The role of the LRP1 receptor in the draining of amyloid beta (Aβ) peptide across the blood brain barrier (BBB)

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Abstract:

A number of recent investigations about the etiology and patophysiology of Alzheimer's disease (AD) emphasize the importance of the intra-arterial perivascular drainage (IPAD) of the amyloid beta (Aβ) from the brain, including the drainage from the blood brain barrier (BBB). They also emphasize the crucial factor in these events, the huge transmembrane low-density lipoprotein receptor-related protein 1 (LRP1). By binding within the BBB capillary wall basement membrane with the specific LRP1 ligand binding domains, Aβ induces a number of mechanical and biochemical events that are essential for the activation of the PICALM/clathrin-dependent endocytosis, transcytosis, and exocytosis, the crucial factors for adequate Aβ drainage from the brain. This drainage is otherwise immesurably important for the proper Aβ homeostasis, because its impairment has a vital role in the pathophysiology and destructive effects of AD. It is evident that the altered Aβ homeostasis induces Aβ elevated aggregation and deposition which are the basis of the disease. A number of recent investigations emphasize that the binding of Aβ induces specific conformational changes in the extracellular and intracellular receptor structures. Extracellular conformational changes of the receptor structure are reflected in the structure of the receptor intracellular tail, with the binding of PICALM protein to the YXXL motif of the tail and with the consequent recruitment of a number of signal proteins, as well as in the onset of PICALM/clathrin-dependent Aβ endocytosis, transcytosis and exocytosis into the capillary circulation. However, a profound insight into the available literature related to these events does not give a satisfactory explanation of these events. Consequently, this study is concerned with the problem of Aβ binding to LRP1, and with local conformational changes on the receptor tail which are activators of Aβ endocytosis, transcytosis, and its exocytosis into the capillary blood. This study, in fact, presents the author's hypothetical approach to the problem, with the aim to contribute to the better understanding of AD.

Keywords: Alzheimer's disease pathophysiology, low-density lipoprotein receptor-related protein 1 (LRP1), intra-arterial perivascular Aβ drainage, PICALM, YXXL motif, endocytosis, transcytosis, exocytosis

Sažetak:

uloga lrp1 receptora u drenaži amiloid beta (ab) peptida kroz krvno-moždanu barijeru (kmb)
Veliki broj najnovijih istraživanja o etiologiji i patofiziologiji Alzheimerove bolesti (AD) ističe važnost intra-arterijalne perivaskularne drenaže (IPAD) amiloida beta (Aβ) iz mozga, uključujući krvno-moždanu barijeru (KMB, BBB, blood-brain barrier), kao i važnost krucijalnog čimbenika u tim zbivanjima, velikog transmembranskog receptora (low-density lipoprotein receptor-related protein 1, LRP1). Vezivanjem unutar bazalne membrane kapilarnog zida KMB sa specifičnim LRP1 domenama

koje vezuju ligande, Aβ izaziva niz mehaničkih i biokemijskih događanja presudnih za aktivaciju PI-CALM/clathrin ovisne endocitoze, transcitoze i eksocitoze, bitnih čimbenika za adekvatnu Aβ drenažu iz mozga. Ta drenaža je inače nemjerljivo važna za pravilnu Aβ homeostazu, jer njen poremećaj ima krucijalnu ulogu u patofiziologiji i destruktivnim učincima AD. Vidljivo je kako poremećena Aβ homeostaza dovodi do njegove povećane agregacije i taloženja, što čini podlogu te bolesti. Veliki broj suvremenih studija ističe kako vezivanje Aβ izaziva specifične strukturalne promjene u ekstracelularnoj i intracelularnoj strukturi receptora. Ekstracelularne strukturalne promjene receptora reflektiraju se na strukture intracelularnog receptorskog repa, uz vezivanje proteina PICALM za YXXL motiv repa, te posljedično nakupljanje velikog broja signalnih proteina, uz početak PICALM/clathrin Aβ endocitoze, transcitoze i egzocitoze u kapilarnu cirkulaciju. Međutim, iscrpan uvid u dostupnu literaturu koja se odnosi na ta zbivanja ne daje zadovoljavajuće objašnjenje. Ova studija je koncentrirana na problem Aβ vezivanja za LRP1, i lokalne strukturalne promjene na repu receptora kao aktivatore Aβ endocitoze, transcitoze i njegove egzocitoze u kapilarnu krv. U biti, studija je autorov hipotetični pristup uz namjeru da doprinese boljem razumijevanju AD.

Ključne riječi: Patofiziologija Alzheimerove bolesti, low-density lipoprotein receptor-related protein 1 (LRP1), intra-arterijalna perivaskularna drenaža Aβ, PICALM, YXXL motiv, endocitoza, transcitoza, egzocitoza

Introduction

Considering the number of recent scientific papers related to the etiology and pathophysiology of Alzheimer's disease (AD), it is evident that events connected with the amyloid beta (Aβ) drainage from the brain have gained special interest. According to the mentioned investigations, the Aβ disordered homeostasis in the brain structures, i. e., the disordered equilibrium between the Aβ production and elimination, with the evident shift to the failured drainage from the brain, not taking into account the Aβ constant unchanged production, has a crucial place in AD pathology and pathophysiology.

A number of explorers emphasize that in all these disordered events, the failures of composit molecular interactions in the intra-arterial perivascular space have a central role, especially in the region of blood-brain barrier (BBB) capillary basement membranes (CBMs). In these membranes, the transmembrane low-density lipoprotein receptor-related protein 1 (LRP1), the member of the great LRP receptor family, is dominantly located on the BMs border at the abluminal cell side of endothelial cells. According to recent investigations, this receptor has a central role in the drainage of Aβ across the BBB endothelial cells into the capillary blood circulation (**Fig. 1**)[1-3].

LRP1 extracellular domain has four ligand binding domains for a great number of ligands, but domains II and IV are dominant for Aβ binding. This binding is primarily induced by the electrostatic attraction between residues His13, His14, and Lys16 on the Aβ β1-chain, and asparagine residue side chains, primarily on the domain IV, i. e., CR (cysteine rich repeats) 2627 of the LRP1. Aβ binding with LRP1 induces biochemical reactions which result in conformational changes in both, extracellular and intracellular segments of the LRP1 receptor. The conformational changes on the point of contact, i.e., binding, are relatively clearly visible and less difficult to explain, but the changes on the receptor tail are in fact mostly unexplained (**Fig. 2**)[2-4].

All recent papers emphasize the conformational changes on the tail region, but nobody gives their explicit description and explanation. Due to this fact, this study has the aim to give a hypothetical explanation of how the initial bonding of Aβ with LRP1 induces signals necessary for the appearance of the mentioned changes, and of the consequent activation of the crucial YXXL motif on the tail, and activation of adaptors, which alll together results in the PICALM/clathrin-dependent endocytosis, transcytosis and exocytosis[2-4].

The approach of Aβ to the LRP1, according to this hypothesis, induces the mutual binding of CR26 and CR27 on the receptor extracellular domain with the mentioned residues on Aβ β1-strand. In this way, it is possible to achieve the electrostatic equilibrium with included residues on both involved elements (Aβ and LRP1). The approach, contact, and binding induce, on the receptor side, the pulling effect on receptor structures which spreads the pulling signal along the receptor toward the abluminal membrane and the tail. In the tail, this results in the pulling of two NPXY and two di-leucine motifs toward the abluminal cell membrane, (ab.l.c.m.) with the consequent spreading of the passage necessary for the PICALM approach to the YXXL motif
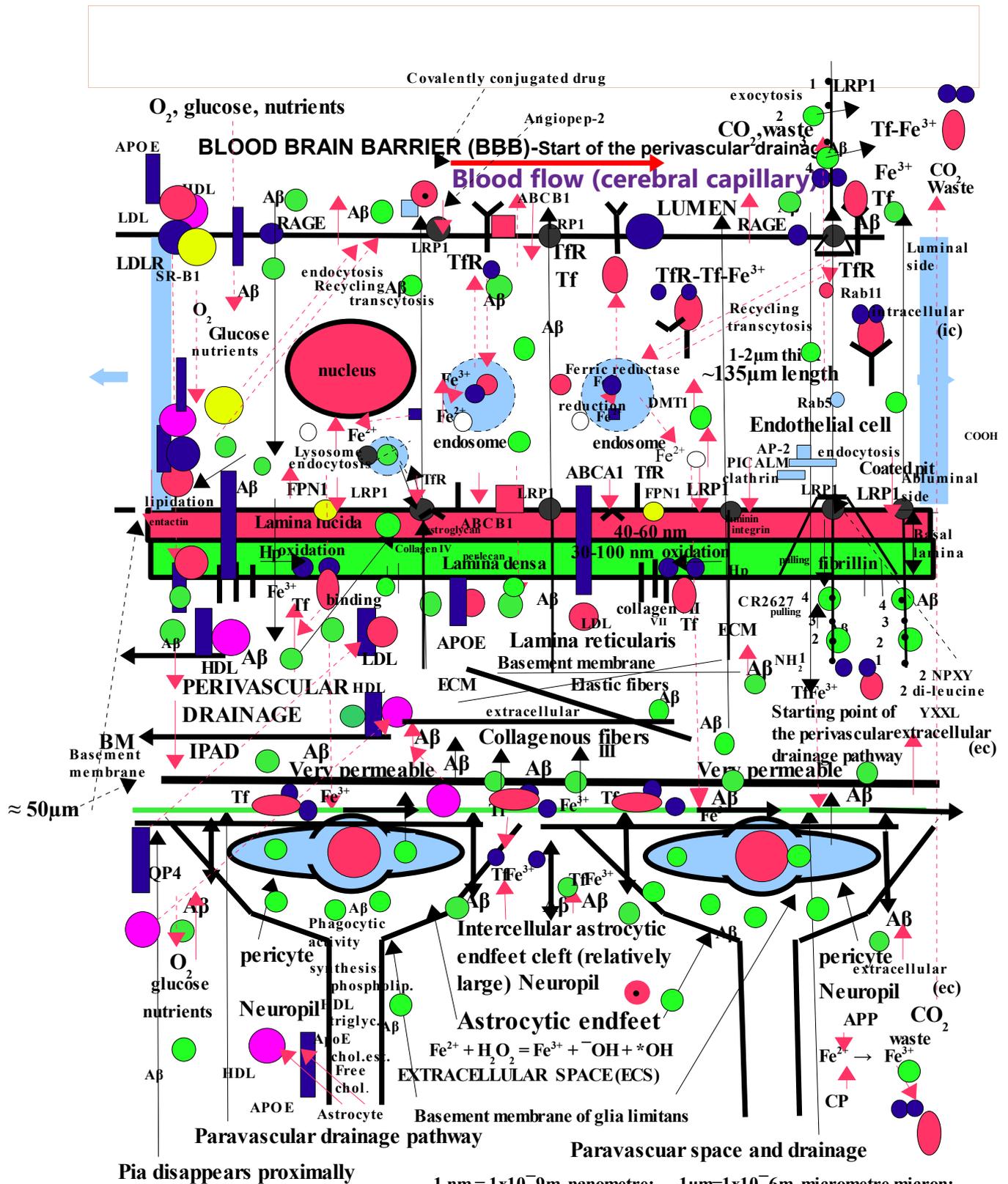
Fig. *1.* **Structure of the cerebral capillary basement membrane**

(longitudinal cross-section)   (effects of aging and AD) (effects of LRP1

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 and lamina densa.

NH₂ fixed
Cysteine-rich module (40 AA in every module)
Peptide bond
Ligand-binding repeats
C I Ligand-binding cluster I
No ligands known to bind

EGF repeats
2 cysteine-rich EGF-repeats

YWTD domains
β-propeller domain (much AA in every domain)

Asp15, Asp26, Glu30, Asp33, Asp36, Glu37, Glu40
(see *Ref.* 36)
Ligand-binding repeats
Ligand-binding cluster II
C II

Ligand-binding domains I-IV contain a net negative charge important for binding positively charged ligands.

The three-dimensional structure of this module has been determined by two-dimensional NMR spectroscopy.
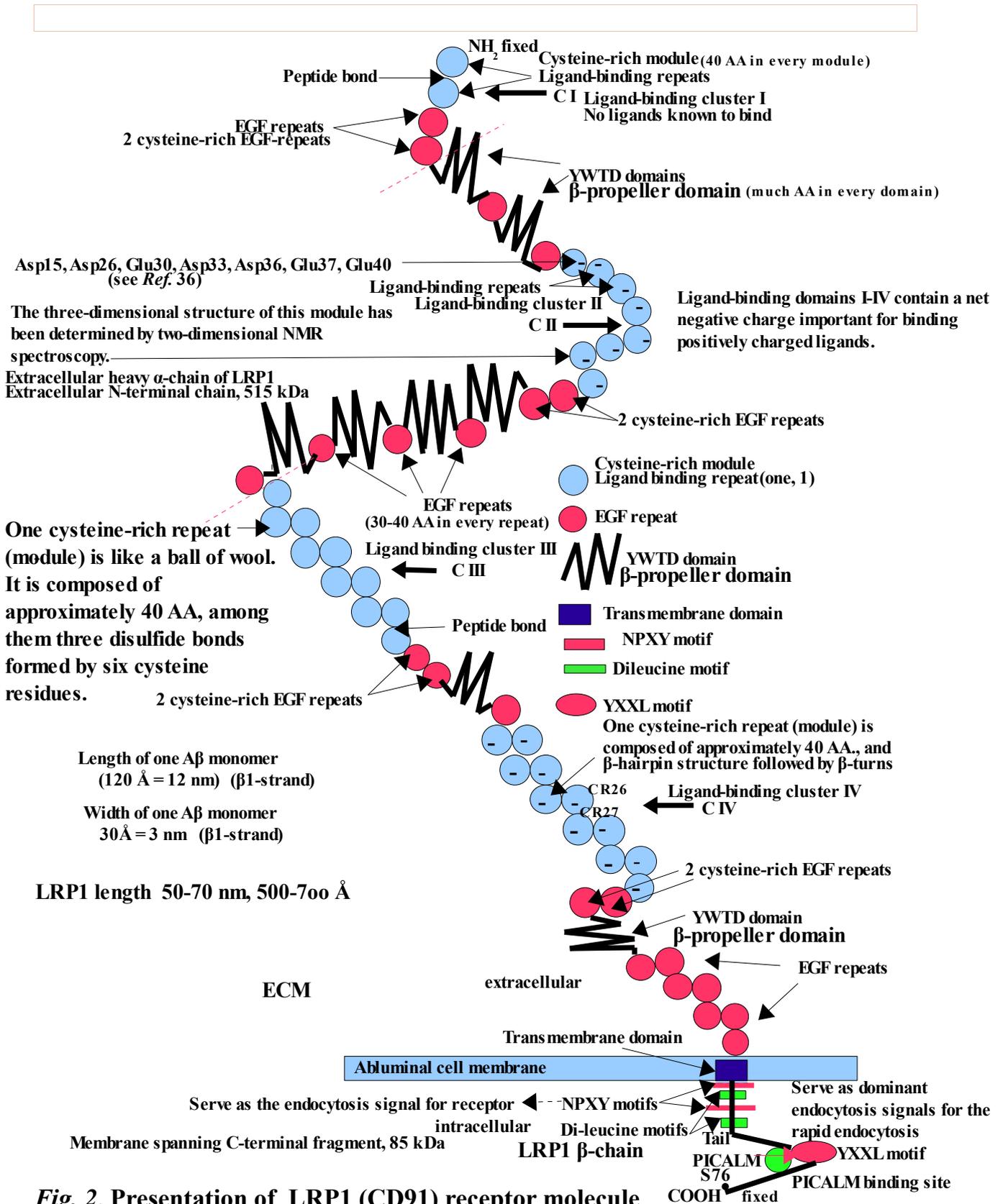Extracellular heavy α-chain of LRP1
Extracellular N-terminal chain, 515 kDa

2 cysteine-rich EGF repeats

Cysteine-rich module
Ligand binding repeat (one, 1)

EGF repeat

One cysteine-rich repeat (module) is like a ball of wool. It is composed of approximately 40 AA, among them three disulfide bonds formed by six cysteine residues.

EGF repeats
(30-40 AA in every repeat)
Ligand binding cluster III
C III

YWTD domain
β-propeller domain

Transmembrane domain

NPXY motif

Di-leucine motif

YXXL motif

Peptide bond

One cysteine-rich repeat (module) is composed of approximately 40 AA., and β-hairpin structure followed by β-turns

2 cysteine-rich EGF repeats

Length of one Aβ monomer
(120 Å = 12 nm) (β1-strand)

Width of one Aβ monomer
30Å = 3 nm (β1-strand)

CR26
CR27
Ligand-binding cluster IV
C IV

LRP1 length 50-70 nm, 500-7oo Å

2 cysteine-rich EGF repeats

YWTD domain
β-propeller domain

EGF repeats

ECM

extracellular

Transmembrane domain

Abluminal cell membrane

Serve as the endocytosis signal for receptor
NPXY motifs
intracellular
Di-leucine motifs

Serve as dominant endocytosis signals for the rapid endocytosis

Membrane spanning C-terminal fragment, 85 kDa
LRP1 β-chain
Tail

YXXL motif
PICALM
S76
COOH fixed
PICALM binding site

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

Second ligand-binding cluster contains 8 cysteine-rich ligand-binding repeats (blue circles);

and its activation. This activation is crucial for the adaptor and scaffold proteins activation, the activation of the protein kinase A (PKA), and serine 76 (S76) phosphorylation. The final result is PICALM/clathrin dependent endocytosis, transcytosis, and exocitosis of the Aβ-LRP1 complex. The pulling also occurs on the other side of the binding place, but for the tail elements/motifs activation, dominantly important is the pulling on the side of the abluminal cell membrane (ab.l.c.m.). According to mechanical laws, it is obvious that the pulling signal and the receptor structure moving have the opposite direction. It is also clear that the signal spread through the LRP1 backbone is incomparably greater than the spread of the structure moving. This difference in both spreads will be explained later in the text (**Fig. 2,3**) [2]. For the better understanding of the problems related to Aβ drainage, and the LRP1 receptor role in these events, it is necessary to present the structure of the receptor, especially domains included in Aβ binding, and intracellular motifs on the tail which are directly involved in the drainage. LRP1 length is about 50-70 nm, i. e. 500-700 Å (nanometers, i. e. angstroms). It is composed of the extracellular heavy α-chain (extracellular N-terminal chain, 515 kDa), and the membrane spanning C-terminal fragment (85 kDa). N-terminal chain is composed of 31 cysteine-rich repeats, 22 cysteine-rich EGF-repeats, and 8 YWTD (β-propeller domains) domains. The cysteine-rich repeat (module) is like a ball of wool, composed of approximately 40 amino acids, among them three disulfide bonds formed by six cysteine residues [1,5-7].

Some researchers have solved the problem of the structure of the first LDL receptor module (CR1, cystaine-rich1, module1) by NMR spectroscopy and X-ray crystallography. This module is composed of a β-hairpin structure followed by a series of β-turns. There are clearly visible 6 disulfide-bound cysteines and a cluster of negatively charged amino acids (Asp15, Asp26, Glu30, Asp33, Asp36, Glu37, and Glu40), primarily clustered on one face of the module. CR module also contains a coordinated calcium ion, important for the structure stability (**Fig. 2,3**) [8,9].

The membrane spanning C-terminal fragment (85 kDa, β-chain) of the receptor is composed of several motifs which have an essential role in Aβ-LRP1 complex endocytosis, transcytosis, and exocytosis. There are two NPXY motifs (N=asparagine, P=proline, X=some other amino acids, Y=tyrosine, two di-leucine motifs), and one YXXL motif (Y=tyrosine, L=leucine, X=some other amino acids, most probably histidine and lysine). NPXY motifs are included in endocytosis and in the multiple signaling ways. YXXL motif is near the second NPXY motif and induces rapid endocytosis. These motifs, the members of the β-chain, interact with scaffolding and adaptor proteins such as postsynaptic density protein 95 (PSD-95), DAB Adaptor-Protein 1(Dab-1), and Fe-65 protein. β-chain can translocate to the cell nucleus and activates the gene transcription and signaling cascades. Analysing a number of figures from various papers, it is

evident that the tail makes specific curves, which connected with neighbouring tissue elements create a blockade for PICALM to approach the YXXL motif with consequent motif activation (**Fig. 3,4**) [1,5,8].

SOME FACTS ABOUT THE LRP1 RECEPTOR BIOSYNTHESIS AND RAP FUNCTIONS

LRP1 gene is located on the long arm of chromosome 12 (12q13.3). LRP1 precursor protein is synthesized in the endoplasmic reticulum (ER) of the endothelial cells (ECs). During the synthesis, LRP1 is linked to one specific protein, the receptor associated protein (RAP). Bound with RAP, LRP1 precursor is transported to the trans-Golgi network, where the low pH induces RAP dissociation. By the locally present protease Furin, LRP1 precursor becomes cleaved to a large α-chain (515 kDa) and shorter β-chain (85 kDa). They both (noncovalently linked) exit from the Golgy and enclosed in the transport vesicule, travel to the cell membrane, primarily abluminal membrane (Ab.l.c.m.), where they are inserted as a united transmembrane functional unit. It is necessary to emphasize that the RAP protein is the LRP1 receptor chaperone, i. e., the protein that assists conformational changes of other macromolecules. Artificially synthetised, it is an important factor in all experiments related to the ligand binding with the LRP1 receptor [1,5-7].

The NMR analysis and X-ray crystalography of the RAP protein indicates that this protein consists of three helical chain complexes, i.e., domains, D1,D2, and D3. D1 domain consists of α1, α2, and α3 helix; D2 consists of α4, α5, and α6 chain; D3 domain consists of α7, α8, and α9 helix. Each domain is completely independent and does not contact with other domains. However, they are mutually linked with long flexible loops. The D3 domain is especially important for this study. It has been proved that RAPD3α8 consists of two extremely positive docking sites for the LRP1 receptor (K256 Lys and K270 Lys). RAPD3α8 in the experiments is analogous to Aβ β1-strand in reality. The distance between these two sites is 21Å. It seems that this distance during the bonding with LRP1 does not diminish. The distance between CR4 and CR3 on the LRP1 receptor before bonding by electrostatic forces is 32Å, but after mutual bonding, this distance declines to 21Å. It is obvious that in this case each CR moves toward the bonding centre approximately 5.5Å. The electrostatic interaction is established between lysine residues and asparagine acid residues that participate in the coordination of calcium with CR3 and CR4. Each CR has three asparagine residues that form the docking site for lysine with D3α8 helix, (CR3: Asp-108, Asp-110, Asp-112, CR4: Asp-147, Asp-149, Asp-151). Each group of three asparagine residues encircles the ε-ammonium group attached to the 4th C atom related to α C lysine residue, forming the tripartite salt bridge (a combination of hydrogen bonding and ionic bonding). The bonding is completed by Phe-105 (phenylalanine) in CR3 and Trp-144 (trypto-
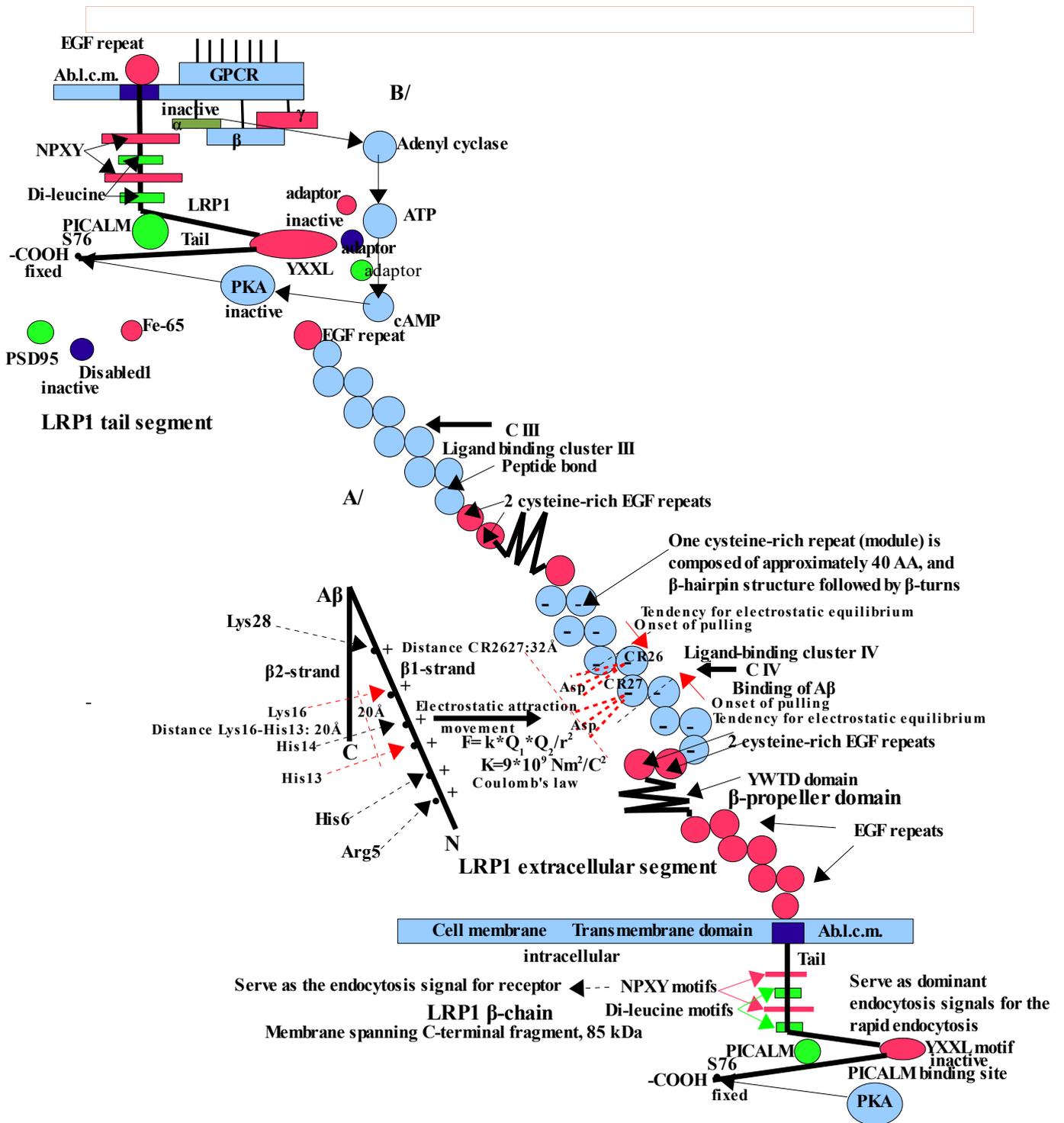
Fig. 3. Partial presentation of LRP1 receptor without complete activation

A and B: The difference compared to Fig.5 is clearly visible; Amyloid beta (Aβ) is free; LRP1

cluster IV is also free; the space for PICALM moving to the YXXL motif is narrowed, and the

PICALM approach to the YXXL is blocked; protein kinase (PKA) is inactive; there is no sign of

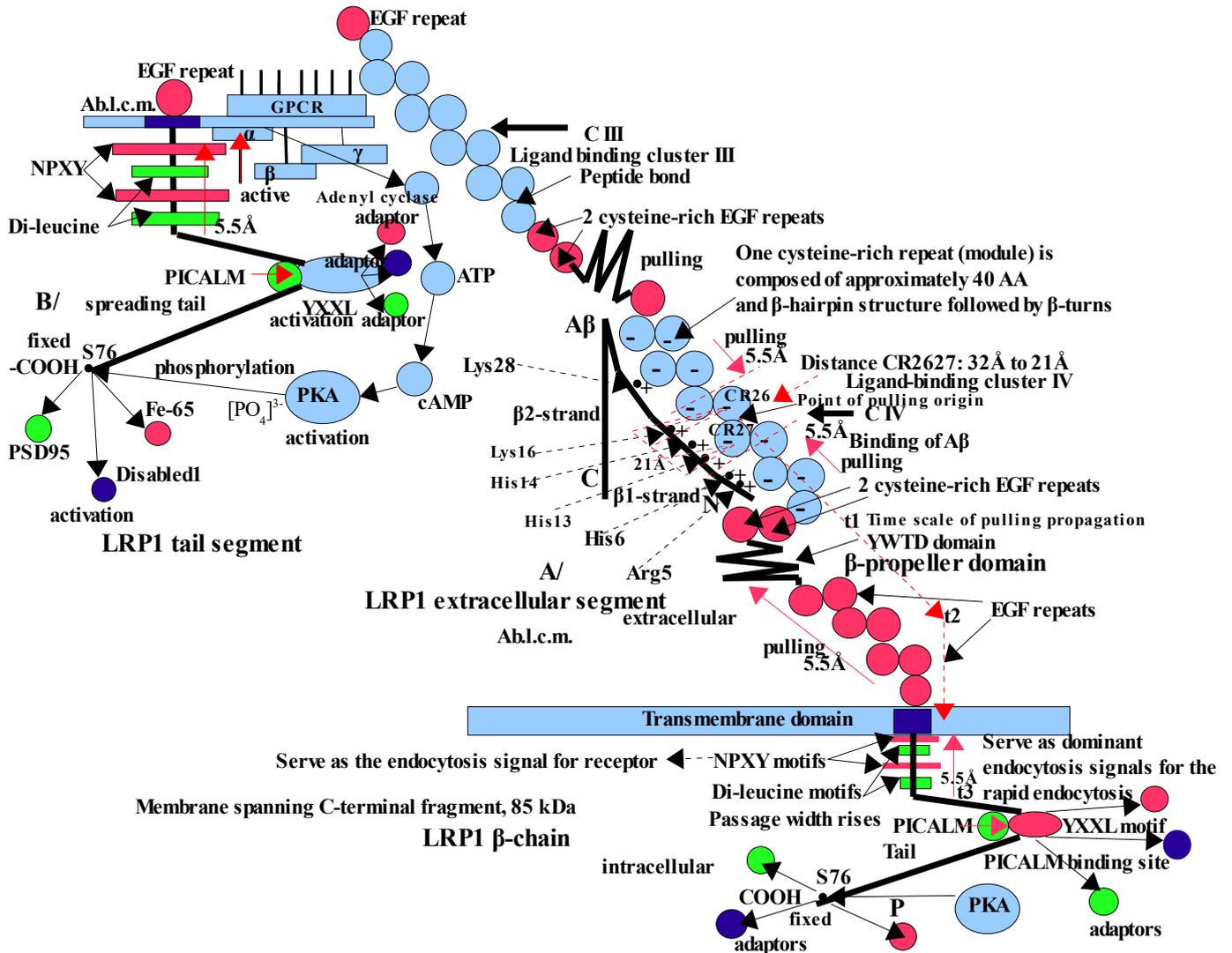S76 phosphorylation; Ab.l.c.m., abluminal cell membrane; adaptor proteins,

Fig. 4. **Partial presentation of LRP1 (CD91) receptor molecule (CIII and C IV)**

Fourth ligand-binding cluster contains 11 cysteine-rich ligand-binding repeats (blue circles); in

the direction to the abluminal cell membrane there are the positions of nine EGF repeats and one

YWTD domain (β-propeller domain); in the tail there are visible 2 NPXY motifs, 2 d-ileucine motifs,

and 1 YXXL motif; every ligand binding domain (cysteine rich domain) consists of a β-hairpin

structure followed by a series of β-turns; one cysteine-rich repeat (module) is like a ball of wool;

it is composed of approximately 40 AA, among them three disulfide bonds formed by six cysteine

residues; PICALM; cell membrane; endocytosis; transcytosis; length of one Aβ monomer is ~120Å

(12nm); width of one Aβ monomer is ~30Å (3nm); LRP1 length ~50-70 nm (500-700Å); PKA,

protein- kinase A; S76, serine residue 76 on LRP1 tail; phosphorylation, attachment of the

phosphoryl group to the molecule; phosphate ion, $[PO_4]^{3-}$, P; Ab.l.c.m., abluminal cell membrane;

adaptor proteins, Fe-65, Disabled1, PSD95; GPCR, G-protein-coupled receptor; His6, membrane

spanning C-terminal fragment, 85 kDa; EGF repeats; YWTD domains.

han) in CR4. The attached shemes made by relevant researchers appropriately present the distance of 21Å between K270Lys and K256Lys (before bonding with LRP1). When the D3α8 (used in experiments) is substituted by Aβ (used in reality), it is evident that their bonding with LRP1 will decrease the distance between CR3 and CR4 from 32Å to the balanced 21Å (**Fig. 5**)[5-7]. These movements (about 5.5Å on both sides of the binding center) induce mutual successive pulling of the following protein segments toward the contact place, while the energy parcel, i. e., the mechanical signal, travels in the direction opposite to the LRP1 segment movement. This spreading of energy towards the tail leads to the transmission of the signal through the abluminal membrane and to the embracing of the tail. The consequence is the movement of two NPXY and two di-leucine motifs toward the abluminal cell membrane, with the spreading of space necessary for the approach and contact of the PICALM with the YXXL motif. The shifted PICALM activates the YXXL motif, with the activation of adaptor and scaffold proteins. The chemical binding of Aβ and LRP1 residues most probably happens between Lys16 on Aβ and three Asp on CR26, and His13 on Aβ and three Asp on CR27 (tripartite salt bridge). The pulling of two NPXY and two di-leucine motifs, due to the close contact with α-unit of the G protein-coupled receptor (GPCR), leads to the distension and separation of α-unit from the other two members of the receptor (β,γ) and to G-protein activation. Following is the activation of adenyl cyclase, the transformation of adenosine triphosphate (ATP) to the cyclic adenosine monophosphate (cAMP), and the activation of protein kinase A (PKA) and serine 76 (Ser76), on the receptor tail (**Fig. 4-7**)[1-4].

The Signal Travelling Through the Receptor Structure

It is necessary to explain some important moments about the signal which travels in the opposite direction, toward the receptor tail. If the signal (energy parcel) travele through peptide links only for four CR, it would have to pass through 160 peptide bonds. The passage of the signal through one β-propeller domain built of six-bladed β-propellers, each composed of four antiparallel β-strands, is also an extremely long way. Each of nine EGF-like domains is composed of 30-40 amino acid residues and six cysteine residues (three disulfide bonds), which all together also create great resistance to the signal propagation. The signal transmission through the total length of this route would surely markedly decline the signal strength up to complete loss of the signal. Obviously, this type of signal transmission is not possible. All this indicates that the signal is transmitted through the complete module structure, abundantly using side chains, and not exclusively using the central direction. The peptide bonds would have the transmission role only at the entrance and at the exit module[2,5-7].

Importance of the Tripartite Salt Bridge Formation

The salt bridge is in fact a combination of two non-covalent interactions, H-bonding and ionic bonding. In this situation it occurs between the carboxilate oxygen atom of three aspartates, and either of His13 imidazole ring two side chain nitrogens, Nδ or Nε. At the same time, the three aspartates, located on the neighbouring RC, surround the ε-amino group of the lysine 16 residue, also in the form of the tripartite salt bridge (hydrogen bond energy, 4-40 kJ/mol; ionic bond energy,170-1500 kJ/mol). It is important to emphasize that the distance between His13 and Lys16 on the Aβ β1-strand, in the case of binding, is about 20-21 Å, as is in the case of the RAPD3/CR34 complex[5-7,10-12].

Explanation of Coulomb's Law

As previously emphasized, Aβ approaches LRP1 by the influence of the electrostatic attractive force, explained by Coulomb's law: $F=k*Q1*Q2/r^2$, where F is the electric force, k is Coulomb's constant (its value is $8.9875*10^9$ in $N*m^2/C^2$ units), Q1 and Q2 are the charges on objects, and r is the distance between charges. Other members of the equation: F, electric force in newtons (N); C, coulomb; m, distance r in meters. The equation clearly shows that the closer two two charges are, the stronger is the force between them. So, by the increased mutual approaching, F rises. The pulling of the two maximally exposed CRs (ligand binding cluster IV, CR26 and CR27), progressively rises up to the occurence of tripartite salt bridges. The critical distance for the occurence of these bridges is ≈ 2.7-3.3Å, with 3.0Å being the most common value for protein. The force F has maximal value just before the occurence of the bridges. In this moment, parallelly to the pulling of both CRs, evolves the strong reversed energy (signal) propagation to the receptor tail [2,11,13].

Conformational Effects on LRP1 After Aβ Binding to Its Structure

Camilla De Nardis, et al.[4], emphasize that the overall conformation of LRP1 remains largely unchanged after RAP binding, as shown by the similarity of the small-single X-ray scattering curves (SAXS) and by the negative-stain electron microscopy (EM) class averages. Conformational changes upon RAP binding might happen at a local level, perhaps influencing the relations of the neighbouring domains (i.e. Cysteine-rich repeats) without generating large structural rearrangements visible at low resolution. The authors emphasize the presence of 515 kDa extracellular right handed α-chain containing the four ligand binding regions. The α-chain is non-covalently associated with the β-chain (85 kDa) composed of the trans-membrane region and the small intracellular domain.

Gonias, et al.[14], also emphasize the α-helical shape of the extracellular N-terminal chain.
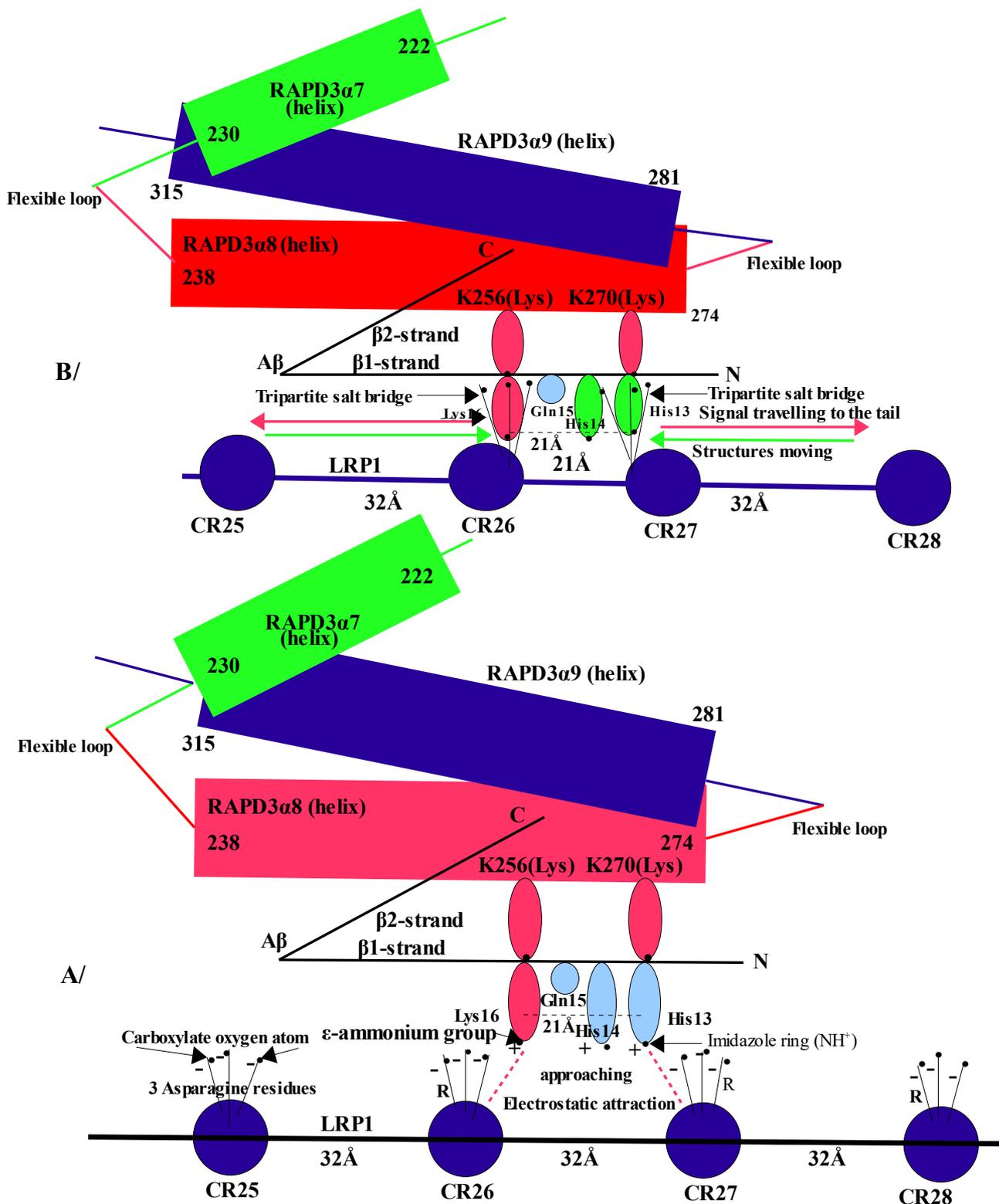
Fig.5. Schematic presentation of the LRP1 and RAP protein molecule

A/ Electrostatic attraction between LRP1 and amyloid beta (Aβ) protein; for better explanation the role of receptor associated protein (RAP) it is inserted in the figure close to the Aβ; B/ It is presented the interaction and binding of Aβ with LRP1 receptor; CR, cysteine-rich repeat

Aβ approach and binding with C II and C IV ligand binding domain; attraction induced by electrostatic forces;

↓

Creation of the electrostatic equlibrium connected with the reduction (32 Å to 21 Å) of the distance between two crucial CR (Cysteine rich repeats, CR26 and CR27), points of binding LRP1 with His13 and Lys16 residues on the Aβ β1-strand;

↓

Reducing of the distance between CR26 and CR27 induces the consequent pulling of the molecular complexes on both sides of the contact points, pulling shift value on each side is 5.5 Å approximately;

↓

Mechanical force in the form of pulling propagates through the LRP1 biomolecular complex in the direction toward the transmembrane domain and receptor tail, inducing the consequent moving of receptor structures in the opposite direction related to the point of initial contact;

↓

The final result is the movement of functional tail motifs (two NPXY and two di-leucine motifs) toward the abluminal cell membrane, with the enlarging of the space necessary for the PICALM free passage toward the YXXL motif and contact/binding with it.

Fig.6. **The first stage of LRP1/ clathrin coated vesicles endocytosis and transcytosis**

Amyloid beta (Aβ; LRP1 ligand binding domains; electrostatic forces; cysteine rich repeats; mechanical force in the form of pulling; transmembrane domain; the movement of functional tail motifs; abluminal cell membrane; two NPXY motifs; two di-leucine motifs; YXXL motif; clathrin coated vesicles; endocytosis; transcytosis; exocytosis.

Pulling of all segments of the LRP1 receptor, i.e. , extracellular part, transmembrane part, and intracellular tail, in the direction toward the contact place;

↓

This pulling and consequent moving of the included receptor segments induce the enlarging of the space necessary for the PICALM free shift toward the YXXL motif and consequently, their mutual contact/binding;

↓

The binding occurs probably between tyrosine, histidine, and lysine on the YXXL side (Y=tyrosine, L=leucine, all other amino acids, probably histidine-X and lysine-X), and aspartic and glutamic acid residues on the PICALM side;

↓

This binding is an exothermic chemical reaction, i. e., during its course occurs the energy discharge in the form of heat. So, the bounded units, probably with the H-bonds, make a compound with the lower energy content, different from the sum of both isolated units. This chemical law is energetically favorable. Molecular theory of the heat explains that the heat is in fact the result of molecular moving or vibration;

↓

The discharged energy, by the direct contact/collision, or with the help of the local water molecules (transporters of energy), elevates the energy of neighbouring adaptor molecules, inducing their chemical activity. The result is LRP1, PICALM/ clathrin-dependent endocytosis, transcytosis and exocytosis.

Fig.7. **Crucial energetic events that precede YXXL motif activation**

Pulling, mechanical force; LRP1, low-density lipoprotein receptor-related protein 1, LRP receptor family; intracellular LRP1 tail; PICALM, protein; YXXL motif; binding between PICALM and YXXL motif; exothermic chemical reaction, energy discharge in the form of heat; molecular theory of the heat; LRP1,PICALM/ clathrin-dependent endocxtosis, transcytosis and exocytosis

Actually, there are no data about the exact nature of the mentioned conformational effect. However, it is evident that this conformational effect has induced the occurence of tension forces in the receptor backbone structure, which propagate along its molecular complex in both directions, especially important for reactions in the LRP1 tail[3,7,14].

Mechanical Force Propagation Through Protein

Young, HT et al.[15], in their study emphasize that mechanical forces are the major factors among regulators of biological functions even at the microscopic level, such as proteins. These forces are connected with protein conformational changes, which on the level of ligand-binding repeats, are the source of bidirectional signal propagation, especially in the form of molecular movements.

By experiments, simulations and theory, a number of researchers have obtained the fundamental characteristics of the dynamic response of a protein complex molecular system to mechanical forces, among them pulling and stretching. In their experiments they have especially explored a mechanically ultrastable multi-domain cellulosome protein complex. They emphasize the great importance of atomistic molecular dynamics (MD) simulations, which is necessary for the understanding of protein dynamics[15-20]. The above described distance shortening (32Å to 21Å) between neighbouring CR repeats evidently induces the movement of molecules on both sides of the contact place (CR2627/His13-Lys16; 5.5Å on both sides), which is manifested on the abluminal cell membrane side, with the consequent pulling of the LRP1 transmembrane portion, as well as of the tail region. It is evident that, the until now blocked PICALM, achieves a lot of free space to come in contact with the YXXL motif, and therefore induces the adaptor protein complex (AP-2) and scaffold recruitment, and enables PICALM/clathrin-coated transcytosis. Obviously, there are no other convincing mechanisms for the explanation of these events. It can be supposed that during the pulling energy transmission from the point of its origin to the tail, there is no evident loss of energy (**Fig. 4,5**)[1,3,20-26].

Schoeler, et al.[17], by the employement of single-molecular spectroscopy with an atomic force microscope (AFM) and steered molecular dynamics (SMD) symulations, have revealed force propagation pathways along a mechanically ultrastable multi-domain cellulosome protein complex. Cellulosomes are multi-enzyme extracellular complexes, associated with the cell surface and they mediate the cell attachment to insoluble substrates. They emphasize that mechanical forces have a crucial role in biological systems. Mechanically active proteins can respond to these forces by conformational changes. The AFM and single-molecule force spectroscopy (SMFS) directly measure the molecular mechanical properties. The mentioned methods can exactly define the pulling geometry of these molecular events.

LRP1 Phosphorylation by Protein Kinase A (PKA)

Yonghe Li, et al.[27], point out that the serine 76 (Ser76) on the end of the receptor tail is the major phosphorylation site within the LRP1 tail. LRP1 phosphorylation, according to experiments, is predominantly mediated by the cAMP-dependent protein kinase A (PKA, a family of enzymes whose activity is dependent on cellular levels of cyclic AMP, cAMP; important for glycogen, sugar, and lipid metabolism) on the serine 76 on the tail. The authors emphasize that the binding of Aβ on the receptor (LRP1) induces the dissociation of the stimulatory heterotrimeric G-protein subunit (Gsa) with the consequent activation of adenyl cyclase (the enzyme that generates the second messenger 3':5'-adenosine monophospate, cyclic AMP, out of ATP) and induces a rise in intracellular cAMP. The final result is enhanced PKA activity and LRP1 phosphorylation (**Fig. 3,4**).

Some researchers emphasize that ligand binding to the LRP1 domain II and IV induces conformational changes on its cytoplasmic tail characterized by the YXXL motif activation and the pulling of two NPXY and two dl-leucine motifs in the directions to the mentioned motifs. These changes are accompanied by the dissociation of heterotrimeric G-protein Gs alpha subunit (one of three heterotrimeric G-protein subunits), which promptly activates enzyme adenyl cyclase (transmembrane protein), and consequent conversion of ATP to cAMP. The consequence is the rise in intracellular cAMP concentration. PKA activation and phosphorylation of the LRP1 tail on Ser76 elevates the interaction between the tail and adaptor proteins such as Dab-1 (**Fig. 3,4**)[28,29].

The pulling of the LRP1 tail enlarges the PICALM passage to the YXXL motif. This change enables the PICALM approach to the YXXL motif and its activation. Activated YXXL motif immediately activates PKA. The mentioned event at the same time induces the breaking of the connection of the Gα subunit from the transmembrane GPCR, and its dissociation and activation. This activation induces the activation of adenyl cyclase, the transition of ATP in cAMP, and PKA activation (**Fig. 3,4**)[29].

LRP1 Ser76 by PKA phosphorylation is crucial for adaptor protein activation and consequent Aβ endothelial transcytosis and clearance[30-34].

Presently, the understanding of the LRP1 role in the brain physiology and AD pathogenesis is of vital importance in the prevention and treatment of this disease. There are a number of actual experiments in animal models and preclinical trials in which researchers make efforts to find optimal pharmaceutical and nonpharmaceutical methods to module LRP1 functions, and improve its effectivenes. Experiments with animals indicate that the treatment with fluvastatin (hydroxymethyl glutaryl-CoA reductase inhibitor) increases LRP1 in mouse cerebral vessels, with the consequent reduction of Aβ levels in the brain. Rifampicin

and caffeine upregulate LRP1 levels at the BBB and enhance Aβ drainage from the mouse brain. In the peripheral tissues, insulin facilitates the hepatic clearance of plasma Aβ by increasing the cell surface LRP1 distribution in hepatocytes. However, this study does not have the tendency to enter such an extensive field of investigations[35-40].

It emphasizes the original model that implies the relation between the Aβ/LRP1 binding and LRP1 conformational changes on the LRP1 tail, and clathrin dependent Aβ endocytosis, transcytosis, and exocytosis. It is necessary that appropriate experiments and studies confirm or reject this concept. Regardless of the concept evaluation, this study has certainly contributed to the better understanding of the Alzheimer's disease pathophysiology, and presents a valuable attempt in the solving of this contemporary society serious problem.

Conclusion

Alzheimer's disease is a severe, chronic, and lethal neurodegenerative disease. It is closely associated with old age and aging. Due to the relative and absolute growth of the worldwide old population (i. e. senectual explosion) there exists the alarming aspect of its incidence and prevalence (data from Croatia: the prevalence of all types of dementia-approximately 86000, 2010;). The exact explanation of its complex polygenetic etiology and effective therapy has not yet been obtained. However, a number of researchers emphasize the crucial importance of intracerebral amyloid beta (Aβ) accumulation and aggregation, as well as its damaged drainage from the brain. A lot of researchers emphasize the important role of lipoprotein receptor-related protein 1 (LRP1) in these pathological events. It is evident that Aβ binding to the LRP1 ligand-binding cluster II and IV is the trigger for conformational changes in the receptor's extracellular heavy α-chain which induce the activation of its tail structures and the present molecules. The consequent result is the PICALM/clathrin-dependent Aβ endocytosis, transcytosis, and exocytosis into the capillary blood circulation and to the points of Aβ degradation and elimination (skin, spleen, and kidneys). The important actual problem is how the binding of Aβ to LRP1 induces conformational changes in the receptor tail and the consequent beginning of the drainage. Experiments and theory emphasize that in these events, mechanical forces, especially pulling forces, have the crucial role in the energy transmission through the protein structure, and in the activation of the tail complex essential for Aβ drainage.

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