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Review article

The drainage mechanism of beta amyloid (Aβ) from the intra-arterial perivascular space by LRP1 receptors of the blood brain barrier endothelial cells

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Abstract

A number of recent research studies related to the significantly important intra-arterial perivascular drainage pathway indicates its crucial role in maintaining the optimal brain homeostasis. Driven by the pulsatile vasomotion forces, this drainage carries and cleans a whole complex of brain metabolism waste products, especially beta amyloid (Aβ). In the case of a markedly increased intracerebral concentration, especially of toxic Aβ, or alterated vasomotion, the drainage pathway undergoes a burdening in the flow, its collapse, and sedimentation of waste particles, among them Aβ aggregates in particular. In addition to the Aβ elimination through this drainage pathway that ends in the neck paracarotide lymph nodes, the drainage from this space also takes place by endothelial receptors and the transcytosis mechanism through the cytoplasm of endothel cells into the capillary blood. The aim of this study is to present a detailed explanation of these drainages, with the special analysis of LRP1 receptor and its functions in these events.

KEY WORDS: intra-arterial perivascular drainage pathway (IPAD), beta amyloid (Aβ), blood brain barrier (BBB), endothelial receptor LRP1

Introduction

Intra-arterial perivascular drainage pathway (IPAD) starts in the capillary basement membranes (BM) of the blood brain barrier (BBB). In this arterial flow segment, through tissue layers, pass in both directions, proteins, lipids, electrolites, water and a number of compounds necessary for the brain functions, as well as for its detoxication. These tissue layers consist of a line of endothelial cells, basal membranes, narrow paravascular space, and astrocytic endfeet of the glial membrane. Aβ first passes through the extracellular space (ECS) and its interstitial fluid (ISF), then across the astrocyte/pericyte membrane, intercellular astrocytic endfeet clefts, and a narrow, almost virtual drainage space, and after passing across a markedly permeabile border of the reticular lamine, it enters the capillary basement membrane. Essentially, this membrane is a continous layer of extracellular matrix composed of the ground substance and fibers. The ground substance is mainly composed of water and in the water immersed collagen fibers (dominantly) and elastine (to a lesser degree). The membrane is 20-50 µm thick on average, and the thickness of the fibers is 3-4 µm. Actually, the BM is a complex originating from the proximal fusion of the outer and inner basement membranes of vascular smooth muscle cells (VSMCs). The study primarily analyses its structure and its relation with the transport activity of the low-density lipoprotein receptor-related protein 1 (LRP1, alpha-2-macroglobulin receptor, A2MR, cluster of differentiation 91, CD91) receptor located on the abluminal and luminal side of the endothelial cell membranes (***Fig. 1***)[1-5].

It is important to emphasize some other elements presented in ***Fig. 1***. In the basolateral endothelial cell membranes, there is embedded ferroportin1 (FPN1, solute carrier family 40 member, SL40A1) receptor, which transports Fe^{2+} out from the cell. Immediately after leaving the cell, Fe^{2+} becomes oxidised in Fe^{3+} by the action of the locally present hephaestin (Hp, HEPH, transmembrane copper dependent ferroxidase), and binds to glycoprotein transferin (Tf), by which it is transported through tissues and blood ($2\,Fe^{3+}$/1 Tf-complex). ***Figure 1*** clearly shows the entrance of this complex into the capillary basement membrane and its transfer into the paravascular drainage pathway. The figure also shows Angiopep-2 (ANG) 19-AA oligopeptide that can bind to LRP1 and penetrate the BBB by receptor-mediated transport. It is a synthetic compound. Its role is to transport certain medications across the BBB

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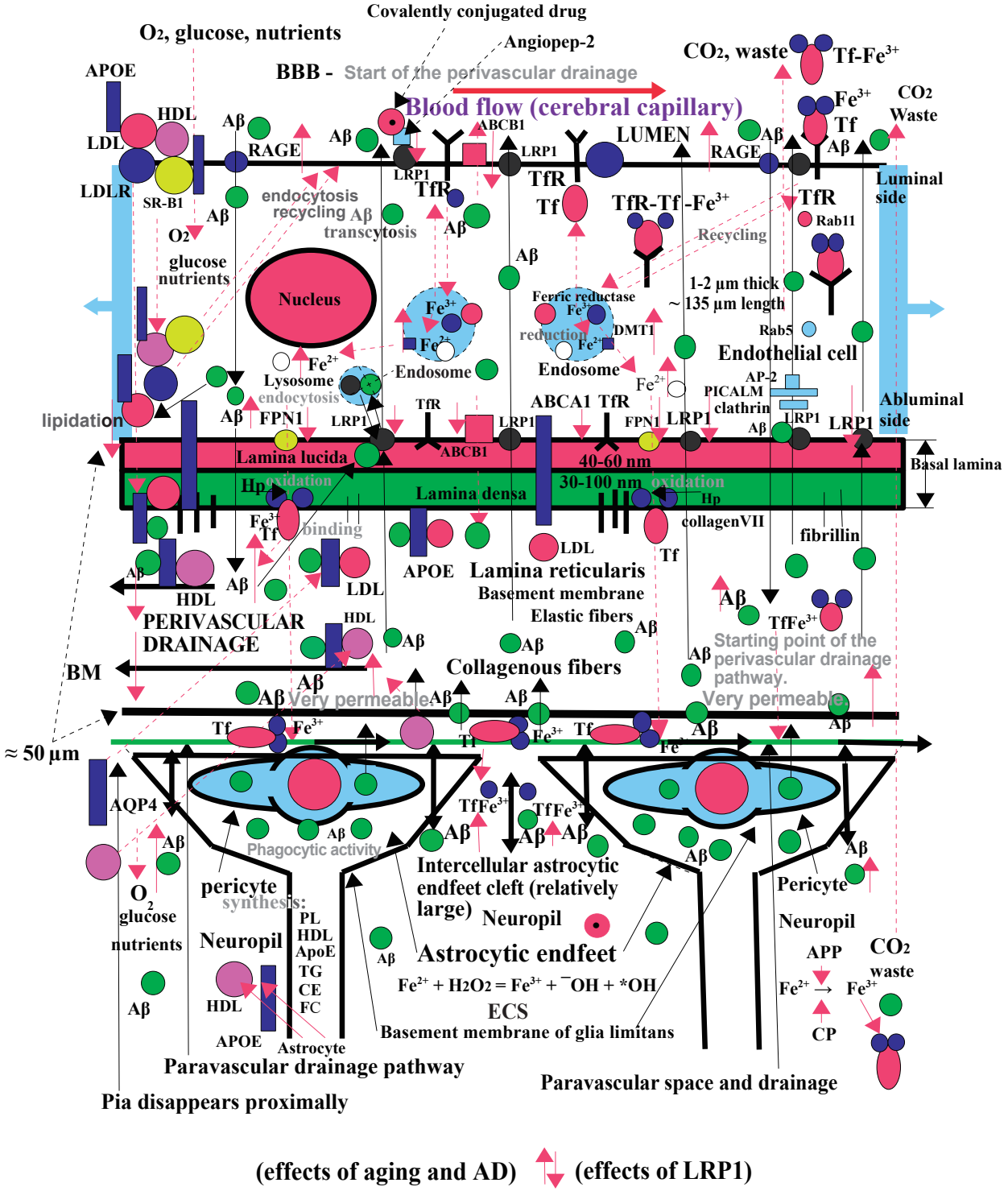

Fig. 1. **Structure of the cerebral capillary basement membrane (longitudinal cross-section)**

Above the luminal side of the cell, by the red arrow is denoted the capillary lumen, anterograde blood flow direction, some receptors (TfR, RAGE, LRP1), and Aβ peptide molecules. Below the cells is visible the basement membrane which is composed of two layers, basal lamina and lamina reticularis. Hp, hephaestin; Basal lamina, bl., is composed of the lamina lucida (below the endothelium), and lamina densa; Angiopep2; conjugated drug; HDL, high density lipoprotein; LDL, low density lipoprotein; LDLR, LDL receptor; LRP1, LDLR -related protein 1; PL, phpsopolipid; TG, triglyceride; CE, cholestrol ester; FC, free cholesterol; CP, ceruloplasmin; AD, Alzheimer's disease; Aβ, beta amyloid; APP, amyloid precursor protein; BBB, blood brain barrier; ESC, extracellular spave; BM, basement membrane; RAGE, receptor for advanced glycation endproducts (AGEs); ApoE, Apolipoprotein E; Tf, transferin; TfR, Tf receptor; Rab, a small GTPase derived from rat brain; FPN1, ferroportin1; ABCA1, ATP-binding cassette transporter A1; ABCB1, ATP-binding cassette protein B1; AP-2, adaptor protein-2; AQP4, aquaporin 4.

into the brain. On the luminal and abluminal membrane, apart from LRP1, there are also marked low density lipoprotein receptors LDLR, SR-B1, RAGE, TfR, ABCA1, and ABCB1. Low density lipoprotein receptor (LDLR) internalizes LDL particles from the blood. Scavenger receptor class B type1 (SR-B1) is important for LDL and HDL internalization. The receptor for advanced glycation endproducts (RAGE) is a multi-ligand receptor that is able to bind several different ligands, among them amyloid beta (Aβ). The binding and internalization of Aβ have a great importance. Transferin receptor (TfR) is the crucial protein for binding and internalization of the TfR/Tf/Fe^{3+} complex. ATP-binding cassette-sub-family B member 1 (ABCB1, P-glycoprotein 1) is a protein that pumps many foreign or toxic substances out from the cell, and among these substances Aβ has a special position. ATP-binding cassette transporter A1 (ABCA1) receptor, expressed at the abluminal side of the endothelium, induces the lipidation of ApoE (Apolipoprotein E), *i.e.* the binding of ApoE (res. 244-272) with molecules on the lipoprotein surface. It is an important factor for efficient binding of Aβ (res. 12-18) with ApoE (res. 136-150). ***Figure 1*** also clearly presents the ECS, astrocytes, the paravascular drainage pathway, capillary basement membranes (BM), perivascular drainage, and endothelial cell cytosol. There is also visible astrocyte synthetic activity, the production of ApoE, and ApoE/HDL/Aβ complex generation and moving [4,5].

In Alzheimer's disease, there are elevated Aβ values in all cerebral tissues. This is especially related to the IPAD. The increased Aβ monomer concentration results in their more frequent colliding and binding. The velocity of their aggregation rises with an increased number of aggregates, and the mechanical resistance to the flow through this pathway increases. On the other side, during the process of Aβ monomer binding, there is a close approach of their two oxido-reductive centers, and electron "hop" from sulphur (S) on Met S35 of one monomer (Aβ1) to the metal binding domain (MBD, Fe^{3+}) on the other already fixed monomer (Aβ2). This results in the reduction (electron gain) of Fe^{3+} into Fe^{2+} (ferrous ion). The increased concentration of hydrogen peroxide (H_2O_2) typical for AD, results in Fenton reaction ($Fe^{2+} + H_2O_2 = Fe^{3+} + {}^{-}OH$ - hydroxyl ion + *OH - hydroxyl radical). *OH is extremely aggressive and toxic for the surrounding molecular structrures, particularly for the cell membranes of the present VSMCs. On these membranes an extremely destructive process of lipid peroxidation occurs. Considering the crucial role of these cells in the vasomotion process, it is evident that there will be an increasing decline of the drainage of Aβ molecules and other waste. Now, the role of LRP1 receptor is obvious, as it tries to compensate the mentioned drainage decline [4,5].

Generally about the function of the endothelial receptors, alternative drainage systems, and driving forces of the perivascular Aβ

The surface of vascular endothelial cells contains a complex of receptors that have various structures and functions. Like LRP1, which is the subject of this study, they all have a transmembrane shape, the outer extracellular part that passes across the cell membrane and the intracellular part. Receptors are actually protein molecules that are located within or on the surface of target cells. Their function is to receive chemical signals that are usually connected to small quite labile and usually soluble molecules named ligands. Endothelial receptors are closely linked to the function of the IPAD and in endothelial cells they provoke endocytosis, exocytosis, or transcytosis of various useful or harmful products, extremely important for brain homeostasis. One of the harmful products is also Aβ.

In further analysis, it is necessary to emphasize some basic characteristics of the IPAD. The brain does not have its standard lymphatic network, and the role of this essential system is taken over by a complex of other drainage systems: the drainage across the BBB, blood cerebrospinal fluid barrier (BCSFB), choroid plexus and arachnoid granulations (AGs), paravascular drainage (along the cerebral vasculature surfaces), perineural drainage (along the cerebral nerve surfaces), drainage through the orbits into the sinus sagittalis superior, and drainage into the nasal cavity (along the olfactory tract). The perivascular drainage system is located within the basement membranes of capillaries, arterioles and arteries. The previously mentioned vasomotion forces are native forces connected to arterial and arteriolar walls, and their direction is opposite to the direction of the blood flow and pulse wave. They are generated by rhythmic intracellular oscilations of Ca^{2+} ion concentrations in smooth muscles (VSMCs) of the vascular walls [5,7,8-12].

The role of LRP1 in Aβ drainage

Low density lipoprotein receptor-related protein 1 (LRP1, alpha-2-macroglobulin receptor, A2MR, apolipoprotein E receptor, ApoER, cluster of differentation 91, CD91) receptor is abundantly expressed in the brain, primarily in neurons, glia and vascular endothelial cells. This is a large transmembrane receptor composed from three parts: the great extracellular subunit (515 kDa) that captures ligands and sends crucial information distally into the intracellular region, the short transmembrane unit, and the intracellular tail (85 kDa). It has been proven that the extracellular part with its four binding regions captures over 40 ligands, and of these regions, domains number 2 and 4 are especially important for capturing Aβ and its further transport. There are four domains, but special attention is given to the 2nd domain from the terminal receptor part. The 2nd ligand binding cluster contains 8 cysteine-rich ligand-binding repeats (blue circles on ***Fig. 2***). This cluster also has 8 epidermal growth factor (EGF) precursor homology domains and 6 YWTD domains (β-propeller- EGF-like domains). One YWTD domain is composed of 6 blades, each of them having 4 β-strands (six-bladed β-propeller domain). Some recent investigations emphasize that the giant LRP receptor (LRP1, LRP1B, LRP2) β-propellers may be active components in ligand binding. The receptor cytoplasmic tail has 2 NPXY motifs, 1 YXXL motif, and 2 di-leucine motifs (LL) (***Fig. 2***). 2 di-leucine motifs interact with α and σ2 subunits of AP-2 adaptor, and YXXL motif with μ2 subunit of AP-2. NPXY

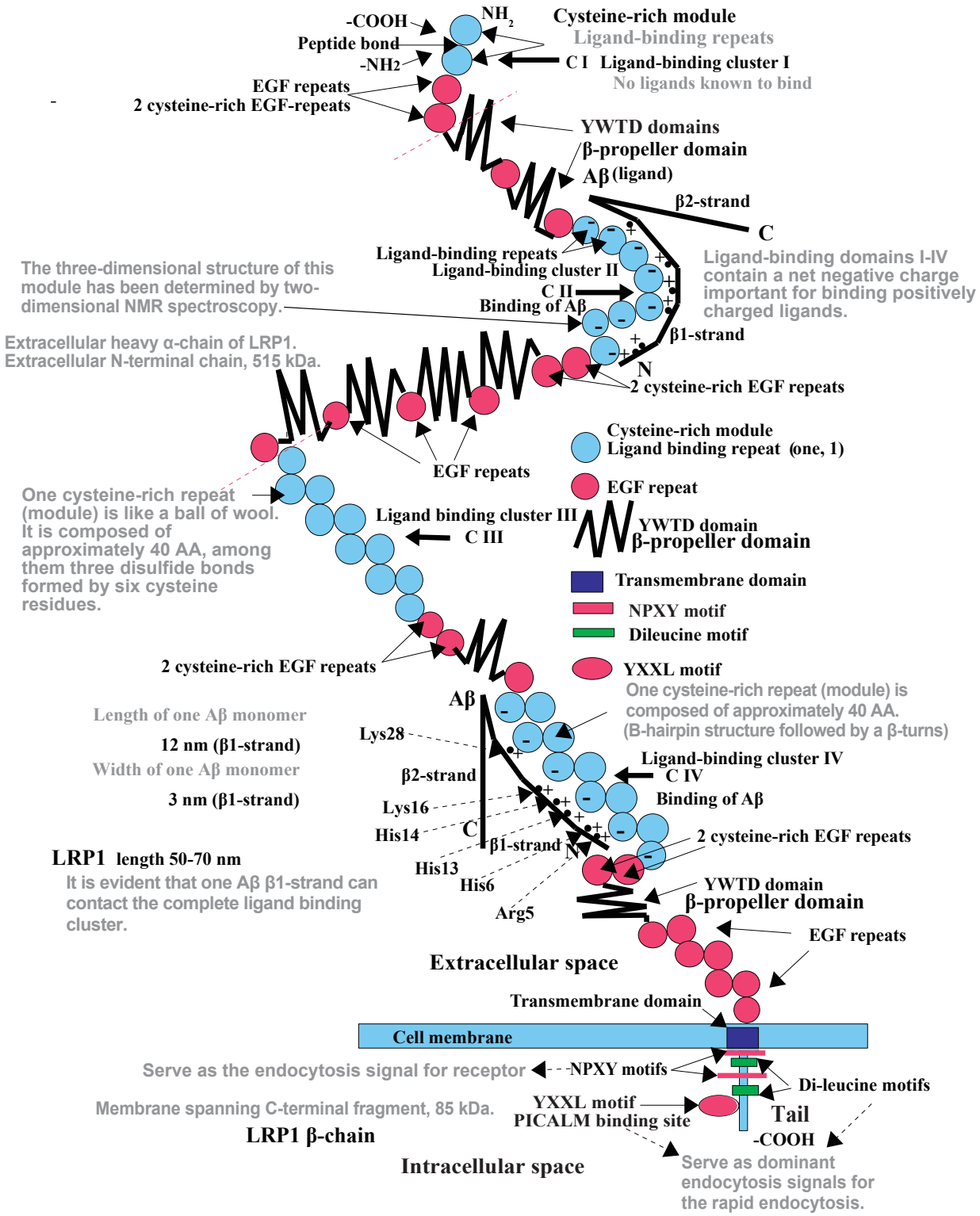

Fig. 2. **Presentation of LRP1 (CD91) receptor molecule.**

The second ligand-binding cluster contains eight cysteine-rich ligand-binding repeats (blue circles). It also contains eight cysteine-rich EGF repeats and six YWTD domains. In the tail there are visible two NPXY motifs, two di-leucine motifs, and one YXXL motif; every ligand binding domain consists of a β-hairpin structure followed by a series of β-turns. Aβ, beta amyloid; AA, amino acid(s); EGF, endothelial grpwth factor; NMR, nuclear magnetic resonance; LRP1, low- density lipoprotein receptor-related protein 1; YWTD, Tyr-Trp-Thr-Asp; NPXY, Asn-Pro-x-Tyr; YXXL, Tyr-x-x-Leu; PICALM, phosphatidyl inositol binding clathrin assembly protein.

motifs recruit clathrin and AP-2 molecules, and act as cargo recognition motifs and transporters of this cargo into endosomes and lysosomes. NPXY motifs initially recruit AP-2 to the plasma membrane and activate μ2 subunit of AP-2. After that, AP-2 β2 subunit binds clathrin to the cell surface, inducing clathrin-mediated endocytosis. In order to understand these events, it is necessary to present the detailed structure of LRP1 and the AP-2 adaptor (***Fig. 2, 3***) [13-16].

AP-2 concentrates the endocytic cargo into clathrin-coated pits (CCPs). AP-2 also serves as an important factor for the recruitment of a number of endocytic accessory proteins: amphiphisin I, epsin 1 and 2, Eps 15, disabled-2, auxilin and huntingtin-interacting protein 1 (HIP). These proteins bind directly to the globular ear domains at the carboxy terminus of the AP-2 subunit. They have multiple functions including catalysing the clathrin assembly, linking endocytic vesicles to the actin cytoskeleton and recruiting regulatory endocytic enzymes such as synaptojanin (it has a crucial role in Ptdlns demolishing and uncoating of clathrin coated vesicle), dynamin and intersectin-1 (***Fig. 2, 3***) [17].

The events prior to the forming of clathrin-coated vesicles are shown in ***Fig. 4***. ***Figure 5*** presents in detail the forming of clathrin-coated vesicle and clathrin scaffold, as well as the uncoating process. The clathrin molecule has a triskelion shape, and is composed of three clathrin heavy chains and three light chains (***Fig. 3***).

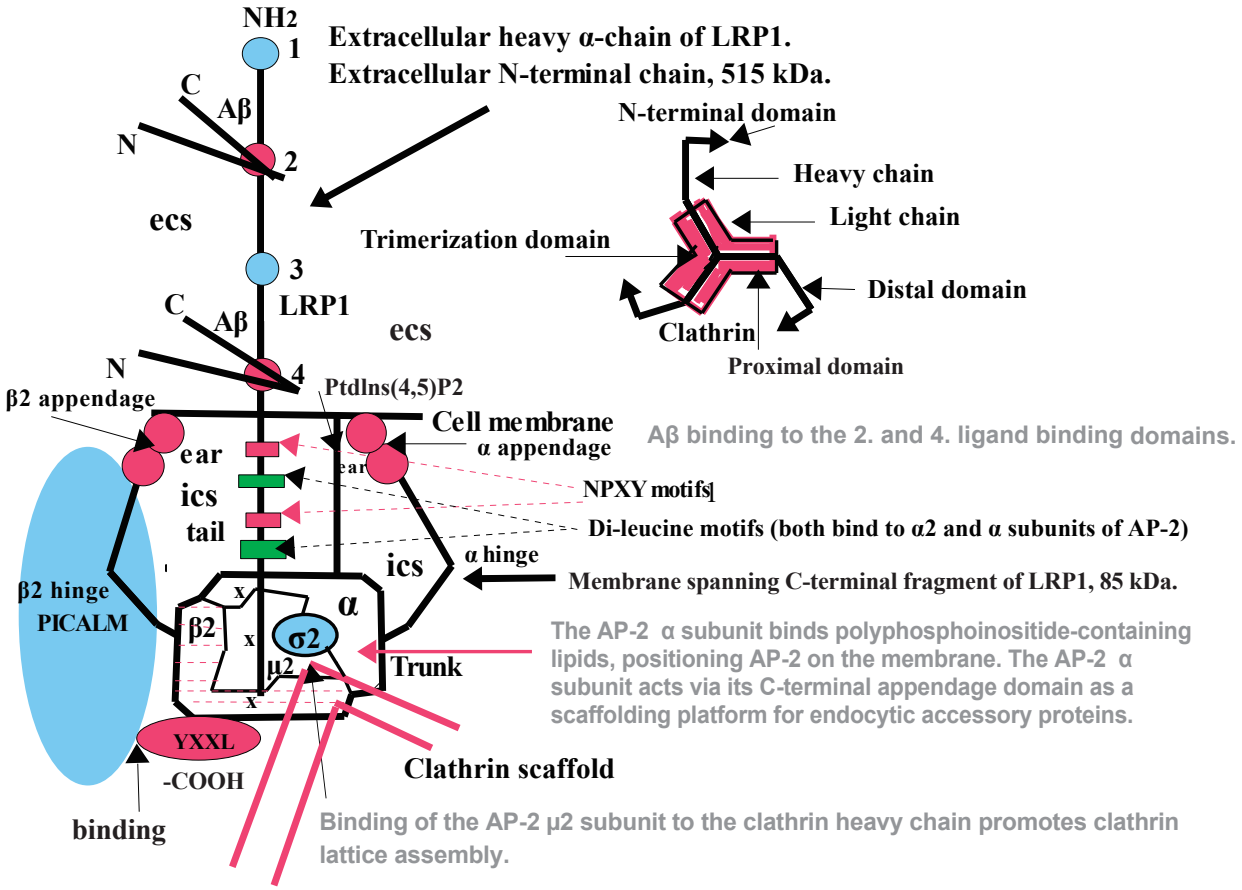

Fig. 3. **Presentation of AP-2 adaptor protein and Aβ binding to LRP1.**

Ear, globular ear domain at the carboxy terminus of AP-2 α-subunit; LRP1, low- density lipoprotein receptor-related protein 1; -NH_2, amine group; -COOH group, carboxyl group; clathrin molecule has a three-legged structure termed triskelion; it is composed of three heavy chains (polypeptides), each of them fused with a light chain; the heavy chains are the units of clathrin lattice structure; the light chains have regulatory domains crucial for clathrin molecules recruitment, assembly and disassembly (clathrin dissociation); clathrin proteins are elementary units of the scaffold that forms a basket-like coat surrounding the coated vesicle. LRP1 YXXL motif is a binding site for PICALM. AP-2, adaptor protein complex 2, heterotetramer, composed of two large adaptins (α and β2), 110 kDa , one medium (μ2) 50 kDa, and one small (σ) 17 kDa; ear domains are recruitment platforms for attraction several AP-2 binding proteins; μ2 subunit is a link between AP-2 and clathrin scaffold; YXXL motif and di-leucine motifs interact with the AP-2; di-leucine motifs bind to the μ2 or β2 subunits; AP-2 recruits diverse proteins important for clathrin coated vesicles formetion and their endocytosis and transcytosis; on the figure the tail is enlarged for better presentation.

Aβ, beta amyloid; ecs, extracellular apace; isc, intracellular space; YXXL, Tyr-X-X-Leu; Ptdins, phosphatidylinositol; PICALM, phosphatidyl inositol binding clathrin assembly protein.

Molecular basis for LRP1/Aβ exocytosis

Generally speaking, the process of exocytosis can be divided in five steps. Essentially, this is also related to the LRP1/Aβ exocytosis. The steps are as follows: vesicle trafficking, vesicle tethering, vesicle docking, vesicle priming, and vesicle fusion. Vesicle trafficking involves movement (recruiting, transporting, transferring) of signal molecules from synthesis and packaging locations to specific release places on the inside of the cell membrane of the involved cell. Vesicle tethering is the initial interaction between a vesicle membrane and a target membrane for the fusion process. Docking is the process in which the vesicle and target membrane line up in a fusion-ready state. Priming prepares the vesicle and target membrane to fuse in response to included physiological mechanisms. Vesicle fusion is the merging of a vesicle with other vesicles or with a part of a cell membrane. The main event in these steps is the throwing of waste (here Aβ) material into a membrane and its fusion with the membrane structures. The fusion opens the endothelial membrane border on the luminal side and expells Aβ monomers into the capillary blood [18].

A detailed presentation of endocytosis,transcytosis and exocytosis is shown in ***Fig. 4, 6***. The binding of Aβ to the cysteine-rich module (blue circles in ***Fig. 2***) on CII and C IV ligand binding clusters induces conformational changes on the receptor tail structure. These changes enhance the binding of locally expressed phosphatidyl inositol binding clathrin assembly protein (PICALM). Its role is to recruit clathrin and adaptor protein 2 (AP-2) to cell membranes at positions of coated-pit formation. It is also an important factor in AP-2 dependent clathrin-mediated endocytosis. The PICALM binding place on the LRP1 tail is on the YXXL motif. By its action, the initial clathrin coated pits transform into clathrin coated vesicles (***Fig. 4***) [13, 14].

Figure 5 presents the next steps of the Aβ transcytosis at the BBB. The transformation of the coated pit is clearly demonstrated first in the incomplete, and upon that in the complete clathrin coated vesicle. The latter is completely separated from the cell membrane. In the middle of the figure, the completely closed LRP1 receptor with two attached Aβ monomers is visible. Around the central cavity, in a circular manner, are the positions of the regularly connected AP-2, PICALM and scaffold elements. The bottom of the figure presents the uncoating process. The dissociation of AP-2 and isolated clathrin molecules is visible. The vesicle transforms into an early endosome. All dissociated AP-2 and clathrin molecules move toward the cell membrane. Hsc70 kD uncoating ATPase removes clathrin coats from the vesicle. The enzyme auxilin is important for the regulation of Hsc70 activity. PICALM is connected to the vesicle further on [13, 14].

Figure 6 shows the completely developed early endosome and its transformation into the late endosome. Early endosomes are primary sorting stations in the endocytic pathway. They evolve from the uncoating of coated clathrin vesicles. They have pH pumps, but not acid hydrolases (aH). Around the PICALM/vesicle complex are visible recruited Ras-related protein Rab5 molecules. Rab5 proteins are small guanosine triphosphatases (GTPases), important for vesicle endocytosis and trafficking. Rab5 protein is necessary for the biogenesis of early and late endosomes. Its overactivation induces the evident enlargement of the early endosome. PICALM directs the uncoated clathrin vesicle which transforms into early endosome, toward Rab5 and Rab11. Rab5, coordinated with PICALM, defines the PICALM-bound vesicle routes toward Rab11, and not toward Rab7 that directs the fusion of late endosomes with lysosomes and ligand degradation. For Rab5 activation guanine nucleotide-exchange factors (GEFs) and SH3-adapter protein SPIN90 are required. The SH3-adapter protein SPIN90 induces the recruitment of both Rab5 and its GEF, Gapex5, to the endosomal membrane. Rab5 is necessary for the biogenesis of late endosomes that have a proton pump (pH) and acid hydrolases (aH). They are subjected to strong acidification, and by the action of Rab7, they can be fused with lysosomes.

By the coordinated action of PICALM Rab5 and now present Rab11, the late endosomes, now in the form of exocytic vesicles, approach the luminal cell membrane, and the complex fusion and exocytic process can begin [13, 14, 19, 20].

According to a number of recent investigations, the analysed fusion is driven by the assembly of functional soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes [17, 21, 22].

The essential SNARE complex consists of four α-helices, one synaptobrevin (vesicle-associated membrane protein or VAMP), one syntaxin and two SNAP-25 (sn1 and sn2). Synaptotagmin, located near the essential SNARE complex and bound with the vesicle membrane, is a calcium sensor which has an important role in SNARE zipping.

The essential function of SNARE protein is to mediate the function of different vesicles with the target membrane. They have a crucial role in the transendothelial drainage of a number of waste compounds, especially Aβ- monomers (***Fig. 6***) [23].

In the detailed study of Han J *et al.* [24], they are especially engaged in the analysis of the coordinated action of SNARE proteins that are the key components of the eucariotic fusion machinery. During the process of exocytosis, synaptobrevin bound to the vesicle, and syntaxin and SNAP-25 both bound to the target cell, all bind together, forming the trans- SNARE complex. According to the authors, this complex has a multifunctional role in different phases of exocyosis (especially priming and fusion), with the final efflux of vesicle content into the ECS. Membrane fusion is the process by which two initially separated lipid bilayers merge to form a single unit. Spontaneous membrane fusion in living organisms is opposed by repulsive forces between approaching bilayers. These forces result from the electrostatic repulsion of equally charged membrane surfaces and from hydration repulsion. The energy, required to overcome the energy barrier for the fusion of biological membranes, is provided by specialized fusion proteins, *i.e.*, in exocytosis the energy results from the assembly of SNARE proteins into the "rod-like" α-helical bundle, termed trans-SNARE complex.

The SNARE motifs, by synaptobrevin and syntaxin, are connected to peptidic transmembrane domains (TMDs) at the C-terminus using a short linker region. These two SNARE proteins are embedded in their respective membranes by means of the anchoring of TMDs. The third interaction partner, SNAP-25, consists of two SNARE motifs that are connected by a linker and attached to the plasma membrane by multiple palmitoyl tails [24].

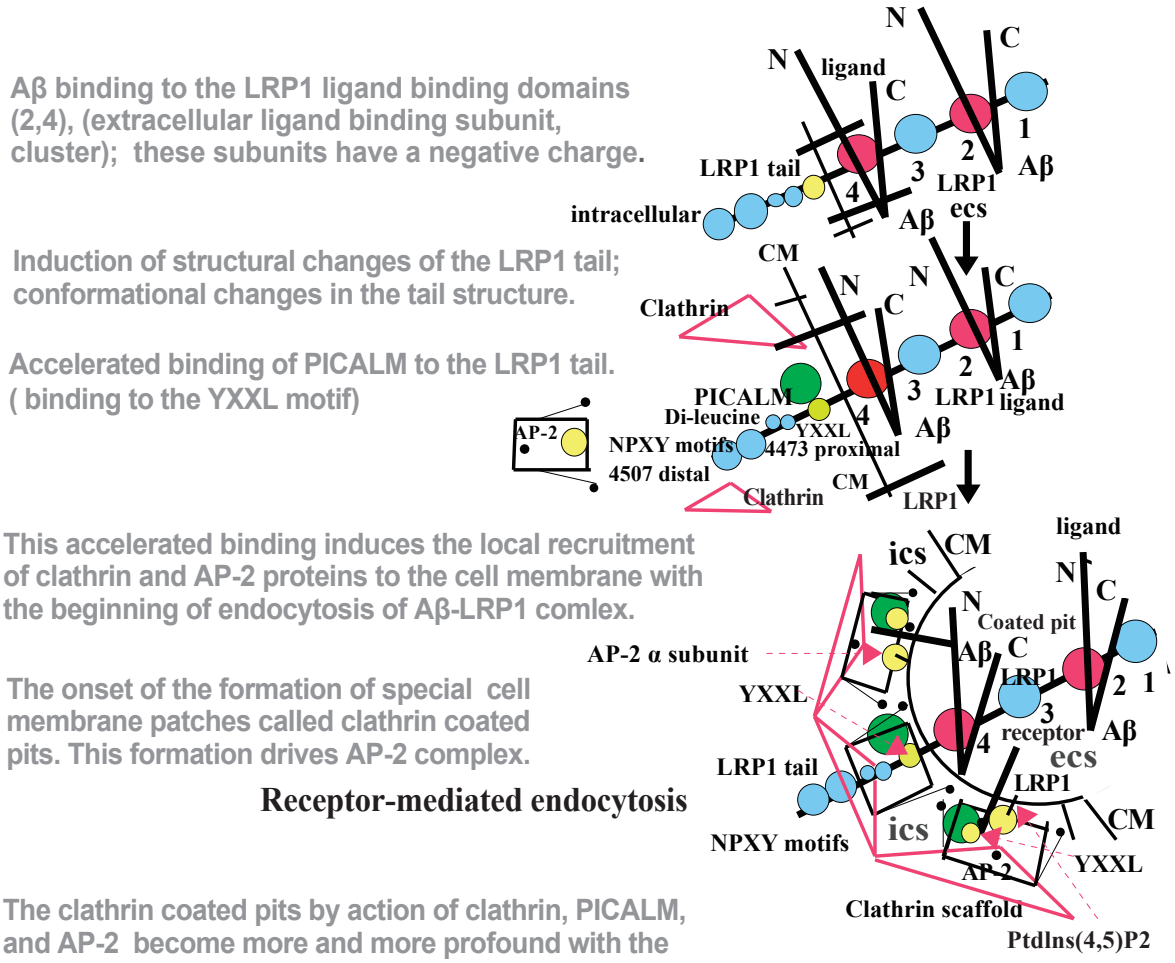

Fig. 4. **Schematic presentation of events that precede the formation of clathrin coated vesicles**

The first event in the process of Aβ internalization, endocytosis and transcytosis is Aβ binding on the 2. and 4. ligand binding clusters of the LRP1 extracellular ligand-binding subunit. The description of the 2. cluster has been previously presented. These clusters have a net negative charge that enables the binding of a number of positively charged ligands. The binding o ligands leads to structural changes of LRP1 tail, resulting in elevated PICALM binding to the tail and the inducing of the PICALM/clathrin-dependent endocytosis of Ab-LRP1 complex (**Ref 13**). It has been established that PICALM binds to the LRP1 tail in the region of YXXL domain (**Ref 13**). The elevated PICALM binding to the tail leads to the local recruitment of clathrin and AP-2 protein to the cell membrane with the begining of endocytosis; Aβ, beta amyloid; ecs, extracellular space; ics, intracellular space; CM, cell membrane; LRP1, low- density lipoprotein receptor-related protein 1; AP-2, adaptor protein complex 2; YXXL, Tyr-X-X-Leu; NPXY, Asn-Pro-x-Tyr; Ptdins, phosphatidylinositol; PICALM, phosphatidyl inositol binding clathrin assembly protein.

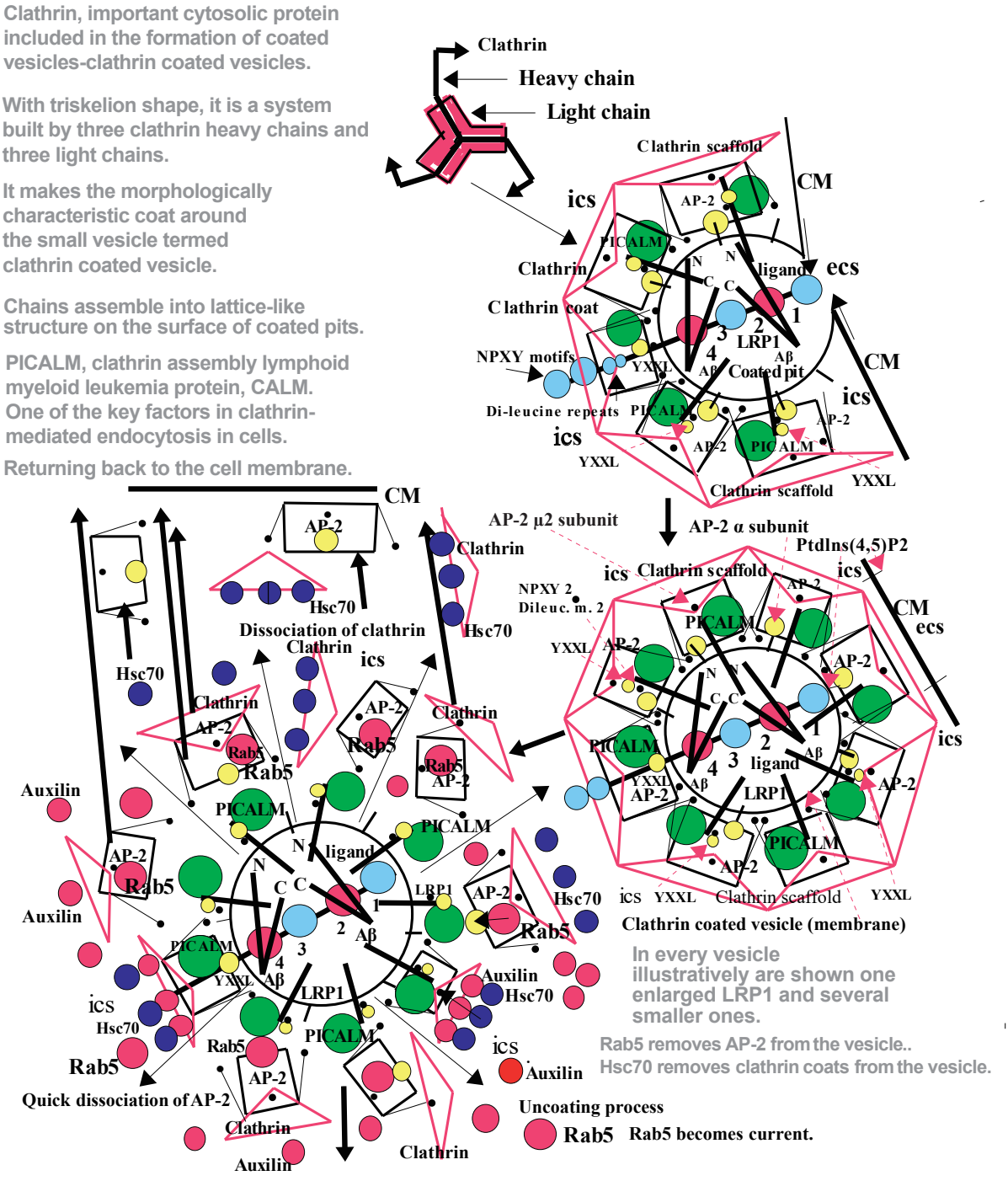

Fig. 5. **The scheme of the Aβ transcytosis at the BBB.**

Hsc70, 70 kD uncoating ATPase, remover of clathrin coats from the vesicle, Hsc70 chaperone; Auxilin, enzyme, it regulates Hsc70 activity by stimulating ATPase activity; CM, cell membrane; ecs, extracellular space; ics, intracellular apace; BBB, blood brain barriar; Aβ, beta amyloid; YXXL; AP-2, adaptor protein complex 2; LRP1, low-density lipoprotein receptor-related protein 1; PICALM, phosphatidyl inositol binding clathrin assembly protein; Ptdins, phosphatidylinositol; Rab, a small GTPase derived from rat brain; YXXL, Tyr-X-X-Leu.

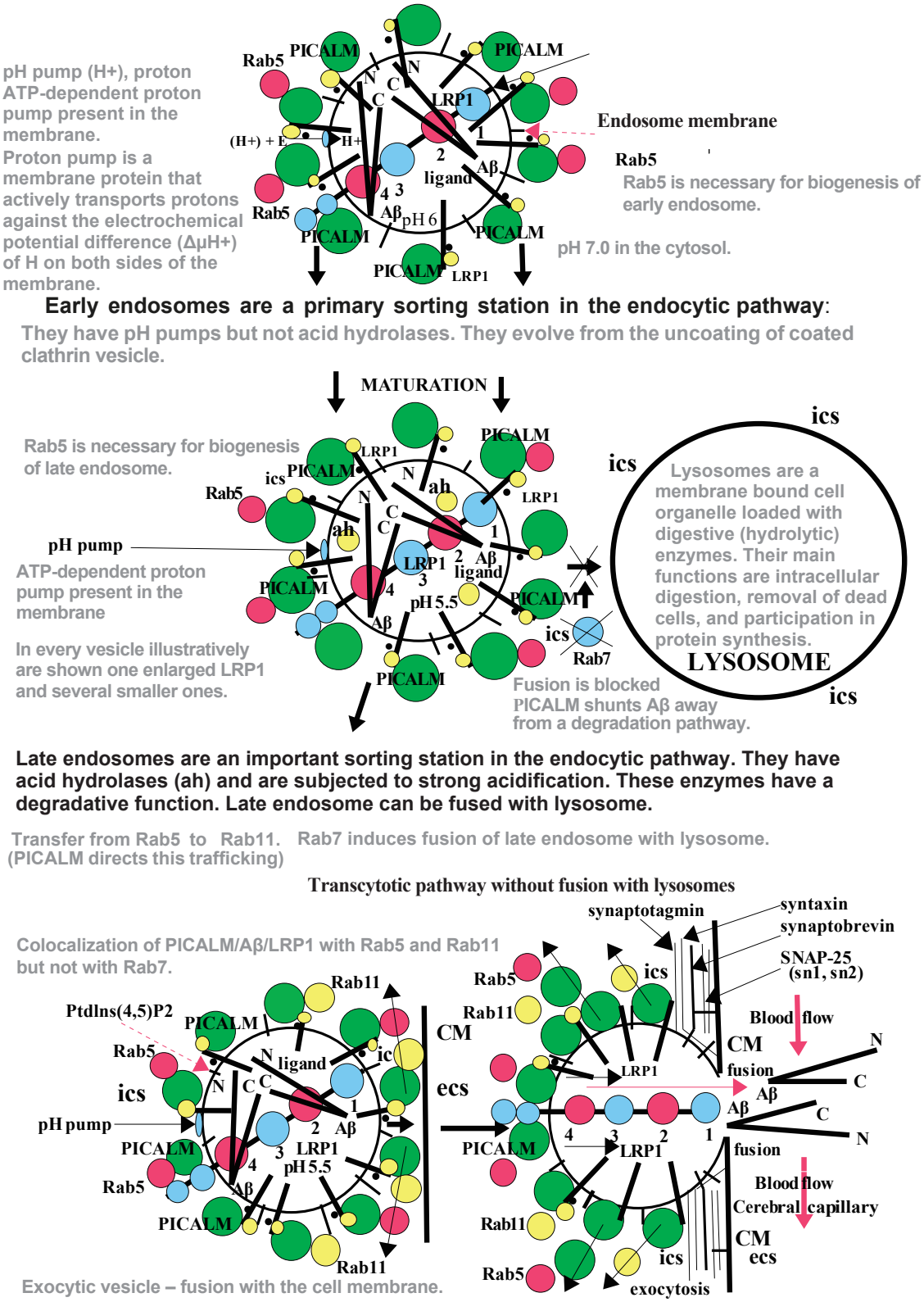

Fig. 6. **Schematic presentation of Aβ transcytosis and exocytosis.**

Aβ is an endocytic protein; Rab proteins, small guanosine triphosphatase regulators of protein transport during the endocytic and exocytic moving inside the cell. PICALM reductions in brain capillar endothelium in Alzheimer's disease. Rab11 depletion retards the exocytic process. Aβ, beta amyloid; CM, cell membrane; ecs, extracellular space; ics, intracellular space; PICALM, phosphatidyl inositol binding clathrin assembly protein; LRP1, low-density lipoprotein receptor-related protein 1; Rab, a small GTPase derived from rat brain; ah, acid htdrolases; SNAP-25, synaptosomal-associated protein, 25kDa; Ptdins, phosphatidylinositol; synaptotagmin, syntaxyn, synaptobrevin, SNAP-25 (sn1, sn2).

The complex (core SNARE complex) formation is accompanied by the energy release which is used to bring the membranes into close proximity. Highly specialized proteins called "fusogens" mediate the merging of two otherwise stable membranes to a single bilayer. It is currently established that widely conserved soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins are the primary fusogens, responsible for nearly all intracellular membrane fusions[22]. The zippering is controlled by regulatory proteins, as is synaptotagmin 1 (syt1) in fast Ca^{2+}-triggered exocytosis in neurotransmitter release[23].

Fang Q *et al.*[25], present practically identical conclusions as Han J *et al.*[24] about the role of SNARE proteins in the vesicle fusion. Mechanisms of the vesicle fusion are of crucial importance in cell biology and neuroscience.

Lakomek NL *et al.*[26], emphasize that optimal flexibility and membrane binding of syb-2 regulate the SNARE assembly and minimize repulsive forces during the fusion process.

It is necessary to point out that the LRP1 is a multi-ligand receptor abundantly expressed in neurons. Investigations have shown that brain LRP1 levels decrease during aging and in Alzheimer's disease[14,27].

Figure 2 shows that the extracellular ligand binding subunit of LRP1 receptor consists of four different ligand-binding clusters (domains) composed of 2, 8, 10, and 11 ligand binding repeats (blue circles) rich in cysteine. Each repeat contains the β-hairpin structure (short β- hairpin near the N-terminal end), which continues with a series of β-strands. Essentially, the repeat is composed of 40 amino acids. Each repeat contains one Ca^{2+} ion and six cysteine residues that form three intra-repeat disulphide bonds. The cluster of acidic residues is an efficient place for Ca^{2+} ion binding (octahedral calcium-binding cage). Each repeat contains a net negative charge that enables it to bind with a variety of positively charged ligands[28-30].

Figures 4 and 5 show some important elements. Yellow circles on the outer side of the AP-2 structure (toward the vesicle membrane) present the AP-2 α subunit, *i.e.*, the AP-2 binding place to the vesicle membrane. The link is presented by the short black line, which presents Ptdlns(4,5) P2 element. This element is abundantly present in vesicular and cell membranes and makes the connection of AP-2 with the vesicular membrane. Black dots in the inner side of AP-2 are AP-2 μ2 subunits. They present the links between AP-2 and the clathrin scaffold. On ***Fig. 6***, there are visible, on cell and vesicle membranes, short black lines which represent Ptdlns(4,5)P2 elements. On ***Fig. 5***, on the clathrin coated vesicle uncoating, still are visible yellow circles on the link between AP-2 and vesicle membranes. Black dots are the link between AP-2 and the clathrin scaffold. Immediately after binding to the clathrin scaffold, auxilin activates Hsc70, and together with Ptdlns(4,5)P2 (PIP2) and Rab5, induces AP-2 and all other mentioned molecule dissociations (uncoatings) from the vesicle membrane. The uncoating of clathrin from the clathrin coated vesicle (CCV) requires Hsc70 and auxilin. Rab5 acts on AP-2 α subunit and dissociates AP-2 from Ptdlns(4,5)P2. Hsc70 induces the dissolution of the clathrin heavy chain from AP-2 μ2 subunit. Auxilin and Hsc70 move around vesicles before binding to the structures of the clathrin coated vesicle. Rab5 induces dephosphorilation of α2 subunit and AP-2 uncoating in vivo. The small yellow circles located on the LRP1 tail end present the YXXL motif crucial for PICALM binding. Immediately near the YXXL motif are visible the black dots which represent two NPXY and two di-leucine motifs.

LRP1-mediated endocytosis regulates cellular Aβ uptake by binding to Aβ either directly or indirectly through its co-receptors or ligands[30].

Shinohara M *et al.*[31], in their thematic review, emphasize that over 40 proteins are reported to be ligands for LRP1, among them are Aβ and ApoE. The actual question; however, is if Aβ directly binds with ligand-binding repeats II and IV (this particularly relates to monomeric Aβ40)[8], or, as Yamada K *et al.*[32] claim, based on a similar experiment, that the cooperation is necessary with other Aβ-binding proteins, such as heparan sulfate proteoglycan.

The analysis of the schematic presentation of two Aβ-monomer β-strands indicates that the monomer is composed of 42 amino acids interconnected by peptide bonds. Of these amino acids, two (Asp, asparagine; Glu, glutamine) have a negative charge, and three (Lys, lysine; Arg, arginine; His, histidine) have a positive charge.

Van den Biggelaar M *et al.*[33], present comprehensive data about the direct binding of various ligands to the LRP1 receptor, mediated by clusters of ligand-binding repeats (complement-type repeat) located on the receptor. Every repeat has three disulfide bonds, octahedral calcium-binding cage, and a short β-hairpin near the N-terminal end. Their experiments with coagulation factor VIII (FVIII) show that the interaction between this factor and LRP1 occurs over the factor extended surface containing multiple lysine residues (negative charge). Interaction starts at the bottom of the C1 domain and winds around the FVIII molecule. Lysine residues are located in the FVIII light chain. This finding can explain the Aβ/LRP1 interaction and Aβ binding to the receptor.

Spuch C *et al.*[34], emphasize that ligand binding repeats contain a net negative charge, which alows the binding of a variety of positively charged ligands.

Experiments show that many side chains of the acidic residues, including Asp-Ser-Glu triad, are clustered on one face of the ligand binding repeat[35].

At the end of the presented study, once again, it is necessary to emphasize the great importance of the role of the LRP1 receptor, which is crucial in the clearing and maintaining of the IPAD. The molecular events during the LRP1 receptor endocytosis, transcytosis and exocytosis are under intensive investigations, and will be continued further on with great intensity.

Conclusion

The role of the inadequate brain lymphatic drainage system has been taken over by some other systems, developed in the course of evolution. Among them, especially important is the IPAD, and the transmembrane drainage of Aβ across the BBB with the active role of the endothelial receptor LRP1. Complex pathophysiological processes can induce damages of these two crucial drainage systems resulting in disorders related to the brain homeostasis, the collapse of a number of vital biochemical and physiological processes, and the development or deterioration of the already

existing Alzheimer's disease. Continuing new findings in the knowledge related to the functions of the IPAD, and the closely related LRP1 receptor activity, lead us to the possibilities for a more efficient prevention and treatment of AD.

Conflict of interest

The author declares no conflict of interest in this study.

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