Biochimica et Biophysica Acta 1813 (2011) 1893–1905

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Urban planning of the endoplasmic reticulum (ER): How diverse mechanisms segregate the many functions of the ER

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ARTICLE INFO

Article history:
Received 20 April 2011
Received in revised form 22 June 2011
Accepted 23 June 2011
Available online 2 July 2011

Keywords:
Endoplasmic reticulum (ER)
Mitochondria-associated membrane (MAM)
Plasma membrane-associated membrane (PAM)
Russell bodies
Lipid droplet

ABSTRACT

The endoplasmic reticulum (ER) is the biggest organelle in most cell types, but its characterization as an organelle with a continuous membrane belies the fact that the ER is actually an assembly of several, distinct membrane domains that execute diverse functions. Almost 20 years ago, an essay by Sitia and Meldolesi first listed what was known at the time about domain formation within the ER. In the time that has passed since, additional ER domains have been discovered and characterized. These include the mitochondria-associated membrane (MAM), the ER quality control compartment (ERQC), where ER-associated degradation (ERAD) occurs, and the plasma membrane-associated membrane (PAM). Insight has been gained into the separation of nuclear envelope proteins from the remainder of the ER. Research has also shown that the biogenesis of peroxisomes and lipid droplets occurs on specialized membranes of the ER. Several studies have shown the existence of specific marker proteins found on all these domains and how they are targeted there. Moreover, a first set of cytosolic ER-associated sorting proteins, including phosphofurin acidic cluster sorting protein 2 (PACS-2) and Rab32 have been identified. Intra-ER targeting mechanisms appear to be superimposed onto ER retention mechanisms and rely on transmembrane and cytosolic sequences. The crucial roles of ER domain formation for cell physiology are highlighted with the specific targeting of the tumor metastasis regulator gp78 to ERAD-mediating membranes or of the promyelocytic leukemia protein to the MAM.

1. Introduction

The endoplasmic reticulum (ER) is a multi-functional organelle that distinguishes itself from other organelles by its size and the plethora of functions associated with it. The size of this organelle and the potential array of targeting mechanisms required to build this little intracellular city were first recognized almost 20 years ago [1]. Initial studies on the ER were done at the beginning of the 20th century by Santiago Ramon y Cajal in neurons [2]. Following its proper discovery in 1945 by Keith Porter and co-workers on electron micrographs [3] and its designation in 1952 [4], microscopic and biochemical observations led to the identification of two distinct domains of the ER: the rough ER, characterized by the presence of ribosomes, and the smooth ER that is devoid of ribosomes [5,6]. Both are found in biochemical preparations first generated in the early 1940s and termed microsomes by Albert Claude [7]. Quite early, it became also clear that the nuclear envelope is another domain of the ER [8]. Recent progress has highlighted how the same set of proteins that shape rough and smooth ER tubules also determine the formation of the nuclear pore complex, a decisive event in the biogenesis of the nucleus [9].

The presence of ribosomes and mRNA on rough ER preparations suggested that this domain of the ER mediates the synthesis of secretory and membrane proteins [5]. Consistent with this hypothesis, the machineries responsible for the translocation of proteins into the ER [10–13] and their glycosylation were found on the rough ER [14]. Sorting to this domain of the ER is thought to involve preferential targeting of rough ER proteins to sheet-like, low curvature domains of the ER that are decorated with ribosomes

Abbreviations: ACS4/FACL4, acyl-CoA synthase 4; ACAT/SOAT, acyl-CoA cholesteryl acyl transferase; AMFR, autocrine motility factor receptor; BAP31, B-cell receptor-associated protein of 31 kDa; BiP, immunoglobulin binding protein; CHOP, CCAAT/enhancer binding protein (C/EBP) homologous protein; Climp63, cytoskeleton-linking membrane protein of 63 kDa; COP, coat protein complex; DGAT acyl-CoA, diacylglycerol acyltransferase; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum associated degradation; ERES, endoplasmic reticulum exit sites; ERK, extracellular signal-regulated kinase; ERMES, endoplasmic reticulum-mitochondria encounter structure; ERQC, endoplasmic reticulum quality control compartment; GFP, green fluorescent protein; GRP, glucose regulated protein; IP3R, inositol 1,4,5-trisphosphate receptor; KDEL, lys-asp-glu-leu; LRP6, low-density lipoprotein receptor-related protein 6; MAM, mitochondria-associated membrane; NADPH, reduced nicotinamide adenine dinucleotide phosphate; OST, oligosaccharyl transferases; PACS-2, phospho-furin acidic cluster sorting protein 2; PAM, plasma membrane-associated membrane; PDI, protein disulfide isomerase; PERK, protein kinase (PKR)-like endoplasmic reticulum kinase; RFP, red fluorescent protein; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SNARE, soluble N-ethylmaleimide sensitive fusion protein attachment protein receptor; SREBP, sterol-regulatory element binding protein 2; SRP, signal recognition particle; STIM1, stromal interaction molecule 1; TIP47, tail-interacting protein of 47 kDa (TIP47); TRAM, translocating chain-associated membrane protein; TRAP, translocon-associated protein; VAPB, vesicle-associated membrane, protein-associated protein B; XBP1, X-box binding protein 1

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doi:10.1016/j.bbamcr.2011.06.011

[15]. However, the functions of the rough ER may not be restricted to secretory protein production, since recent studies have shown that autophagosome formation involves membranes of the rough ER [16,17], suggesting that this domain of the ER may exhibit heterogeneity under certain circumstances.

Contrary to these numerous breakthroughs in the understanding of the functions of the rough ER, progress on the smooth ER has been slower. Initially, this portion of the ER was identified by the absence of ribosomes on its surface [6]. The past two decades have seen the identification and characterization of subdomains of the smooth ER, including ER exit sites (ERES), the cortical ER (also known as peripheral ER or plasma membrane-associated ER, PAM), the mitochondria-associated membrane (MAM), and the ER quality control compartment (ERQC). Our image of the smooth ER as the lesser cousin of the rough ER is therefore changing quickly and the smooth ER emerges as a heterogeneous mix of highly specialized subdomains. Moreover, the existence of these membrane domains within the smooth ER supports the hypothesis that smooth ER proteins are actively sorted away from rough ER proteins [15], but the deciphering of such putative mechanisms is not yet very advanced.

Biochemical characterizations of the smooth ER determined that this domain is rich in enzymes involved in drug detoxification such as NADPH cytochrome c reductase [18] and epoxide hydrolase [19]. In the presence of high amounts of toxins such as ethanol, the smooth ER increases in size [20,21], concomitant with an increased production of smooth ER-associated detoxifying enzymes such as cytochrome P450 2E1 [22]. Similar expansion of the rough ER is observed when protein secretion increases, for example, during the differentiation of B cells into plasma cells, coinciding with an increase of proteins involved in the production of secretory proteins [23]. Together, these findings demonstrate not only the existence of rough and smooth ER-specific transcriptional responses, but also the ability of the cell to specifically expand either ER membrane system, including mechanisms that keep rough and smooth ER separate in a state of ER expansion. Interestingly, the site of lipid synthesis during ER expansion is the smooth ER [24], suggesting that ER expansion may first result in naked, smooth ER that can later be decorated with ribosomes [25].

Research during the past decade has shown that both rough and smooth ER enzymes rely on the precision of intra-ER targeting mechanisms. For example, triacylglycerol hydrolase becomes inefficient in the mobilization of lipids when its ER localization motif is mutated from a histidine-isoleucine-aspartic acid-leucine (HIEL) to a classical lysine-glutamic acid-aspartic acid-leucine (KDEL) sequence. Although both motifs allow the interaction with the KDEL receptor, only the HIEL motif leads to proper targeting of triacylglycerol hydrolase to the peripheral smooth ER [26]. Intra-ER targeting is also relevant for the study of the ER using fluorescent proteins in vivo. Studies had initially indicated that ER-targeted green fluorescent protein (GFP) is freely diffusible within the ER lumen, albeit at a slower speed than in aqueous solutions and three- to sixfold slower than in the cytoplasm [27]. However, red and green fluorescent proteins (RFP/GFP) when fused to the KDEL motif do not co-localize within the ER of neuronal cells [28]. This finding corroborates that intra-ER sorting is distinct from ER retention. Similarly, GFP fusions with presumed ER proteins have led to spurious results, such as in the case of the oxidoreductase ERp44, which targets to the rough ER when fused to GFP, although its endogenous form is found close to ER exit sites and the Golgi [29,30]. Another example is GFP-tagged Sec61β that loses its specific targeting to the rough ER seen for the endogenous protein [31]. This review aims to outline what is known about the composition of individual domains of the ER and the intracellular sorting mechanisms that give rise to the unique architecture of the ER. We will usually refer to what is known about ER domain enrichment in mammalian cells, unless noted otherwise. Individual domains discussed in the text and known bona fide markers are summarized in Fig. 1.

2. Targeting to rough ER domains

The distinctive feature of the rough ER is the presence of ribosomes and protein translocation channels, also called translocons. Together, these proteins mediate the production of secretory proteins and proteins to be inserted into the membranes of the secretory pathway. Translocons are made up of more than 20 polypeptides that span the membrane individually multiple times [32]; associated with them are the translocon-associated protein (TRAP) complex, translocating chain-associated membrane protein (TRAM), oligosaccharyltransferases (OST), signal peptidase, the signal recognition particle (SRP) receptor and accessory proteins [33,34]. Within the translocons, the Sec61 α, β, and γ proteins are thought to capture and bind ribosomes [32,35,36] that are also associated with translation initiation factors and mRNAs [37,38]. Formation of these interactions is thus a critical determinant of rough ER formation. Moreover, the interaction between the Sec61 proteins and ribosomes depends on the formation of a complex between the ER-associated signal receptor and the SRP that recognizes mRNAs giving rise to signal peptides [39,40]. However, secretory protein mRNA localization to the ER does not require the presence of SRPs, suggesting these mRNAs are additionally equipped with so far unknown targeting information [41,42]. A special case of ER targeting of mRNAs is observed with the mRNA for the yeast transcription factor Hac1, the substrate of the ER-stress activated endonuclease Ire1p. Here, the 3′ untranslated region element is sufficient for the targeting to localized accumulation of Ire1 activity [43].

Overall, association with the translocon depends frequently on direct protein–protein interactions with translocon and ribosome components. This mechanism is utilized in particular by the OST complex [44], composed of ribophorin I and II, OST48, STT3A, STT3B, and DAD1 in mammals [45,46], which can bind to the translocon in a ribosome-dependent or -independent manner [46,47]. These divergent OST targeting mechanisms stem from the sequence of events in the glycosylation process, which comprises ribosome-requiring cotranslational N-glycosylation, but also posttranslational N-glycosylation of newly synthesized proteins [48]. For some subunits of the OST complex, specific targeting information has been described: ribophorin I requires luminal domains for its retention within the ER, whereas cytosolic and transmembrane sequences are needed in the case of ribophorin II [49,50]. The rough ER-localized Hsp40 family protein ERj1 interacts with ribosomes that mediate its targeting to the rough ER via a positively charged motif in its cytosolic domain [51]. Additional translocon-associated proteins are the Sec62/Sec63 complex proteins [52]. The phosphorylation of Sec63p by protein kinase CK2 is required for the association of this complex with the protein translocation apparatus in yeast [53].

In addition to the directly involved translocation machinery, the rough ER also contains ER chaperones and oxidoreductases, which mediate the folding of newly synthesized polypeptides. Not all proteins of this group target tightly to the rough ER; some perform important and in some instances predominant roles on other parts of the ER [54]. Another caveat is that some chaperones and oxidoreductases associate with translocons that are not bound to ribosomes, suggesting they are preferentially associated with retro-translocation of misfolded proteins on domains of the smooth ER (see below). GRP78/BiP is among the chaperones that interact with active, importing translocons and serves to close the translocon during protein integration into the ER membrane [55–57]. In yeast, it has been shown that this interaction boosts the ATPase activity of GRP78/BiP and depends on the binding to Sec63p, a member of the DnaJ family [58]. ER oxidoreductases are also found on ribosome-associated translocons, among them in particular ERp57 [59]. Another example is the lectin chaperone calnexin, a transmembrane protein, which associates with incompletely translocated polypeptides at the translocon [60,61]. Like Sec63, calnexin is a substrate of protein kinase