chapter **TWO**

The endoplasmic reticulum as a central organelle organizer

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Abstract

The Endoplasmic Reticulum (ER) fills the cytosolic space and is by far the largest intracellular compartment. The ER is traditionally considered a site for protein synthesis, folding and export while other functions are usually ignored. This may be a limited view. Due to its size and omnipresence, the ER functions as a membrane source for organelles such as autophagosomes, peroxisomes and lipid droplets and is able to contact all intracellular organelles to control lipid transfer, signaling and intracellular transport. Here we postulate a more central role of the ER in distributing software between intracellular compartments to control their biochemistry, transport, signal transduction and without doubt many other processes yet to be uncovered. The ER is not large just for protein synthesis, but applies its intracellular corpulence for organelle contacts and control.

From its first description in 1945, the Endoplasmic Reticulum (ER) has been one of the most studied cellular organelles. In fact, it represents an interconnected network of tubules and cisternae that occupies the entire cytosolic space. Rough ER (ER lined by ribosomes) defines sites of protein translation, whereas the smooth ER is considered a site of lipid production and storage of lipids and ions. A third ER 'subdomain' recognized is the nuclear envelope. The ER is by far the largest intracellular organelle containing about 70% of cellular lipids and contributes to generation of other organelles such as the Golgi complex. By specific interactions between ER resident and organelle resident proteins, the ER can generate membrane contact sites (MCSs) with other compartments. MCS's resemble intracellular synapses where membrane proteins from two compartments can directly interact and regulate processes as diverse as lipid and ion transfer but also microtubule-motor binding. Given its omnipresence in the cytosol, the ER is the basic compartment to integrate and coordinate communication with and between different intracellular compartments. Here we will discuss how the ER guides the biogenesis and maintenance of other, apparently unrelated organelles and how information is exchanged between the ER and these organelles. We will elaborate on how the ER can integrate, rank and distribute information gathered from the different organelles and discuss the molecular mechanisms as understood at present. These uncover a new role for the ER in cellular homeostasis: distributing biochemical information from organelles to control life and location of other intracellular compartments.

The ER as an Organelle birth ground

Considering the origin of membranes for organelles touches the basis of cellular life. Membranes in the endocytic system are derived from the plasma membrane and the Golgi is derived from the ER. Yet, the origin of membranes for organelles such as peroxisomes, autophagosomes and lipid droplets (LD) has long been elusive. As the ER holds most cellular lipids, it may be the prime candidate for donating membranes to other organelles. The ER is indeed a major contributor to the formation of autophagosomes, peroxisomes and LD and the mechanisms are beginning to be elucidated, as illustrated below.

As a first step in LD biogenesis, neutral lipids accumulate between ER bilayer leaflets at defined sites[1]. Subsequently the hemi-membrane will bud into the cytosol to provide the phospholipid monolayer, while lipids accumulate in the interior before the mature LD releases itself from the ER. An alternative model suggest that the ER and LD are not continuous but that the ER membrane is tightly wrapped around the lipid droplet resembling an egg cup (the ER) holding an egg (the lipid droplet)[2]. Proteins involved in lipid metabolism such as the ER resident FATP1/acyl-CoA synthetase and the lipid droplet protein DGAT2/diacylglycerol acyltransferase interact at these ER-LD contact sites[3] (figure 1). Another ER protein, Seipin, also localizes to these inter-organellar contact sites and determines the morphology of lipid droplets and their metabolism[4]. The ER is thus involved in the control of various steps in the birth and life of lipid droplets.

Autophagosomes are compartments induced by starvation and require membranes to encapsulate cytosol before fusion to lysosomes for the degradation of cytosolic material. But what is the origin of the membranes for this compartment? PI3P is required to initiate autophagosome

formation[5] and is produced by Atg14. Atg14 localizes to the ER and the phagophore (the autophagosome precursor). The ER is critical in this process as a mutant of Atg14 unable to bind the ER impairs autophagosome formation[5,6]. The specialized regions of the ER that provide the autophagosomal membranes are termed "omegasomes" [7]. Omegasomes are continuous with the ER membrane and cradles between two ER membranes during early stages of growth [8] (figure 2). How scission of the omegasome from the ER occurs is -like for other ER derived compartments- unclear, as is the role of the ER in later stages of autophagosome maturation and fusion with lysosomes.

A third compartment originating from the ER is the peroxisome[9-11]. Two subpopulations of pre-peroxisomal vesicles (each containing half of the peroxisomal translocon machinery) bud from the ER[12]. These two populations fuse later to assemble a functional translocon allowing the peroxisome to import matrix proteins for its maturation.

The ER is the largest membrane-containing compartment in the cell and contributes to the early life of different compartments. As in normal adult-child interactions, the ER stays in close contact with these (and other) organelles long after their initial birth.

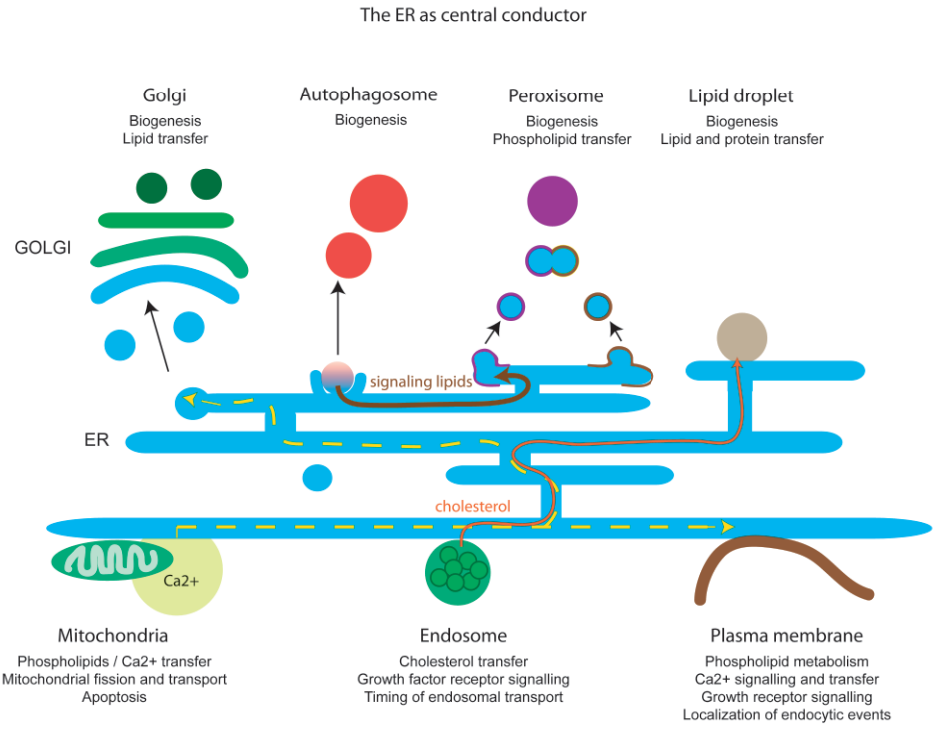


Figure 1. The ER as a central conductor. The ER is the largest intracellular compartment. Illustrated are new functions of the ER that relate to the birth of organelles (shown at top) and direct interactions in membrane contact sites- with organelles and the plasma membrane (bottom). The transfer of lipids and cholesterol by lateral diffusion in ER membranes is depicted. These and other molecules can use the ER to distribute to different compartments without exposure into the free cytosol. Some of the functions of organelles related to the ER is depicted in the figure.

Staying in touch

Membrane contact sites (MCS) are defined as sites where membranes of two organelles locate within 10-30 nm (for comparison, the thickness of a lipid bilayer is ~10 nm while a small protein like GFP is ~5 nm) (figure 3). Proteins of opposite compartments can interact to form functional inter-compartment interactions within this space[13,14]. MCS's are only recently recognized and their function (if only one) not fully specified. We asked leaders in this developing area on their opinion of the function of MCSs, and their quotes are summarized in Box 1. One dominant feeling is that information exchange between compartments occurs at MCS (as described below). In a way, the ER might be considered the cell's central nervous system, able to gather,

Box 1

Will Prinz National Institute of Diabetes and Digestive and Kidney diseases, NIH, Bethesda	"Membrane contact sites may be regions were signals and small molecules are effectively channeled between organelles, increasing the rate and efficiency of exchange."
Clare Futter UCL institute of Ophthalmology, London, UK	"The close apposition of the ER membrane with the membranes of other organelles generates a discrete micro-environment with the potential for lipid transfer and protein-protein interactions."
Scot Emr and Chris Stefan Cornell University, Ithaca, USA	"The endoplasmic reticulum is the foundation of the secretory pathway, as it serves essential roles in protein and lipid biosynthesis. Intracellular signaling at ER-organelle junctions provides an elegant system to coordinate protein and lipid synthesis in the ER, in response to cues from various compartments along the secretory and endocytic pathways."
Rik van der Kant and Jacques Neefjes, The Netherlands Cancer Institute, Amsterdam NL	"The ER controls the location of other compartments by affecting motor protein binding. This inter-organelle control is likely controlled by the transfer of lipids within the membrane contact sites.".
Tim Levine UCL institute of Ophthalmology, London, UK	"If lipids do not cross between organelles at contact sites by the action of lipid transfer proteins then I'll have been proved absolutely wrong."

integrate and distribute information between all organelles[13,15,16]. This information often comes in the form of (signaling) lipids and calcium that are exchanged at MCS between the ER and other organelles. The ER can subsequently distribute lipids via membrane diffusion and ions through the ER luminal space to other compartments thus acting as a passive, long-range pan-cellular distribution system[15]. MCS probably not only function as sites of substance transfer, but may also play a more active role in cellular homeostasis by sensing the need for ions, lipids (and other undefined substances) of individual organelles. The ER is thus a major distributor of molecules that are difficult to selectively distribute by other means. As a result, the ER senses the homeostasis of the different organelles in a cell. But what is transferred at MCS and how?

Information transfer at Membrane Contact Sites

Hydrophobic lipids can be distributed by vesicular transport or by lipid carriers[15]. Alternatively, lipids can use the ER for lateral diffusion to other compartments before actual transfer in MCS by lipid transfer proteins (summarized in figure 2). Examples of lipid transfer proteins are Golgi proteins such as CERT, Nir2, OSBP and possibly ORP9. These proteins possess an FFAT motif to contact the ER protein VAP (figure 2) for lipid transfer[17-19]. Cholesterol transport between endosomes and the ER also involves MCS containing ER protein ORP5 and endosomal proteins HRS and NPC-1[20,21]. Membrane contact sites between the ER and late endosome are established by another OSBP family member, ORP1L, that senses but may not transfer cholesterol[22]. Triglyceride synthesis in lipid droplets is controlled by the ER protein PATP1 [3]. In addition, the ER/Golgi resident PI/PC exchange protein Nir2 can appear on lipid droplets possibly to support lipid transfer at ER-lipid droplets contact sites [23]. Other lipid transfer proteins enriched in MCS between the ER and LD are Seipin (in yeast)[4], Adiphophilin[2] and possibly ORP2 [24]. The exchange of phospholipids between peroxisomes and the ER [25] likely also involves peroxisome-ER contacts sites[10,16]. In general, a theme emerges where the ER provides a distribution system for lipids and other compounds that cannot efficiently be delivered by simple diffusion in the hydrophilic milieu of the cytosol. The ER then dynamically contacts other compartments for specific exchange and delivery.

Data integration by membrane contact sites

While some lipids are membrane building blocks, other lipids can act as second messengers in signal transduction. If such lipids are transferred at MCS between compartments, signals are very specifically delivered in an undiluted manner. At present, there are two compartments defined for inter-compartmental signal control by the ER: the cell surface and mitochondria. Further studies may broaden this concept to other compartments.

How does the ER control signaling at the cell surface and mitochondria? Contact sites between ER and plasma membrane (PM) are particularly obvious in muscle where the sarcoplasmic reticulum (SR) (ER found in smooth and striated muscle) is positioned within 10-20 nm of the plasma membrane [26]. Upon ligand binding, many membrane receptors initiate the production of Inositol-1,4,5-trisphosphate (IP_3) at the PM. IP3 activates the IP3 receptor (IP3R) in the ER to release Ca^{2+} that acts as second messenger in signaling. After the depletion

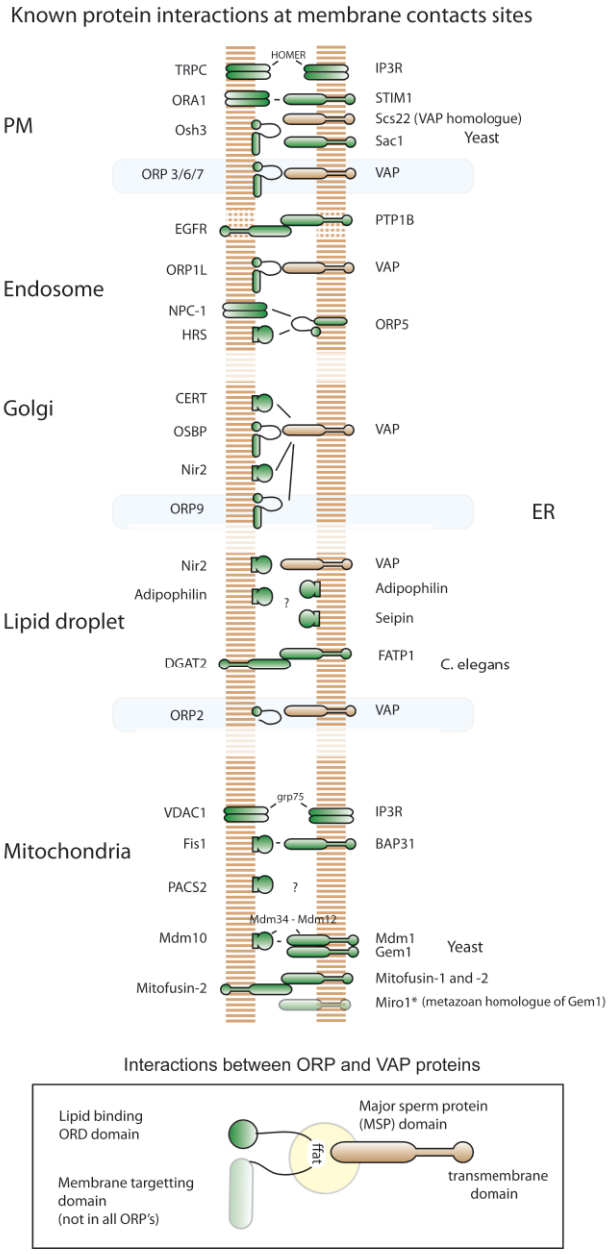


Figure 2. Protein-protein interactions in ER-organelle MCS's. ER protein interactions (Right) with proteins of other compartments as indicated (Left). These proteins meet in MCS's or MCS are formed as the result of these interactions. Proteins involved in these interactions are diverse. Bottom. Box illustrates a common interaction in MCS's where the integral ER protein VAP (**A** and **B**) (right) interacts with proteins containing a FFAT motif (meaning 'two phenylalanines in an acidic trail (Aspartate and Glutamate rich trail') (left). These proteins belong to from the ORP (oxysterol binding protein related protein, Osh in Yeast) family that are (often) lipid transfer proteins through the ORD domain. The FFAT motif is essential for interacting with the MSP domain of VAP (in yellow circle).

of Ca^{2+} from the ER lumen, Ca^{2+} channels in the PM open to allow entry of extra-cellular Ca^{2+} to fill the ER. Efficient directional transfer without long distance diffusion of Ca^{2+} in the cytosol is solved by MCS between plasma membrane and ER.

The molecular mechanisms are understood to some degree. The ER protein STIM1 is enriched at the PM-ER interface following depletion of Ca^{2+} from ER stores[27]. STIM1 then interacts with the ORA1 subunit of CRAC channels at the plasma membrane to open these for Ca^{2+} inflow[28]. IP3R in the ER also contacts other calcium (TRPC) channels in the plasma membrane via an adapter protein called Homer1[29]. Of note, Homer1 binds to a wide variety of receptors at the plasma membrane yielding probably a broader signaling response than currently anticipated[29]. As stated above, the first step in the response to calcium signaling is the generation of IP3. Also this may be cross-compartmentally controlled as studies in yeast suggest that ER-PM contact sites actually regulate plasma membrane PI metabolism[30], possibly controlling PI flux and calcium signaling at the plasma membrane via the ER. The plasma membrane controls Ca^{2+} release from the ER and the ER controls Ca^{2+} inflow via the plasma membrane to spatially control second messengers signaling.

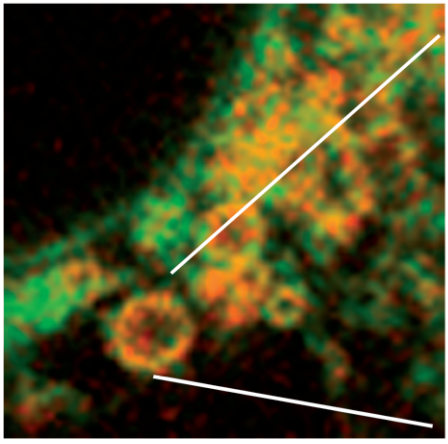
Is cross-talk between ER and plasma membrane the exception or does it exemplifies a broader concept? Other important mediators in Ca^{2+} signaling are mitochondria. Mitochondria store Ca^{2+} from the cytosol, participate in Ca^{2+} signaling and make frequent contacts with the ER to regulate Ca^{2+} transfer[31-35]. In addition, phospholipids (PS and PC) and probably cholesterol are also transported from the ER to mitochondria utilizing MCS[33,36]. How formation of these MCS is controlled is understood at some level of detail and involves the ERMES complex in yeast that includes Gem1[33,37]. Gem1 is a small GTPase with Ca^{2+} sensing abilities controlling phospholipid exchange between the ER and mitochondria[36]. The ERMES complex is absent in metazoans, but possibly replaced by another ER-mitochondria tethering complex of mitofusins that may interact with Miro1, the mammalian homologue of Gem1, to regulate ER-mitochondria MCS[37-39].

Local Ca^{2+} transfer between ER and other organelles has not yet been described. However, Ca^{2+} is involved in the dynamics of lysosomes[40], Golgi[41] and peroxisomes[42] and all of these organelles are also contacted by the ER. How the ER integrates lipid and Ca^{2+} signaling at MCS with these and other organelles, remains an intriguing question.

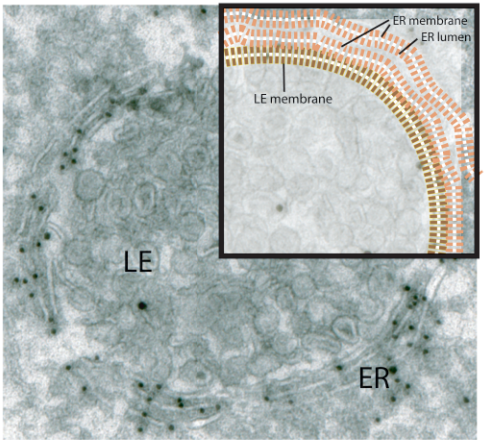
Textbook pictures suggest that a cell contains isolated organelles connected through vesicular transport. This view is now challenged by the concept of dynamic inter-organel interactions that serve to exchange lipid, ionic and other information between different compartments. The ER might serve as the central nervous system of the cell integrating signals acquired through the various MCS, acting as a middleman for different compartments to control intracellular dynamics and signaling.

The ER as terminator of extracellular signaling

The EGF-receptor (EGFR) is strongly phosphorylated following binding of EGF. Phosphorylation induces signal transduction that can be terminated by protein-tyrosine phosphatase 1B (PTP1B). A topological problem occurred when PTP1B was shown to reside on the cytosolic side of the ER implying the ER in control of EGFR signaling. Recent data indicate that the ER contacts



Confocal micropy
Green: VAP-A (ER protein)
Red: ORP1L (Late endosomal protein)



Immuno-EM
small gold: VAP-A (ER protein)
large gold: ORP1L (Late endosomal protein)

Figure 3. Contact site or fusion? Many organelles make transient - non-continuous - contacts with the ER. These can be easily observed by confocal microscopy (Left) when one compartment (late endosomes) is labeled differently than the ER. When ORP1L (a late endosomal protein) is expressed, the ER protein VAP perfectly colocalizes suggesting fusion of late endosomes with ER. The resolution of light microscopy is usually >240 nm leaving details below this unresolved. When the same cells are analyzed by electron microscopy, the formation of MCS between late endosomes and ER is observed with the two markers labeling exclusively their respective compartments. MCS appear as one membrane by light and as two by electron microscopy. No evidence for fusion of ER and late endosomes was observed at sufficient resolution (for details, see [22]).

endosomes carrying phosphorylated EGFR to induce contacts with PTP1B [43]. As PTP1B is involved in more signaling processes, the ER may have a broader involvement in the spatial and temporal control of these and other processes.

The ER as an organelle organizer

Is the ER only mediating inter-compartmental lipid and Ca^{2+} transfer at MCS or is the ER also actively controlling organelle behavior? Lipids and Ca^{2+} are known to affect the location of organelles, a system that might be co-regulated by the ER. The inter-compartmental control of these processes has only recently been realized and is illustrated by two examples that are understood in molecular detail: scission of mitochondria, and dynein motor-mediated transport of late endosomes and lysosomes.

One of the most spectacular examples of ER control of dynamics of other organelles is the fission of mitochondria[44]. The ER tightly wraps around mitochondria during fission events marking the site of mitochondrial division. The ER may further support fission by depositing Ca^{2+} at these (now scission) sites required for the recruitment of cytosolic Drp1 to mitochondrial membranes[45]. Drp1 is a dynamin-related protein that provides the mechanical force for fission[46].

Ca^{2+} also regulates mitochondrial transport via the calcium binding protein Miro1/Rhot1, a GTPase that interacts with motor proteins to regulate transport[47]. In yeast, Miro1 is found at the ER-mitochondrial interface, indicating a role for MCS in mitochondrial trafficking[39]. Of note, another protein enriched at the ER-mitochondrial interface is Fis1, a protein involved in apoptosis by controlling cytochrome C release. The ER-mitochondria MCS's may thus control apoptosis (figure 2)[48,49]. Since Fis1 and Drp1 are also involved in peroxisomal dynamics, a similar mechanism involving the ER may be at work there as well [50,51].

Endosomes move due to the activities of microtubule and actin-based motor proteins that determine their intracellular location[52]. The dynein motor transports vesicles such as late endosomes to the microtubule minus-end (where the microtubule organizing center (MTOC) resides). The dynein motor binds to late endosomes through the Rab7 effector RILP[53,54]. Rab7 also binds the FFAT motif containing cholesterol sensor ORP1L that changes conformation dependent on late endosomal cholesterol content [22]. Low LE cholesterol results in a conformation change of ORP1L allowing the FFAT motif in ORP1L to interact with ER-resident protein VAP-A. VAP-A then removes the dynein motor from late endosomes effectively allowing kinesin motors for transport to the cell periphery. Niemann-Pick and other lysosomal storage diseases such as Gaucher patients have lysosomes clustered around the MTOC and also high lysosomal cholesterol levels[55-57]. The cholesterol alters the conformation of ORP1L to not expose the FFAT motif thus preventing interactions with ER protein VAP-A. As a result, the dynein motor remains on the Rab7-RILP receptor and lysosomes cluster around the MTOC[22]. De-regulation of interactions within MCS's might also underlie other disease phenotypes such as Amyotrophic lateral sclerosis ALS[58], further illustrating the importance of inter-compartmental contacts in cellular homeostasis.

Concluding remarks

ER-organelle contact sites were first observed some 40 years ago [59]. Recent years have seen a burst of new observations and the beginning of a "molecular era of organelle contact sites" [60]. The ER is not only a site for protein synthesis and folding but is also a major organizer of other intracellular compartments, as a puppeteer controlling the marionettes. It is involved in the distribution of information in the form of Ca^{2+} and signaling lipids and the delivery of other lipids such as cholesterol between different compartments. In addition, the ER controls major cell biological events such as mitochondrial fission, organelle birth and transport of other compartments. As the ER fills the cytosolic space, it is probably the only compartment able to contact and connect every other organelle. The role of the ER as a master conductor that controls the nature of other intracellular compartments is only beginning to be appreciated and it is anticipated that many more processes involve this large and dynamic organelle. We are only at the beginning of understanding and appreciating the complexity of the ER as a central organizer of other intracellular compartments.

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