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Review article

Conformational changes in the LRP1 receptor related to the amyloid beta binding on the receptor's II and IV ligand binding domains

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Abstract

Related to the great importance of the intra-arterial perivascular drainage (IPAD), the drainage function of the lipoprotein receptor-related protein-1 (LRP1) that is a huge transmembrane LRP receptor member, is increasingly becoming the subject of comprehensive researches. Two important questions thus emerged: 1. How does the binding of the whole ligand complex members, among them amyloid beta (Aβ), the crucial factor in the pathology and pathophysiology of Alzheimer's disease (AD), lead to conformational changes in the extracellular receptor structure? 2. How do these changes reflect in the structure of the receptor intracellular tail, with the binding of PICALM (phosphatidylinositol binding clathrin assembly protein) to the YXXL tail motif, and how do they reflect in the recruitment of a number of signal proteins, and in the onset of PICALM/clathrin Aβ endocytosis, transcytosis and Aβ exocytosis into the capillary circulation? Both questions require an exact explanation. The profound insight into the available literature related to these events does not give a satisfactory explanation to these questions; consequently, the present knowledge of the AD pathophysiology requires additional comprehensive research. This study is not concerned with the details of the AD pathology and pathophysiology; it is primarily concerned with the problem of Aβ binding to the LRP1, with local conformational changes in the tail, and with the activators of Aβ transcytosis and Aβ exocytosis into the capillary blood.

***KEY WORDS*:** lipoprotein receptor-related protein1 (LRP1), intra-arterial perivascular drainage (IPAD), Alzheimer's disease (AD), PICALM, YXXL motif, endocytosis, transcytosis, exocytosis

Introduction

The available literature related to the amyloid beta (Aβ) drainage from the brain often emphasizes the conformational changes on the lipoprotein receptor-related protein-1 (LRP1) tail after Aβ binding to the receptor. Changes on the tail are obviously the result of induced events on the extracellular N-terminal chain. It can be supposed that both, -COOH and -NH2 ends of LRP1, are fixed to the surrounding structures in the cell (tail elements) and to the elements of lamina reticularis (extracellular matrix, ECM). Aβ binding to the LRP1 ligand binding domain, conformational changes on LRP1 extracellular heavy α-chain (ectodomain) of the receptor (induced by the aligning of the electrostatic forces of both participants) lead to mild LRP1 contraction. This contraction with the fixed LRP1 ends (ECM and tail elements), probably induces the discrete pulling of the distal di-leucine towards the receptor extracellular part, with the widening of the accessed space to the PICALM (phosphatidylinositol binding clathrin assembly protein) for the contact with the YXXL motif. This space in which PICALM is trapped, is bordered by the fixed -COOH on one side and the less fixed di-leucine on the other side. Essentially, the LRP1 activation results in the zigzag wrinkling of its structure with the accompanying mild shortening. The result is the LRP1-PICALM/clathrin-dependent endocytosis of Aβ-LRP1 complex (***Fig. 1***) [1-6].

Daly NL *et al.* [7], by NMR (nuclear magnetic resonance) spectroscopy, solved the problem of the structure of the first LDL receptor module (CR1, cysteine-rich1). This module is composed of a β-hairpin structure followed by a series of β-turns. In its structure are clearly observable 6 disulfide-bound cysteines and a cluster of negatively charged amino acids (Asp-15, Asp-26, Glu-30, Asp-33, Asp-36, Glu-37, and Glu-40), primarily clustered on one face of the module. Between the fourth and sixth cysteine, there was found the region for high-affinity binding of positively charged sequences in LDLR's ligands. The CR module also contains about 40 amino acids and a coordinated calcium ion important for the structure stability (***Fig. 1***).

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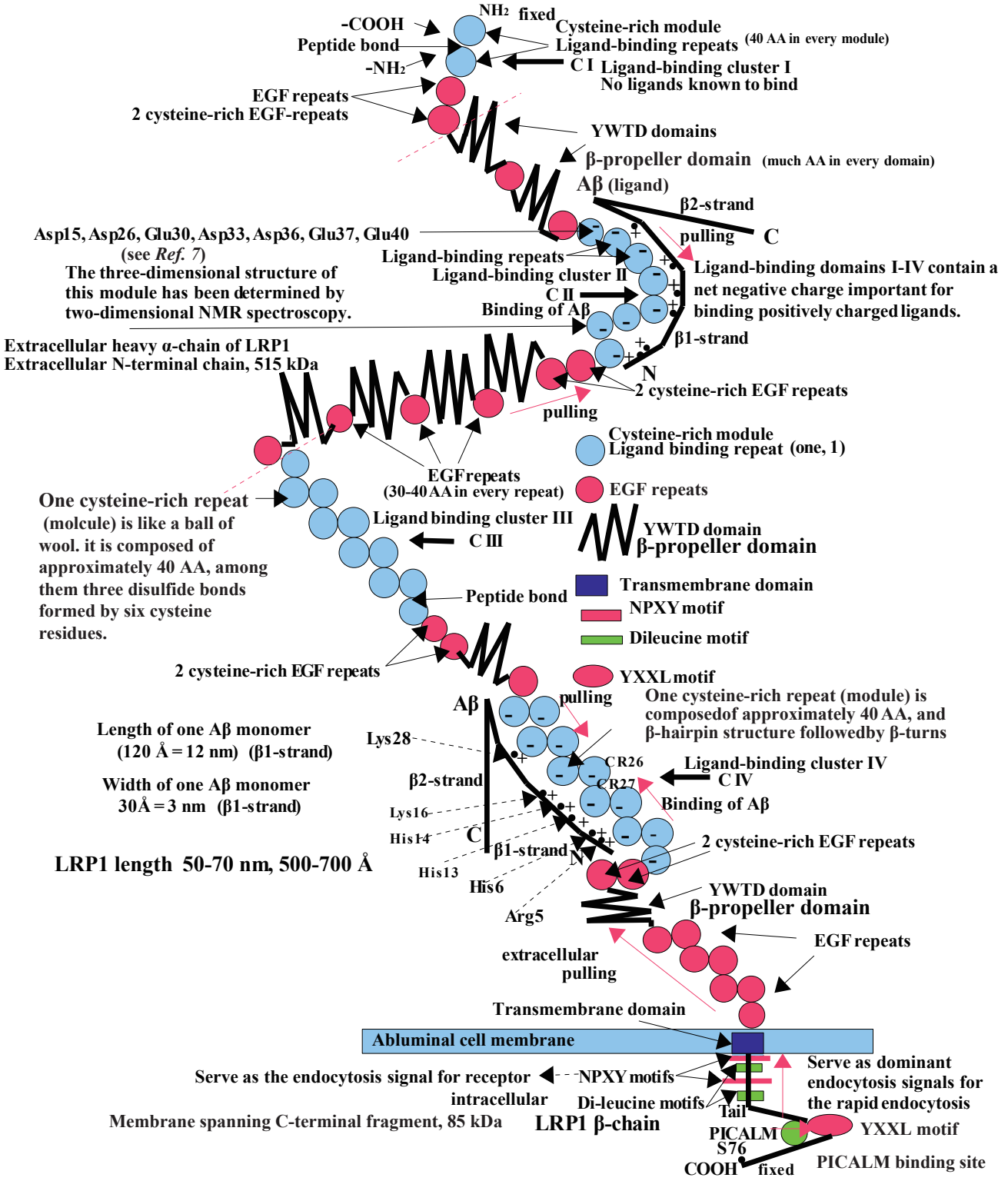

Fig. 1. **Presentation of LRP1 (CD91) receptor molecule.**

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 m motifs, two dileucine motifs, and one YXXL motif; every ligand binding domain consists of a β-hairpin structure followed by a series of β-turns. LRP1, lipoprotein receptor-related protein-1; EGF, epidermal growth factor; Aβ, amyloid beta; AA, amino acids; NMR, nuclear magnetic resonance; PICALM, phosphatidylinositol binding clathrin assembly protein.

RAP role in LRP1 biosynthesis

Before further analysis of the above mentioned events, it is important to explain the structure and function of the receptor-associated protein (RAP), the LRP receptor chaperone (protein that assists the conformational changes of other macromolecules). Its artificially synthethysed form is the crucial factor in all experiments related to the ligand binding to LRP receptor. On account of this, it is necessary to primarily emphasize some facts about the LRP1 generation. *LRP1* gene is located on the long arm of chromosome 12 (long arm chr. 12Q13.3). Biosynthesis of the LRP1 protein (location ER, endoplasmic reticulum) proceeds by the 39 kDa receptor-associated protein (*RAP* gene, short arm, 4p16.3 gene), normally the resident in the endoplasmic reticulum (ER). Naturally, RAP is a chaperone which is the key factor for the conformational folding and unfolding, as well as assembly and disassembly of many macromolecules, among them LRP1. Its crucial role is to prevent the binding of superfluous ligands on the LRP1 during its formation, and to prevent the aggregation of formed structure in the nonfunctional shape. RAP, which is bound with LRP1, after entering into Golgi apparatus dissociates from LRP1 by effects of low pH. After this, RAP returns back to the ER and continues its function as a blocker of the superfluous ligands and aggregation. A dissociated LRP1 exits from Golgi, enters the transport vesicle, and continues its way to the cell membrane, primarily on the abluminal side. On account of its molecular structure and affinity for binding to LRP1, RAP artificial synthetic form is optimal for the mentioned experiments (***Fig. 2***) [7, 8-10].

The importance of the CR34 distance shortening in the structure of LRP1

Prasad JM *et al.* [8] in their study present experimental results related to the distance between LRP1 CR2 and CR7 in the endoplasmic reticulum (ER), which ranges from 20-43 Å. The distance between CR3 and CR4, measured in conditions of the neutral endosomal, is 32 Å, which is quite different from the distance of 21 Å between the same modules in the RAPD3/CR34 structure. The difference between these two conditions indicates obvious narrowing of the CR34 distance after the adequate electrostatic attraction and binding (H-bonds, salt bridge, isopeptide bonds) Hys13, Hys14, Gin15, Lys16 (positive charge cluster - *e.g.*, on the Aβ monomer) and Asp residue (on CR34 LRP1 receptor). It is evident that the decrease of this distance has a direct effect on the pulling of the extracellular LRP1 part, on the structural change on the tail, and on the widening of the passage for PICALM towards the YXXL motif. All this indicates the marked CR repeat flexibility in the LRP family.

Generally, the study presents the probable events after Aβ binding to LRP1, which, however, require a detailed and critical examination. The electrostatic forces that attract Aβ and LRP1 residues induce a certain movement (about 5.5 Å) on both sides of the contact region (interaction). These movements induce mutual successive pulling of the following protein segments toward the contact place (onset of a certain chemical bond), while the energy packet, or the signal, travels in the direction opposite to the LRP1 segment movement. It is supposed that the energy released by the chemical reaction (between Aβ and LRP1) with the probable smaller resistance of LRP1 structure to this spreading of energy towards the tail, leads to the transmision of the signal (packet of energy) through the membrane and to the embracing the tail (enclosing). The result is the movement of two NPXY and two di-leucine motifs toward the abluminal cell membrane, with the spreading of space necessary for the contact of PICALM with YXXL motif. The -COOH part of the receptor is most probably firmly fixed to the neighboring structures, so that the intracellular part distends and enables the above mentioned widening. The shifted PICALM activates the YXXL motif, with the activation of the adaptor proteins, which leads to the PICALM/clathrin dependent endocytosis, transcytosis, and exocytosis. The analysis of the ligand binding cluster IV (C IV) contributes to the understanding of these events. 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 (tripatite salt bridge). With the beginning of the electrostatic balance between the involved residues, the earlier distance CR2627 with 31 Å decreases to 20 Å. This induces the above mentioned mutual shift of 5.5 Å. The pulling of two NPXY and two di-leucine motifs, due to the close contact with α-unit of the G protein-coupled receptor, leads to the distension and separation of α-unit from the other two members of the receptor (β, γ) and its activation. Following is the activation of adenyl cyclase, transformation of adenosine triphosphate (ATP) to the cyclic adenosine monophosphate (cAMP), and the activation of protein kinase A (PKA) and serine 76 (Ser 76) on the receptor tail (***Fig. 3, 4, 5***) [1-3].

The analysis of Aβ and LRP1 binding indicates the most probable participation of CR26 and CR27 that with His13 and Lys16 contact with three Asp residues from each CR. The retrograde pulling signal (energy packet) that travels to the tail, must pass through four CR modules, one YWTD motif, and nine EGF (epidermal growth factor) repeats. If the signal travelled 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 a 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. This type of signal transmission obviously is not possible. All this indicates that the signal is transmitted through the complete module structure, abundantly using side chains, and not exclusively the central direction. The peptide bonds would have the transmission role only at the entrance and at the exit module point [8-10].

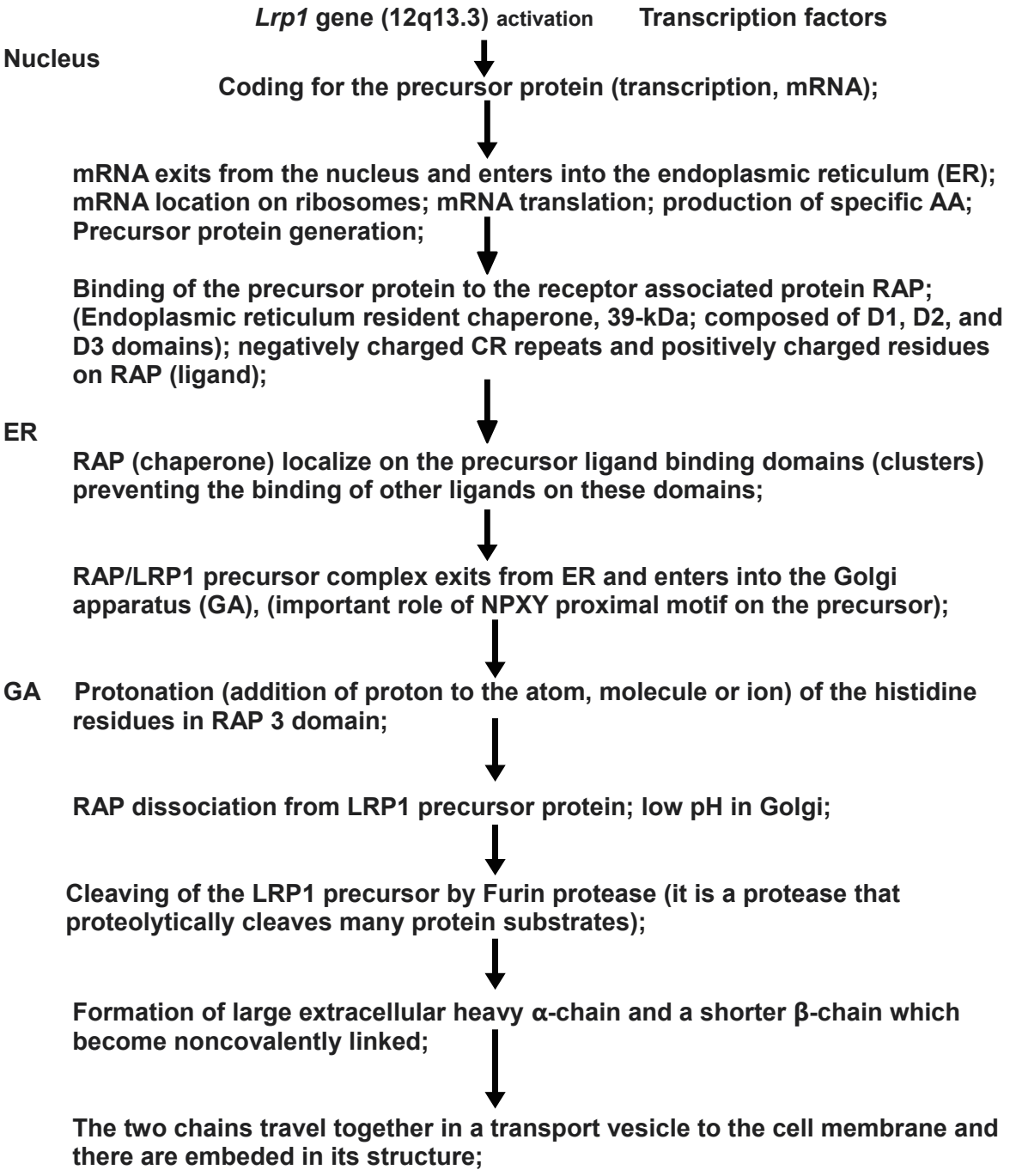

Fig. 2. **Coding for the LRP1 precursor protein and its transformation into the mature LRP1.**

According to a number of recent investigations, transcription of the Lrp1 gene can be activated, among the number of different transcription factors, particularly by sterol regulatory element binding protein 2, hypoxia-induced factor 1, and nitric oxide-dependent transcription factors; *Lrp1* gene activation, coding for the LRP1 precursor protein, and mRNA generation occurs in the cell nucleus. Transcribed mRNA exits from the nucleus and immediately enters into ER and contacts with ribosomes. Now, on the ribosome occurs the translation during which the mRNA is decoded with the production of a specific amino acid (AA) chain, or polypeptide. LRP1, lipoprotein receptor-related protein-1; Rap, Ras proximate; RAP protein, chaperone; protonation, RAP dissociation; CR, cysteine-rich repeat on LRP1; ER, endoplasmic reticulum.

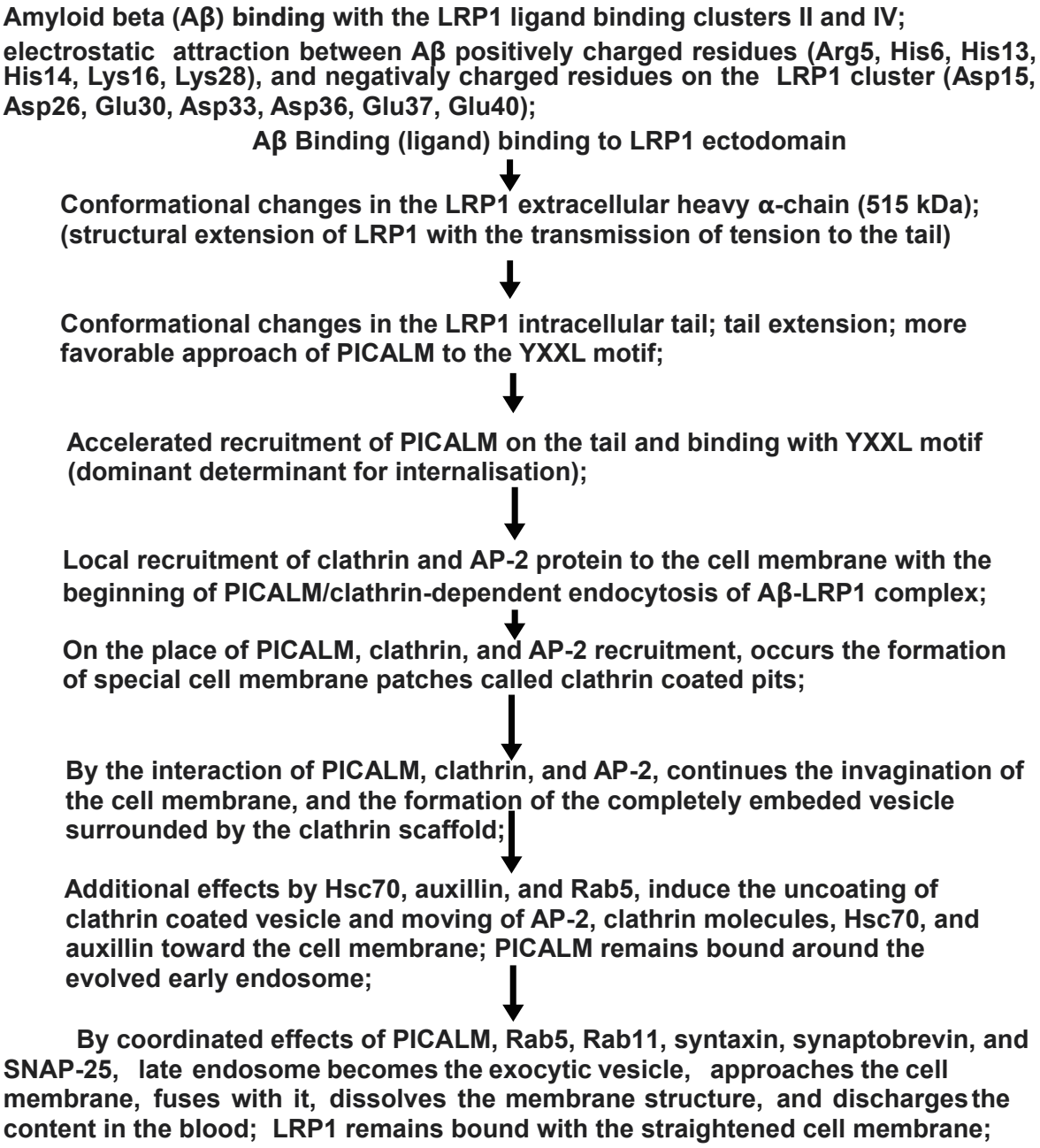

Fig. 3. **Events typical for LRP1/clathrin coated vesicles endocytosis and transcytosis.**

Aβ binding to the LRP1 cluster II and IV induces conformational changes into the LRP1 extracellular and intracellular segments. Changes in the intracellular segment induce the receptor slight elongation and concomitant pulling forces which propagate through its structure, pass across the cell abluminal membrane, and enter into the receptor's tail segment. Now, it follows the structural change of the tail's structure, which results in PICALM/ clathrin induced Aβ endocytosis and transcytosis. The crucial importance in these events has the protein PICALM, whose binding with YXXL motif is the first step in all events. LRP1, lipoprotein receptor-related protein-1; Aβ, amyloid beta; PICALM, phosphatidylinositol binding clathrin assembly protein; AP-2, adaptor protein 2; HSC70, 71 kDa heat shock cognate protein; Rab5, Ras analog in brain 5; SNAP25, synaptosome associated protein 25.

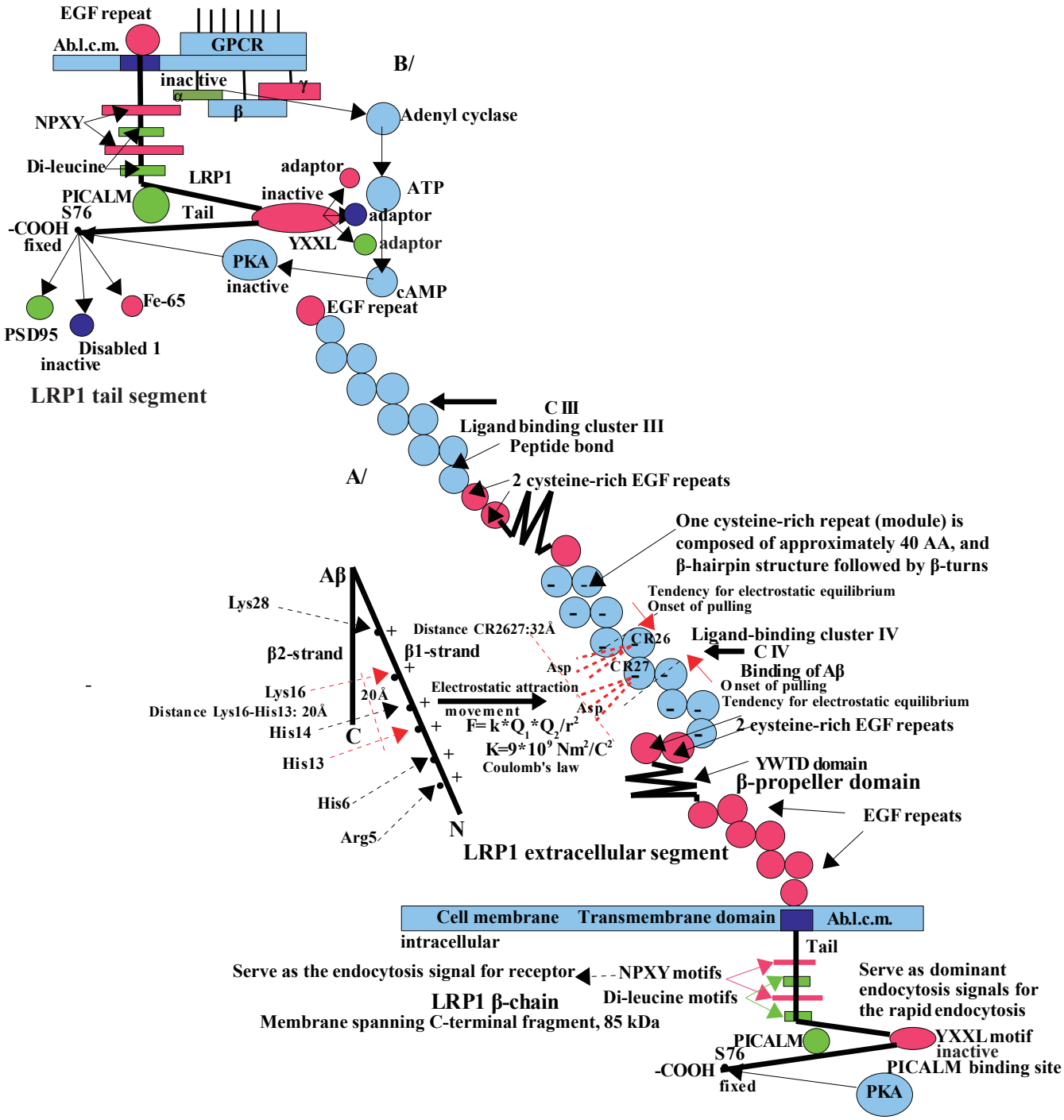

Fig. 4. **Partial presentation of LRP1 receptor without complete activation.**

A and B: The difference compared to **Fig. 5** is clearly visible; 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; PKA is inactive; there is no sign of S76 phosphorylation; Ab.l.c.m., abluminal cell membrane; adaptor proteins, Disabled1, Fe-65, PSD95; NPXY motifs, Di-leucine motifs, LRP1 tail; **B**/Aβ positive residues, Lys28, Lys16, His14, His13, His6, membrane spanning C-terminal fragment, 85 kDa; EGF repeats; YWTD domains, Tyr-Trp-Thr-Asp (YWTD)-repeat; LRP1, lipoprotein receptor-related protein-1; EGF, epidermal growth factor; Aβ, amyloid beta; AA, amino acids; PICALM, phosphatidylinositol binding clathrin assembly protein; PKA, protein kinase A; YXXL, Tyr-x-x-Lue; NPXY, Asn-Pro-x-Tyr, a sequence often found in cytoplasmic tails; PSD95, postsynaptic density protein 95; GPCR, G protein-coupled receptor; CR, cysteine-rich repeat on LRP1.

Importance of the tripartite salt bridges formation

In the case of Aβ and LRP1 binding, the appearance of electrostatic equlibrium between their opposite charges creates the tripartite salt bridge (combination of two non-covalent interactions: H-bonding and ionic bonding) 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 neighboring RC) surround the ε-amino group of the lysine16 residue, also in the form of the tripartite salt bridge (the average value of covalent bond energy, 415 kJ/mol; hydrogen bond energy, 4-40 kJ/mol; ionic bond energy, 170-1,500 kJ/mol). As in the presented experiments with RAP in the study of Prasad JM et al.[8], the binding is linked with the narrowing of the distance between two included neighboring CRs and the occurence of pulling forces *i.e.*, pulling signals. It is important to emphasize that the distance between His13 and Lys16 on the Aβ-strand, in the case of binding, is about 20-21 Å, as is in the case of RAPD3/CR34 complex[9,10].

Aβ approaches LRP1 by the influence of the electrostatic attractive force. This force is explained by the Coulomb's law: $F = K*Q1*Q2/r^2$, where F is the electric force, k is the Coulomb's constant ($k=8.9875*10^9$ $N*m^2/C^2$), Q1 and Q2 are the charges on objects, and r is the distance between the charges. Other members of the equation: F, electric force in newtons (N); C, coulomb; m, distance r in meters (m). The equation clearly shows that the closer 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, ***Fig. 4, 5***) progressively rises up to the occurrence of tripartite salt bridges. The critical distance for the occurrence of these bridges is ≈2.7-3.3 Å, with 3.0 Å being the most common value for protein. The force F has the maximal value just before the occurence of the bridges. In this moment, parallel to the pulling of both CRs, evolves the strong reversed energy (signal) propagation to the receptor tail (***Fig. 4, 5***)[11].

Fisher C *et al.*[9] present the 1.26 Å X-ray structure of the complex composed of two module regions of the LDLR ligand binding domain (D), and the third RAP domain (escort protein for LDLR family members). RAP has three domains: D1, D2, and D3. The RAPD3 (positively charged) domain has three-helix bundles (α7, α8, α9), and two docking sites, one for each LDLR module. Three calcium-coordinated Asp residues (negatively charged) from each LDLR module encircle a lysine side chain (positively charged) protruding from the RAPD3 α8 helix. The first explored LDLR module region CR3, with its aspartates, surrounds Lys 270 (K270), and module region CR4 also with its aspartates surrounds Lys256 (K256). In this way, the three Asp at each contact position surround the ε-amino group (NH^{3+} attached to the fourth C atom from the α-carbon) of the lysine side chain $(CH2)_4NH_2$. LDLR aromatic residues, F105 (Phe-105, Phenylalanine, essential aa) of CR3 and W144 (Trp-144, Triptofan, essential aa) of CR4, complete the pocket surrounding. The surrounding of the ε-amino groups is in fact a tripartite salt bridge. The authors mark the LRP module as LA module. There are electrostatic interactions between complementary charged surfaces at each docking site. The authors emphasize that Aβ peptide corresponds to RAPD3. Strongly positive RAPD3 potential is concentrated around K256 (Lysine) and K270 (Lysine). These two docking sites are separated by four helical turns. The positively charged side chains of RAP K253 and R285 correspond to Aβ peptide His13, His14 and Lys16.

Lillis AP *et al.*[10] in their research strongly confirm the results of Fisher C *et al.*[9]. They present in detail the RAPD3 structure in the complex with two CRs from the LRP receptor, and emphasize the importance of further analysis of ligand interaction with LRP1 receptor. They also confirm that the YWTD repeat (β-propeller domain) forms a six-bladed β-propeller that packs tightly against C-terminal EGF module. This observation leads to the conclusion that the β-propeller domain functions as an alternate ligand for CR4 and CR5, which bind in a calcium-dependent manner promoting ligand release.

The analysis of the distance between the middle of residue His13 and the middle of residue Lys16 shows the value of ~20 Å. This strikingly corresponds to the distance between CR3 and CR4 in the structure RAPD3/CR34, which is 21 Å. The distance between Lys16 and Lys28 is significantly larger than 21 Å. This indicates the purposeful adaptation of LRP1 extracellular N-terminal chain (515 kDa), *i.e.*, heavy α-chain, to electropositive residues of Aβ monomer. The resulting link is in the form of a tripartite salt bridge.

De Nardis C *et al.*[2] emphasize that the overall conformation of LRP1 remains largely unchanged after RAP binding, as shown by the similarity of the SAXS curves and by the negative-stain EM class averages. Conformational transitions upon RAP binding might happen at a local level, perhaps influencing the relations of the neighboring domains (*e.g.*, Cysteine-rich repeats) without producing large structural rearrangements visible at low resolution, as supposed by Migliorini MM *et al.*[12]. According to the authors, the low-resolution electron spectroscopic microscopy images of full-length LRP1 purified from human placenta, suggested an elongated "zigzaged" shape ranging 50-70 nm in length. Detailed structural studies of the full LRP1 molecule have been hindered by the difficulties in generating LRP1. The authors frequently emphasize the presence of 515 kDa extracellular right handed α-chain containing the ligand binding regions. It is non-covalently associated with the β-chain (85 kDa) composed of the trans-membrane region and the small intracellular domain (***Fig. 1***).

Gonias SL *et al.*[13] present the molecular models of the organization of structural domains in LRP1. They also emphasize the helical shape of the extracellular N-terminal chain (515 kDa).

As it was mentioned in the introduction, the binding of Aβ to the LRP1 ligand binding domains (especially II and IV) induces a certain conformational effect on the LRP1 cytoplasmic C-terminus with subsequent expressive binding of PICALM on the YXXL motif, and induction of the PICALM/clathrin-dependent endocytosis of the Aβ-LRP1 complex. There are no data about the exact

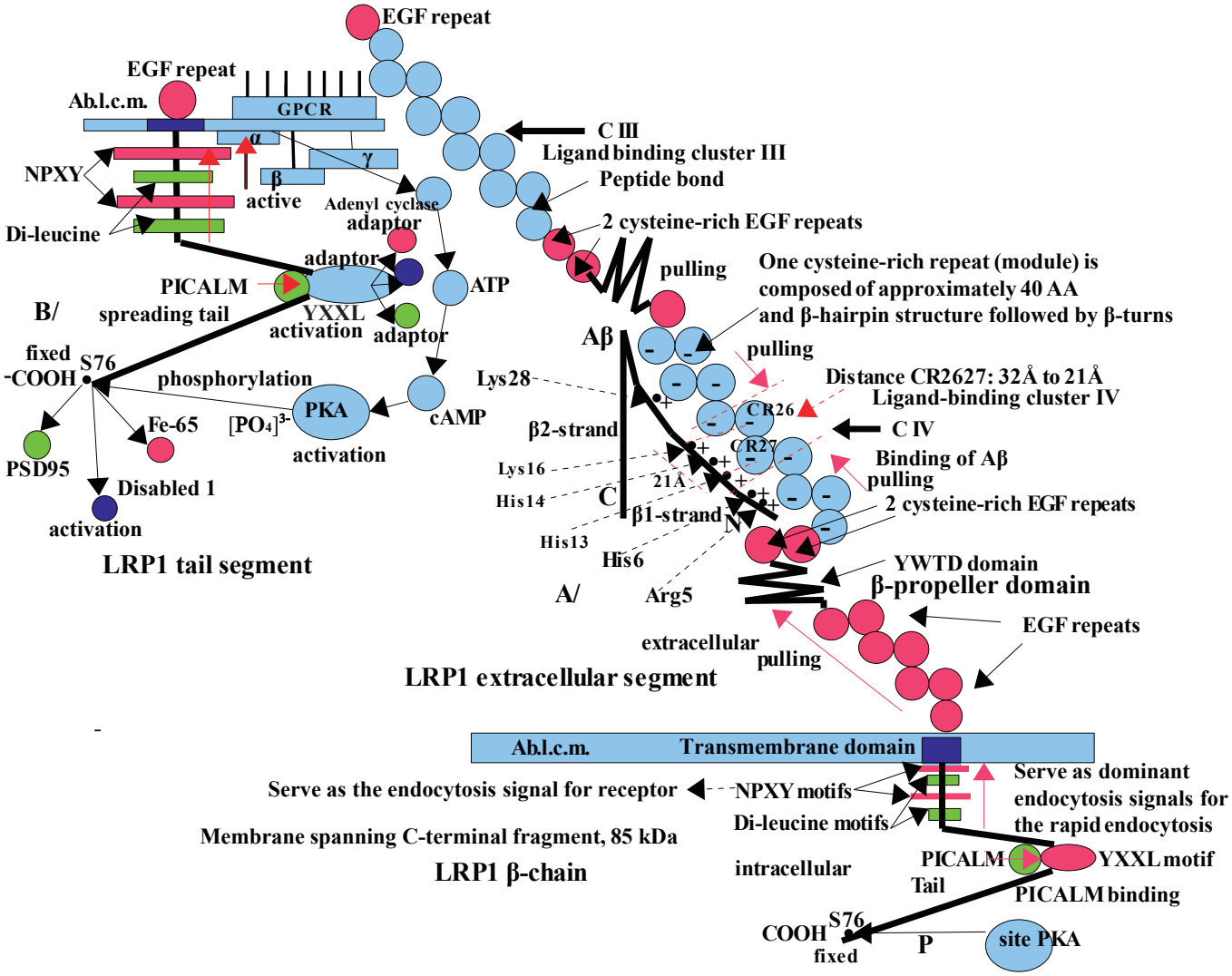

Fig. 5. **Partial presentation of LRP1 (CD91) receptor molecule (CIII and C IV).**

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 two NPXY motifs, two d-ileucine motifs, and one 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 (12 nm); width of one Aβ monomer is ~30 Å (3 nm); LRP1 length ~50-70 nm (500-700 Å); S76, serine residue 76 on LRP1 tail; phosphorylation, attachment of the phosphoryl group to the molecule; phosphate ion, [PO4]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. Tyr-Trp-Thr-Asp (YWTD)-repeat; LRP1, lipoprotein receptor-related protein-1; EGF, epidermal growth factor; Aβ, amyloid beta; PICALM, phosphatidylinositol binding clathrin assembly protein; AA, amino acids; PKA, protein-kinase A; NPXY, Asn-Pro-x-Tyr; YXX, Tyr-x-x-Lue; PSD95, postsynaptic density protein 95.

nature of this conformational effect. It is evident that the approach of positively charged Aβ to the negatively charged ligand-binding LRP1 repeats, their mutual contact, and the formation of tripartite salt bridges, is all the result of electrostatic interactions. As mentioned earlier, the optimal electrostatic connection is possible by the changes in the LRP1 backbone structure, which include its total shortening (32Å to21 Å, distance between CR3 and CR4). It is evident that this conformational change has induced the occurrence of tension forces in the backbone structure, which propagate along its molecular complex in both directions, especially important for reactions in the LRP1 tail. Here it is also important to emphasize that hydrogen bond energy varies between 1-40 kcal/mole, (4.184-167.54 kJ/mole), covalent bond energy is 415 kJ (99.2 KCAL; 111 kcal/mole, 464 kJ/mole), and ionic bond 700-4,000 kJ (167.30-956.02 kcal/mole). (Energy in kilojouls is equal to the kilocalories multiplied by 4.184) [1, 8, 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.

Like Young HT *et al.*[15], a number of other researchers have presented the results of their investigations of the mechanical force propagation through biomolecular complexes. By experiments, simulations and theory, they have obtained the fundamental characteristics of the dynamic response of a protein complex molecular system to mechanical forces, among them pulling and stretching. All the researchers emphasize the great importance of steered molecular dynamics (SMD) simulations for revealing force propagation pathways through a mechanically ultrastable multi-domain cellulosome protein complex. They also add the great importance to the atomistic molecular dynamics (MD) simulations which is necessary for the understanding of protein dynamics[15-20].

The mentioned distance shortening between neighboring CR repeats practically induces the movement of molecules on both sides of the CR2627 (5.5 Å on both sides), which is manifested on the abluminal cell membrane side, with the evident pulling of LRP1 transmembrane portion and also of the tail region (5.5 Å). Based the assumption that the receptor COOH region is tightly fixed to the local tissue structures (ECM), and YXXL motif is practically only connected with the distal dileucine motif (which is movable), it is evident that, the until now blocked PICALM, has now a lot of free space to come in contact with YXXL motif, and therefore induces the adaptor protein complex (AP-2) and scaffold recruitment, and enables the clathrin-coated transcytosis. It is possible that the NH_2 end of the LRP1 is also fixed. It is evident that for the explanation of these events there are no other possible mechanisms. 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, 20-25].

Schoeler C *et al.*[16], by the employment of single-molecule spectroscopy with an atomic force microscope (AFM) and steered molecular dynamics (SMD) simulations, 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 cell attachment to insoluble substrates. According to the authors, 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) makes it possible to measure directly the molecular mechanical properties. The mentioned methods can exactly define the pulling geometry of these molecular events. In this situation, the conventional bioinformatics approach is completely insufficient. For the exact explanation, the molecular dynamics (MD) is very important as well. SMD simulations are also essential in studying the force propagation through protein structures, and in this case, the force distribution analysis (FDA) is very useful.

Once again, the adequate re-establishment of the equilibrium between Aβ charges and LRP1 CR charges induces the decrease (from 32 Å to 21 Å) of the interspace between the two neighboring CR repeats. The consequence of this decrease is the onset of intermolecular pulling in the LRP1 backbone structure directed on both sides to the center of the mentioned interspace. Pulling, on the side of abluminal cell membrane, as a signal, also propagates in the direction to the abluminal cell membrane, and after passing across it, arrives to the distal Di-leucine motif and YXXL motif. The structural consequences are explained above. It is clear that contrary to the direction of the pulling propagation as a signal, forces induced by pulling have an inverse direction to the CR/CR interspace. It is evident that in these events the intermolecular attractive forces have a crucial role[15-17].

LRP1 phosphorylation by protein kinase A (PKA)

Li Y *et al.*[26] point out that the serine 76 is the major phosphorylation site within the LRP tail. Serine73 and serine 79 most probably function as the docking site of the kinase and are the part of the phosphorylation motif of LRP tail. The results of their experiments indicate that LRP phosphorylation is predominantly mediated by the cAMP-dependent protein kinase A (PKA) on the serine 76 on its tail. They also emphasize that LRP/ligand endocytosis is primarily induced by the YXXL and the distal di-leucine motifs, and that the two NPXY motifs and the proximal di-leucine motif are not included in the initial endocytosis. The binding of certain LRP ligands (possibly Aβ) induces dissociation of the stimulatory heterotrimeric G-protein subunit (Gsα) with consequent activation of the adenylate cyclase and a rise in intracellular cAMP. The result is an enhanced PKA activity and LRP phosphorylation (***Fig. 5, 6***)[27].

Goretzki L *et al.*[28], at the beginning of their exposition, specify that LRP-mediated ligand internalization is dependent on cAMP-dependent protein kinase (PKA) activity. Ligand binding to the LRP1 cluster II and IV induces conformational changes on its cytoplasmic tail characterized by the YXXL motif activation and the pulling of two NPXY and two di-leucine motifs in the direction to the clusters. These changes induce the dissociation of heterotrimeric G-protein Gs alpha subunit (one of three heterotrimeric G-protein units), which promptly activates enzyme adenyl cyclase (transmembrane protein), and consequent conversion of adenosine triphosphate (ATP) to 3,5-cyclic adenosine monophosphate (cAMP). This reaction raises the intracellular cAMP concentration. PKA becomes activated and phosphorylates the LRP1 tail on Ser76. Phosphorylation of Ser76 elevates the interaction between the tail and adaptor proteins such as Dab-1 (***Fig. 5, 6***).

It is possible to suppose that the pulling of the LRP1 tail changes the direction of di-leucine – YXXL motif connection. This change enables the PICALM approach to the YXXL motif and its activation. Activated YXXL motif activates PKA. The mentioned change at the same time induces the breaking of the connection of the Gα subunit from GPCR, and its dissociation and activation. This activation induces the activation of adenyl cyclase, the transition of ATP in cAMP, and PKA activation (***Fig. 5, 6***)[29].

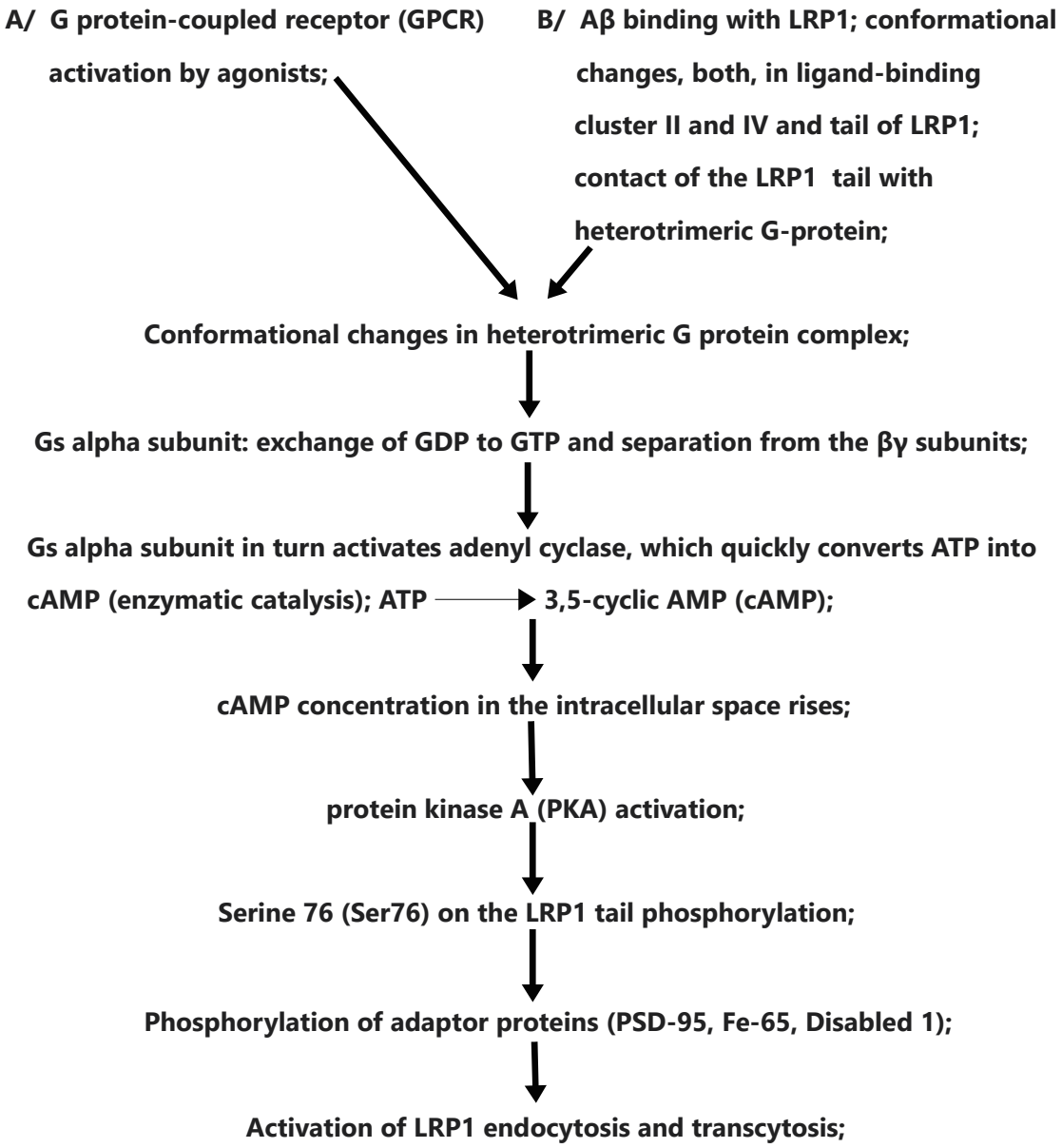

Fig. 6. **The role of GPCR receptor, conversion of ATP into cAMP, and PKA activation.**

LRP1; adaptor proteins (PSD-95, Fe-65, Disabled 1); ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate, second messenger included in many biological processes; GPCR, G-protein coupled receptor, transmembrane receptor, has three intracellular units (α, β, γ); PKA, protein kinase A, a family of enzymes, its activity depends on cellular levels of cAMP, cAMP dependent protein kinase, its α-subunit binds to adenyl cyclase which converts ATP into cAMP, important function in LRP1 endocytosis and transcytosis. LRP1, lipoprotein receptor-related protein-1; PSD-95, postsynaptic density protein 95.

LRP1 Ser76 by PKA phosphorylation is crucial for adaptor protein activation and consequent Aβ endothelial transcytosis and clearance [30-33].

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

Conclusion

The importance of Alzheimer's disease, this severe, chronic, and lethal neurodegenerative disease, is continually becoming an increasing medical and social problem. This is due to the relative and absolute growth of the worldwide elderly population and the alarming aspect of its incidence and prevalence. The exact explanation of its complex polygenetic etiology and effective therapy has not yet been obtained. However, a lot of researchers emphasize the crucial importance of intracerebral amyloid beta (Aβ) accumulation and its damaged drainage from the brain. They especially emphasize the important role of lipoprotein receptor-related protein1 (LRP 1) in these disturbed events. A number of complex experiments confirm that Aβ binding to the LRP1 ligand-binding cluster II and IV are 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. This activation is the inductor of the PICALM/clathrin Aβ endocytosis, transcytosis, and exocytosis into the capillary circulation and to the points of Aβ degradation. The important actual problem is how the binding of Aβ to LRP1 induces the conformational changes in the tail and the consequent beginning of the drainage. Experiments emphasize that in these events the 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.

Conflict of interest

The author declares no conflict of interest in this study.

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