Regulation of nucleo-cytoplasmic transport

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Eukaryotic cells control many biological processes by regulating the movement of macromolecules into and out of the nucleus.
Steady-state localisation of a protein is 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. In contrast, 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.

Movement of proteins across the nuclear envelope can be regulated in several ways:

1. modification of the cargo can affect its ability to bind to import or export receptors
2. the cargo-receptor complex can be tethered to an insoluble cellular component
3. the activity of the soluble transport machinery can be regulated
4. the NPC itself might be modified in a way that affects its transport properties

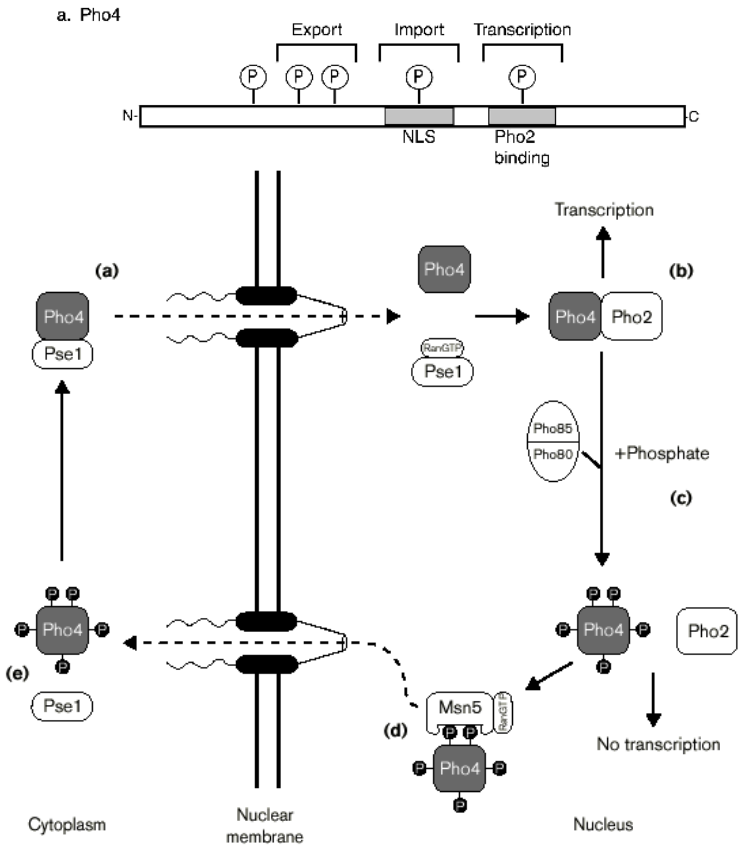
1. Regulating Cargo-Receptor Complex Formation

Regulating the affinity of the cargo for its receptor is achieved in most cases by phosphorylation of the cargo or by the intermolecular association of the cargo with accessory proteins. Phosphorylation and intermolecular association can either enhance or decrease the affinity of a cargo for its receptor.

1.1. PHOSPHORYLATION

1.1.1. Pho4

Pho4 is a yeast transcriptional activator involved in the phosphate starvation response. Under limiting phosphate conditions Pho4 is unphosphorylated, localised to the nucleus and, along with another transcription factor, Pho2, Pho4 activates transcription of a set of genes that allow yeast to grow under conditions of limited inorganic phosphate availability.
Nuclear localisation of Pho4 is regulated by its phosphorylation. In the presence of phosphate, a kinase complex (Pho85/Pho80) is activated and phosphorylates Pho4p on five serine residues causing its rapid nuclear export.



The import and export receptor for Pho4 have been identified.
Under low phosphate conditions, Pho4 is unphosphorylated and imported into the nucleus by the importin Pse1p that preferentially binds the unphosphorylated form of Pho4.
In the presence of phosphate, Pho4 becomes phosphorylated and dissociates from Pho2. The phosphorylated form of Pho4 is then recognised by the exportin Msn5 and exported out of the nucleus.

Phosphorylation inactivates Pho4 by dissociating it from Pho2, promoting its export, and preventing its re-import.

1.1.2. NF-AT

The nuclear factor of activated T cells (NF-AT) plays an important role in T-cell biology. Activation of T cells results in the translocation of NF-AT from the cytoplasm to the nucleus. This translocation process is coupled to the subsequent active maintenance of NF-AT in the nucleus and critical for the induction of expression of several genes encoding cytokines and membrane proteins that modulate immune responses.

Phosphorylation of the transcription factor NF-AT is thought to affect its subcellular localization by modulating both its import and export rates. Stimulation of the T-cell receptor causes an **elevation in cytosolic Ca^{2+} levels**, which activates the phosphatase calcineurin leading to **dephosphorylation of NF-AT**. A complex consisting of dephosphorylated NF-AT and calcineurin is then translocated to the nucleus, allowing for transcriptional induction of genes required for T-cell activation. Some of the most potent immunosuppressive drugs, such as cyclosporin A and FK506, act by inhibiting the ability of calcineurin to dephosphorylate NF-AT, thereby preventing the nuclear accumulation of NF-AT.

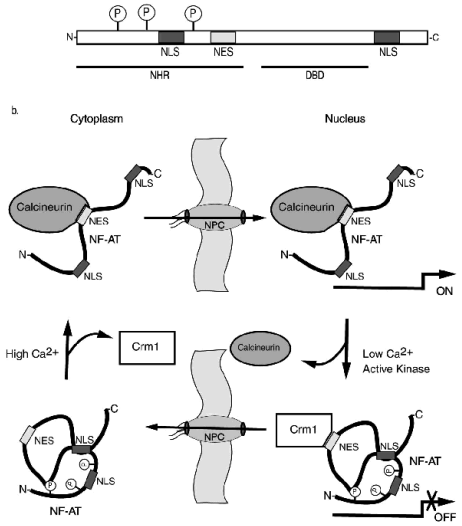


Figure 3 The subcellular localization of nuclear factor of activated T cells (NF-AT) is regulated by phosphorylation and calcium-induced masking of its nuclear export signal (NES), mediated by calcineurin. (*a*) Schematic of localization signal sequences and phosphorylation sites (P) important for regulating the subcellular localization of NF-AT2. (*b*) Phosphorylation is thought to enhance nuclear export of NF-AT and to block nuclear import of NF-AT by masking its two nuclear localization signal (NLS) sequences. In addition, calcium-induced binding of calcineurin to the NES in NF-AT prevents binding of the export receptor Crm1 to NF-AT, thereby blocking its export. NHR, NF-AT homology region; DBD, DNA-binding domain; NPC, nuclear pore complex.

Phosphorylation inhibits the rate of import of NF-AT by inducing an intramolecular conformational change that makes its two NLS sequences inaccessible for binding by the import machinery.
The interaction between NF-AT and its exportin Crm1 is dependent on two leucine-rich sequences within NF-AT. These two NESs overlap precisely with the calcineurin-binding site on NF-AT, suggesting that calcineurin and Crm1 compete for binding to NF-AT. Active calcineurin prevents Crm1-mediated export of NF-AT. When NF-AT enters the nucleus, its export by Crm1 is inhibited because its NES is masked by the activated, bound calcineurin.

1.2. COMPLEX FORMATION

Nuclear localisation can also be regulated through intra- and intermolecular interactions. Association of the cargo with itself, with RNA or with other proteins illustrate how complex formation can influence the ability of the cargo to bind to the soluble transport machinery. In some cases, complex formation is required for nuclear targeting, whereas in others, complex disassembly is required for nuclear localization. Complex formation or disassembly can be regulated in many different ways, such as by phosphorylation-dependent degradation or by binding to a ligand.

1.2.1. Mitogen activated protein kinase (MAPK)

Numerous signal transduction cascades transduce signals from the plasma membrane through the cytoplasm and into the nucleus. Many signaling pathways use a kinase cascade involving a MAPK. MAPK is activated in the cytoplasm by phosphorylation on conserved threonine and tyrosine residues, causing it to translocate into the nucleus. A block in translocation of the activated MAPK leads to a defect in signal transduction, which suggests that translocation is required for proper transmission of the signal. In the nucleus, activated MAPK phosphorylates downstream targets to induce the appropriate transcriptional response. MAPK is activated by a cytoplasmic MAPK kinase, referred to as MEK. The cytoplasmic localization of MEK requires a leucine-rich NES at its N terminus. MEK in turn is phosphorylated and activated by a membrane-associated MEK kinase.

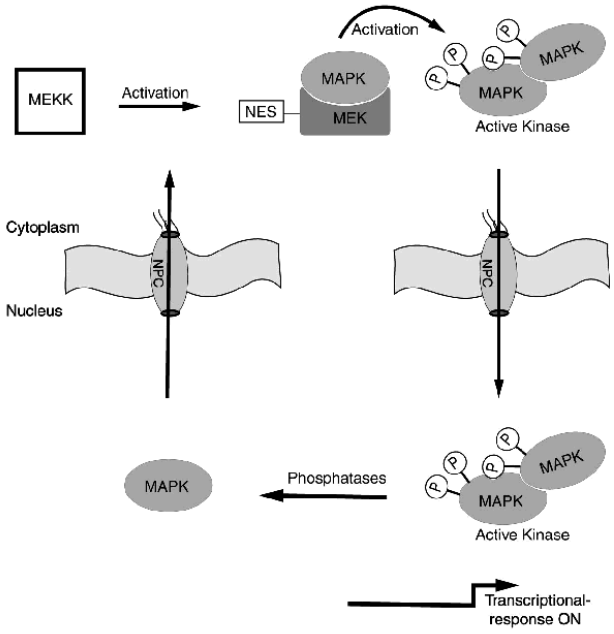


Figure 4 Activation of mitogen-activated protein kinase (MAPK) causes its entry into the nucleus. Phosphorylation of MAPK by the cytoplasmic MAPK kinase (MEK) causes activation of MAPK, release from MEK, dimerization, and nuclear accumulation. Nuclear entry of activated MAPK leads to phosphorylation of downstream targets, leading to induction of a transcriptional response. MEKK, MEK kinase; for other abbreviations, see legend to Figure 3.

The family of MAPKs is divided into two large subfamilies. The ERKs, which include ERK1 and ERK2, are involved mainly in cell proliferation and differentiation. The stress-activated protein kinases, which include p38 and the Jun N-terminal kinase (JNK), are activated in response to cellular stresses such as UV irradiation, heat, and high osmolarity.

Studies with yeast have shown that, for the p38 homologs—Hog1 in budding yeast and Spc1 in fission yeast—phosphorylation on the activating tyrosine and threonine residues is necessary for nuclear translocation; mutants that cannot be phosphorylated on these sites are unable to enter the nucleus However, the kinase activity of MAPK is not required, because mutants lacking kinase activity are translocated to the nucleus.

Phosphorylation of ERK2 induces it to form homodimers, and phosphorylated ERK2 mutants that fail to dimerize do not enter the nucleus. From these results it was proposed that phosphorylation of ERK2 triggers its dimerization, which is required for its nuclear localization. This has meanwhile however been questioned.

The mechanism of MAPK nuclear entry has been puzzling because no sequences resembling NLSs have been identified. The recent discovery that Hog1, the p38 homolog in budding yeast, requires the small GTPase Ran and the importin β homolog **Nmd5p** for its nuclear import may help to explain why MAPK does not have a classical NLS. Quite recently, it has been shown that Drosophila ERK requires **importin 7** (the higher eukaryotic homolog of Nmd5p) for nuclear uptake.

Phosphorylation of MAPK accomplishes three tasks:
(a) it turns it into an active kinase
(b) it promotes dissociation from the cytoplasmic MEK
(c) it may promote dimerization that might be required for its nuclear localization

Phosphorylation-induced dimerization followed by nuclear translocation is not a unique feature of MAPK and is seen also in a large family of transcription factors known as signal transducers and activators of transcription (STATS).

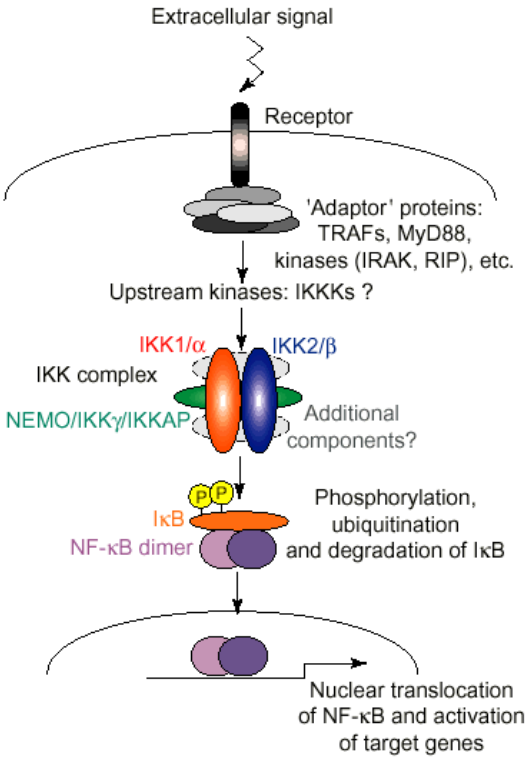
1.2.2. NF-kB

NF-kB localisation is regulated by controlling its association with the transport machinery using intermolecular association. NF-kB is a heterodimeric transcription factor composed of the **RelA (p65)** and the **p50** subunits. NF-kB is activated by numerous stimuli, enabling it to induce transcription of a large number of genes involved in the immune, inflammatory and apoptotic responses. Among the most important activators are the pro-inflammatory cytokines (e.g. Interleukin 1) and tumour necrosis factor α (TNF) that are produced in response to pathogenic stimuli.

The swiftness with which NF-kB responds to a vast number of environmental stimuli and its ability to activate a large array of genes are thought to demand tight control over its transcriptional activity. This is achieved by maintaining NF-kB in **an inactive form in the cytoplasm in a stable complex** with the **small NF-kB inhibitor, IkB**. IkB inactivates NF-kB by restricting its access to the nucleus and by inhibiting its DNA-binding activity. Cytoplasmic localization of NF-kB is accomplished by the ability of IkB to both block import and to promote export. Different environmental stimuli activate a kinase **(IKK)** that

phosphorylates IkB on two serine residues and targets it for rapid degradation. Degradation of IkB allows for the rapid translocation of NF-kB into the nucleus and the onset of a transcriptional response. Taken together, **nuclear localization of NF-kB is controlled by regulating its association with other proteins (IkB), which in turn controls its access to the soluble transport machinery.**

The ability of IkB to block import has been demonstrated by showing that antibodies directed specifically against the NLS of RelA (p65) and p50 fail to recognize the complex when IkB is bound, but are able to recognize NF-kB in its absence. In addition, mutations in the NLS of the RelA (p65) subunit abolish its ability to bind IkB, consistent with a model in which IkB binding masks the NLS on RelA (p65).



Schematic diagram of the NF-κB activation pathway. In response to an extracellular signal, the IKK complex becomes activated after a series of poorly characterized membrane-proximal events. These events involve a number of proteins that constitutively or transiently associate with the intracellular region of a receptor (these proteins are here called 'adaptors' to indicate that their exact role, interacting partners and epistatic relationships are poorly understood). The final result is the activation of the IKK complex by phosphorylation of the kinase subunits; IKK kinases might be responsible for this phosphorylation but such proteins have not been identified. The IKK complex comprises the two kinase subunits (IKK1/α and IKK2/β), probably present as homo- or heterodimers, and the structural/regulatory subunit NEMO/IKK-γ/IKKAP. The difference between the apparent mass of the purified complex and the total molecular mass of the known subunits suggests that other components of the complex might exist. After activation, the IKK complex phosphorylates the IκB molecules (three species of which exist in the cells: IκBα, IκBβ and IκBε), which are then ubiquitinated and degraded, releasing NF-κB dimers that translocate to the nucleus and activate transcription of their target genes. Additional post-translational modifications of the NF-κB complexes themselves are also induced by signalling.

In addition, NF-kB bound by IkB is unable to bind DNA. The addition of IkB to NF-kB complexed with DNA results in dissociation of the NF-kB–DNA complex, indicating that DNA and IkB binding to NF-kB are mutually exclusive. IkB contains a leucine-rich NES and microinjection of IkB into the nucleus enhances NF-kB export.
IkB transcription is induced by NF-kB, and new protein synthesis is required for inactivation of NF-kB. Thus, IkB seems to play an important role in terminating the NF-kB transcriptional response by triggering dissociation of NF-kB from DNA and promoting its nuclear export. Because IkB is degraded upon activation of NF-kB, it must be re-synthesized to turn off the transcriptional activity of NF-kB. This requirement for new protein synthesis makes NF-kB inactivation rather slow.

1.2.3. Glucocorticoid receptor (GR)

Regulated localization of the GR illustrates how ligand binding allows a cargo to bind its import receptor, presumably by disrupting association of the cargo with accessory proteins.

GR belongs to the steroid hormone receptor family and is responsible for the coordination and regulation of cellular responses such as stress and metabolic homeostasis. In unstimulated cells GR is inactive in the cytoplasm and is complexed with a number of proteins such as a 90-kDa heat shock protein (Hsp90) and the immunophilin 56-kDa heat shock protein (Hsp56).
Binding of these proteins accomplishes three tasks important for the regulation of GR:
(a) restricting GR localization to the cytoplasm,
(b) inhibiting its DNA-binding activity and
(c) maintaining GR in a state of high affinity for its ligand

Binding of GR to its ligand causes the dissociation of these accessory proteins from GR, allowing for rapid nuclear translocation and the activation of gene expression.
GR contains two NLS sequences. The central region of GR contains the DNA-binding domain adjacent to a functional NLS (NL1), whereas the ligand-binding domain (LBD) is found at the C terminus and is equipped with a second NLS (NL2). NL1 resembles a bipartite NLS and is required for the rapid nuclear localization of GR induced by association of GR with a ligand. Fusion of a heterologous protein to NL1 leads to constitutive nuclear localization that is ligand independent. In addition, importin α binds GR, and this binding requires NL1, which suggests that NL1 directs import of GR via the classical import pathway.

Upon ligand withdrawal, the ligand is released from GR causing dissociation of GR from chromatin thereby terminating the ligand-induced transcriptional response. GR is exported back to the cytoplasm in a process that is currently poorly understood. The slow movement of the ligand-free GR from the nucleus to the cytoplasm suggests that the steady-state cytoplasmic localization of ligand-free GR, in unstimulated cells, is probably not caused by rapid export.

The mechanism proposed for maintaining GR in the cytoplasm is controversial.
Several studies indicate that import of GR is regulated by masking its NLS. Removal of the LBD renders GR constitutively nuclear even in the absence of a ligand, which suggests that the presence of the LBD inhibits the activity of NLS1 in the ligand-free GR. In addition, binding of a ligand causes the dissociation of Hsp90 from this domain, and this release correlates with the accessibility of NLS1 to NLS1-specific antibodies. Based on these studies, it has been proposed that binding of ligand to the LBD unmasks NLS1, triggering import of GR.
However, in vitro binding assays demonstrate that the affinity of importin α for GR is similar in the presence or the absence of a ligand, indicating that binding of importin α to GR may not be inhibited by the presence of Hsp90. From these results the authors proposed that a mechanism distinct from masking of the NLS is responsible for the cytoplasmic localization of GR. Further studies are required to establish whether importin α is required for the rapid import of GR and whether association between GR and importin α is regulated by a ligand in vivo.

If NLS masking is not a major determinant in maintaining GR in the cytoplasm, the mechanism responsible for its subcellular localization needs to be identified.

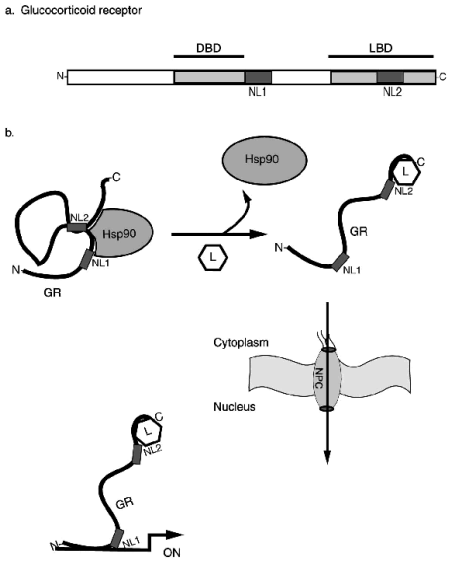


Figure 6 Two distinct nuclear localization (NL) signal sequences in the glucocorticoid receptor (GR) mediate its entry into the nucleus. (*a*) Functional domains and NL signal sequences in GR. (*b*) Association of GR with accessory proteins (illustrated here with Hsp90) determines its subcellular localization. Binding of a ligand (L) to the ligand-binding domain (LBD) causes dissociation of these accessory proteins, leading to nuclear accumulation of GR. Nuclear import of GR is mediated by two NLS sequences with distinct import properties. DBD, DNA-binding domain; NPC, nuclear pore complex; Hsp90, 90-kDa heat shock protein.

1.2.4. Period and Timeless

The regulated nuclear entry of Period (Per) and Timeless (Tim) represents a unique example of how regulated nuclear localization is used by cells to construct biological clocks. The requirement of the two proteins to form a complex before their movement into the nucleus serves as another example of how intermolecular association can be used to regulate nuclear entry.

Circadian rhythms are daily changes observed in the behavior and physiology of many organisms. These changes are controlled by internal clocks, which share a central component known as the oscillator. The oscillator is composed of a protein or a protein complex that oscillates with the same daily periodicity, and this oscillation is required for the formation of daily rhythms. All oscillatory systems studied to date are characterized by a transcriptional negative-feedback loop, in which the oscillator protein shuts off transcription of its own messenger RNA.

However, oscillation of such a simple negative-feedback loop will dampen over time until the oscillations stop altogether. Biological clocks circumvent this problem by introducing a delay between RNA accumulation and the transcriptional inhibition caused by the oscillator protein.

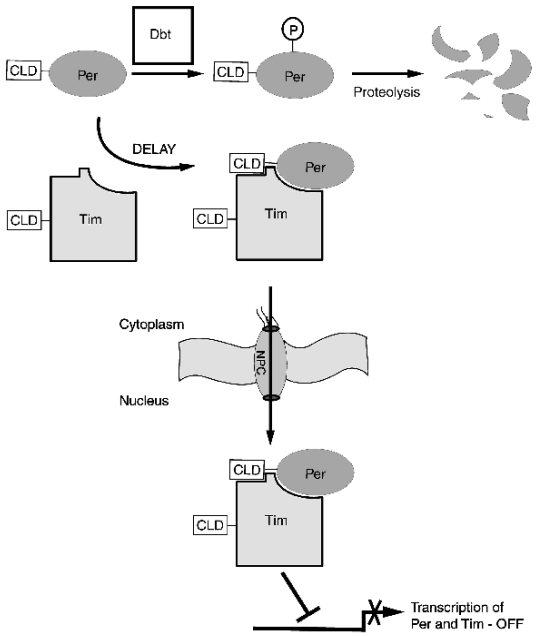


Figure 5 A delay in nuclear entry of Per and Tim is required for persistent oscillation of the biological clock of *Drosophila melanogaster*. Per and Tim enter the nucleus as a complex. The formation of a Per-Tim complex that can enter the nucleus is thought to be regulated by degradation of Per and cytoplasmic localization domain (CLD) sequences on Per and Tim that prevent their nuclear localization. Upon entering the nucleus, Per and Tim act together to inhibit their own transcription. NPC, Nuclear pore complex; Dbt, doubletime; (P) phosphorylation sites.

The Per and Tim proteins are required for circadian rhythms in D. melanogaster, and these proteins have been proposed to form the functional subunits of the Drosophila oscillator. The delay between Per and Tim synthesis and their ability to repress their own transcription is thought to occur by regulation of the nuclear translocation of Per and Tim. The Per protein accumulates in the cytoplasm several hours before it is triggered to enter the nucleus, and its nuclear entry requires the presence of Tim. In addition, mutations in Per that lead to a longer period of oscillation have reduced affinity for Tim and enter the nucleus at later time points, which suggests that the affinity of Per for Tim determines the rate of nuclear entry and the period length.

The presence of both proteins is required for nuclear entry, because expression of each protein alone leads to their cytoplasmic accumulation. Deletion analysis revealed a domain in each of these proteins that is responsible for their cytoplasmic localization; deletion of this **cytoplasmic localization domain (CLD)** leads to nuclear accumulation of Per and Tim even when each is expressed alone. These data suggest that the CLD on each protein is required for cytoplasmic localization and that coexpression of the two proteins relieves the block in nuclear accumulation caused by these domains. The CLD on Per has been shown to bind Tim, which suggests that complex formation might mask the CLD on Per, leading to nuclear localization of the complex.
It is interesting that nuclear accumulation of the coexpressed Per-Tim complex is slower compared with nuclear import of an individually expressed, CLD-deleted protein. This observation is consistent with the idea that the slow formation of a productive complex that is capable of entering the nucleus provides a delay necessary to establish robust oscillation. The mechanism by which the CLD causes cytoplasmic localization is not known.

DOUBLETIME was identified as another gene required for circadian rhythms in D. melanogaster. Doubletime (Dbt) is essential for viability and shares homology with casein kinase epsilon. Dbt binds Per and is required for its phosphorylation. Phosphorylation of Per is thought to destabilize it, providing a delay in its cytoplasmic accumulation and subsequent nuclear entry.

1.3. PROTEIN CONFORMATION (yAP1)

The yeast transcription factor Yap1 is rapidly localized to the nucleus in response to oxidative stress and exposure to a variety of toxic compounds. In the nucleus, yAP1 induces transcription of antioxidative 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 CRD has been shown to be necessary and sufficient for the regulated localization of yAP1. Deletion of the CRD leads to nuclear accumulation of yAP1, and fusion of the CRD to a heterologous protein is sufficient to impose regulated localization on this protein in response to oxidative stress. The CRD contains three conserved cysteines that are required for its proper function. These observations led to the proposal that the **cysteines act as an oxidation sensor** able to control CRD activity and the subcellular localization of yAP1.

It was later shown that the CRD contains a leucine-rich NES and that the cytoplasmic localization of yAP1 requires the activity of the export receptor Crm1. yAP1 shuttles rapidly between the nucleus and the cytoplasm and is cytoplasmic because of the efficient export mediated by Crm1. The CRD acts as a redox sensor whose oxidation (disulfide bond formation within yAP1 leads to a masking of the NES) inhibits the interaction between yAP1 and Crm1, leading to rapid nuclear accumulation of yAP1 and activation of the antitoxic transcriptional response.

2. CYTOPLASMIC ANCHORING

In this form of regulated localization, a receptor-cargo complex can form but cannot be targeted to the NPC, because the cargo is tethered to an insoluble cellular component. This mechanism has been proposed for several cargoes.
Some of the best-characterized examples of cytoplasmic retention include the sterol response element-binding protein, Notch, PKA and β-catenin. Sterol response element-binding protein and Notch are integral membrane proteins found in the endoplasmic reticulum (ER) and plasma membrane, respectively, of unstimulated cells. Upon stimulation, both proteins undergo proteolytic cleavage to produce a soluble fragment that enters the nucleus and can activate transcription. This type of regulation is irreversible because the mature soluble fragment cannot be anchored back to the cytoplasm by the same mechanism.
The regulated localization of β-catenin provides an interesting example of how cytoplasmic anchoring and cytoplasmic instability are combined to coordinate nuclear localization and activity of this protein.

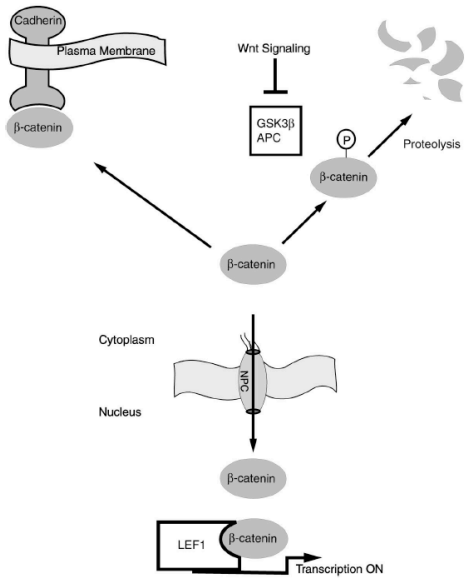


Figure 7 Nuclear entry of β-catenin is governed by the combined action of cytoplasmic anchoring to cadherins and the degradation of β-catenin in the cytoplasm. Degradation is promoted by glycogen synthase kinase 3β (GSK3β) and adenomatous polyposis coli protein (APC). Wnt signaling inhibits the cytoplasmic degradation of β-catenin, leading to its accumulation in the cytoplasm and entry into the nucleus. In the nucleus, β-catenin associates with the transcription factor lymphoid enhancer factor 1 (LEF1), and together this complex activates transcription of genes that are important for cell fate determination. NPC, nuclear pore complex; (P), phosphorylation site.

β-catenin in vertebrates and its homologue Armadillo in D. melanogaster are multifunctional proteins that play an important role in the Wnt-signaling pathway. In Xenopus laevis, β-catenin plays an important role in body-axis formation.
β-catenin is able to serve at least two different functions:
(a) It is an adaptor linking cadherins, which are surface membrane proteins involved in cell adhesion, to the actin cytoskeleton
(b) β-catenin is also required for coactivating transcription with the lymphoid enhancer factor 1/T-cell factor (LEF1/TCF), which presumably induces a transcriptional program required for cell fate determination and dorsoventral axis formation

The Wnt protein is a secreted glycoprotein that binds a membrane-bound receptor on the target cell and activates a signal transduction cascade leading to translocation of β-catenin from the cytoplasm into the nucleus. In the nucleus, β-catenin associates with LEF1, and this complex activates transcription of genes that are required for cell-fate determination. Inappropriate regulation of β-catenin stability triggers its nuclear localization and correlates with certain forms of cancer.

Two mechanisms are thought to ensure that β-catenin is maintained in the cytoplasm in unstimulated cells.
(a) β-catenin is tethered to membrane-bound cadherins
(b) free, cytoplasmic β-catenin is maintained at a low level by its constitutive degradation mediated by the proteasome
This is a good example of how cytoplasmic anchoring and degradation in the cytoplasm can act together to prevent nuclear entry of a protein.

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

The soluble transport machinery, which includes importins, exportins, and components of the RanGTPase cycle, could also be targeted for regulation. Data to support this mode of regulation come from several studies that are mainly correlative in nature.

The matrix protein from vesicular stomatitis virus **(VSV-M)** inhibits protein import and nuclear export of almost all forms of RNA. Vesicular stomatitis virus is an RNA virus that replicates in the cytoplasm . Inhibition of nuclear export reduces the concentration of cellular messenger RNA in the cytoplasm and thus may enhance the transcriptional and translational efficiency of viral RNA. It was proposed to do so by inhibiting the Ran GTPase cycle. If this was the case, the matrix protein seems to act catalytically, because it inhibits transport at a concentration that is 1000-fold lower than the concentration of Ran.
However, meanwhile it has been demonstrated that **VSV-M interacts with the nucleoporin Nup98** suggesting that VSV-M inhibits host cell gene expression by targeting a nucleoporin and primarily blocking nuclear export.

Another example of the regulation of the transport machinery is the change in localization of **importin** α in response to **cell cycle** position. In D. melanogaster, the importin α homolog Pendulin enters the nucleus at the onset of mitosis with kinetics similar to those observed for cyclin B. This regulated nuclear entry occurs mainly in proliferating cells and is thought to be important in regulating cell proliferation .The mechanism responsible for this change in localization is not known, and it might be related to the ability of importin α to be phosphorylated.

Results from several studies have suggested that the transport properties of the NPC itself might be regulated.

1. **Quiescent cells show a slower rate of nuclear import** of large gold particles coated with nucleoplasmin, compared with proliferating cells.
It is interesting that smaller gold particles are imported with the same efficiency in both types of cells, indicating that the difference in import rates was size specific. To determine whether this reflects a change in the activity of the NPC, a heterokaryon consisting of proliferating and quiescent cells was obtained by fusing the two cells together. Injection of large gold particles into the cytoplasm of this heterokaryon shows that import into the nucleus of proliferating cells is significantly more efficient compared with import into the quiescent nucleus. Because the two nuclei share the same soluble machinery, it was proposed that the NPC is modified to allow more efficient import.

2. A component of the NPC in budding yeast, Nup53, has been shown to be phosphorylated during mitosis. This cell cycle-dependent phosphorylation correlates with the inability of the importin β homolog Pse1 to associate with the nuclear pore. Because Nup53 seems to be important for the ability of Pse1 to bind the NPC and to transport cargo, these results suggest that phosphorylation of Nup53 during mitosis regulates import mediated by Pse1 in a cell cycle-dependent manner. Because import mediated by Pse1 is not completely blocked in cells lacking Nup53, the significance of this regulation still needs to be determined.