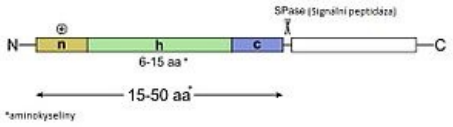
Post-translational modifications and protein targeting

In both bacteria and eukaryotes, some proteins are destined for the cytosol, others are part of the membranes, or are secreted out of the cell. In eukaryotic cells, some proteins are sent to cellular organelles after completion of their synthesis, such as lysosomes, mitochondria, of the cell nucleus and in plants to chloroplasts. Sending new proteins to their destination is called **targeting** (target = destination).

Polypeptide signal sequences, free and bound ribosomes

Cytosolic proteins are synthesized on **free** cytosolic **ribosomes**, while membrane proteins, organelle proteins, and proteins released outside the cell are synthesized on ribosomes bound to the rough endoplasmic reticulum (ER). Free and bound ribosomes are structurally and functionally exactly the same, their binding to the ER is determined by the sequence of the synthesized chain. Most proteins determined outside the cytosol have a so-called 13 –16 amino acid **signal sequence** at the N-terminus. Although these sequences vary from protein to protein, the presence of several hydrophobic amino acid residues is characteristic. The sequence is recognized by an **SRP particle** (signal recognition particle), consisting of six protein subunits and 7SL RNA. It binds to the signal sequence of the synthesized protein and stops translation at an early stage. The ER membrane contains receptors for SRP. Once the ribosome-SRP complex binds to them, proteosynthesis continues with the participation of two other membrane proteins, **riboforin I and II**, and at the same time, the peptide chain passes through the membrane into the ER tank. The SRP is again released from the receptor into the cytosol.

The signal sequence is crucial for translocation. If it is attached to a cytosolic protein, such as hemoglobin, by gene manipulation, then this protein is released outside the cell. Peptide translocation is an active energy-requiring membrane process (ATP). Permeation is not driven by translation, the ribosome. Theoretically, it could take place even after the synthesis of the free ribosome chain has been completed. However, early binding of the synthesized protein and ribosome to the ER is advantageous and mostly necessary because, after synthesis on the free ribosome, the protein could assume a conformation that would prevent translocation across the membrane.



Signal peptidase

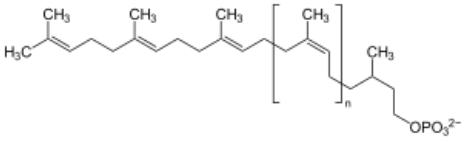
The tight space between the translation site and the translocation will not allow the chain to conform before or on the other side of the membrane. Some proteins remain anchored in the membrane, which is also due to their primary sequence.

In fact, in addition to the signal sequence, membrane proteins also have an **anchoring, stop-transferase sequence**, which terminates translocation across the membrane and the protein remains an anchored part of the membrane. The signal sequence of these proteins may also be somewhat distant from the N-terminus. Some even have several such sequences, alternating with stop-transfer sections, so that they are anchored in the membrane in several ways, sometimes several times (see figure). The signal sequence of the secreted proteins is still cleaved by the membrane **signalase** during translocation. The protein penetrates the ER tank. Here and especially in the Golgi apparatus, it is covalently modified (see Post-translational glycosylation of proteins) and then transported to the site of its function.

Post-translational glycosylation of proteins

After translocation into ER cisterns, many proteins are further modified. The signal peptide is cleaved, disulfide bonds are formed. Later, a certain section can be cleaved from the polypeptide chain proteolytically, and thus the protein is functionally activated (hormone, enzyme). The function of several proteins can be modified by phosphorylation, acetylation, or ADP-ribosylation (p.OOO). Many proteins acquire oligosaccharide residues in the ER and Golgi, making them **glycoproteins**. These changes in the finished peptide chain are called **post-translational modifications of proteins**, or also their covalent modifications. This structural and functional maturation of the protein is very important for the regulation of biochemical processes.

Oligosaccharides bind by either an N-glycoside bond to an asparagine residue or an O-glycoside bond to a serine or threonine residue of a protein. Oligosaccharide precursors are synthesized on an isoprene support – **dolichol phosphate**, contained in the ER membrane. If its phosphate group is on the cytosolic side of the membrane, two N-acetylglucosamines and five mannoses sequentially bind to it. The dolichol phosphate with this heptasaccharide is then oriented in the membrane so that the oligosaccharide is on the luminary side of the membrane, heading to the ER tank. Here, four more mannose and three glucose are transferred to it from another dolichol phosphate precursor (see figure).



Dolichol phosphate

The oligosaccharide thus activated is transferred to the Asn peptide, the phosphatase cleaves one of the dolichol pyrophosphate phosphates and the regenerated dolichol phosphate can re-enter the reaction cycle. The antibiotics **bacitracin** blocks this phosphatase. The attachment of Glc-NAc to dolichol phosphate is inhibited by the antibiotic **tunicamycin**.

In the ER, three glucose and one mannose are cleaved from the N-linked oligosaccharide. The protein is then transferred to the **Golgi apparatus (GA)**. In its vesicles, oligosaccharides also bind to the protein through an O-glycoside bond. The N-linked oligosaccharides are further modified. Six mannoses are sequentially cleaved and additional GlcNAc, galactose, fucose and finally **sialic acid (N-acetylneuraminic acid)** are added. These modifications in the Golgi apparatus are called **terminal glycosylations**, in contrast to the **basic glycosylations** (core glycosylations) already taking place in the ER. Lysosome-derived glycoprotein oligosaccharides are specifically phosphorylated.

During all processes after transfer of the protein to the ER tanks, the peptides in the membranes are oriented so that the oligosaccharide residues are on the luminal side of the membrane (in the tank, in the GA vesicles, in the transport vesicles). When the transport vesicles merge with the plasma membrane, the glycoprotein oligosaccharides reach the outer, extracellular side of the membrane. Some **asymmetry of the membranes** is maintained.

The glycoprotein part of the oligosaccharides have sometimes been shown as a signal or an *address*, to which proteins from the Golgi apparatus are sent to their proper function. There is **mucolipidosis** (I-cell disease), which is caused by a genetic error in the modification of oligosaccharide residues of lysosomal enzymes. In patients, instead of mannose-6-phosphate, there is only mannose. As a result of this variation, lysosomal enzymes are not transferred to lysosomes, but out of the cell and can be detected in blood plasma. In contrast, undecomposed glycosamines and glycolipids accumulate in lysosomes. The patient suffers from psychomotor retardation and skeletal deformities.

However, glycosylation of most proteins probably has a different function than providing the molecule with a targeting signal. Oligosaccharide residues of glycoproteins increase their solubility and help orient the protein molecule towards the aqueous phase. Another role of oligosaccharides is to protect the protein (e.g. immunoglobulin) from the proteases' activity. Carbohydrates are a marker for the uptake and subsequent degradation of plasma glycoproteins in the liver. Another significance is seen in the fact that the kinetics of glycoprotein modifications in ER and GA indicate a step in the passage of these proteins through cellular organelles, thus ensuring the time required for accurate sorting of synthesized proteins.

Glycosylation-independent targeting

Plasma membrane proteins and secretory proteins do not need an oligosaccharide signal to localize properly. Other types of signaling are assumed (conformation of the protein, a certain three-dimensional motif in its structure). These proteins can be "sent" to the apical or basolateral part of the plasma membrane in some way, or they are classified for two types of secretion: **constitutive secretion**, which is constant, rapid and proteins do not condense in secretory vesicles, or **controlled secretion**, when proteins are stored and concentrated in the vesicles and can be released from the cell only on a hormonal impulse. Then do the vesicles merge with the cytoplasmic membrane and their contents are released outside the cell. Examples of controlled secretion are the release of digestive enzymes from acinar cells of the pancreas or the release of peptide hormones from endocrine cells.

Targeting of proteins to mitochondria

Most mitochondrial proteins are synthesized on free cytosolic ribosomes and posttranslationally incorporated into mitochondria. Some proteins are intended for the outer/inner mitochondrial membrane, for the intermembrane space, and for the matrix. The localization of the protein is determined by the sequence of the N-terminal region of the chain, the so-called mitochondrial input sequence, which is rich in basic amino acid residues and serine and threonine. If the protein is to anchor in the outer mitochondrial membrane, then the input sequence is followed by an anchoring sequence and a second positively charged region.

A proton transmembrane gradient is required for the protein to pass through the inner mitochondrial membrane. Passage through the outer membrane does not require this energy source. The input sequence is proteolytically cleaved after passing through the inner (not outer) membrane.

The protein transferred from the cytosol to the matrix first binds by its presequence to the receptor on the outer mitochondrial membrane. At the site of permeation, the outer and inner membranes abut each other and the protein permeates both at the same time. In the matrix, the transferred protein is cleaved from the membrane-anchored presequence.

Intermembrane proteins (e.g. cytochrome b) are first anchored in the inner mitochondrial membrane and a special protease cleaves them from the intermembrane space. Some intermembrane proteins (cytochrome c) remain bound to the inner membrane.

During passage through the membrane, mitochondrial proteins fully unfold and then restore the tertiary structure.

Bacteria also distribute synthesized proteins using signal sequences. Some of their proteins are destined for the plasma membrane, for the outer membrane, for the periplasmic space, or are rarely released outside the cell. The translocation is driven by a proton gradient. The analogy with mitochondrial targeting is thus obvious.

Nuclear targeting of proteins

Nuclear proteins (histones, polymerase, etc.) are synthesized on free cytosolic ribosomes. They enter the nucleus through the pores in the nuclear membrane. These pores open to a special signal, which is usually unknown. In the case of the SV40 virus T-antigen, an amino acid sequence was found between positions 127 and 131, which is crucial for the entry of the protein into the nucleus (nuclear localization sequence). Replacing a single amino acid in this region prevents the protein from leaving the cytosol. If this sequence is experimentally inserted into the structure of another protein, this protein will appear in the nucleus even if it is not a nuclear protein.

Decision-making mechanism for the destruction of non-functional proteins

Proteins destined for destruction and removal from the cell are also subject to specific targeting. The biological half-life of cytosolic proteins varies widely, from a few minutes to more than twenty hours. The length of existence of these proteins is determined by their N-terminal amino acid. Met, Gly, Ala, Ser, Thr, and Val is amino acid No. 1 of more stable proteins (half-life longer than 20 hours). The N-terminal with Ile or Glu signals about half an hour of peptide *survival*. Pro, Leu, Phe, Asp, Lys, and Arg provide a half-life of only a few minutes. Such a short half-life is significant for regulatory peptides, such as hormones, so that regulation changes can be fast. This signaling originated in the early stages of life, as it is known in bacteria, yeasts and mammals. The mechanism of the described targeting is not fully elucidated. **Ubiquitin** (Mr=8500), a protein which is present in all eukaryotic cells, plays an important role. The C-terminal Gly of ubiquitin is covalently bound to the ε-NH_2 group of lysine of the protein to be degraded. Interestingly, ubiquitin is first activated by ATP and three enzymes and is attached to their –SH groups. Thus, this activation resembles the activation of fatty acids or the synthesis of aa-tRNA (amino acid activation), which is one example of a general principle that we encounter more often in biochemistry.

Receptor-mediated endocytosis

Previous chapters have focused on targeting intracellular proteins. However, the principle of regulation also applies to the uptake of proteins from the extracellular space by endocytosis, which is mediated by the interaction of the protein with a membrane receptor on the cell surface.

Receptor

The mentioned receptor is a glycoprotein, located in special places of the membrane, so-called coated pits. On the cytosolic side of these sites is a clathrin coat. Due to its three-armed structure, **clathrin** is able to form a reticulate mantle around the wells of the membrane or around various cytoplasmic vesicles, vacuoles. Upon delivery of ATP, the clathrin network can be enzymatically disrupted and clathrin can be used for further interactions.

After binding of the absorbed protein to the receptor, the well is deepened, and the clathrin eventually forms a closed network, releasing a **coated vesicle** from the membrane into the cytoplasm. It then quickly loses its clathrin mantle and turns into an **endosome** or **receptosome**. It usually enlarges by merging with other endosomes. The function of these organelles is to decide where to transport the absorbed protein. An important mechanism is the **acidification of the endosome content**. This is done by the action of the ATP-dependent H^+/K^+ pump in the endosome membrane.

Absorbed transferrin in a more acidic environment releases Fe and transfers it to the cytosol for ferritin. The endosome then merges with the cytoplasmic membrane. The apotransferrin receptor complex appears on the cell surface, in a higher pH environment in which apotransferrin is released for re-use.

Proteins after absorption

Other proteins taken up by receptor-directed endocytosis face a different fate in endosomes. Cholesterol-transporting **LDL-apoprotein** is transferred to the lysosome upon binding to the membrane receptor and after endocytosis. Here, it is degraded by lysosomal proteases while the receptor is reused on the cell surface. **Immunocomplexes, insulin,** or some **growth factors** are degraded in lysosomes with their receptors. This is an example of modulating the effect of protein hormones, as this reduces their blood levels and the number of their receptors in the target cells.

In human

Receptor-mediated endocytosis transports IgG from breast milk through the intestinal enterocytes of the newborn. On the other side of the cell adjacent to the capillary, endosome carrying the IgG-receptor complex fuses with the cytoplasmic membrane and the antibody is released into the bloodstream of the child with a fragment of the receptor called the **secretory component**.

In viruses

The described receptor-mediated endocytosis is also involved in the **entry of some viruses into the host cell**. After endosome + lysosome fusion, the acidic environment results in the fusion of the virion envelope with the lysosomal membrane, thereby releasing the nucleocapsid with the viral nucleic acid into the cytosol.

In the final stage of viral reproduction, newly synthesized viral nucleocapsids are released from the cell by *erupting* from it, encasing it in a plasma membrane.

In bacteria

Bacterial toxins (diphtheric and cholera toxin) also enter the cell through receptor-mediated endocytosis.

References

Related Articles

- Translation of membrane and secretory proteins (protein sorting, targeting)
- Translation, post-translational processing of proteins in eukaryotes

Source

- ŠTÍPEK, Stanislav. *Stručná biochemie : Uchování a exprese genetické informace.* 1. edition. Medprint, 1998. ISBN 80-902036-2-0.

References

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