

## Intracellular transport in eukaryotes

---

### *Overview*

Compartmentalization and inner membranes enables eukaryotic cells

- to be 1000-10000 times larger than prokaryotes
- to isolate specialized chemical processes in specific parts of the cell
- to produce “packages” (vesicles) of chemical components that can be shuttled around the cell actively

Membrane-enclosed **organelles** take up ~50% of the volume of eukaryotic cells:

- **nucleus** – genomic function
- **endoplasmic reticulum** – synthesis of lipids; on the border with the cytosol, synthesis of proteins destined for many organelles and the plasma membrane
- **Golgi apparatus** – modification, sorting, and packaging of proteins and lipids for specific intracellular destination (akin to a mail sort facility)
- **lysosomes** – degradation
- **endosomes** – sorting of endocytosed (engulfed) material by the cell
- **peroxisomes** – oxidation of toxic species
- **mitochondria**, chloroplasts – energy conversion

Cells contain  $10^{10} - 10^{12}$  protein molecules that are constantly being synthesized and degraded

Proteins are synthesized in the **cytosol**, but not all proteins remain there and many must be transported to the appropriate compartment

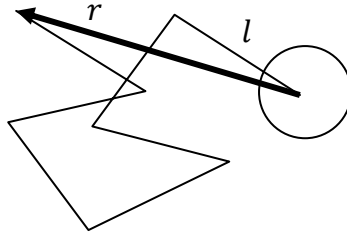
### *For comparison: transport by diffusion*

Even without **active transport** requiring free energy transduction, movement of molecules in the cell is rapid by diffusive motion

Consider a sea of molecules. Pinpoint one molecule and note its starting position at time 0.

Due to thermal motion, the particle on average makes a random jump of length  $l$  every  $\tau$  units of time. The jump is random in the radial direction. This is called a **random walk**.

Repeat this process for many jumps  $n$  and interrogate the final distance of the particle from its starting point



We could imagine doing many such experiments. What would be the expected  $\langle x \rangle$ ,  $\langle y \rangle$ ,  $\langle z \rangle$  as a function of  $n$ ?

$$\langle x \rangle = 0 \quad \langle y \rangle = 0 \quad \langle z \rangle = 0$$

since the process is spherically symmetric.

What about  $\langle r_n^2 \rangle$ ?

$$r_n^2 = x_n^2 + y_n^2 + z_n^2$$

Consider the case in going from step  $n$  to  $n+1$ :

$$\begin{aligned} r_{n+1}^2 - r_n^2 &= x_{n+1}^2 - x_n^2 + y_{n+1}^2 - y_n^2 + z_{n+1}^2 - z_n^2 \\ &= (x_n + \Delta x)^2 - x_n^2 + (y_n + \Delta y)^2 - y_n^2 + (z_n + \Delta z)^2 - z_n^2 \\ &= (x_n + \Delta x)^2 - x_n^2 + (y_n + \Delta y)^2 - y_n^2 + (z_n + \Delta z)^2 - z_n^2 \\ &= 2x_n \Delta x + \Delta x^2 + 2y_n \Delta y + \Delta y^2 + 2z_n \Delta z + \Delta z^2 \end{aligned}$$

Here,  $\Delta x, \Delta y, \Delta z$  are the random amounts by which we change the length at one step. Notice that we have the constraint  $\Delta x^2 + \Delta y^2 + \Delta z^2 = l^2$ . Therefore

$$r_{n+1}^2 - r_n^2 = 2x_n \Delta x + 2y_n \Delta y + 2z_n \Delta z + l^2$$

Now, we average over all possible (random) trajectories for the same starting point:

$$\langle r_{n+1}^2 - r_n^2 \rangle = \langle 2x_n \Delta x + 2y_n \Delta y + 2z_n \Delta z \rangle + l^2$$

However, since the  $\Delta x, \Delta y, \Delta z$  are random with zero mean, and uncorrelated to the current position of the molecule, the average on the RHS becomes

$$\langle r_{n+1}^2 - r_n^2 \rangle = l^2$$

Or

$$\langle r_{n+1}^2 \rangle = \langle r_n^2 \rangle + l^2$$

By recursion, therefore, we can write

$$\begin{aligned} \langle r_n^2 \rangle &= nl^2 \\ &= \frac{t}{\tau} l^2 \end{aligned}$$

The LHS is called the **mean squared displacement**. It tracks the average squared distance of a particle at time zero from its random location at time  $t$ .

This kind of random movement is called **Brownian motion** and is a kind of **diffusive** process. Here, diffusive means that the motion is dominated by random fluctuations. In contrast, **activated** processes require concerted movements over free energy barriers.

In fact, we can define the diffusion constant  $D \equiv \frac{l^2}{6\tau}$ . Then,

$$\langle r^2 \rangle = 6Dt$$

In the limit that both the step length and the step time go to zero, the random walk can be described by the diffusion equation:

$$\begin{aligned} \frac{\partial \wp(x, y, z, t)}{\partial t} &= D \left( \frac{\partial^2 \wp(x, y, z, t)}{\partial x^2} + \frac{\partial^2 \wp(x, y, z, t)}{\partial y^2} + \frac{\partial^2 \wp(x, y, z, t)}{\partial z^2} \right) \\ &= D \nabla^2 \wp(x, y, z, t) \end{aligned}$$

Here,  $\wp(x, y, z, t)$  gives the probability a molecule is at location  $x, y, z$  at time  $t$ . You can think of it in the same sense as a concentration.

### *The path of a protein: transport and sorting*

In reality, proteins do not move by simple diffusion

- **free energy transduction** is used to power **active transport** of molecules
- proteins are selectively transported to different parts of the eukaryotic cell → called **sorting**

Three basic mechanisms by which proteins can move selectively to different parts of the cell

- **gated transport** – large openings that allow passage of folded proteins, e.g., nuclear pore
- **transmembrane** – translocator protein channels move unfolded proteins through membranes
- **vesicular** – lipid vesicles with contents pinch off and fuse with other organelles

## Protein synthesis

Start of a protein: synthesis takes place

- in the **cytosol** on **free ribosomes** → released to the cytosol
- in the cytosolic side of the rough ER on **membrane-bound ribosomes**

Many proteins stay in the cytosol, but for the others they must carry **signal sequences** at either the N or C terminal, or both, to direct to which compartment they go

- **recognition** of signal sequences by pores and transport proteins
- often removed from the protein after it has reached its final destination

## Proteins entering the nucleus

Incoming: histones, polymerases, regulatory factors

Outgoing: rRNA, mRNA

**Nuclear pores** are giant protein gateways that cross the nuclear envelope and allow selective passage of proteins destined for the nucleus

- ~50 proteins
- unstructured, entangled protein strands in the middle, like a kelp bed → sieving property
- 5-10 kDa molecules can pass through simple diffusion, larger requires active transport
- **nuclear transport receptor** proteins bind to the nuclear signal sequence on cytosolic proteins and navigate them through this tangle
- requires hydrolysis of GTP
- proteins are transported in fully folded form

## Proteins entering the mitochondria and chloroplasts

Though mitochondria and chloroplasts have their own genome, many of the genes for the required proteins have evolved into the nuclear DNA, and thus these proteins must be imported

- signal sequence binds to receptors on the outer membrane of the organelle
- receptors diffuse laterally in the membrane until finding a contact site
- protein unfolds and passes through the contact site, to the interior of the organelle
- **chaperones** inside the organelle help pull the protein through and refold it

## Proteins entering the ER

Proteins that are eventually sent to the Golgi apparatus, endosomes, lysosomes, and cell surface all must first enter the ER

Proteins can diffuse from the cytosol to the ER surface, but most are synthesized at the ER with membrane-bound ribosomes

- ER signal sequence directs a ribosome to attach to the ER by a binding mechanism to a **translocation channel**
- other ribosomes bind to the first membrane-attached ribosome, forming a **polyribosome**

Two kinds of proteins can be synthesized by membrane-bound ribosomes:

- **soluble proteins** are released to the interior of the ER (**lumen**)
- **transmembrane** proteins are left inserted into the membrane of the ER by the translocation channel
  - hydrophobic **start and stop transfer sequences** tells the channel how to insert the protein in the membrane
  - orientation with respect to the membrane is subsequently fixed because flipping is so kinetically slow

## *Vesicular transport*

Once in the ER, proteins can be sent to many different intracellular and extracellular locations, but in all cases transport occurs by **vesicles**

ER → Golgi apparatus → “-somes”, plasma membrane, or extracellular space

### Basic features of transport

Specificity: vesicles carry specific cargo and drop it off at specific locations → **recognition** events during packaging of materials and in vesicles interacting with other molecules

Proteins involved in the formation and loading of vesicles

- **clathrin** is a protein that self-assembles into basket-like cages to help bud off and stabilize a small vesicle
- **dynamin** helps pinch off the end of the budding vesicle by constraining the “neck”
- transmembrane **receptors** bind specific cargo along the inner vesicle membrane
- **adaptins** bind and help assemble specific kinds of receptors for pinching off with clathrin

How does a vesicle find the correct destination?

- **Rab** proteins on the vesicle surface are recognized and bound by complementary cytosolic tethering proteins in the destination organelle
- **SNARE** proteins on both the vesicle and organelle also recognize each other, wrapping around like a twist-tie and pulling the vesicle close to the destination organelle

Vesicle **fusion** occurs with the help of proteins that assemble at the fusion site

### Pathways of transport from the ER

Proteins that are synthesized and remain in the cytosol are typically not post-translationally modified

Proteins that enter the ER, however, are covalently modified:

- **disulfide bonds** are formed → used to increase stability of proteins that might need to be released to the harsh extracellular environment
- **glycosylation** begins → sugars help prevent degradation, sometimes assist folding, and can be recognition or transport signals

Typically glycosylation occurs in chunks of sugars that are attached to the amino acid **asparagine**

Glycosylation continues in the Golgi apparatus

Exit from the ER serves as a quality-control mechanism for protein folding and assembly

- unfolded proteins or multimeric structures not properly assembled are not allowed to exit
- **chaperones** in the ER help fold proteins correctly so they can exit
- proteins that don't fold correctly are eventually degraded in the ER
- if too many unfolded proteins are present, the **unfolded protein response** program causes the production of more ER and its components

Protein-containing vesicles exit the ER and fuse with the **Golgi apparatus**

- vesicles enter and fuse on the **cis** face
- vesicles bud off and exit with cargo on the **trans** face

In the Golgi apparatus

- proteins are further covalently modified, notably by addition of complex **oligosaccharide** groups
- proteins bud off in transport vesicles destined for specific locations
- location in the Golgi apparatus where budding occurs determines what kinds of modifications are made and to where the proteins are sent

**Secretion pathways** for protein-containing vesicles

- **default pathway** → no signal required, to the extracellular space
- **constitutive exocytosis pathway** → contents used to supply plasma membrane with proteins and lipids
- **regulated exocytosis pathway** → vesicles wait at the cell membrane for release triggered by changes in pH, ion concentration, or binding proteins

### How do vesicles move fast from one part of the cell to another?

Vesicles do not simply diffuse around, but are **actively transported** to other parts of the cell

**Motor proteins** “walk” along actin filaments and microtubules in the cell cytoskeleton, pulling attached vesicles

- requires ATP hydrolysis
- conformational changes due to ATP binding and hydrolysis produce net movement
- proteins that walk along microtubules are **kinesins** (outward from the centrosome) and **dyneins** (inward)

### Pathways of import into the cell: endocytosis

**Endocytosis** necessary for take up of fluid and materials that can be digested to create building blocks for macromolecules

Extracellular space → small vesicle → fusion with **endosome** → transfer to lysosome for degradation → cytosol

Two mechanisms for endocytosis:

- **pinocytosis** → small vesicles for take up of fluids and molecules → all eukaryotic cells do this
- **phagocytosis** → cells engulf and digest large particles or other, small cells → specialized eukaryotic cells (e.g., white blood cells) and bacteria

Endocytosis can be non-specific or specific

- membrane-bound receptors detect concentrations of particular species
- clathrin and other proteins help form an endosome in response to receptor binding

**Lysosomes** degrade ingested molecules

- contain digestive enzymes for proteins, lipids, nucleic acids, oligosaccharides
- lower pH ~5 versus ~7 in the cytosol
- transporter proteins in surrounding membrane that pump out components: amino acids, simple sugars, nucleotides

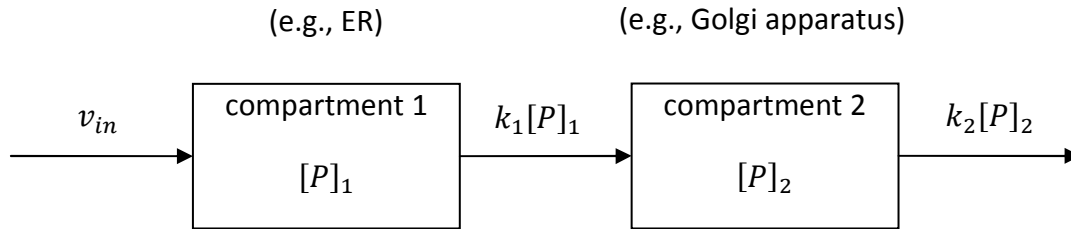
### *Modeling protein sorting*

We can model the movement of proteins between different cellular compartments using the following assumptions

- compartments are well-mixed volumes



- constant rate of synthesis of the protein initially
- the rate of leaving a compartment is proportional to the protein concentration times a rate prefactor



Here,  $v_{in}$  is the synthesis rate of the protein in molecules / time / volume

The  $k$ 's are such that  $k[P]$  gives the rate of exit of the protein in molecules / time / volume

Consider first compartment 1

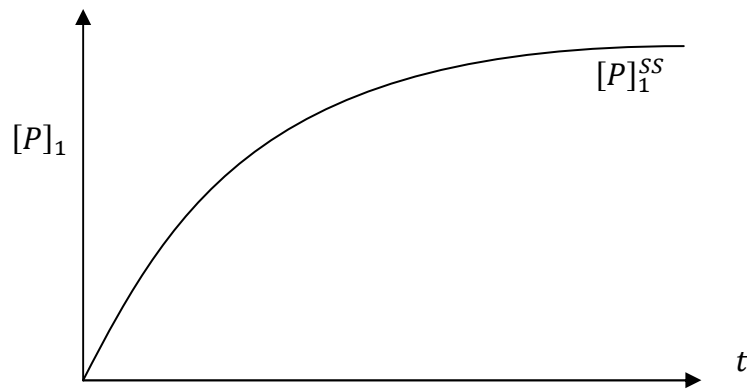
$$\frac{d[P]_1}{dt} = v_{in} - k_1[P]_1$$

At steady state, the LHS is zero and we have

$$[P]_1^{SS} = \frac{v_{in}}{k_1}$$

If the cell suddenly begins synthesizing protein, with an initial zero concentration, we can solve for the concentration profile

$$\begin{aligned}
 [P]_1 &= \frac{v_{in}}{k_1} (1 - e^{-k_1 t}) \\
 &= [P]_1^{SS} (1 - e^{-k_1 t})
 \end{aligned}$$



What about compartment 2?

$$\frac{d[P]_2}{dt} = k_1[P]_1 - k_2[P]_2$$

At steady-state,

$$\begin{aligned} [P]_2^{SS} &= \frac{k_1}{k_2} [P]_1^{SS} \\ &= \frac{k_1}{k_2} \frac{v_{in}}{k_1} \\ &= \frac{v_{in}}{k_2} \end{aligned}$$

Time-dependent solution requires first the solution for  $[P]_1(t)$

$$\begin{aligned} \frac{d[P]_2}{dt} &= k_1 \frac{v_{in}}{k_1} (1 - e^{-k_1 t}) - k_2 [P]_2 \\ &= v_{in} (1 - e^{-k_1 t}) - k_2 [P]_2 \end{aligned}$$

The solution to this equation

$$[P]_2 = [P]_2^{SS} \left( 1 + \frac{k_1 e^{-k_2 t} - k_2 e^{-k_1 t}}{k_2 - k_1} \right)$$

