Intracellular Compartments & Protein Sorting

Compartmentalisation of Cells

- a shift from bacterial to eukaryotic cells ⇒ ↑ in volume, and ↓ in surface area to volume ratio.
  ↳ leads to necessity of internal membrane system.

Nucleus | ER | Golgi Body | Mitochondria | Chloroplast

↳ deals with membrane-dependent functions

⇓

different components specialise in different functions.

Movement of Proteins between compartments

Synthesis of proteins begins on ribosome surface

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Amino acid sequence contains sorting signals that direct delivery locations outside cytosol.

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Without sorting signals, proteins remain inside the cytosol.

Three kinds of transport mediate protein sorting:

- Gated transport – Nuclear pores act as specific gates for macromolecular assemblies b/w cytosol and nucleus. Also allow free

diffusion of small molecules.

- Transmembrane transport – direct transport of specific proteins across membrane from cytosol to topologically distinct space – protein has to unfold to pass through translocator
- Vesicular transport – small/large membrane enclosed spherical vesicles ferry proteins from one compartment to another. (only topologically equivalent ones)

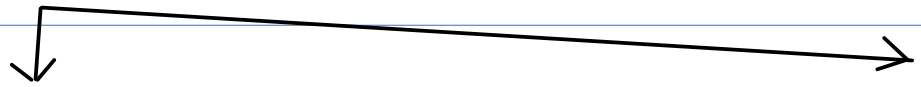
Each mode of protein transfer – signalled by sorting receptors that guide its transport into destination.

Signal Sequences

- Most proteins – sorting signals in stretch of amino-acid sequences – Signal sequences found at N-terminus.
- Signal peptidases remove signal sequence from finished proteins.
- Each signal sequence specifies particular destination in the cell
- Signal sequences recognised by complementary sorting receptors – guide proteins to their appropriate destination and after one round of unloading, return to current location.

Transport of Molecules b/w Nucleus and Cytoplasm

- nuclear envelope encloses DNA & defines nuclear compartment



inner nuclear membrane (anchoring sites for specific proteins like chromatin & nuclear lamina)

← different protein compositions →

outer nuclear membrane (continuous with ER, proteins made on these ribosomes, are transported into perinuclear space)

Transport b/w nuclear pore

- bidirectional Traffic
- proteins that function in the nucleus (histones, DNA & RNA polymerases, gene regulatory proteins, RNA processing proteins) selectively imported into nuclear compartment from cytosol (cytosol → inside)
- tRNAs & mRNAs synthesised in nuclear compartment & transported into cytosol (nucleus → cytosol)

Transport in both ways is selective;
- mRNA is transported only after RNA-processing rxn
- ribosomal Proteins transported after assembling with rRNA

Nuclear Pore Complex (NPC)

- large elaborate complex – 30 different NPC proteins (nucleoporins)
- perforate nuclear envelope of all eukaryotes – around 3000 - 4000 NPCs
- each NPC transports 500 macromolecules/s - handling of ↳ bidirectional transport traffic is unknown
- each NPC contains one or more aqueous passages – small molecules pass through effectively, passively
- for large molecules, receptor-bound – active transport through NPC (passive transport is slow for large molecules due to limited size of pore)

Nuclear Localisation Signals (NLs)

How are nuclear proteins directed to the nucleus?

Sorting signals – nuclear localisation signals

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responsible for selective import of active nuclear import process

- Signals precisely defined using recombinant DNA technology (for numerous nuclear proteins
  ↳ one or two short sequences that are rich in positively charged amino acids lysine and arginine

- precise sequence of NLS vary for different proteins
- NLs form loops or patches on the protein surface.
  ↳position within the AA seq is not imp.

Nuclear Import Receptors (Importins)

- each specialised for the transport of a subset of cargo proteins – bind to NLS
- initiates nuclear transport by binding to both protein NL and FG repeat (phenylalanine & glycine) on NPC protein fibril that enter the cytosol
- soluble, cytosolic proteins
- FG repeats line the path through NPCs taken by import receptors and their bound cargo proteins
- receptors move by repeatedly binding and dissociating to the FG repeats along the path.
- might not bind to proteins directly, may be adaptor proteins forming a bridge between NL and importin

Ran GTPase imposes directionality on NPC Transport

- import of nuclear proteins → concentration ↑ → order ↑
  → entropy ↓ → Energy reqd!
- energy reqd. obtained by cells through GTP hydrolysis by GTPase Ran
- Ran required for both nuclear import & export

- molecular switch - conformation varies based on whether GTP or GDP bound (RAN-GTP & RAN-GDP)
  - conformational switch controlled by two Ran-specific regulatory proteins

GAP → RAN-GDP ← GEF

RAN-GDP → RAN-GTP

- RAN-GTP $\xrightarrow{GAP}$ RAN-GDP (triggers GTP hydrolysis)
  (found mainly in cytosol)
- RAN-GDP $\xrightarrow{GEF}$ RAN-GTP (guanine exchange factor)
  (found mainly in nucleus)
- If nuclear transporter arrives at nuclear side with cargo → RAN-GTP binds to importin and releases cargo

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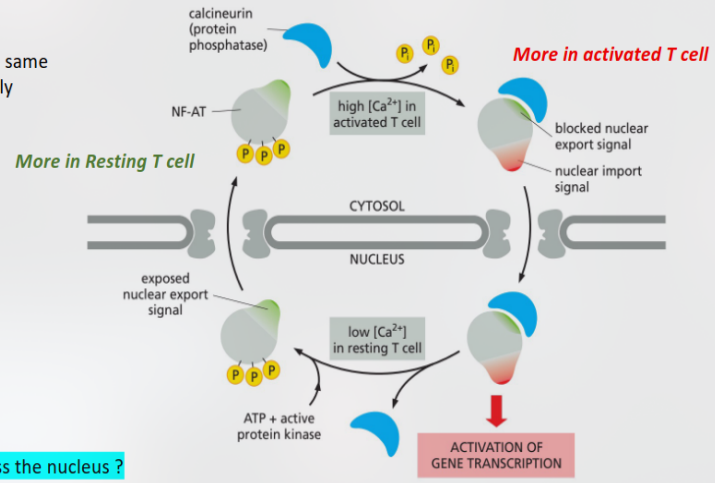
creates directionality of transport

Nuclear transport and gene regulation

Both NLS and NES present in the same protein and exposed conditionally



Transport Through npCs Can Be regulated by **Controlling access to the Transport machinery**

Can you keep Ca^{2+} gradient across the nucleus ?

Nuclear Lamina

- located on nuclear side of inner nuclear membrane
- meshwork of interconnected protein subunits called nuclear lamins
- lamins are a special class of intermediate filament proteins, that polymerise into 2D-lattice
- nuclear lamina gives shape and stability to the nuclear envelope – connected to NPC and integral memb. prot. of inner nuclear membranes
- lamina interacts with chromatin – chromatin interacts with integral memb. prot. – structural links b/w DNA and nuclear envelope
- On the onset of mitosis:

nucleus disassembles, nuclear lamina depolymerises

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due to direct phosphorylation of nuclear lamins, by CdK activated on onset of mitosis

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nuclear envelope proteins no longer tethered to pore complexes

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nuclear envelope proteins disperse through ER membrane

The Endoplasmic Reticulum

- more than half of the total membrane of cell
- netlike labyrinth of branching tubules and flattened sacs that extends throughout the cytosol
- continuous sheet enclosed by ER, called ER lumen or ER cisternal space

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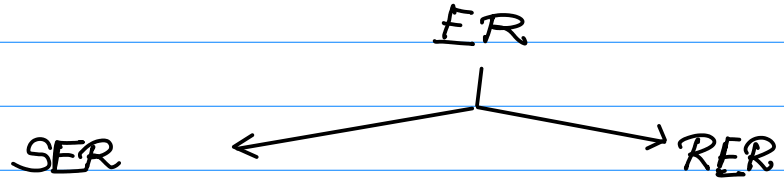
central role in lipid and protein biosynthesis, serves as an intracellular Ca^{2+} store

- protein synthesis on cytosolic surface of RER
- in ER lumen, proteins fold and oligomerise, only then can leave ER, otherwise transported back to cytosol and degraded in proteasomes.
- misfolded proteins in excess in ER → unfolded protein response which activates appropriate genes in the nucleus to help ER cope
- mammalian cells begin to import most proteins into ER before complete synthesis of polypeptide chain (co-translational) while import of proteins into mitochondria, chloroplast, nuclei, peroxisomes → post-translational



- only proteins that carry a special ER signal sequence are imported into ER
- signal sequence is recognised by a signal-recognition particle (SRP) — bridge between growing polypeptide chain and ribosome, and directs them to a receptor protein on cytosolic surface of RER

- This binding to the ER membrane initiates the translocation process that threads a loop of polypeptide chain across the ER membrane through the hydrophilic pore of a protein translocator. **Soluble proteins**—destined for the ER lumen, for secretion, or for transfer to the lumen of other organelles—**pass completely into the ER lumen**.
- Transmembrane proteins destined for the ER or for other cell membranes are translocated part way across the ER membrane and remain anchored there by one or more membrane-spanning α-helical segments in their polypeptide chains.
- These hydrophobic portions of the protein can act either as **start-transfer or stop-transfer signals** during the translocation process.
- When a polypeptide contains multiple, alternating start-transfer and stop-transfer signals, it will pass back and forth across the bilayer multiple times as a **multipass transmembrane protein.**
- The asymmetry of protein insertion and glycosylation in the ER establishes the sidedness of the membranes of all the other organelles that the ER supplies with membrane proteins