Cellular Biochemistry

PART 1

25.9.2017

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The membrane layer is liquid but not really elastic. Molecules can diffuse within the membrane and interact with other molecules in a non-fixed way.

Features: Membranes are thin (5-7 nm), strong, non-elastic, self-sealing, flexible, deformable, hydrophobic barriers between aqueous compartments, composed of amphiphilic lipids (phospholipids) arranged as a bilayer. Membranes also contain non-membrane forming lipids and proteins.

Pulling on a membrane will not be like a tent, but rather a very small pipe will stick out of the place where it is being pulled so that it can minimize the increase in surface since it is liquid.

Dogma on membranes: Membranes come from membranes, they cannot be de novo biosynthesized. Therefore, they grow first and then divide, or they can undergo fission and fusion (or growth of one molecule at a time). E.g. they come from sperm and oocyte.

How can we distinguish membranes? How can cells know on which membranes to perform chemical reactions? What is the specificity?

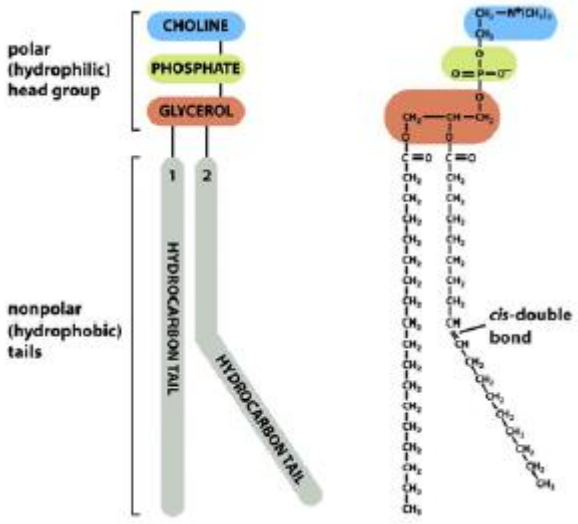
Selectivity: Permeability: ions, metabolites, proteins, lipids. Ex. Ca2+ is interesting: in the cell, the concentration is 1nM and outside it is 1mM (difference of the order of 10**6)

They allow compartmentalization and favour 2D diffusion over 3D diffusion.

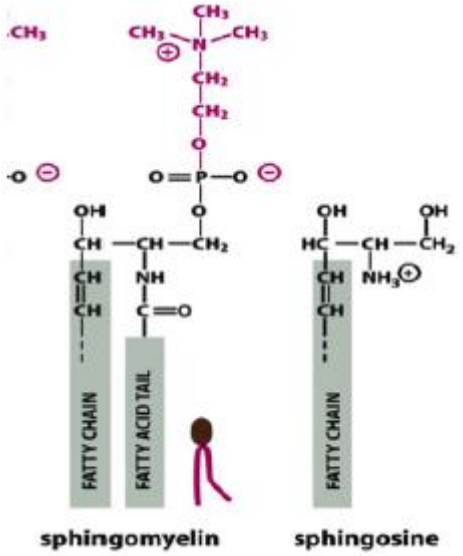
Lipids

Glycerolipids and sphingolipids (both are phospholipids), sterols, isoprenoids.

Glycerolipids: Glycerol with two ester bonds and carbyl rest (non-polar) and phosphate head group with rest R that is polar.

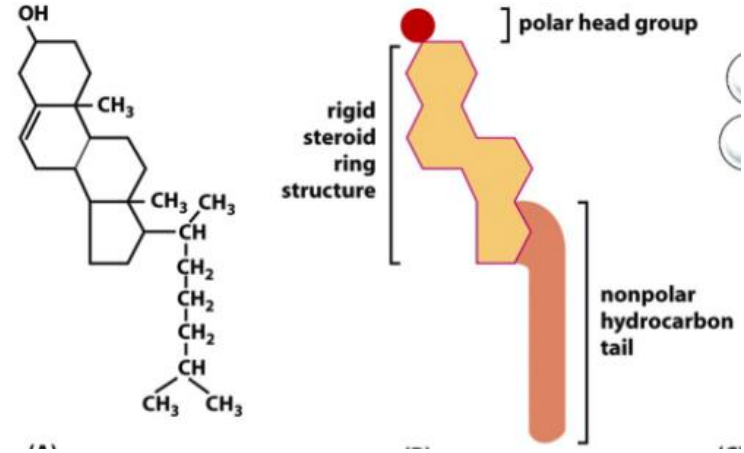


Sphingolipids: A fatty acid with instead an ester bond, there is an amide bond.



Sphingosine: generated from serine and a fatty acid CoA. Ceramide is generated through amide bond with free amino groups.

Sterols: Cholesterol in membranes (rather hydrophobic). Sterol in circulation. Ex.: corticosterone, testosterone, progesterone, cortisone.



Isoprenoids: Examples are ubiquinone, retinol, carotene, prenyl lipids (can be added to proteins so that the proteins can stick to proteins), squalene (basis for sterol synthesis such as cholesterol). Sterols are made from isoprenoids.

Membrane Proteins

Monotopic transmembrane proteins only traverse the membrane once. Polytopic transmembrane proteins traverse the membrane more than once.

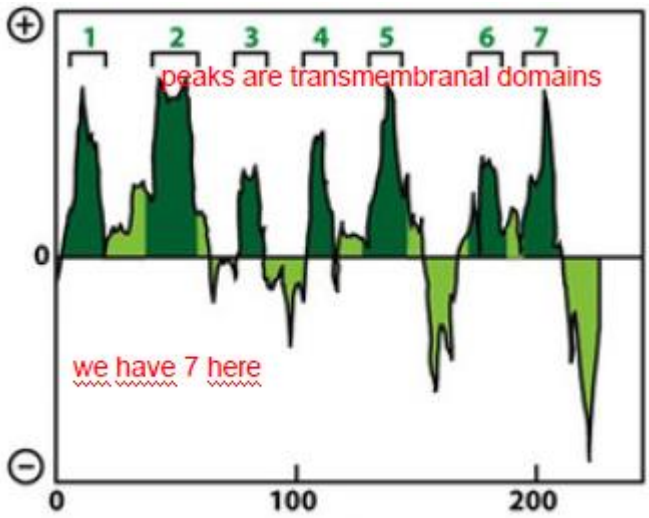
There are proteins that are covalently attached to lipids on the membrane (they are not TM proteins), we calll them lipidated proteins.

These two types summarize to integral proteins (can be either TM or lipidated protein).

A sister group are peripheral proteins (a protein that can attach to a integral protein or do NOT bind to a lipid like lipidated proteins).

The membrane is 6nm thick, 3nm of that are hydrophobic, so TM proteins they must be able to interact with the hydrophobic domain. Those interacting are exclusively composed of hydrophobic amino acids (this is called the alpha-helical segment).

Hydropathy plot lets us identify hydrophobic regions in a TM protein (alpha-helices):



Exceptions are beta-barrels. They let hydrophlilic proteins go through the membrane. Beta barrels are found in the outer membrane of bacteria, mitochondria and chloroplasts. They are made of beta-sheets.

Why are they only found in mitochondria in our cells, but in bacteria everywhere? Endosymbiosis.

The functions of beta-barrels is to make pores. Mitochondria has lots of them, where actually all the proteins pass through (via machinery, that is absent in eukaryotic membranes).

Lipidated proteins

Protein originally made without a lipid and then post-translationally modified by a lipid (added to the protein).

Examples: Myristylation (amido bond between H3N terminus and COOH-terminus of myristyl (is C14)) is permament.
Palmitoylation (fatty acid, looks just like myristyl, but it is C16; thioester bond) is reversible.
Prenylation, prenyl group, is either geranyl geranyl or farnesyl (farnesyl is just slightly shorter than geranyl). They are added via a thioether bond between Cys and prenyl-OH. Placed at a CaaX box (C = cysteine, aa = two long alipathic amino acids, X = whatever C-terminus)

Myristylation	C14 fatty acid via amide bond to amino group of N-terminal glycin	Added to co-translationally to cytosolic non membrane proteins, a permanent modification.	Sufficient for membrane binding only when combined with:1) Positive charge cluster,2) FA alkyl group, or 3) protein-protein interactions	Arf1 and c-src Exposed or hidden after reversible conformational change.
Fatty acylation	Typically C16 i.e. palmitic acid By thioester to cystein	Reversible modification in cis-Golgi, on soluble proteins and endodomains of trans-membrane proteins	Double FA-acyl group direct many proteins to lipid rafts	Caveolin, influenza hemagglutinin.
Prenylation	Farnesyl (C15) or geranylgeranyl (C20) By thioether to cystein	Added to cytosolic non membrane proteins. So called CaaX box usually at the C-terminus, a permanent modification	Often combined with nearby FA acyl groups	Two Ras isoforms: H ras farnesyl plus two FA acyl chains. K-ras Farnesyl plus cluster of positive charges

Lipidation takes place in the cytosolic side. There is one case that takes place in the extracellular side: **GPI anchoring** – proteins are just attached to it (on the extracellular side).

Importance of lipidation: support dynamic processes such that proteins can come on and off the membrane during signal transduction, molecular sorting, membrane bending, vesicle formation, membrane recognition etc. Regulation of dynamics is in time and space. Allow interaction of proteins with specific membranes only and with specific lipid microdomains such as lipid rafts.

Peripheral proteins

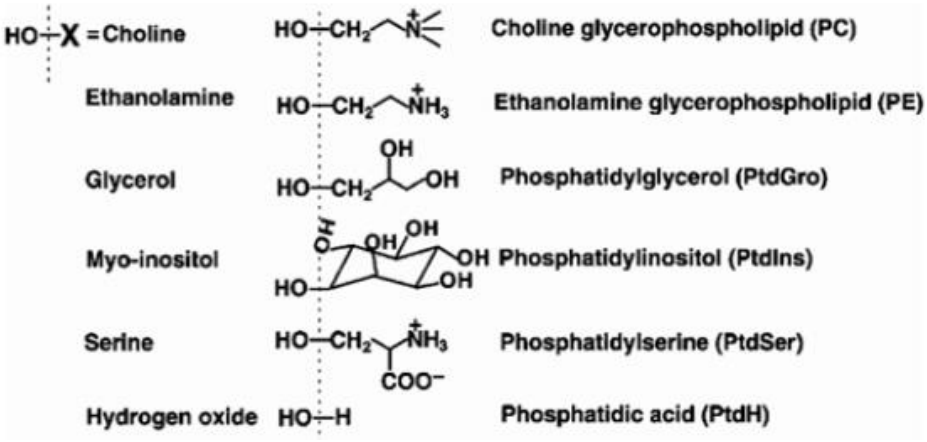
Clathrene coated pits occur in the endocytosis of proteins etc. Non-covalently attached to lipid head-groups or proteins in the membrane; Complex mixture of proteins on both sides of a membrane; Interactions are often transient and regulated; Cytosolic side of PM is particularly rich in peripheral proteins: an extensive, dynamic 'cortex' of actin, adaptor proteins, and other proteins (needed for membrane stability; local membrane specializations; connections with cytoskeleton; transmission of signals; trafficking of vesicles; cell shape and polarity determination; membrane curvature; endocytosis)

Lipid diversity

We have 4 main classes, but within the classes there are lots of different types that are combinatorial.

In fatty acids for instance, length varies from 14-22C, insaturations (0 – 6 poly unsaturated fatty acids (=: PUFA)). A FA can also change the polar head (positively or negatively charged).

Various glycerol heads can be:

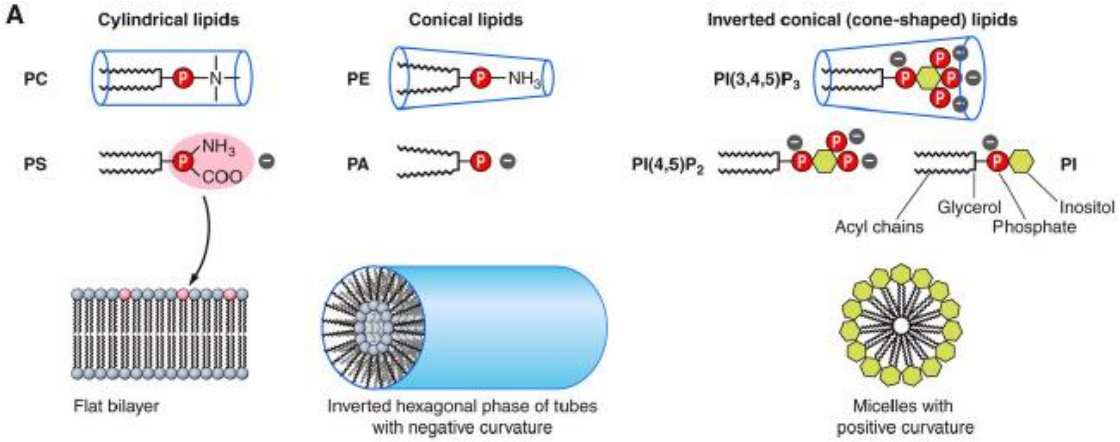


Different heads have different biophysical properties.

Difference between ethanolaime and choline: Ethanolamine can get rid of H+ in the acid for, while choline cannot.

It is estimated that there are around 100'000 different lipids (different heads and different tails lead to many combinations).

What determines if a lipid will for a bilayer or a micelles? Shape and size determine it.



Detergents form micelles.

Oil does not have a polar head which is why they do not form a bilayer on water. They create droplets that can also occur in cells.

Insaturations

When there are kinks in the lipid, we get a more liquid membrane, because the interactions of the hydrophobic and hydropilic ends to not take place that well, which is why they don't pack so nicely.

Sterols

Sterols make the membrane more rigid as they fill out the voids of the kined lipids for example.

Gradients: length and thickness of the membrane: ER < golgi < plasmamembrane.

Also, there is a asymmetric distribution in the monolayers, so the outer layers have a different composition than the intracellular membrane, which is logical due to the environment. As a rule, negatively charged lipids are more abundant (in the cytosol?).

Lipids that tend to have an affinity to each other tend to cluster together. It is due to van der Waals forces that is due to insaturation (that pack nicely together and that are chaotic).

Segregation in lipids: High temperature favors high entropy (=> chaos) and when you lower the temperature the degree of disorder will decrease, leading to the clustering phenomena.

Lateral segregation: In the ER, there is lots of PI, in golgi PI4P, in endosome PI3P and in plasmamembrane PI4,5P2 (abbreviated to PIP2).

How are lipids transported? It is not yet understood.
Osh4 is a protein that binds a sterol in it. Osh4 can kick out the sterol and take up a PI4P. This transport occurs against the concentration gradient.

Flippases and Scramblases: They can transport a lipid from one leaflet to the other leaflet. Flippases use energy to act against the concentration gradient, while scramblases …

Both basically work like ion channels. Ion pumps example: P-type ATPases.

2.10.2017

Shape defines function and function defines shape.

In mitochondria, cristae are very important for mitochondrial function, so how do you get it? The crista has the cristae junction which is where all the F_1/F_0 ATPases are.

Proteins that bend membranes

BAR-domain proteins: Have a banana shape. When they attach to membranes (peripherally attached proteins), will cause a little increase in curvature in the membrane.

Coat proteins: cop1, cop2, clathrine are the best studied coat proteins.

Wedge-like insertions of reticulon proteins: Important for the tubular nature of the ER.

Nucleus and its membrane: So there are protein complexes on the nucleic site of the membrane and the cycplasmic site of membrane. There are receptors on the cytoplasmic site that go through the nuclear membrane “touching” the receptors attached to the nucleic site (that also go through the membrane). Cyplasmic stimuli can induce a response within the nucleus this way (?).

Membrane fusion and fission

There is fission, fusion and hemifusion (see fission and fusion in cell biology summary).

On fission

Liposomes won’t sponaneously fuse together due to the water (cytoplasm actually) between them:

Cell division: It requires energy, since polar side of membranes is hydrated, which is why the cell needs to overcome hydration energy. Also, the lipid bilayer is stable and it needs to be destabilized for cell divison.

Machineries for fission: 1) dynamins (and dynamin related proteins). Dynamin can spiral around an endocytic vesicle (dynamin is a GTPase), so it can like squeeze where it's bound and "cut it apart". Dyamins related proteins are important for mitochondrial fission (see cell biology summary).

2) ESCRT := endosomal sorting complex required for transport: important for hiv budding, used in last step of cytokineses and used for multi vesicular bodies. Some components of ESCRT are ATPases.

Dynamin does fission from the outside, ESCRT occurs from the inside (fission from inside). So, dynamin is cytoplasmic and ESCRT is non-cytoplasmic.

On fusion

Examples for fusion: fusion of gametes, mating in yeast, enveloped virus infection, mitochondrial fusion, vesicular trafficking and exocytosis. It also requires energy.

SNAREs: SNAP receptor, soluble NSF attachment protein, unstructured when not in a complex.
There are SNAREs on the protein called v-SNAREs and on the outer membrane there are t-SNAREs, when they interact they fold and basically intertwine with the other SNARE. Neither one of them and ATPase or GTPase. The folding though is energetically more favorable that favors the folding (it only uses the folding energy). Reopening them again costs energy. NSF is the protein that provides the energy from reopening (NSF is a triple ATP, AAAATPase) at the expense of ATP hydrolysis.

There are further proteins helping fusion to happen: viral -> hemaglutinin and also dynamin related proteins (unknown why, because they are actually involved in fission, but apparently in fusion too.)

Cell fusion proteins (machinery unknown): mitofusin and OPA1 for inner and outer mitochondrial membrane fusion, atlastins for ER fusion, GTPases.

Organelle Biogenesis

On mitochondria: it is endosymbiotic, has two membranes, more han 4 compartments, the mitochondrial membrane is divided into (from cytoplasmic to matrix): OMM, IMS, IMM, matrix/cristae.

In yeast, mitochondria make FeS clusters. So, in a glucose rich medium, yeast actually uses energy (and does not produce energy) to make FeS clusters. Moreover, it also triggers apoptosis by rapturing the outer membrane of mitochondria, they are also involved in Ca2+ signalling and in innate antivirus defense.

Fission is catalyzed by the Dynamin related protein 1 (=: DRP1). It assembles around OMM as a contractile ring and also requires GTPases and is recruited by OMM-resident adaptor proteins to the site.

Fusion of IMM is catalyzed by Opa1 (and requires GTPases and dynamin related proteins).
Fusion of OMM is catalyzed by Mitofusin (and requires GTPases and dynamin related proteins)

Biogenesis of mitochondria: There is a genome in mitochondria (mtDNA), also proteins, metabolites, lipids. Other facts: 20 kb, always maternally inherited (oocyte), has 12 proteins, encodes 20 tRNAs, 1000-2000 proteins are imported from the cell into the mitochondria, it also has rRNA.

How do lipids get into the mitochondria? The ER mitochondria encounter structure is a protein complex that is bound to the OMM and ER and the lipids like jump/are transported between them that way for transport (?). This protein complex seems to be very important, shape of mitochondria changes and the model organism (yeast in the experiment) becomes very sick.

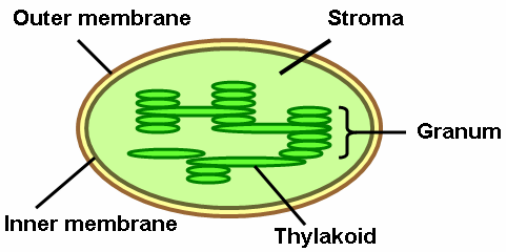
How proteins are imported in the mitochondria: TOM for the outer membrane (TOM40 main subunit) and TIM for the inner membrane. Protein needs to be unfolded, have to go both through TOM and TIM (studied in vitro mostly). The unfolded protein has a MTS sequence (mitochondria target sequence) which will be imported into the mitochondria (the site with MTS goes in first).

TIM22 imports many IMM proteins. TIM23 imports many matrix proteins. SAM inserts beta barrel complexes from IMS to OMM. OXA1 inserts matrix-translated or matriximported proteins in the IMM. Import works in vitro, so no cytosolic factors are required.

How to check if protein really has been imported in the mitochondria: western blots and centrifugation. Or also, since the experiment is normally in a test tube, you can also add a protease that cannot access the mitochondria (MTS can be cleaved off, so that we can measure the amount of imported proteins and non imported proteins (since the protease will cleave the MTS off when it tries to enter and not when it is simply floating in the tube)).

On chloroplast

It is basically the same thing as in mitochondria: The outer membrane is called POM and the inner PIM. For the transport, we have TOC and TIC respectively, which are needed for protein import. Inside, there is the granae and thylakoid.



In a test tube, there are no chaperones and the protein is in a badly folded form (needs chaperones and a favourable folding environment) before it enters the chloroplast. In the chloroplast, there are chaperones to help it unfold.

There are 3 pathways for import: Sec, SRP, TAT pathway.

Sec pathway: Translocon homologous to bacterial SecYEG (eukaryotic Sec61).
SRP pathway: Oxa1 related translocon, no RNA moiety, homologous to bacterial SRP.
TAT pathway: twin-arginine translocon, relies on two adjacent arginines close to C-terminal.

On peroxisomes

Peroxisomes are not necessary for growth in yeast. How proteins are imported into peroxisomes is still unknown. Proteins imported are fully folded (no need for unfolding). It can even import gold particles. It imports proteins from ER or cytosol (cytosolic proteins have a C-terminal SKL sequence).

Functions: beta-oxidation, breakdown of fatty acids, innate viral defense, break down of ethanol, detoxification (generate H_2O_2 from O_2), contains catalase (break down H_2O_2 to water and oxygen or use it for peroxidation).

Pink1 (PTEN-induced putative kinase 1) is constantly imported into mitochondria where it is degraded. When mitochondria lose membrane potential, protein import is inhibited. Therefore Pink1 accumulates on the mitochondrial surface. There it recruits Parkin, a ubiquitin ligase, which ubiquitylates many proteins on the OMM. This leads to the specific recruitment of the autophagy machinery and the subsequent degradation of mitochondria in autolysosomes.

Trafficking

9.10.2017

Overview of trafficking: sorting – budding – transport – recognition – fusion – recycling.

ER to Golgi, Golgi to ER, Golgi to PM and early endosome, early endosome to late endosome to lysosome

All of them are basically reversible except for cargo that is in the lysosome. What is in the lysosome stays in the lysosome. Also, PM to golgi does not really happen.

Methodologies: electron microscopy, light microscopy, pulse-chase approach, genetics (SEC screen, VPS screen), biochemistry approaches.

Observe secretory pathways with for example radiolabelled amino acids: radiohistochemistry: EM pictures allow us to see where radio label accumulates. Often in the ER.

Principle of pulse-chase: A **pulse-chase analysis** is a method for examining a cellular process occurring over time by successively exposing the cells to a labelled compound (pulse) and then to the same compound in an unlabelled form (chase).

Observe secretory pathways using genetics: **Sec screen ("Sheckman" in 80s)**: Yeast that cannot secrete proteins normally die. At 37 degrees, they cannot secrete, filling up secretion vesicles within them. Take them and centrifuge them on a sucrose gradient (changes the density of the medium, so the cells float and they can be separated using this neat trick). (those are cells on the verge of dying).
Then, put them back at 25 degrees – the mutants become normal again. Sediment them by centrifugation. This way, we can find all the sec-mutants.

Vesicle genesis:

Clathrin for endocytosis and Golgi → PM trafficking. CopII for ER → Golgi (anterograde). CopI for Gogi → ER and intraGolgi (retrograde trafficking).

Vacuolar protein sorting screen: From ER, proteins go to golgi, then can go to PM or to endosome-lysosome:

Invertases: ER-golgi-PM, yeast cannot absorb sucrose, yeast can secrete invertases to make glucose out of sucrose and absorb it finally.

Cyp carboxypeptidase: ER-golgi-endosome-lysosome

Note: cyp-invertase fusion proteins go to the lysosome. Therefore, yeast cannot metabolize sucrose.

Observe secretory pathways using biochemistry: Principles:
There are cargo receptors of a vesicle folded into the cell, so to say (increases cargo concentration locally).
We have: Cop2 is anterograde (ER to golgi), Cop1 is retrograde (golgi to ER), clathrin at the PM for endocytosis and at the golgi that go towards PM and at the early endosome that go the PM.
Retromer takes cargo from early endosome to golgi.

Cargo sorting:

How can we favour cargo disassociation, when the cargo should leave the cargo receptors? Take advantage of inherent pH value differences: ER>golgi>EE>LE>lysosome with ER quite neutral and lysosome quite acidic. So, when cargo enters a more acidic environment it will normally disassociate, but sometimes the opposite can also occur.

How can we recycle the cargo receptor so it goes back to the PM and the cargo goes to the lysosome?

Vesicles are not just spheres, they also have tubes sticking out of them. The receptor will normally go to the tube side (low volume, high surface) and cargo stays in the sphere part (high volume, lower surface) and the tubes will the sort of cleave off and become a (late) endosome with the receptors inside it.

Retrieval:

Sometimes, an ER resident protein can accidentally go into a vesicle and this vesicle fuses with the golgi. Such a protein has a signal sequence called KDEL (4 amino acid sequence, so these proteins that have to stay in the ER have evolved such a sequence). There is a KDEL receptor (pointing its receptive site inside the golgi) which can take proteins with KDEL as cargo, so it can be brought back.

Conservation of membrane identity:

How can an ER stay ER and not become a golgi for example?

PIPs: most important one for endocytosis: PIP4,5-P2 at the PM: Important for making the clathrin coat. There are synaptoganins in the coat, which is a phosphatase that destroys PI4,5P2 (:= PIP2) to PI.

So, PIP2 is in the PM and can always diffuse into the clathrin coat with the synaptojanin. Synaptojanin converts PIP2 to PI, which leads to disassembly of the clathrin coat

GTPase: Sor1 important for making Cop2. Arf important for making Cop1. Rab used in endocytosis amongst others.

GTPases will assemble at the membrane and travel to a GAP (GTPase activating protein – changes GTPase to GDPase). At the donor membrane (here the travelling membrane), there is a GEF, so that GDPase becomes GTPase (lecturer said: D form becomes converted to T form).

Early endosomes have Rab7 and late endosomes have Rab5.

Exocytosis: Process of making protein in the ER and finally bringing it to the PM:

Journey of a protein in secretory pathway: Protein is translocated by Sec61. Protein folds and it requires PTMs (post-translational modification) which are N-linked glycosylation, disulphide bonds.
For folding, calnexin and calreculin are used – they are chaperons that bind glycan groups. If misfolded, then glucosyl transferase so that calnexin again affects the protein until proper binding occurred: This prevents a misfolded protein to leave the ER.

If you treat cells with an inhibitor with Cop2, then we get no golgi. We can wash off the inhibitor and here will be de novo creation of the golgi.

PM: constitutive exocytosis. Secretory granules: signal-mediated exocytosis (for example insulin signalling or more generally hormones and histamine and neurotransmitters). A very important signal is Ca2+ that activates synaptotagmin and this activates SNARE complex.

Endocytosis:

Functions: take up nutrients, downregulation of receptors (desensitation/adaption), eat up foreign objects (killing by phagocytosis), recycle proteins from PM.
Examples for nutrients: LDLs, irons (transporter: transferrin).

HRP: not receptor mediated, linear increase of endocytosis with concentration of HRP.
EGF: receptor mediated, logarithmic increases, can be saturated when no receptors are "free" anymore, effective at low concentrations already.

Early endosome: looks like a UFO, geometrical sorting, recycling of TM proteins. They can mature into a **late endosome**: is a multi-vesicular body. It will also fuse with a lysosome, which releases a lot of hydrolytic enzymes into the late endosome such as hydrolases and lipases that destroy the little vesicles within the late endosome.

How come the membrane of the lysosome and of the late endosome are not degraded too? Because they are very rich in glycolipids (glycocalyx). The glycolipids are in the lysosome and diffuse in the late endosome in order to protect the late endosome from destruction.

Lysosomes: degradative organelles (contains nucleases, proteases, lipases, phospholipases), acidic, terminal (no recycling of lysosomal content).

Lysosomes fuse with late endosomes/multivesicular bodies to generate an endolysosome. More than one lysosome can fuse with endolysosome. After digestion of the content, the endolysosome matures into a lysosomes, that can, again, fuse with a late endosome → the lysosome cycle.

Lysosomes also fuse with autophagosomes (double-membraned organelles product of autophagy) to form autolysosomes.

Lysosomal acidification: Mediated by the vacuolar H+-ATPase (v-type ATPase). Electrogenic: each proton pumped adds one + charge into compartment → build-up of + charges limits the acidification. Chloride channels (CLCs): Cl-/H+ exchangers, neutralize the charge of H+ → allow further drop of pH.

PART 2

Chromatin packaging is affected by: underlying sequence, histones (variants and modifications), chromatin remodelling enzymes, other DNA binding factors, architectural proteins.

DNA double helix is wound around a core of eight histone proteins: two H2A/H2B and H3/H4 dimers each. Nucleosomes is further packed into secondary structures (30 nm fibres). Euchromatin is accessible, heterochromatin is inaccessible.

Histone modifications: acetylation, methylation, ubiquitylation, phosphorylation (see GGB summary).

DNA methylation: In eukaryotes, DNA is methylated at the cysteine residues by DNA methyltransferases, which typically results in DNA repression. DNMT3A and 3B are de novo methyltransferases. DNMT1 is required for maintaining DNA methylation. DNA methylation typically results in gene repression.

Telomere functions: protect DNA from being recognized as DNA double strand breaks and from being degraded; act as tumour suppressors by limiting the proliferative effect of normal human cells; telomeres shorten after every cell division. When a certain critical shortness threshold is reached, it induces a cell death response (senescence, apoptosis). Telomeric human DNA is double-stranded TTAGGG repeats.

Telomerase enzyme is a reverse transcriptase that can add telomeric repeats to chromosomes ends de novo in each cell cycle and it is highly expressed in stem cells and in embryonic cells.

Acetylation makes the chromatin more open.

RNA polymerase II: Transcribes telomeric DNA into TERRA (ncRNA).

TABLE 6-3 The General Transcription Factors Needed for Transcription Initiation by Eukaryotic RNA Polymerase II

Name	Number of subunits	Roles in transition initiation
TFIID TBP subunit TAF subunits	 1 ~11	 Recognizes TATA box Recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP
TFIIB	1	Recognizes BRE element in promoters; accurately positions RNA polymerase at the start site of transcription
TFIIF	3	Stabilizes RNA polymerase interaction with TBP and TFIIB; helps attract TFIIE and TFIIH
TFIIE	2	Attracts and regulates TFIIH
TFIIH	9	Unwinds DNA at the transcription start point, phosphorylates Ser5 of the RNA polymerase CTD; releases RNA polymerase from the promoter

TFIID is composed of TBP and ~11 additional subunits called TAFs (TBP-associated factors); CTD, C-terminal domain.

Non-conventional promoter elements: CpG islands: region with CpG content of over 70%. These regions are generally associated with house-keeping or ubiquitiously expressed genes. Several TSS are used.

On mediator (complex): (might be very important since we answered an edu app question on it.) Mediator is a multi-subunit complex that provides a large interaction surface to contact DNA-bound transcription factors, general transcription factors, RNA Pol II and ncRNAs. It coordinates input from different TF and is important for gene loop formation. Mediator also regulates the transcriptional activity of RNA polymerase II. (**ask: how precisely do we need to know mediator and RNA polymerase 2.**)

Mediator does not bind to DNA and neither does mediator recognize DNA.

On chromosome organization: Important in development, differentiation and diseases.

Techniques:

Microscopy: Light microscopy, Electron microscopy, Live-cell imaging, superresolution microscopy: e.g. hetero- vs. euchromatin, subnuclear compartments, etc.

Chromosome conformation capture (3C) techniques: Use crosslinking to capture spatial proximities of the genome: e.g. new structural features of chromosomes, improved understanding of transcriptional regulation, etc.

ChIP-sequencing to analyze protein binding sites in the genome. Techniques to study chromatin organization in the nucleus: Hi-C.

Chromosomes have preferred positions, which may vary between cell types but are conserved between humans and primates. Gene-rich chromosomes are more central while gene-poor chromosomes are around nuclear periphery. Longer chromosomes and chromosomes with higher transcriptional activity occupy larger nuclear volumes, since they are open. Chromsome compartments are not conserved between cell types. They preferentially interact with each other (gene-rich/high activity with gene/rich-high activty or low-gene/low-activity with low-gene/low activity).

Topologically associated domains (TADs) are large genomic regions (~1 Mb on average) that display high levels of chromatin interactions within this region. TADs are often similar across different cell types, and partially conserved across species.

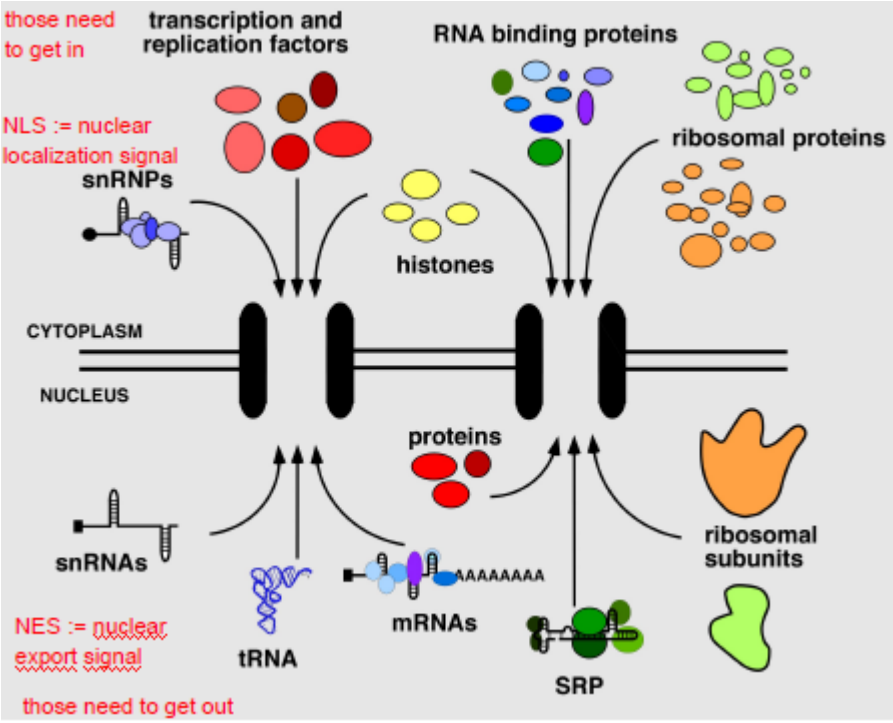
Hierarchy: chromosome territories, chromatin deparments (50kb resolution), TADs (10kb resolution), chromatin loops (5kb resolution).

Lamin-associated domains (LADs) are large genomic regions (0.1-10 Mb) that associate with the nuclear lamina. They contain few genes with low or no expression and are Strongly enriched for repressive histone marks. Constitutive LADs (cLADs) are maintained across a wide range of cell types and are highly conserved. Facultative LADs (fLADs) show variable association with the nuclear periphery (e.g. gene activation during differentiation).

23.10.2017

PART 3 – U.Kutay

Nuclear pore complexes (=: NPC) are the gateways to the cell nucleus. FG repeat domain nups (nups = nucleoporins) are the binding sites for nuclear transport receptors and they define the permeability characteristics of NPC.
For now, we look at nucleus – cytoplasm situations.



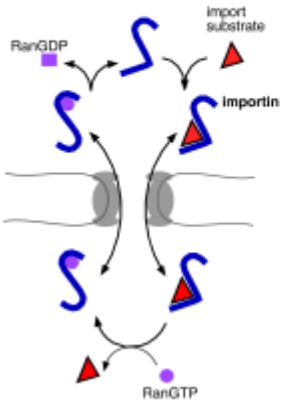
In the cytoplasm, we have importins, which are proteins for importing substrate and exportins , which are proteins for exporting ist substrate.

Ran is a GTPase: RanGTP + RanGAP (GAP = G-protein activating protein) => RanGDP. RanGDP + RanGEF (RCC1) => RanGTP.

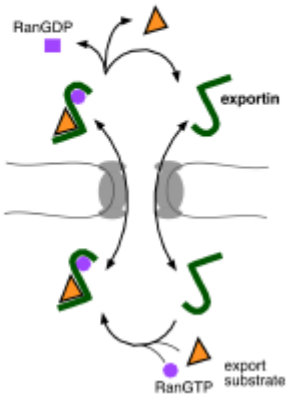
Asymmetry: in nucleus, RanGTP concentration is very high and in cytoplasm it is low.

RanGTP binds to importin in the nucleus and the substrate is removed and is freely floating in the cell nucleus. The importin-RanGTP complex leaves the nucles where it can unbind from importin and become RanGDP. The same concept is applicable for exportins.

- high affinity for RanGTP
- RanGTP binding dissociates import substrate



- low affinity for RanGTP
- RanGTP and export substrate bind cooperatively



Importin alpha binds to importin beta and they can carry cargo together (NLS is on importin alpha) from cytoplasm to nucleus. Importin alpha is exported with CAS and importin beta is bound to RanGTP and exported then.

Two models for the mechanisms of translocation through the NPC

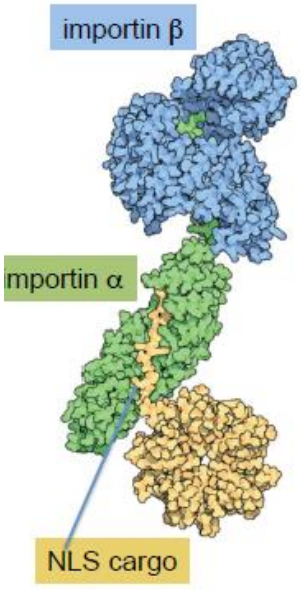
Virtual gate model: Unstructured **non-cohesive** FG-repeat-containing filaments fill the NPC channel to **entropically exclude cargo** not bound by nuclear transport receptors. Binding of transport receptors to FG repeats would significantly speed up translocation.

Selective phase model: The formation of a "**saturated hydrogel**" within the NPC, in which all the FG domains engage in a maximum number of interactions to form a highly ordered mesh with very even pore size. Transport receptors are thought to dissolve the FG mesh and thus catalyze the entry and translocation of cargo through the NPC channel. The mesh size would define the limit for passive diffusion.

Functions of the small GTPase Ran: nucleo-cytoplasmic transport, mitotic spindle assembly, nuclear envelope reassembly after mitosis. RanGTP marks the position of chromatin in the cell in both interphase and mitosis.

Effectors of Ran: interact more tightly with the GTP-bound form. For Ran: importins and exportins as well as RanBP1 family members.

Importin-beta (=: imp-beta) has HEAT-repeat structures. Imp-beta can bind to importin alpha with the cargo. Imp-alpha has an N-terminal stretch that binds to a C-terminal in imp-beta.



The RanGTP/cargo/exportin interaction: Why is export complex formation, i.e. binding of RanGTP and cargo to the exportin cooperative? Two ideas: RanGTP and cargo contact each other through release of extra binding energy. Or: Allosteric activation of conformational change. (those ideas are not mutually exclusive.)

Take home message 2

- N-terminal helical repeat motifs of nuclear transport receptors interact with switch 1 and 2 regions of Ran

- **Importins:** interaction of RanGTP sterically interferes with binding of import cargo molecules and changes their helicoidal pitch
  (release of import cargo upon RanGTP-binding)

- **Exportins:** extensive interactions between exportin, RanGTP and cargo and allosteric changes ensure cooperative complex formation (example CAS)

- allosteric communication between distant RanGTP and cargo binding sites can ensure cooperative complex formation (example XPO1)

- binding of RanGTP to transport receptors results in the inhibition its enzymatic activity and does not allow attack of its cofactors
  - intrinsic and RanGAP induced GTP hydrolysis
  - RanGEF stimulated nucleotide exchange

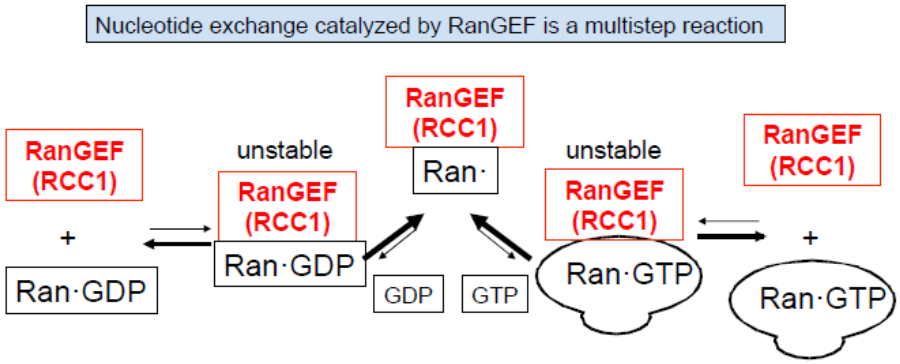
  result from overlap of binding sites

RanBP1 specifically reacts with RanGTP in its bound form.

RanBP1 binds to C-terminal of Ran which is bound to its substrate (receptor) and they form a dissociation intermediate. Finally, dissociation occurs.

Take home message 4

- Ran is resistant to RanGAP-mediated GTP hydrolysis when bound to importins/ exportins, due to the inaccessibility of the switch regions in such a complex.
- When transport receptor/RanGTP complexes reach the cytoplasm, GTP hydrolysis on bound Ran is stimulated by the concerted action of RanBP1 or RanBP2 and RanGAP.
- The RanBDs can access RanGTP even when it is in a complex with importins or exportins. The RanBD recognizes the C-terminal helix of Ran, which is exposed to solvent in RanGTP–transport receptor complexes and dissociates RanGTP from the transport receptor.
- Ran becomes susceptible to RanGAP-mediated GTP hydrolysis when bound to the RanBD of RanBP1 or RanBP2.
- RanGAP stimulates GTP hydrolysis on Ran by 5 orders of magnitude.



1. a ternary G protein•GEF•nucleotide complex of low nucleotide affinity is formed
2. the mutual competition of GEF and nucleotide leads to the formation of a stable binary G protein•GEF complex
3. this complex reverts back to the binary G protein•nucleotide complex in the presence of nucleotide

The reaction goes this specific way due to the concentration ration of GTP:GDP – cells invest a lot of energy to keep nucleotide concentration high.

The RanGTPase system overview

Factor	Interaction RanGTP/ GDP	Localization	Function
Ran	n.a.	Nuc/Cyt	Directionality of nuclear transport
Transport receptors superfamily	RanGTP	Nuc/ NPC/ Cyt	Translocation of macromolecules through the NPC
RanGAP	RanGTP	Cyt/ NPC	Stimulation of GTP hydrolysis on Ran
RanGEF (RCC1)	Nucleotide free form of Ran	Nuc	Nucleotide exchange
RanBP- family: RanBP1 RanBP2 RanBP3	RanGTP	 Cyt NPC (cyt. side) Nuc	 Export complex dissociation Export complex dissociation Formation of spec. export complexes
NTF2	RanGDP	Cyt/ NPC	Nuclear import of Ran

Regulation of nucleo-cytoplasmic transport

Means to regulate nucleo-cytoplasmic transport: regulation of cargo-transport receptor complex formation (e.g. phosphorylation of cargo), regulation of cytoplasmic or nuclear anchoring/retention (also often by phosphorylation), regulation of the soluble transport machinery, regulation of the NPC (very uncommon).

Regulation of cargo-receptor complex formation

Example: Pho4: …

Example: NF-AT: …

6.11.2017

Ribosome biogenesis

(**FOR FURTHER REFERENCE – SEE PART 2 ZEMP_RIBOSOME-BIOGENESIS.PDF**)

Modification or rRNAs: …

60S maturation: …

40S maturation: …

Also, know their differences and similarities.

Differences and similarities between bacterial and eukaryotic ribosome biogenesis:

Differences: in vitro assembly possibly/impossible, bacteria in one compartment vs. in nucleolus, nucleoplasm and cytoplasm, only few vs very many trans-acing factors.

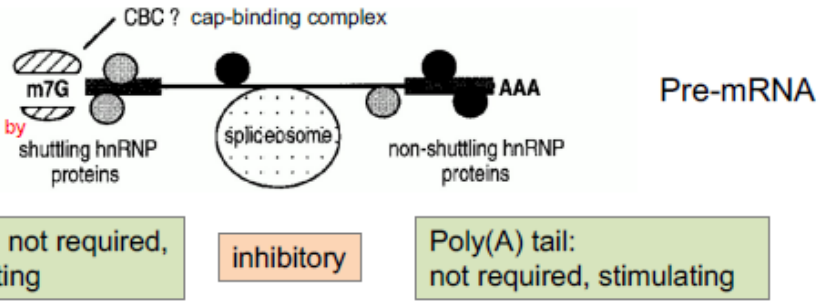
Similarities: pre-rRNA produced as a polycistronic precursor, rRNA is modified, role of ribosomal proteins in stabilizing secondary structures, mediating tertiary structures, etc., trans-acting factors that have similar activities (e.g. helicases etc.).

Rough order of the following steps in rRNA maturation: transcription, modification, processing/secondary structure formation (locally: processing first, globally: can differ), binding to ribosomal proteins.

13.11.2017

cap not needed for export as well as tail, even if both are removed, mRNA export will still occur.

all mRNAs that contain introns and recognized by the spliceosome will not be exported



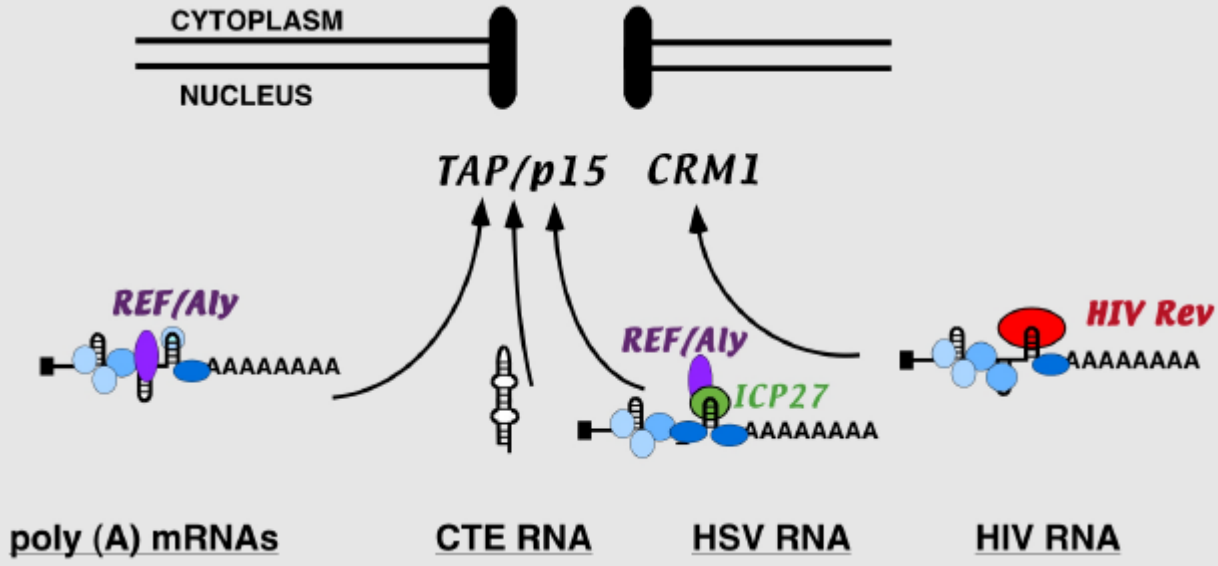
Viral mRNA export strategies

Mason-Pfizer Monkey virus: CTE domains…

HIV: REV and RRE…

Herpex Simplex Virus (HSV): DNA virus, ICP27…

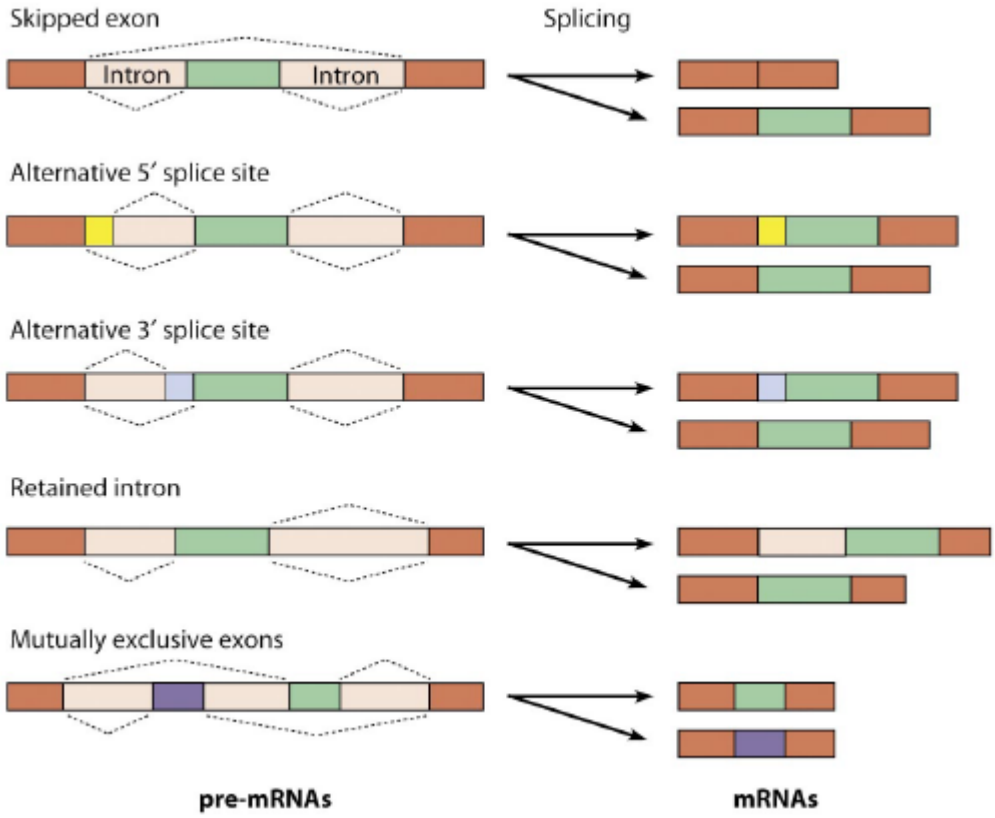
Overview



Post-transcriptional regulation of gene expression

Pre-mRNA processing

Exon definition: …



Mechanism of alternative splicing

Splicing is regulated by **trans-acting proteins** (repressors and activators).

These bind to **cis-acting RNA elements** regulatory sites (silencers and enhancers) on the RNA. Such elements may also influence how splicing will occur independently of proteins by their secondary structures.

Together, these elements form a "splicing code" that governs how splicing will occur under different cellular conditions.

Positive regulation: …

Traditional enhancer model: …

Negative regulation: …

20.11.2017

Sxl gene: How it works and what it controls

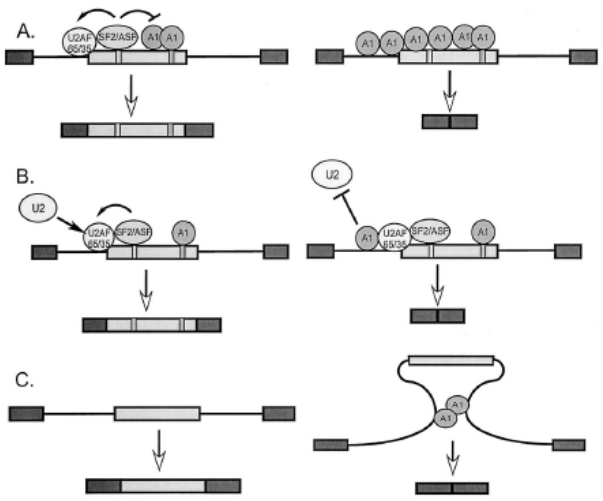
…

Models for splicing repression by hnRNP A1

(A) A1 binding nucleates the assembly of additional A1 molecules along the RNA, creating a zone of RNA where spliceosome assembly is repressed

(B) an additional A1 binding site adjacent to the branch point blocks splicing. A1 binding to this intronic element does block U2 snRNP binding to the branch point

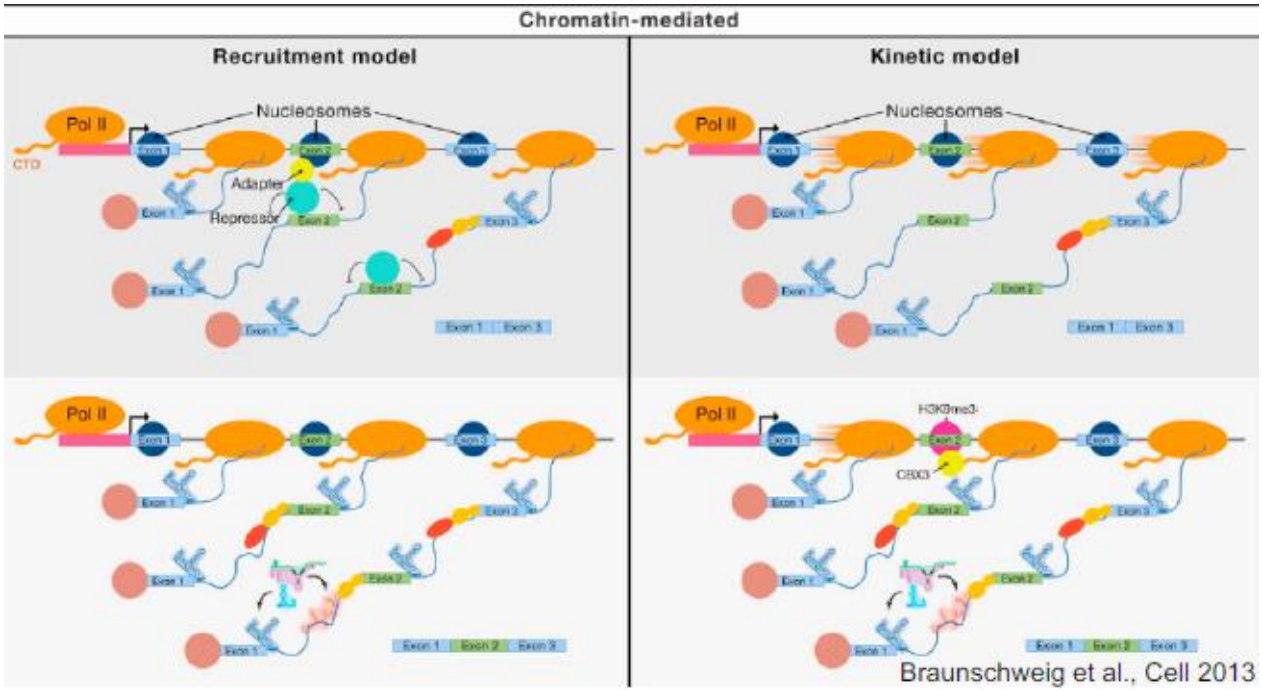
C) intronic binding sites for A1 allow A1 to multimerize, thus looping out the exon and causing exon skipping



Alternative splicing concepts

1. **Influence of transcription on splice-site choice by use of different promoters that affect**
   a) recruitment of splicing factors (e.g. SR proteins)
   b) kinetics of transcription elongation

2. **Influence of chromatin configuration on splice-site choice by**
   a) chromatin-mediated recruitment of splicing repressor
   b) effects of chromatin status on the transcription elongation rate

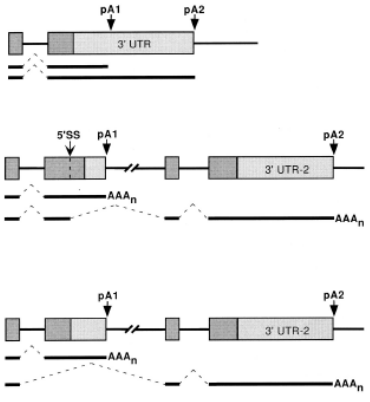


Regulation of pre-mRNA 3' end processing

Regulation of 3' end formation

1. whether to process the transcript or not (valid for single poly(A) sites)
(transcripts which are not polyadenylated, will be degraded or not transported efficiently into the cytoplasm, and the amount of protein expressed from that gene will decrease)
Example: U1 snRNP component U1A blocks polyadenylation of its own pre-mRNA

2. where to process and to place the 3' end (if there are two poly(A) sites)



a) tandem arrays of poly(A) sites within a single 3' UTR

b) composite exons whose 3' end is formed by either a 5' splice site or a poly(A) site

c) alternative 3' exons

Degradation of mRNA and mRNA stabilty

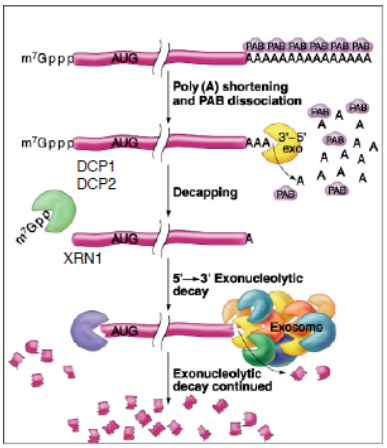
Global decay: mechanism

- as the mRNA ages in the cytoplasm, the length of the poly(A) tail gradually decreases, releasing the poly(A) binding proteins

- the poly(A) begins to be degraded by 3' to 5' exonucleases (CCR4/NOT complex)

- as the RNA can no longer be circularized, the RNA also looses its 5' cap (DCP1/2)

- without the physical interactions between the 5' and 3' ends and the protecting proteins, the RNA is rapidly degraded by 5' to 3' exonucleases (XRN1) and by 3' to 5' exonucleases (exosome)



Pollard 15-10

Nonsense-mediated decay: …

No-go decay: …

Non-stop decay: …

How to recognize premature stop codons: two models

Downstream marker model: …

Faux 3'-UTR model: …

27.11.2017

mRNA stability – summary

- The **stability of different mRNAs in the cytoplasm varies widely**.

- Generally, mRNAs are **degraded by exonucleases** after poly(A) tail shortening and decapping.

- RNAs containing **premature termination codons (PTCs)** are funneled into the **NMD pathway**.

- Recognition of PTCs **requires translation**.

- **Downstream marker model:** selection for degradation involves the EJC for spliced mRNA, whereas DSEs are involved for intronless mRNAs.

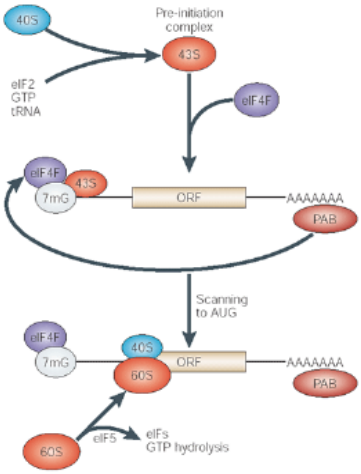
- **Faux 3' UTR model:** an abnormally long distance between the termination site and poly(A)-tail is recognized and initiates NMD

- Some cellular mRNA have short half-lifes because they contain **AU-rich elements (AREs), which bind ARPs**.

- ARPs can have destabilizing or stabilizing functions.

- The rate of **degradation of some specific mRNAs can be regulated**. The transferrrin receptor mRNA, for example, contains iron response elements that can protect the mRNA from degradation if iron uptake is required.

Regulation of translational initiation



1. **40S assembles with eIF2, GTP and initiator tRNA** to form the 43S pre-initiation complex

2. **5' cap of the mRNA attracts the eIF4F complex** (composed of eIF4E (cap-binding protein) eIF4A (RNA-dependent ATPase) eIF4G (recruits the 40S subunit)

3. The **43S complex then scans the mRNA until the AUG codon is recognized**.

4. This triggers eIF5 to hydrolyze GTP thereby promoting the other eIFs to dissociate and **the 60S subunit to join** resulting in a fully functional ribosome

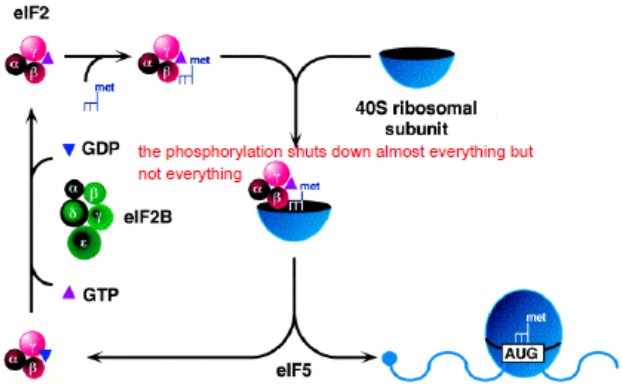
- eIF2 consists 3 subunits: α, β, γ

- in mammals, **phosphorylation** of the **alpha-subunit (eIF2α)** is induced in response to a number of different stress conditions:
  - iron (haem) deprivation
  - heat-shock
  - viral infection
  - interferon

Four different **kinases for eIF2α** have been identified:

1. heme-regulated inhibitor (**HRI**)
2. **PKR** (protein kinase activated by dsRNA)
3. **GCN2** (monitors uncharged tRNAs - starvation for amino acids)
   GCN general control non-derepressing
4. **PERK** (PKR-like kinase activated by ER stress)

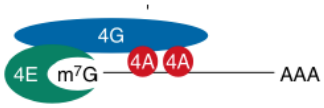
eIF2 is a key target in translational regulation.



Phosphorylation of eIF2α inhibits guanine nucleotide exchange by eIF2B which is the responsible GEF.
The result is a general **down-regulation of protein synthesis under various stresses**.

Regulation of eIF4E activity

eIF4E is the cap-binding subunit of eIF4F that facilitates 43S complex binding to the mRNA



3 ways of regulation:

1. limiting concentrations of eIF4E

2. **phosphorylation of eIF4E** (activation)
   - phosph. eIF4E has a 3-fold greater affinity for both the cap structure and eIF4G
   → phosphorylation in response to hormones and growth factors
   → dephosphorylation in response to heat-shock and viral infection
   (exception: HSP mRNAs are efficiently translated after heat-shock as initiation of their synthesis is not strictly dependent on eIF4E)

3. two **translational repressors (4E-BP1 and 2)** inhibit eIF4E function
   → 4E-BP1 becomes phosphorylated in cells treated with hormones or growth factors and subsequently dissociates from eIF4E

• Translational regulation can be **global or mRNA-specific**, and most examples of translational regulation that have been described so far affect the **rate-limiting initiation step.**

• **Global control** of translation is frequently exerted by regulating the **phosphorylation or availability of initiation factors**. Two of the most well-known examples are the regulation of **eukaryotic initiation factor (eIF)4E** availability by 4E-binding proteins (4E-BPs), and the modulation of the **levels of active ternary complex by eIF2 phosphorylation.**

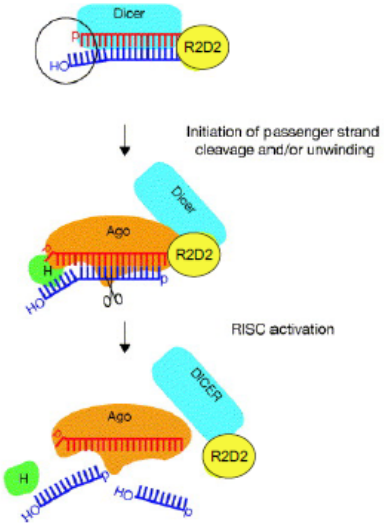
• **mRNA-specific translational control** is driven by **RNA sequences and/or structures** that are commonly located in the untranslated regions of the transcript. These features are usually recognized by regulatory proteins or microRNAs (miRNAs).

Post-transcriptional gene silencing by siRNAs and miRNAs

Single strand selection

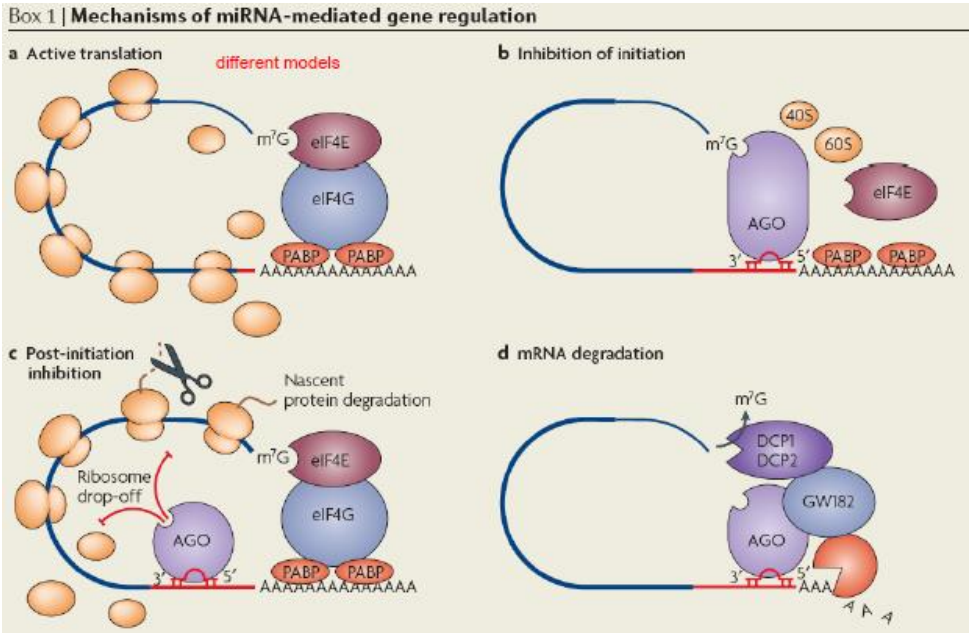
How are siRNA duplexes converted to single-chain forms and how is one strand selected for loading onto the RISC?

Asymmetric RISC formation is governed by the relative thermodynamic strength of the first four nucleotide-pairs of the 5′ termini of an siRNA.



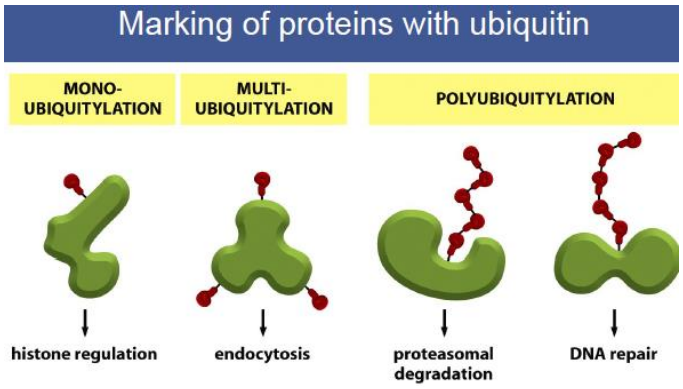
GC bonds more stable than AT bonds - nucleotide pairs determine stability.
how is this figured out by the cell?

Dicer forms a heterodimer with R2D2 (name of fly protein).
R2D2 binds to the thermodynamically more stable end of an siRNA.
Dicer recruits Ago2.
The thermodynamically non-preferred strand is eliminated by Argonaute.



04.12.17

PART 4 – The ubqiuitin-proteasome system

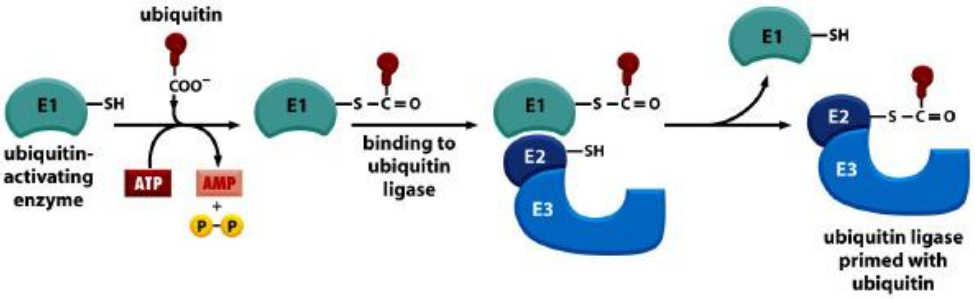


Ubiquitination often occurs at lysine side chains, but sometimes also at non-lysine side chains.

There are monoUb, modified monoUb, homotypic, modified homotypic, mixed and modified mixed, branched and modified branched ubiquitinations. They can also be acetylated and phosphorylated.

Three important enzymes in ubiquitination: E1/2/3. There are two types of E3 ligases: HECT domain and RING finger ligases.

Marking of proteins with ubiquitin



Substrate recognition

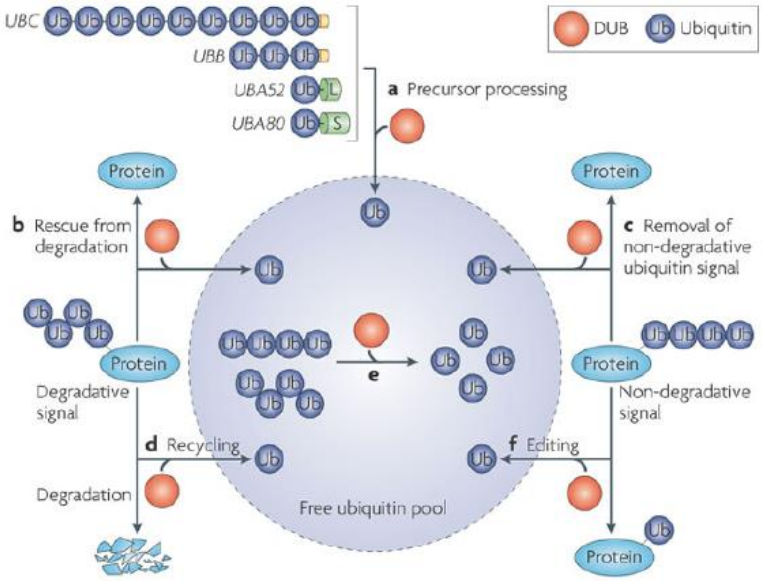
constitutive substrates → degradation signals

occasional substrates → e.g. misfolded proteins

Structural motifs that target proteins for ubiquitination

1. the N-terminal residue (F,L,W,Y,R,K,H) (N-end rule)
2. phosphorylation (e.g. transcription factors like beta-catenin)
3. PEST elements (regions rich in P,S,T,E)
   often linked to phosphorylation
4. destruction boxes (e.g. cyclins)
   R (A/T) (A) **L** (G) x (I/V) (G/T) (N)

Deubiquitylating enzymes (DUBs)

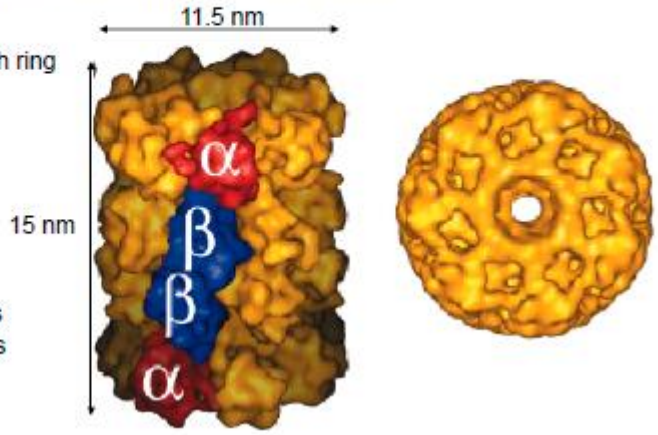


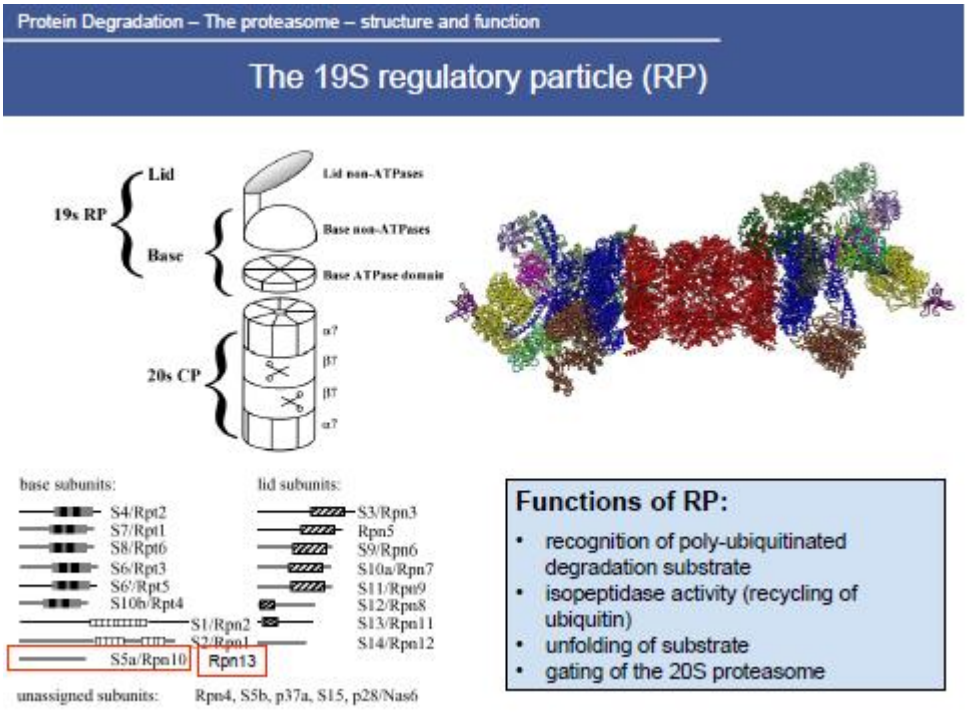
The degrading machinery, the proteasome

Occurence:
- in pro- and eukryotes
- in higher eukaryotes: up to 1% of total cellular protein

Structure of the 20S proteasome core

- 700 kDa
- consists of 4 rings, each ring contains 7 subunits
- cylinder





Autophagy

Cellular homeostasis: recycling of cellular building blocks.
Organelle homeostasis: degradation of superflous and damaged organelles.
Adaptation to chaning environmental conditions.
Protection and defense: removal of protein aggregates and intracellular viruses and bacteria.
Biosynthetic functions such as the transport of vacuolar enzymes.

Autophagy has important functions in the development of an organism (e.g. autophagy and apoptosis of neurons during early development). Other: differentiation, development, cellular homeostasis, adaptive response to starvation, cellular defense, quality control.

Complexes involved: Atg1 (protein kinase in vacuole), Atg9 (transmembrane protein of vacuole), Vps34 complex (lipid protein kinase), Atg2/18 (PI3P binding), Ubl protein system (Atg8 lipidation).

Identification of ATG proteins: Screen yeast for absence of autophagy bodies.

Types of autophagy: mitophagy (neurodegenerative diseases), lipophagy, virophagy (infectious diseases), ERphagy (only small parts, because too big parts are problematic for the autophagosome), aggrephagy (neurodegenerative diseases), nucleophagy, pexophagy, bacteriophagy (infectious diseases).

Bulk/macroautophagy is nutrient regulated. In selective autophagy, the cargo signals its own degradation.

Bacteriophagy: Ubiquitination of bacteria (bacteria tries to get rid of it as a response), binding of p62 and optineurin, recruitment of autophagy machinery, ATG8 recruitmen on p62 and optineurin (and NDP52), TBK1 kinase recruitment (promotes autophagosome formation by co-recruiting WIPI2 + phosphorylation of optineurin to enhance ATG8 binding affinity), membrane formation.

Reduction of mitochondria: reduced metabolic demand, cone cells during hibernation, quality control and prevention of toxic mitochondrial products.

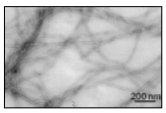
Mitophagy: upon mitochondrial damage Pink1 accumulates in the outer mitochondrial membrane, Pink1 phosphorylates ubiquitin, phospho-ubiquitin recruits the E3 ubiquitin ligase Parkin, Pink1 further activates Parkin by phosphorylating its Ubl domain, high Parkin ubiquitination activity plus Pink1 mediated phosphorylation of transferred ubiquitins, recruits cargo adaptors with ubiquitin binding domains (e.g. TAX1BP1, p62, NDP52 and Optineurin), TBK1 recruitment, which lead to autophagosome formation and selective elimination of damaged mitochondria.

18.12.2017

Protein aggregation diseases and physiological protein aggregation

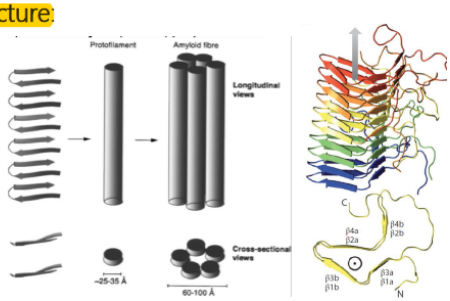
Disease	Protein
Parkinson's disease	α-Synuclein
Alzheimer's disease	Aβ, Tau
Prion Diseases (e.g. Kuru, CJD, BSE)	PrP
Amytrophic lateral sclerosis	SOD1
Huntington's disease	Huntingtin
Frontotemporal lobar degeneration	TDP-43, Tau
Spinocerebellar ataxias	Ataxin-1,2,3
Amyloid polyneuropathy I	Transthyretin

Morphological – Fibrillar appearance on EM, 7-13 nm in diameter, 2-8 filaments, 2-7 nm in diameter, often twisted or associated laterally as ribbons up to 30 nm wide



Structural - Cross-β structure:

β-Strands oriented perpendicularly to the fibril axis and assembled into β-sheets that run the length of the fibril.



Tinctorial properties:

Binding of specific dyes:
Congo Red and Thioflavin T

those dyes can enter the amyloid structure, changing their spectoscopic structure

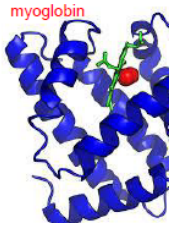
Insolubility in water and buffer in the absence of detergents

Extreme stability to heat, proteolysis and chemical denaturants. Due to H-bonds between sheets and between protofilaments.

1. Genetic alteration (Mutations, gene multiplication)
2. Proteolysis (or introduction of stop codon)
3. Increase in concentration — prinicipally, every protein can be pushed to form amyloid fibers
4. PTMs (e.g. phosphorylation)
5. Binding of other molecules (e.g. RNA, metabolites, lipids)
6. Alterations of quality control systems
7. Environmental changes (pH, T, oxid. stress…)

→ the myoglobin example

myoglobin



Prions: Infectious agents, transmissible between individuals, and tractable with microbiological techniques—including, e.g., titer determinations.

(both are amyloids obviously)

Prionoids: Infect neighboring molecules and neighboring cells, propagate within an organ, but do not propagate within communities. Not found to cause macroepidemics.

The aggregation propensity of a protein is determined by different factors. Extrinsic factors: interactions with cellular components, physico-chemical properties of the environment. Intrinsic factors: charge, hydrophobicity, secondary structures preferences, polar residues. About 40% of any eukaryotic proteome is disordered. It is another regulatory mechanism of protein activity. Protein aggregates can also perform certain functions in the cell. Disordered regions also exert additional functions in cells.