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Review Article

The essential functions of endoplasmic reticulum chaperones in hepatic lipid metabolism

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ABSTRACT

The endoplasmic reticulum (ER) is an essential organelle for protein and lipid synthesis in hepatocytes. ER homeostasis is vital to maintain normal hepatocyte physiology. Perturbed ER functions causes ER stress associated with accumulation of unfolded protein in the ER that activates a series of adaptive signalling pathways, termed unfolded protein response (UPR). The UPR regulates ER chaperone levels to preserve ER protein-folding environment to protect the cell from ER stress. Recent findings reveal an array of ER chaperones that alter the protein-folding environment in the ER of hepatocytes and contribute to dysregulation of hepatocyte lipid metabolism and liver disease. In this review, we will discuss the specific functions of these chaperones in regulation of lipid metabolism, especially *de novo* lipogenesis and lipid transport and demonstrate their homeostatic role not only for ER-protein synthesis but also for lipid metabolism in hepatocyte.

1. Introduction

Hepatocytes contain unusually large amounts of smooth and rough endoplasmic reticulum (ER) that serve a myriad of metabolic functions, including plasma protein synthesis and secretion, sterol biosynthesis, lipogenesis, lipoprotein production and secretion, detoxification [1]. The ER especially provides protein folding environment to ensure that protein folding occurs in a stable and constant state. If the protein-folding homeostasis is perturbed, the stress is induced in the ER. Thus, the ER homeostasis is extremely important to maintain metabolic functions in response to extracellular stimuli and intracellular physiological changes in hepatocyte. The unfolded protein response (UPR) constituted by series of signalling molecules and a network of protein chaperones efficiently coordinates cellular changes to maintain protein-folding fidelity in the ER to preserve normal hepatic lipid homeostasis [2,3].

2. Hepatic protein synthesis in the ER

The ER is classified by its association of ribosomes. Ribosome-associated ER is termed rough ER (rER) otherwise it is termed smooth ER (sER). Rough ER is mainly responsible for protein synthesis in hepatocytes to supply large quantities of plasma secretory proteins including different coagulation factors and apolipoproteins [4]. Newly synthesized polypeptides are in unfolded states and undergo energy-dependent folding processes mediated by a series of folding enzymes and molecular chaperones in the ER lumen to make them folded appropriately before they exit ER into secretory pathways [5] (Fig. 1). Proteins that cannot fold properly are recognized by a quality control system in the ER, which either retained them in the ER for refolding or targeted unfolded or misfolded proteins for degradation [5]. Since the ER synthesizes over 70% proteins for hepatocytes, ER homeostasis is extremely important for hepatic physiology [6]. Accumulating evidence demonstrates increased occurrences of perturbed ER homeostasis in several human liver diseases such as alcoholic fatty liver diseases, non-alcoholic fatty liver diseases and hepatic carcinoma [7,8].

Protein folding requires an ER chaperoning network to provide a platform coordinating a large number of proteins uploaded into the ER and *en route* to the Golgi. This ER chaperoning network consists of at least 5 groups of proteins based on their functions [9,10]: (1) chaperones and co-chaperones of heat shock protein (Hsp) family, which include glucose regulated protein (GRP) 78 (GRP78, also known as Bip) and its co-chaperones such as GRP170 and Sil1 (BiP exchange factor), ERdj1, ERdj3, ERdj4, ERdj5 and P58ipk (Bip co-chaperone); GRP94 and its co-chaperone such as CNPY3. They facilitate protein folding by selectively binding with certain structural elements, such as hydrophobic patches, to lead

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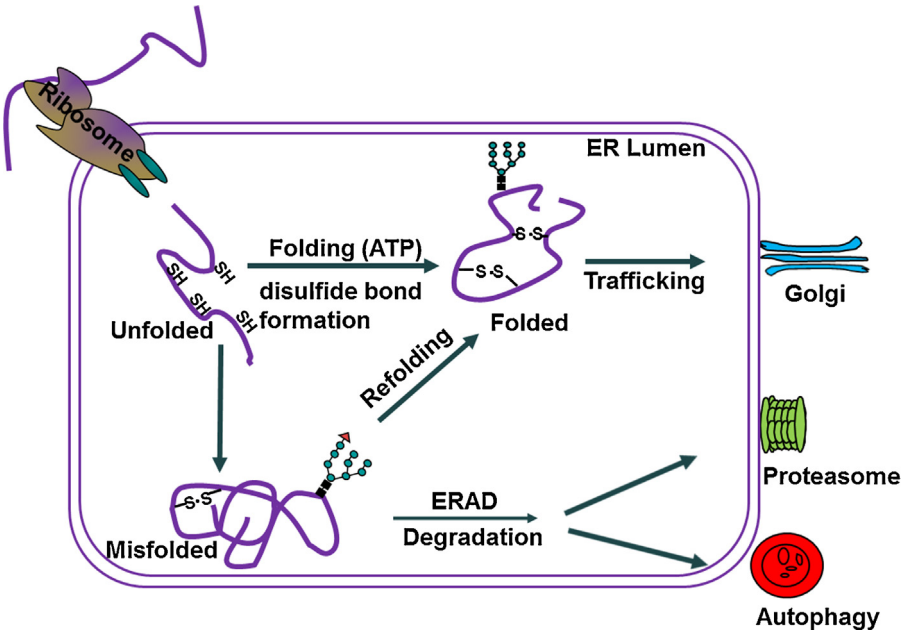

Fig. 1. Hepatic Lipid Metabolism in the ER.
The ER in the hepatocyte is the major site where TG synthesis, VLDL assembly and lipid droplet formation occur. TG synthesis starts with FFAs synthesis mediated by another two ER-localized enzymes SCD and ELOVL. TG synthesis is catalyzed by a series of ER-localized enzymes including GPAT, MGAT or DGAT. The majority of TG is stored in lipid droplet, which is also produced by the ER. TG partitioned in the ER is used for VLDL assembly mediated by MTP complex. TG partitioned in the ER is also subjected to hydrolysis by ER lumen-localized lipases, which can be re-esterified to TG as lipid supplies for VLDL.

protein folding in its particularly favourable folding pathways. (2) chaperone lectins like calnexin, calreticulin, UGT1 and UGT2 (Glucosyl-transferase) and ER degradation enhancer mannosidase (EDEM). They play major roles in ER quality control system that recognizes unfolded proteins to either put them back for refolding or dispose them for degradation. (3) thiol oxidoreductases of the protein disulfide isomerase (PDI) family such as PDI1, ERp57 and ERp72. They stabilize proper intermediates for disulfide bond formation and resolve aberrant disulfide bonds and in the meanwhile destabilize disulfide bond of unfolded protein before they are exacted out of the ER for proteasome-mediated degradation. (4) peptidyl prolyl isomerases (PPIs) [11] that interconverts the cis and trans isomers of peptide bonds with the amino acid proline; (5) substrate-specific chaperones such as Hsp47 for collagen that inhibit collagen aggregation by binding procollagen in the ER and facilitate triple helix formation [12].

The most interesting perspective of those chaperones is that they can bind with different substrates through different cooperation with other chaperons to assist their client-protein folding. Some of these chaperons also have multiple functions in the folding process for different client proteins. It is common to find members of isomerase to serve a role as co-chaperon for their client proteins [9]. Recent development in gene knockdown or knockout technology allows functional analysis of specific ER chaperone in hepatic lipid metabolism studies [13].

3. Hepatic lipid metabolism in the ER

The ER plays an important homeostatic role for lipid metabolism in hepatocytes. The major lipid metabolic pathways, including fatty acid synthesis, triglyceride synthesis and storage, apolipoprotein assembly and secretion, lipolysis and fatty acid oxidation are all present in hepatocytes [1]. The ER is the major site for lipid synthesis and apolipoprotein assembly and secretion [14] (Fig. 2).

First, *de novo* lipogenesis (or sterol synthesis) is controlled by a family of ER membrane-localized transcription factor, the sterol regulatory element binding proteins (SREBP1c for fatty acid synthesis and SREBP2 for sterol synthesis) [15,16]. When the lipogenesis is inhibited, the SREBP precursor is localized in the ER membrane in a complex with SREBP cleavage activating protein (Scap) and the insulin-induced gene protein (Insig). When hepatocyte senses the reduced levels of cholesterol or free fatty acids, Insig dissociates from SREBP/Scap complex, which allows the SREBP/Scap complex to the *Golgi* apparatus where SREBP1c undergoes regulated intramembrane proteolysis (Rip) by site-1 protease (S1P) and site-2 protease (S2P) to release a cytosolic transcription factor that traffics to the nucleus to induce expression of lipogenic programs including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), the long-chain elongase (LCE), and stearoyl-CoA desaturase (SCD) and elongase (ELOVL) [17]. Thus, ER homeostasis appears important to regulate the activation the SREBP [18].

Second, triglycerides (TG) are also synthesized from fatty acids and glycerol by a group of ER-localized acyltransferase enzymes including glycerol-3-phosphate acyltransferase (GPAT), acylglycerolphosphate acyltransferase (AGPAT), monoacylglycerol acyltransferase (MGAT) or diacylcglycerol acyltransferase (DGAT) [19,20]. In addition, phospholipids such as phosphatidylcholine or phosphatidylethanolamine are also synthesized in the ER by ER-localized enzyme choline/ethanolaminephospho-transferases (CEPT) [21,22]. TG synthesis is coupled with lipid droplet formation with involvement of protein such as adipocyte differentiation-related protein (ADRP) and perilipin in the ER. As major source of lipid storage, lipid droplet formation also plays a homeostatic role in hepatic lipid metabolism. Thus, perturbed ER function affects lipid droplet formation and morphology [23,24].

Third, hepatic lipoprotein particles are all synthesized and assembled in the ER. Lipids stored in the hepatocyte are secreted as very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) [25]. VLDL particle is composed of structural protein apoB and other apolipoproteins, which are all translated in the rER to initiate the VLDL synthesis [25]. ApoB is further lipidated by a hydrophobic lipid core