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Review

Low-density lipoprotein receptor structure and folding

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Abstract. The endoplasmic reticulum (ER) is a major cellular 'production factory' for many membrane and soluble proteins. A quality control system ensures that only correctly folded and assembled proteins leave the compartment. The low-density lipoprotein receptor (LDLR) is the prototype of a large family of structurally homologous cell surface receptors, which fold in the ER and function as endocytic and signaling receptors in a wide variety of cellular processes. Patients with familial hypercholesterolemia carry single or multiple mutations in their LDLR, which leads to malfunction of the protein, in most patients through misfolding of the receptor. As a result, clearance of cholesterol-rich LDL particles from the circulation decreases, and the elevated blood cholesterol levels cause early onset of atherosclerosis and an increased risk of cardiac disease in these patients. In this review, we will elaborate on the structural aspects of the LDLR and its folding pathway and compare it to other LDLR family members.

Key words. LDL receptor; structure; folding; chaperones; disulfide bonds.

Introduction

To be functionally active, proteins need to adopt their proper three-dimensional (3D) conformation. In principle, the native, biologically active conformation of a protein is determined by its sequence of amino acid residues encoded by the DNA. Upon translation, polypeptide chains of proteins destined for the secretory pathway are targeted to the endoplasmic reticulum (ER), where folding of the primary amino acid sequence into secondary and tertiary structure starts. An ER quality control system ensures passage of only correctly folded and assembled proteins to the Golgi compartment (reviewed in [1, 2]). Here, N-linked glycan chains attached to proteins in the ER are modified, serine and threonine residues may become decorated with O-linked glycans, and proteins may be proteolytically cleaved, for example by the enzyme furin. From the trans-Golgi network, matured proteins may be directed to the plasma membrane or to organelles of the endomembrane system such as endosomes or lysosomes. Due to compartmentalization of the cell, the conditions for folding of proteins in the ER differ importantly from those in the cytosol: the ER supports formation of disulfide bonds and incorporation of calcium ions into a protein's structure. In most compartments, including ER and cytosol, a large number of chaperones and folding enzymes are available to assist formation of the proper, biologically active structure of proteins.

The low-density lipoprotein receptor (LDLR) is the first member of a still increasing family of structurally and functionally related cell surface receptors (reviewed in [3, 4]). The fact that LDLR relatives can be found in species ranging from nematodes to insects and mammals indicates that the genes have originated from an evolutionary ancestor gene. Based on structural properties, the mammalian LDLR family can be subdivided into at least four groups (fig. 1). The first group comprises LDLR, very-low density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2 or LRP8). The giant LDLR-related protein 1 (LRP1), LRP1B and Mega-

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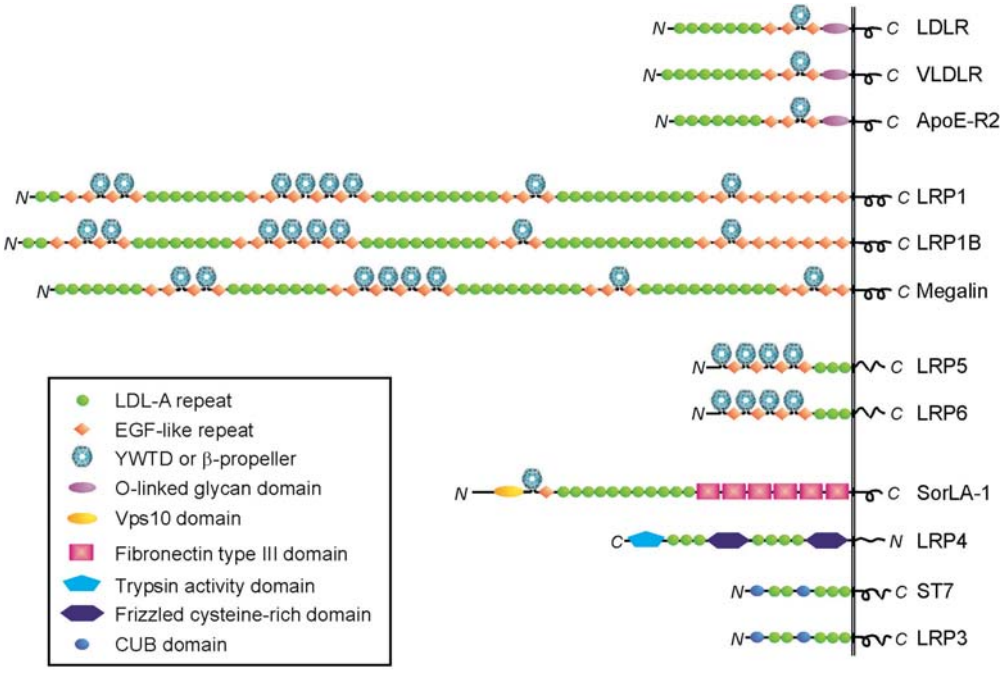

Figure 1. Domain organization of human LDLR family members. Domains characteristic for the LDLR family are discussed in detail in the text. In the more distantly related family members (i.e. SorLA-1, LRP4, ST7 and LRP3), additional domains have been identified that may function as structural (e.g. fibronectin type III domain), protein-protein interaction (e.g. Vps10 and CUB domain) or enzymatically active elements (e.g. trypsin activity domain). All family members have a type I topology (i.e. N-terminus in the ER lumen or the extracellular space and the C-terminus in the cytosol) with the exception of LRP4. The cytosolic domains contain one or multiple copies of NPXY, YXXL and dileucine based sequences that mediate internalization and intracellular sorting (not indicated but discussed in the text). The proteins are not drawn to scale.

lin (also known as gp330 or LRP2) belong to the second group. LRP5 and LRP6 are members of the third group. Recently, a fourth subgroup was identified which contains novel, more distantly related members, including SorLA-1 (or LR11), LRP4, ST7 and LRP3. All family members show a modular domain organization and characteristically contain one or multiple copies of a conserved cysteine-rich domain referred to as the LDLR class A (LDL-A) repeat (see below). Despite their structural homology, these receptors are involved in a wide range of cellular processes. Many LDLR relatives were found to function as endocytic receptors for a variety of ligands, including lipoproteins, protease/protease-inhibitor complexes as well as vitamin carriers. More recent findings, however, show that some members are (also) involved in signaling events that play a role in neuronal migration, synaptic transmission or embryonic development (for reviews see [5, 6]).

Function of the LDLR

The LDLR mediates clearance of cholesterol and cholesteryl ester-containing low-density lipoprotein (LDL) particles from blood [7]. Upon binding, the LDL-LDLR complex is taken up by the cells via clathrin-mediated endocytosis (fig. 2). In endosomes the ligand dissociates from the receptor due to the local low pH, after which the LDLR recycles back to the cell surface. The LDL particle is targeted to lysosomes where cholesterol is made available again by hydrolysis of the cholesteryl esters. LDLR expression is transcriptionally regulated in response to intracellular cholesterol concentrations. When cholesterol levels are low, membrane-bound precursors of sterol regulatory element-binding proteins (SREBPs) are transported from the ER to the Golgi ([8] and reviewed in [9]). Following a two-step proteolytic cleavage event, SREBPs are released from the Golgi membrane and translocated to the nucleus where they activate transcription of genes, including the LDLR and HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. When cholesterol levels are elevated, ER-to-Golgi transport of SREBP is prevented. The cholesterol-sensing chaperone SCAP (for SREBP cleavage-activating protein) that escorts SREBPs towards the Golgi plays a key role in this regulation mechanism [10].

The crucial role of the LDLR in cholesterol homeostasis is indicated by the more than 900 mutations that have been found in patients with familial hypercholesterolemia (FH) ([11, 12]; mutation databases available on World Wide Web URL www.umd.necker.fr and www.ucl. ac.uk/fh). FH is a common inherited disorder

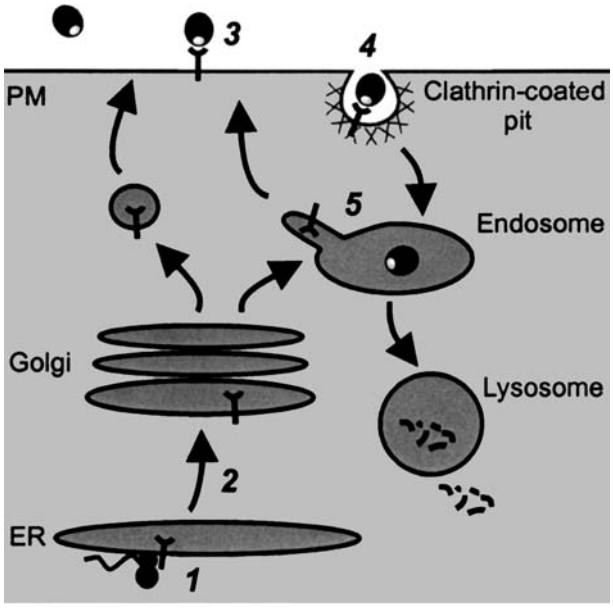

Figure 2. Schematic overview of the LDLR life cycle. Folding of newly synthesized LDLR molecules starts in the ER. The LDLR is transported then via the Golgi complex to the plasma membrane. At the cell surface, LDL particles (black spheres) can bind to the receptor. Following clathrin-mediated endocytosis, the LDL-LDLR complex reaches endosomes. Due to the low pH, LDL dissociates from the LDLR, which can then recycle back to the plasma membrane. LDL is further transported to lysosomes and degraded. Italic numbers refer to the classification of LDLR mutations identified in FH patients. The position in the figure indicates at which step in its life cycle LDLR function is impaired. PM, plasma membrane.

that is associated with elevated blood levels of cholesterol (LDL in particular), xanthomas (i.e. deposits of cholesterol in skin tissue) and the early onset of atherosclerosis with the accompanying risk of coronary heart diseases. Patients with a mutation in both LDLR alleles (FH homozygotes) are more severely affected than patients with a single mutant allele (FH heterozygotes). Homozygous and heterozygous mutations occur with a frequency of 1 in a million and 1 in 500, respectively [13]. In socially or geographically isolated communities the frequency can be higher due to founder effects. Mutations in the LDLR gene that have been identified to date comprise small deletions, insertions, duplications, missense mutations as well as large splicing defects. Analysis of the LDLR mutations has led to a classification as depicted in figure 2 [11]. Class 1 mutations are characterized by the absence of protein, irrespective of the mechanism, which may range from lack of synthesis to lack of entry into the secretory pathway. Class 2 mutations affect transport of the LDLR from ER to Golgi and can be grouped into class 2A and 2B, indicating either a complete block or a reduced rate of transport, respectively. Class 3 mutant receptors can reach the cell surface, but are defective in LDL binding. Mutations that prevent the internalization of the ligand-receptor complex belong to class 4. Other mutations affect recycling of the LDLR from endosomes back to the plasma membrane [14, 15]. As a consequence, the LDLR is targeted for degradation in the lysosomes together with the ligand. These mutants represent class 5. Currently, it is unclear whether the phenotype is the result of a defect in ligand release or a defect in the recycling signal. Recently, a sixth group of LDLR mutants was identified, which mislocalize to the apical instead of the basolateral plasma membrane in polarized cells [16].

Although many FH mutations have been identified, most have not yet been classified. Because over 50% of the classified mutations are class 2, extrapolation to the total pool of mutants suggests that most FH patients suffer from a folding disease. The quality control system in the ER ensures that newly synthesized proteins leave the compartment only when certain folding criteria are met. Exposure of hydrophobic amino acid residues in a protein or the presence of a particular N-linked glycan usually is indicative of incorrect or incomplete folding. A repertoire of chaperones in the ER is available to assist during folding of these proteins. If unsuccessful, the misfolded proteins may be targeted for degradation, which involves retrotranslocation to the cytosol followed by ubiquitylation and degradation by the 26S proteasome. Considering the above scenario, class 1 mutants include the ones where protein is synthesized but rapidly degraded. The class 2 mutations are likely to induce global changes in the LDLR that interfere with its proper folding in the ER, whereas mutations of classes 3, 4 and 5 must cause more subtle changes in the LDLR that do not affect the overall conformation of the receptor, because they do not lead to its retention in the ER. Instead, the latter mutations are likely to represent local changes that interfere with specific functions of the receptor after it has folded, such as binding of ligand or association with adaptor proteins required for endocytosis and/or recycling. Classification of mutations is needed for predicting the effect of a mutation on LDLR function. Addition of this information to the currently available databases on the web would be beneficial for many researchers.

Structure of the LDLR

The human LDLR is encoded by a gene of ~45 kb located on chromosome 19p13.1–13.3 [17]. The 18 exons of the gene are translated into an 860-amino acid type I transmembrane protein including a signal sequence of 21 amino acids, which is cleaved during translocation into the ER [18]. A striking correlation exists between exon and protein domain organization, suggesting that these exons can act as functionally independent modules. Indeed, in LDLR family members these modules are organized in

variable sequence. The LDLR has five functionally distinct regions: an N-terminal ligand-binding region, an epidermal growth factor (EGF)-precursor homology region, a region containing O-linked sugars, a transmembrane domain and a C-terminal cytosolic domain (fig. 3). These regions are described in more detail below.

Ligand-binding region

The ligand-binding region consists of seven cysteine-rich repeats (R1–R7) of ~40 amino acids, the so-called LDLR class A repeats (LDL-A, also known as complement-type repeats) [18] (figs 1 and 3, green). Nuclear magnetic resonance (NMR) structures of R1 [19], R2 [20] and R6 [21, 22] as well as the crystal structure of R5 [23] revealed a two-loop conformation stabilized by three disulfide bonds. Characteristically, disulfide bond formation occurs between cysteine residues I and III, II and V, and IV and VI (Roman numerals indicate the relative position of the cysteine residues in the repeat). In addition, a conserved sequence of acidic amino acids (C**D**XXX**D**CX**D**XS**DE**; acidic residues indicated in bold and X representing any amino acid) is present in the C-terminal part of each LDL-A repeat. Originally, these acidic residues were proposed to mediate the interaction with basic amino acid residues of apolipoprotein B-100 (apoB-100) and apolipoprotein E (ApoE) (see below). Confusion arose when crystallographic analysis demonstrated that many acidic residues were involved in coordination of a calcium ion [23]. In the calcium-bound state, however, not all negative charges are occupied, still allowing their involvement in ligand binding [22]. The incorporation of a calcium ion in the structure explains the calcium requirement for correct folding and disulfide bond formation of LDL-A repeats [24, 25] and for binding of lipoproteins to LDLR [26].

In mammals, LDL is one of the major cholesterol-containing lipoproteins in blood (for a recent review on lipoprotein physiology see [27]). The LDL particles are composed of an inner core of esterified cholesterol surrounded by a layer of phospholipids. In addition, each LDL particle contains a single molecule of the ~550 kD apoB-100, which increases solubility of the LDL particle in the aqueous environment of the blood and mediates its binding to the LDLR. Besides LDL, the LDLR can bind VLDL particles that contain several copies of the ~33 kD apoE protein in addition to cholesteryl esters and a single apoB-100 molecule. The structural requirements for binding LDL and VLDL differ: LDL binds its receptor via apoB-100, VLDL via apoE. Whereas LDL binding depends on the presence of R2-R7 and EGF repeat A, only R5 appears essential for interaction with VLDL [28, 29]. The importance of R5 is underscored by the high number of FH mutations that have been localized to this repeat. Only a minor number of FH mutations locate to R1, which is not important for binding of either ligand to the LDLR.

EGF-precursor homology region

The second region in the LDLR ectodomain contains amino acid stretches with homology to the EGF-precur-

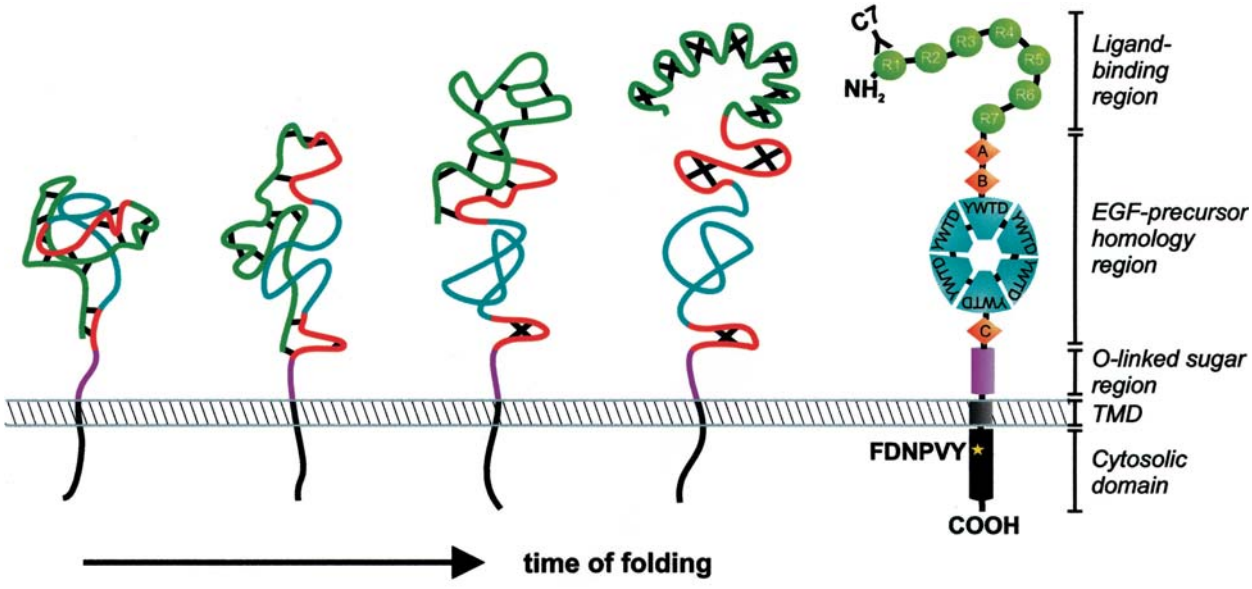

Figure 3. Folding pathway of newly synthesized LDLR molecules. Upon translation the LDLR forms a compact structure including many non-native disulfide bonds. With time, the disulfide bonds are reshuffled (isomerized) during continued folding and extension of the molecule. In the native conformation only disulfide bonds within individual domains remain. On the right, a schematic representation of the regional composition of the LDLR is depicted. TMD, transmembrane domain.

sor protein [30]. This region consists of three EGF-like repeats (A, B and C) of ~40 amino acids (figs 1 and 3, red). Although these repeats contain six conserved cysteine residues similar to the LDL-A repeats, they differ in the pattern of disulfide bond formation, which occurs between cysteines I and III, II and IV, and V and VI. The repeats surround an ~260-amino acid domain containing six YWTD motifs (also known as LDL-B repeats) which adopts a β-propeller structure [31, 32] (figs 1 and 3, blue). Deletion of the EGF-precursor homology region does not affect VLDL binding, but instead prevents the acid-dependent dissociation of ligand in endosomes [33]. Rudenko and colleagues suggested a plausible molecular mechanism for the acid-dependent release of ligand [34]. Their crystallographic analysis of the LDLR ectodomain at endosomal pH revealed that the ligand-binding region folds back onto the β-propeller. This conformation is stabilized by interactions between repeats R4/R5 and the β-propeller and implicates the β-propeller in displacement of the ligand from the ligand-binding region.

O-linked glycan region

The third region in the LDLR ectodomain is enriched in serine and threonine residues that function as acceptor sites for O-linked sugars (figs 1 and 3, purple). The role of O-linked glycosylation in LDLR function is still unclear. LDLR molecules lacking this region behave like wild-type receptors with respect to ligand binding, endocytosis and degradation [35]. Possibly, the glycosylation region provides a rigid stalk allowing the ectodomain to extend into the extracellular space. Alternatively, glycosylation may protect the receptor from denaturation during recycling through the slightly acidic endosomal compartments [36]. As a third function, O-glycosylation may modulate the rate of proteolytic cleavage of the ectodomain by metalloproteases at the cell surface. Alternative splice variants of VLDLR and apoE-R2 lacking the O-linked glycosylation region are more prone to proteolytic cleavage than their full-length counterparts [37, 38]. Ectodomain 'shedding' has been reported for LRP1 [39], SorLA-1 [40], VLDLR [41], ApoE-R2 [38] and recently also for LDLR, albeit to a lesser extent [42]. Of these, only LRP1 and SorLA-1 lack an O-linked sugar region. Whether the released ectodomains of LDLR family members have any physiological function remains to be determined. Alternatively, they may be side products of a two-step proteolytic event resulting in the release of their intracellular domains into the cytoplasm, analogous to the release of the Notch cytosolic domain, which acts as a transcription regulator [43].

Transmembrane domain

A hydrophobic domain of 24 amino acids anchors the LDLR in the lipid bilayer. Only a small number of FH patient mutations were found in the transmembrane domain of the LDLR, perhaps because these mutations do not affect the topology of the protein and are therefore not identified in screenings for FH mutations. On the other hand, the relatively small size of the transmembrane domain reduces the chance that spontaneous mutations will be found. This aspect is often not taken into account in comparisons of the number of mutations in the different domains.

Cytosolic domain

Endocytosis and intracellular transport of the LDLR are regulated via its cytosolic domain. Mutational analysis has revealed a dominant role for the FDNPVY sequence in recruitment of the LDLR to clathrin-coated pits [44]. A single copy of this motif has also been found in VLDLR, the ApoER2 and LR11/SorLA-1, whereas LRP1, LRP1B and Megalin each contain two copies. In addition to the two NPXY motifs, LRP1 contains a YXXL motif which serves as the dominant internalization motif [45]. Strikingly, LRP5, LRP6, ST7 and LRP3 lack the characteristic NPXY motif. Instead, they contain YXXL and/or dileucine-based endocytosis motifs.

The NPXY motif adopts a tight hairpin conformation that serves as a binding site for a variety of adaptor proteins and signaling molecules. The autosomal recessive hypercholesterolemia (ARH) gene encodes an adaptor protein that binds via its N-terminal phosphotyrosine binding (PBD) domain to the FDNPVY motif of the LDLR [46]. Via a canonical clathrin box sequence (LLDLE) in its C-terminal domain, ARH binds directly to clathrin, whereas a conserved 27-amino acid sequence interacts with the β2-subunit of the clathrin-binding AP-2 adaptor complex [47]. Mutations in the ARH gene lead to reduced internalization of the LDLR and a clinical phenotype indistinguishable from FH (reviewed in [48, 49]). In fibroblasts from ARH patients, however, the LDLR can be internalized [50]; this tissue specificity suggests that other adaptor proteins can compensate for the loss of ARH function. Accordingly, the β-arrestin-2 adaptor protein was recently shown to mediate clathrin-dependent internalization of the LDLR but not of a mutant LDLR with a Y-to-A substitution in the FDNPVY motif [51]. Another clathrin- and AP-2 binding protein, Disabled-2 (Dab-2), binds peptides corresponding to the FDNPXY motif and co-localizes with the LDLR in clathrin-coated pits [52, 53].

In polarized cells, the LDLR is directed towards the basolateral plasma membrane. A weak basolateral targeting determinant overlaps with, but is structurally distinct from, the FDNPVY endocytosis motif [54]. Another dominant sorting signal is found in the C-terminal region of the cytosolic tail. The activity of both of these determinants depends on a tyrosine residue and a downstream cluster of three acidic residues. Comparable tyrosine-based basolateral sorting signals have been found in LRP1 [55].

In contrast to the internalization process, little is known about proteins involved in intracellular LDLR trafficking. Sorting nexin-17, a protein located in early endosomes, binds the LDLR NPXY motif, but its precise role in intracellular trafficking remains unclear [56, 57].

Folding of the LDLR

Although structural and functional information for individual LDLR domains and combinations thereof is available, it is still unclear how, in the context of a full-length protein, these different domains fold into a functional receptor. Disulfide bond formation and calcium incorporation are two dominant characteristics of LDLR folding in the ER, and recent data imply an exciting new mechanism for LDLR folding in vivo [58]. As many of the LDLR family members differ only in the number and organization of individual domains, a similar mechanism of folding could be applicable to other family members as well.

Non-native disulfide bonds and isomerization

Because of the modular structure and the observation that individual modules can fold into native structures both in vitro (in a test tube) and in vivo (in cultured cells) [19, 20, 22, 23], folding of the complete receptor has long been considered to occur in a vectorial manner, i.e. domain by domain, starting with the most N-terminal cysteine-repeat R1. Using an electrophoretic mobility based assay in combination with metabolic radiolabeling, however, we demonstrated that the newly synthesized LDLR polypeptide chains fold rapidly into compact structures containing non-native disulfide bonds linking distant regions of the protein (fig. 3) ([58] and reviewed in [59]). With time, the non-native disulfides are reshuffled (i.e. isomerized), allowing extension of the molecule. Ultimately, in the native conformation, disulfide bonds only exist between cysteine residues within individual repeats [34]. The conformation-specific antibody C7, which exclusively recognizes the first cysteine repeat (R1) in its native conformation [60], interacts mainly with late folding intermediates [58], fitting with a nonvectorial folding pathway. Although being translated first, the R1 repeat apparently gains its native conformation only during later stages of folding. Despite the extensive formation of non-native disulfide bonds during folding, the LDLR rarely aggregates. We can assume therefore that non-native disulfide bond formation and isomerization are part of the normal LDLR folding pathway. The rapid collapse may prevent unfavorable aggregation and interaction with other cysteine-containing proteins or LDLR ligands [61].

Liver cells are capable of producing both apoB-100 and LDLR. Secretion of apoB-100 (as a component of VLDL particles) is dependent on microsomal triglyceride transfer protein (MTP) and lipid availability [62]. Under hypolipidemic conditions a major portion of newly synthesized apoB-100 is degraded intracellularly in an LDLR-dependent manner [63, 64]. A mutation in the ligand- binding region of the LDLR that precludes binding of apoB-100 and apoE prevents this presecretory degradation of apoB-100 [65], indicating that intracellular interactions between the LDLR and its ligand can occur.

Calcium incorporation

Incorporation of calcium ions is the second major characteristic of LDLR folding. As discussed above (and reviewed in [66]), the cysteine repeats of the ligand-binding region have high-affinity calcium binding sites. NMR studies on the folding of repeat R5 demonstrated a strict correlation between native disulfide bond formation and calcium binding [67]. In the absence of disulfide bonds, the calcium binding capacity is negligible. When the two distal disulfide bonds (between cysteines II and V, and IV and VI) are formed, calcium will bind, but maximum affinity requires a native structure, including the disulfide bond between cysteines I and III. The first two EGF-like repeats A and B but not C (figs 1 and 3, red) contain calcium binding sites as well; coordination of a calcium ion stabilizes the linker region between repeats A and B [68, 69]. In contrast to the cysteine repeats of the ligand binding region, which are flexible and not influenced by each other when expressed as tandem repeats [70–72], EGF-like repeats A and B form a rigid structure in which repeat A is in a fixed orientation with respect to repeat B.

Folding enzymes and chaperones

The remarkably efficient folding of the LDLR may be caused by assistance of chaperones and folding enzymes in the ER. Two classes can be distinguished: general and private chaperones.

General chaperones

General chaperones recognize and interact with unfolded, partially folded or misfolded proteins due to common features such as exposed stretches of hydrophobic amino acid residues, thereby preventing aggregation. A well-known chaperone is BiP, an ER-localized Hsp70 family member, also named Grp78, for 78-kDa glucose-regulated protein. BiP binds transiently to the LDLR and more persistently to LDLR mutants that are retained in the ER [73]. BiP may improve LDLR (mutant) folding, but prolonged interaction of the chaperone with the LDLR mutants may also be merely indicative of their misfolded state. If folding is successful,

the LDLR can proceed to the Golgi. If, on the other hand, the protein is misfolded permanently, it is retained in the ER and may be targeted for retro-translocation to the cytosol and subsequent degradation by the 26S proteasome (reviewed in [1]), as recently suggested for LDLR class 2 mutants [74]. Whereas overexpression of BiP slows down LDLR folding in the ER, steady-state cell-surface LDLR levels appear unaffected [73].

Calnexin and calreticulin are lectin-like ER chaperones that interact specifically with monoglucosylated N-linked glycoproteins and play an important role in glycoprotein quality control (reviewed in [1, 75]). In addition to the O-linked sugar moieties that are attached in the Golgi complex, the LDLR is decorated with N-linked glycans [76]. Using tunicamycin, an inhibitor of N-linked glycosylation, Filipovic and colleagues showed that N-linked glycosylation is more important for LDL binding than for transport of the LDLR to the cell surface [77]. A crucial role for calnexin/calreticulin in LDLR folding therefore is not likely.

The relatively oxidizing environment of the ER may support disulfide bond formation, but the actual redox reactions are catalyzed by oxidoreductases of the protein disulfide isomerase (PDI) family (reviewed in [78–80]). In mammals, about a dozen members have been identified. PDI, the most abundant one, can catalyze formation of disulfide bonds, but also their isomerization or reduction, depending on redox conditions and substrate [81]. Because formation and isomerization of disulfide bonds are major events during LDLR folding (as described above), a role for PDI or one of its relatives is more than plausible and currently under investigation.

Private chaperones

In addition to general chaperones, certain proteins or protein families require the action of specific, so-called private chaperones. The 39-kDa receptor-associated protein (RAP) facilitates folding of numerous LDLR family members, including LRP1 [82], Megalin and VLDLR [83, 84]. Although LRP1 and VLDLR folding is impaired in RAP knockout mice, folding of the LDLR is unaffected [85]. This finding is thought to be in accordance with the lower affinity of RAP for the LDLR as compared to LRP1 and VLDLR [86], although low affinities usually are required for chaperones to function properly. Perhaps RAP is only required under certain circumstances or the LDLR uses an alternative private chaperone.

Recently, two labs simultaneously identified an evolutionarily conserved ER protein that acts as a chaperone for LDLR relatives. In *Drosophila*, Boca is required for intracellular trafficking of Arrow (analogous to mammalian LRP5/6) and Yolkless (homologous to a portion of mammalian LRP1 and Megalin) [87]. The mouse homologue of Boca, encoded by the mesoderm development (Mesd) gene, promotes plasma membrane localization of LRP5 and LRP6 [88]. Mesd/Boca reduces disulfide-linked aggregation and thereby may improve trafficking of these LDLR relatives. When the LDLR is expressed in insect S2 cells, it needs Boca to reach the cell surface [87]. Whether Mesd/Boca functions as a chaperone for the LDLR in mammalian cells remains to be established. As suggested by Hsieh and colleagues [88], RAP and Mesd/Boca may facilitate folding of different domains of LDLR relatives (reviewed in [89]). Whereas RAP can improve folding of LRP minireceptors whose ectodomain is almost exclusively composed of LDL-A repeats [82, 85], Mesd/Boca seems to act on proteins primarily containing EGF-like repeats and β-propeller structures [88, 90]. Because these latter structures are also found in a wide variety of other proteins [32], Mesd/Boca eventually may turn out to be less specific for LDLR relatives than currently anticipated.

Perspectives

The recent developments described in this review have dramatically changed our view on the structure and folding of a modular protein such as the LDLR. Whereas interactions between domains within the protein were barely appreciated in the past, the current view of LDLR folding (including non-native disulfide bonds and isomerization) and function (including the release of ligand in the endosomes) is based on extensive interplay between distant domains. Now that the mechanisms are roughly understood, further studies will be required to fill in the details. What prevents folding of the first cysteine repeat (recognized by the C7 antibody)? Which domains interact during folding? What is the function of the initial collapse? And which chaperones, folding enzymes and oxidoreductases are involved in the folding process? Whether the characteristic folding pathway of the LDLR is a general feature of its family members is another intriguing question.

We envision that more molecular players in the LDLR folding pathway will be discovered. Depending on the specificity of these molecules, they may be useful tools for improving expression of (mutated) LDLR molecules at the cell surface. Whereas LDLR gene replacement therapy generally is regarded as the most logical treatment for FH patients in the future, induction of humoral and cellular immune responses against the vector (i.e. adenovirus) and LDLR in animal model systems still is a major drawback for this approach (reviewed in [91]). Because the majority of FH mutations affect folding and hence transport of the LDLR to the cell surface, exogenous expression of molecular chaperones that specifically improve LDLR folding may turn out to be useful as an alternative tool in the battle against elevated cholesterol levels and cardiac disease.

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