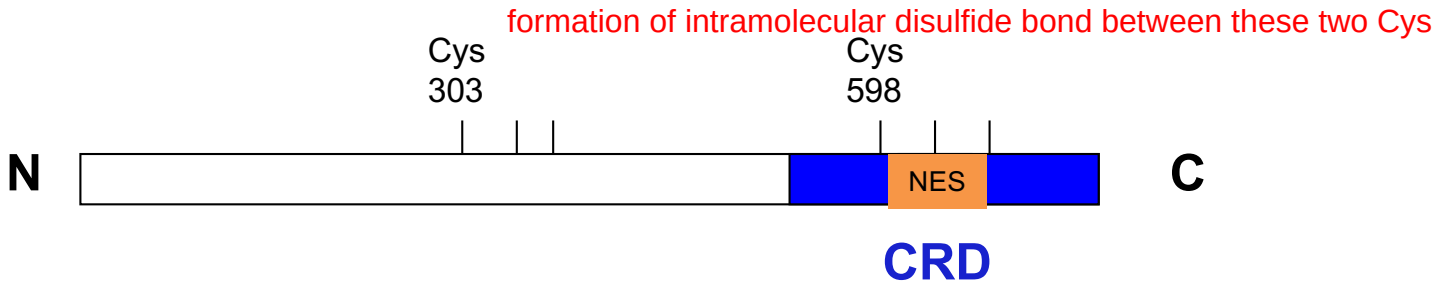
- restricting its access to the nucleus (NLS shielding to block import)
- promoting NF-kB export (NES in IkB)
- inhibits its DNA-binding activity

A conformational change triggers nuclear import of yAP1 in response to oxidative stress

- yeast transcription factor yAP1 translocates to the nucleus in response to oxidative stress and a variety of toxic compounds

- yAP1 induces transcription of anti-oxidative genes (e.g. thioredoxin and catalase) and genes that belong to the multidrug-resistance family to provide protection against toxic compounds

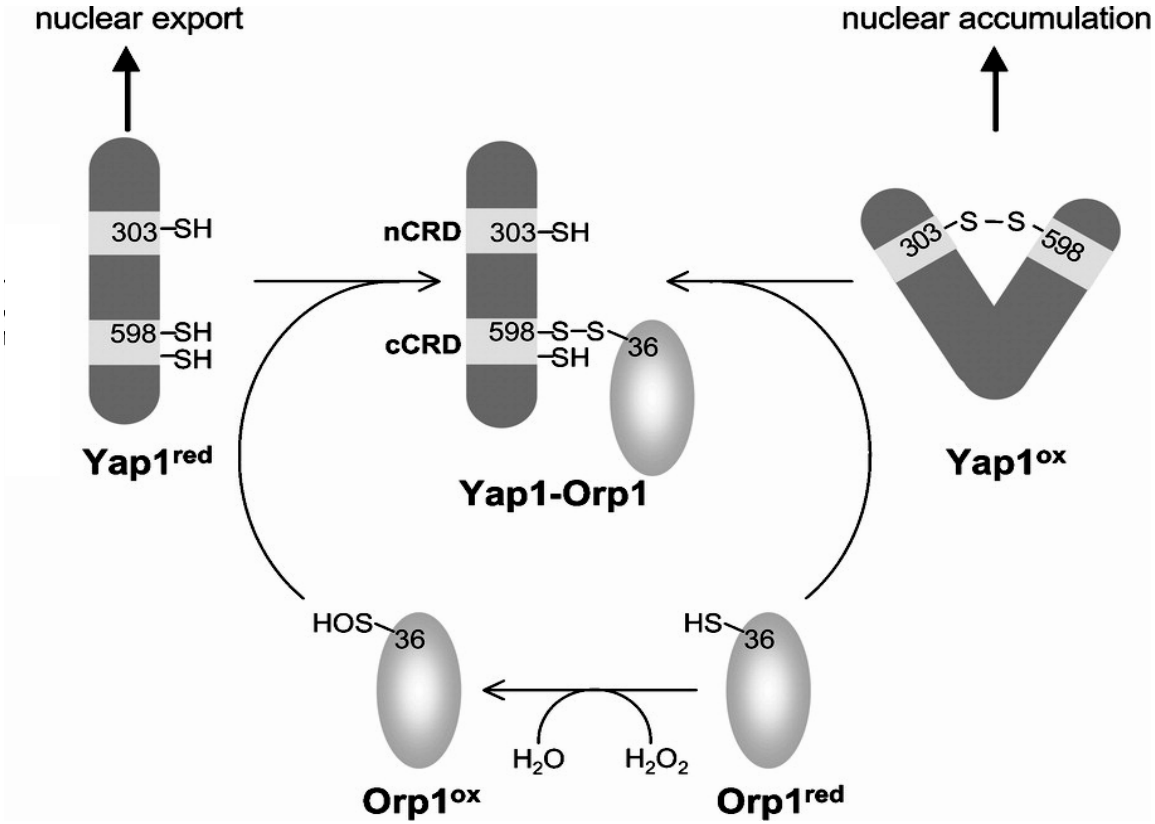
- cytoplasmic localization of yAP1 in unstressed cells requires a small region known as the **cysteine-rich domain (CRD)**



The activity of Yap1 is controlled primarily at the level of nuclear localization.

The CRD acts as a **redox sensor.**
Oxidation (disulfide bond between Cys 303 and 598) leads to a masking of the NES.

A conformational change triggers nuclear import of yAP1 in response to oxidative stress



In response to H_2O_2, a disulfide bond is formed between Yap1 C303 and C598.

This involves **the oxidant receptor protein** Orp1, which is oxidized to sulphenic acid.

The resulting NES masking leads to nuclear accumulation of Yap1 and activation its target genes.

Oxidation (disulfide bond between Cys 303 and 598) leads to a masking of the NES.

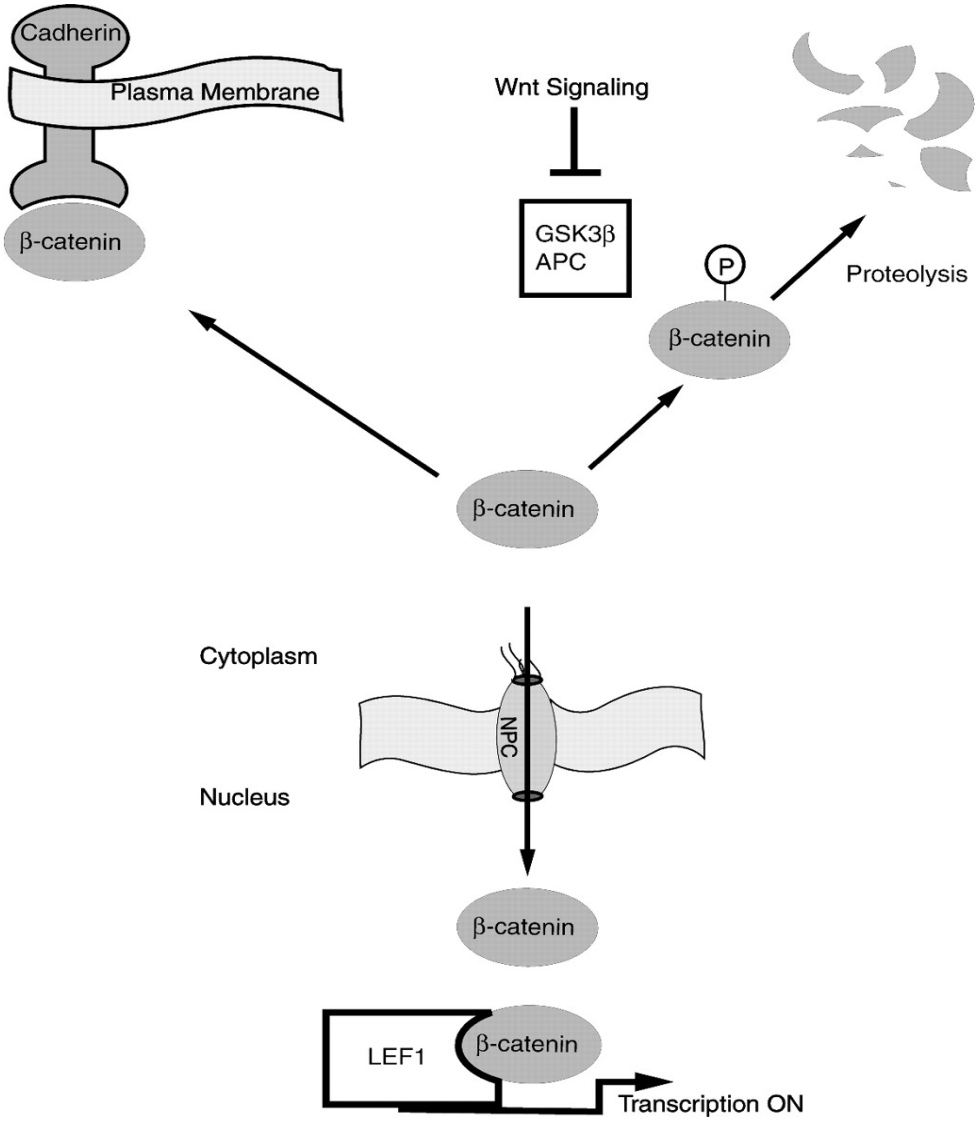
This inhibits the interaction between yAP1 and Crm1, leading to rapid nuclear accumulation of yAP1 and activation of the antitoxic transcriptional response.

2. Cytoplasmic anchoring: Example beta-catenin

we skipped this example

(a) adaptor linking cadherins, which are surface membrane proteins involved in cell adhesion, to the actin cytoskeleton

(b) role in the Wnt-signaling pathway as a transcriptional co-activator to induce a transcriptional program required for cell fate determination and dorso-ventral axis formation



without Wnt signal:
β-catenin is bound to cadherins and free β-catenin is phosphorylated and degraded

with Wnt signal:
β-catenin is no longer phosphorylated and degraded; any β-catenin released from retention is imported into the nucleus

3./4. Regulation of the soluble transport machinery and of the nuclear pore complex

1. **Stress-induced regulation of nucleo-cytoplasmic transport**
   - downregulation by several stresses (if ATP/GTP levels decrease)

2. **Regulation of nucleo-cytoplasmic transport by modification of transport receptors or NPCs**
   - S-nitrosylation of CRM1 in response to nitric oxide (NO) inhibits its activity
   - Phosphorylation of certain importins suggested to inhibit their activity

3. **Regulation of transport receptors or the NPC by viruses**

in yeast

Example VSV-M (matrix protein of vesicular stomatitis virus)
- VSV is an RNA virus that replicates in the cytoplasm
- VSV-M inhibits nuclear export of almost all forms of cellular RNA
- VSV-M interacts with the nucleoporin **Nup98** suggesting that VSV-M inhibits host cell gene expression by targeting a nucleoporin

Recommended reading

- Sekimoto and Yoneda (2012) Intrinsic and extrinsic negative regulators of nuclear transport processes. *Genes to Cells.* 17: 525-535
- Macara, I.G. (2001) Transport into and out of the nucleus. *Microbiol. Mol. Biol. Rev*. 65: 570-594
- Hood, J.K. and Silver, P.A. (2000) Diverse nuclear transport pathways regulate cell proliferation and oncogenesis. *Biochim Biophys Acta*, 1471, M31-41.
- Kimura and Imamoto (2014) Biological Significance of the importin-beta family-dependent nucleocytoplasmic transport pathways. *Traffic*, 15, 727-748.
- **Kaffman, A. and O'Shea, E.K. (1999) Regulation of nuclear localization: a key to a door. *Annu Rev Cell Dev Biol*, 15, 291-339.**
- Christie, M. and .... and Kobe, B. (2015) Structural Biology and regulation of protein import into the nucleus. J Mol Biol. 2015 (15) doi: 10.1016/j.jmb.2015.10.023.

Overview

CARGO	TRIGGER FOR TRANSPORT	EFFECT ON IMPORT	EFFECT ON EXPORT	EFFECT OF PHOSPHORY-LATION	COMPLEX FORMATION	BIOLOGICAL FUNCTION
Sc Pho4p	Phosphate starvation Phosphate supply	⇑ ⇓	⇓ ⇑	Import: ⇓ Export: ⇑	Pho2p	TK – phosphate starvation response
NF-AT	Raise in Ca^{++} conc	⇑	⇓	Import: ⇓;Export: ⇑	Calcineurin: Import ⇑, Export: ⇓	TK- e.g. immune response
MAPK	Signaling through RTK, GPCR	⇑	-	Import ⇑	Homodimerisation ?	Gene expression
NfkB	Diverse, e.g. interleukin1, TNFα	⇑	⇓	of IkB: Import ⇑	IkB: Import: ⇓;Export: ⇑	TK- e.g. immune and stress response
Sc Yap1p	Oxidative stress	-	⇓	-	-	TK –response to oxidative stress
GR	glucocorticoid hormone	⇑	-	-	HSP90: Import: ⇓	TK
Beta-catenin	Wnt signaling	⇑	-	stability	cadherins	TK

TK - transcription