

## Review

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# Cellular microcompartments constitute general suborganellar functional units in cells

**Abstract:** All cells are compartmentalized to facilitate enzymatic reactions or cellular dynamics. In eukaryotic cells, organelles differ in their protein/lipid repertoire, luminal ion composition, pH, and redox status. In addition, organelles contain specialized subcompartments even within the same membrane or within its lumen. Moreover, the bacterial plasma membrane reveals a remarkable degree of organization, which is recapitulated in eukaryotic cells and often linked to cell signaling. Finally, protein-based compartments are also known in the bacterial and eukaryotic cytosol. As the organizing principle of such cellular subcompartments is likely similar, previous definitions like rafts, microdomains, and all kinds of ‘-somes’ fall short as a general denominator to describe such suborganellar structures. Within this review, we will introduce the term *cellular microcompartment* as a general suborganellar functional unit and discuss its relevance to understand subcellular organization and function.

**Keywords:** cellular microcompartment; membrane domain; microdomain; molecular scaffold, organelle.

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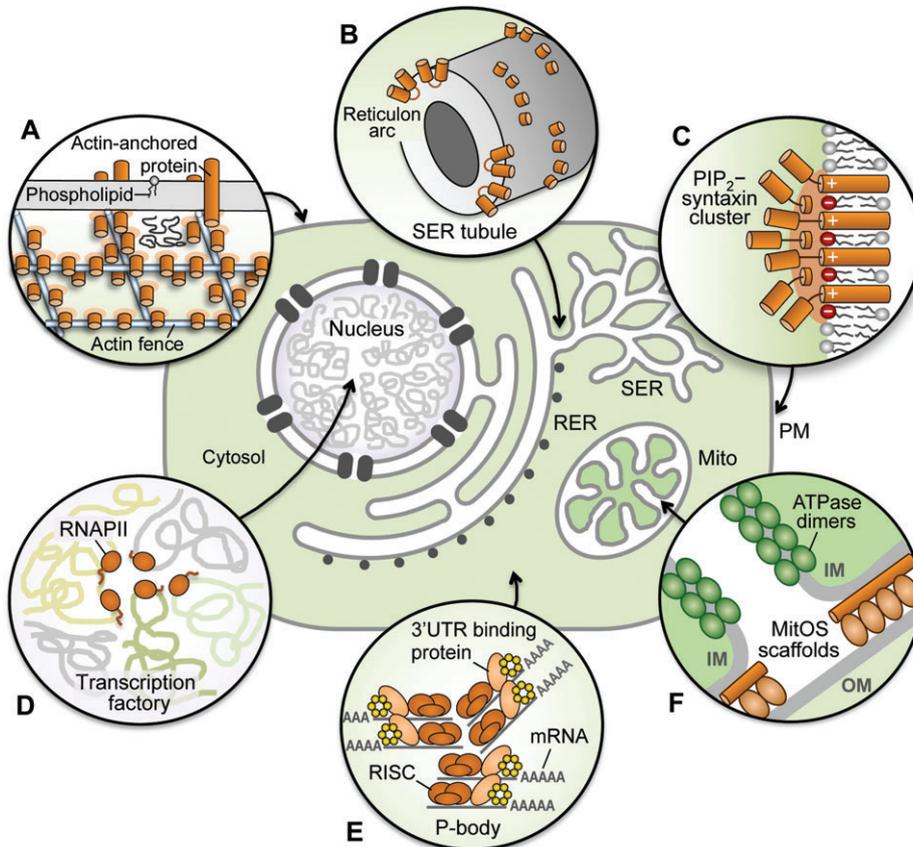
## Introduction

Cells have adapted in several ways to optimize reactions and responses to external and internal stimuli. Most obvious, eukaryotic cells are compartmentalized into organelles with defined protein composition and a dedicated enzyme repertoire for a particular metabolic reaction. However, even within the bacterial plasma membrane or eukaryotic organelle membranes, several subcompartments exist and provide distinct functions

(Shapiro et al., 2009). For instance, the mitochondria are subdivided into the inner and outer membrane, the intermembrane space, and the matrix. Recently, the inner mitochondrial membrane was further functionally subdivided into the inner boundary membrane and the cristae, and a novel protein complex seems to separate the two segments, indicating that specialized zones exist even within one organellar membrane (Shapiro et al., 2009; Harner et al., 2011; Hoppins et al., 2011; Malsburg et al., 2011). Importantly, the understanding of a particular reaction cascade within such a membrane will fall short, if only the individual enzyme complexes are analyzed without appreciating the microenvironment, in which they act. We therefore use here the term *cellular microcompartment* to describe the principle that underlies the organization of proteins in their microenvironment, which can be based on proteinaceous or membranous scaffolds. We thus extend a term, which was initially coined for bacterial protein-surrounded compartments, to all cells.

Microcompartments can be subdomains on an organelle membrane such as subdomains on endosomes (de Renzis et al., 2002), the plasma membrane (Kusumi et al., 2012), the ER (Stefan et al., 2011), the Golgi (Bankaitis, 2009), or protein-lipid-based supercomplexes of the mitochondrial respiratory chain (Schagger and Pfeiffer, 2000). They also include contact zones between distinct membranes (Elbaz and Schuldiner, 2011) or defined regions within the bacterial plasma membrane (Shapiro et al., 2009). Even the cytosol of bacteria and eukaryotic cells, and the lumen of the nucleus contain subcompartments, which use protein scaffolds such as the cytoskeleton, or RNA and DNA polymers as an assembly platform (Eulalio et al., 2007; Kaganovich et al., 2008; Shapiro et al., 2009).

This review will provide an overview of our understanding of cellular microcompartments in the context of cell function and physiology. We will use selected examples to initially discuss microcompartments based on protein-protein and protein-DNA/RNA interactions within the cytosol and nucleus, as well as membrane-bound microcompartments (Figure 1). Within the second part,



**Figure 1** Schematic diagram of an eukaryotic cell depicting a diversity of microcompartments.

(A) Actin skeleton-based subcompartmentment of the plasma membrane; (B) Reticulon-mediated segregation of ER in the tubular/smooth ER and peripheral/rough ER; (C) Syntaxin-1/PIP<sub>2</sub> cluster at the plasma membrane; (D) RNAPII transcription factory in the nucleus; (E) RNA processing or P-body in the cytosol; (F) MitOS scaffold-based subcompartmentment of the inner mitochondrial membrane. See text for details. ER, endoplasmic reticulum; RER, rough ER; SER, smooth ER; Mito, mitochondrion; OM, outer membrane; IM, inner membrane. PM, plasma membrane.

two examples, the endosomal system and the membrane contact sites will be presented in more detail to highlight some organizing principles that have emerged so far.

## Microcompartments within the cytosol and nucleus

Initially, the cytosol was considered a homogenous protein mixture with several enzymes involved in metabolic reactions. With the advent of electron microscopy and modern cell biology, it became clear that also the cytosol contains defined large structures (Palade, 1964). This includes large multiprotein complexes such as the fatty acid synthase or the ribosome. In evolution, the fatty acid synthase complex changed from eight individual polypeptide chains to just two large polypeptides with multiple activities, which surround a central

reaction chamber (Maier et al., 2008). Beyond such large polypeptide complexes, it is known that proteins can be organized into dynamic substructures by taking advantage of protein or nucleic acid scaffolds, along which proteins are organized into functional units. Here, we will provide a brief overview of some microcompartments of the cytosol and nucleus as an entry to the overall topic.

The term microcompartment initially referred to bacterial reaction centers such as the carboxysome (Cheng et al., 2008; Yeates et al., 2008). A protein scaffold forms a rigid structure around ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase to promote carbon fixation. Such an arrangement concentrates enzymes that act in consecutive reactions in a closed environment and maintains continuous exchange of metabolites between the lumen of the carboxysome and the surrounding cytoplasm. From a structural point of view, bacterial microcompartments are in essence in

their structure similar to a virus shell and, indeed, rely on similar very simple building blocks (Yeates et al., 2008; Parsons et al., 2010; Tanaka et al., 2010).

The clustering of metabolic enzymes is also well known for glycolytic enzymes in muscle cells of *Drosophila*. Here, enzymes like glycerol-3-phosphate dehydrogenase (GAPDH) and aldolase localize close to the Z-line of the muscle cell. This GAPDH localization is essential for flight muscle function as mutant flies with enzymes lacking the respective localization sequence cannot fly (Wojtas et al., 1997; Sullivan et al., 2003). In other organisms such as the salamander *Necturus*, pyruvate kinase is localized close to the potassium channel of the basolateral membrane, again suggesting a tight coupling of glycolytic enzyme localization and intracellular function in a metabolic microcompartment (Dubinsky et al., 1998). It is possible that microcompartments allow for a fast and efficient substrate channeling in response to metabolic needs.

Besides metabolic enzymes, regulatory protein complexes seem to exist in plant cells and likely in all meta-zoan cells. In the plant nucleus and the cytoplasm, redox-based microcompartments have been postulated that control flower development and have been localized in association with the cytoskeleton and within the nucleus at the promoter regions of key proteins involved in development (see Zachgo et al., 2013). As redox alterations are tightly linked to cellular stress responses, it will be important to analyze such cascades in the future. Within mammalian cells, this remains a poorly explored issue.

Nuclear and cytosolic scaffolds are responsible for several microcompartments. Two microcompartments are the juxtannuclear quality control (JUNQ) and the insoluble protein deposit (IPOD) compartment, where cellular deposits such as aggregates have been detected (Kaganovich et al., 2008). As they are found proximal to the vacuole and nuclear ER, they may include membrane-binding domains. Another microcompartment involved in cellular organization is the polarisome, which is also involved in retrograde transport of misfolded proteins back to the mother cell (Liu et al., 2010). Both JUNQ, IPOD, and polarisome seem to be critical for the protein homeostasis of the cell, which avoids the accumulation of unfolded proteins by all means. The identification of Hsp104 in the IPOD and the proteasome in JUNQ indicates that protein deposits will have a defined, but dynamic composition and are likely tightly regulated. Whether the IPOD compartment, which is proximal to the lysosomal vacuole in yeast, and contains *bona fide* autophagy markers such as Atg8/LC3 (Kaganovich et al., 2008), is eventually consumed by autophagy, is not yet known.

Likewise, the particular proteins responsible for the accumulation of the IPOD and JUNQ to their subcellular localization have not been identified.

The DNA- and RNA-based scaffolding seems to be a common principle for several cellular microcompartments. This could include the storage and turnover of mRNA in cytosolic P bodies (Figure 1E) (Eulalio et al., 2007), where mRNAs may be both cargo and scaffold or platform for its assembly, the rearrangement of the genome into dynamic microcompartments to fine-tune gene expression, the congregation of RNA polymerase II complexes into transcription factories (Figure 1D), chromosome clustering due to chromatin modification, the clustering of tRNA genes at the nucleolus, and the assembly of nuclear PML and Cajal bodies, which participate in various RNA-related metabolic processes (Nicodemi and Prisco, 2009; Meldi and Brickner, 2011). For cytosolic clusters, the association with the cytoskeletal structures or membranes is also probable as discussed for the glycolytic enzymes (Wojtas et al., 1997; Sullivan et al., 2003) (see Zachgo et al., 2013). For most of these structures, the turnover and spatiotemporal dynamics of its constituents remains unclear. Both likely determine the size of the respective microcompartment.

It is very likely that each of these microcompartments will be subject to tight regulation and will rearrange in response to redox changes, starvation, or other cellular stress situations. This set of examples show that microcompartmentation within the nucleus and cytoplasm is a widespread phenomenon to coordinate physiological reactions in a compact environment.

## Microcompartments within membranes

Membranes define the boundaries of cells and their internal organelles and, consequently, are fundamental to the compartmentalization of cellular metabolic pathways and networks. Importantly, cellular membranes do not behave as homogenous two-dimensional liquids. On the contrary, they are heterogeneous entities that contain various structures on nano-mesoscale (5–500 nm), where particular membrane molecules stay together for limited periods of time to influence the fundamental cellular processes such as nutrient uptake, signaling, and endo/exocytosis (Kusumi et al., 2012). The molecular principles that govern the formation of the microcompartments in the cellular membranes are complex and only partially understood.

One level of complexity is that the cellular membranes not only comprise a rich variety of proteins but also contain hundreds of different lipid species that are non-randomly distributed between organelles and even within individual organellar membranes. Studies with model membranes revealed that mixtures of lipids mimicking the composition of the outer leaflet of the plasma membrane exhibit liquid-liquid immiscibility, creating laterally segregated lipid assemblies enriched in sphingolipids and sterols (Simons and Vaz, 2004; Lingwood and Simons, 2010). Whether these self-organizing lipid assemblies, termed rafts, also form in membranes of living cells has been subject of much debate (Munro, 2003). In spite of overwhelming evidence for co-migration of sphingolipids and sterols in cells, there is no consensus on the size and lifetime of rafts. There are also contradictory views on how rafts would organize themselves in the presence of proteins. One model proposes that some proteins serve as nucleation sites for raft domains by organizing ‘shells’ of raft lipids around their membrane spans (Anderson and Jacobson, 2002; Sharpe et al., 2010). Recent identification of a conserved sphingolipid-binding signature in a variety of membrane proteins (Contreras et al., 2012) is consistent with the idea that specific lipids may influence the lateral organization of proteins and vice versa. In addition, there is evidence that electrostatic protein-lipid interactions can generate membrane microdomains enriched in phosphoinositide-4,5-bisphosphate (PIP<sub>2</sub>) and the SNAP-receptor syntaxin-A1 (Figure 1C). These amorphous protein-lipid assemblies form independently of rafts and may serve to enhance the efficiency of membrane fusion during exocytosis (van den Bogaart et al., 2011).

Further complexity is added by the fact that the two leaflets of cellular membranes can have very different protein and lipid compositions, so that protein-lipid assemblies present on one side could, in principle, be independent of those formed on the other side. High-speed single molecule tracking experiments on the surface of live cells revealed that the plasma membrane is compartmentalized for the diffusion of both proteins and lipids (Fujiwara et al., 2002; Kusumi et al., 2012). This has been ascribed to the filamentous actin meshwork underneath the plasma membrane and to the transmembrane proteins anchored to and aligned along the actin filaments, with the latter forming rows of pickets that confine the free diffusion of lipids and lipid-anchored proteins in the exoplasmic leaflet (Figure 1A). It is believed that this spatial confinement plays important roles in clathrin-coated pit formation,

B-cell receptor signaling, and EGF receptor signaling in tumors (Kusumi et al., 2012).

Besides being parceled up in zones of confined diffusion by the membrane-associated actin skeleton, the plasma membrane is studded with morphologically distinct domains like microvilli, cell-cell junctions, caveolae, and coated pits. Each of these suborganellar structures has a specialized function, such as nutrient uptake, cell-cell communication, and endocytosis. Yet, their formation generally follows the same basic principle, involving the assembly of peripheral or integral membrane proteins into a molecular scaffold that influences the local composition of the bilayer by attracting some additional proteins and lipids while excluding others. The biogenesis of caveolae, for instance, relies on the incorporation of caveolins and cavins into multimeric membrane-embedded protein complexes that create flask-shaped invaginations of the plasma membrane enriched in sphingolipids and cholesterol (Bastiani and Parton, 2010). Caveolae have been implicated in a wide variety of cellular processes, including fatty acid uptake, endocytosis, cell signaling, and mechanosensing.

Protein scaffolds also play a key role in the functional compartmentalization of the mitochondria (Figure 1F) (Harner et al., 2011; Hoppins et al., 2011; Malsburg et al., 2011; Appelhans et al., 2012), endosomes, and the endoplasmic reticulum (ER). The latter organelle is subdivided into a tubular network of smooth ER (SER) involved in calcium storage and lipid synthesis, peripheral sheets of rough ER (RER) specialized in protein translocation, ER exit sites where protein cargo is collected for departure to the Golgi complex, and the nuclear envelope with nuclear pores allowing the exchange of water-soluble molecules between the nucleus and cytosol. ER tubules are created by reticulons. These proteins harbor long hydrophobic segments that form hairpins, which may adopt a wedge shape that curves the membrane (Figure 1B) (Voeltz et al., 2006). Reticulons also assemble into homo- and heterooligomers that create arc-like scaffolds around the ER tubules. Interestingly, these oligomers are also found on the edges of ER sheets (Shibata et al., 2010). Reticulon-mediated stabilization of sheet edges may enable the upper and lower membrane of the sheets to adopt planar shapes. The latter would provide a better surface for the binding of large polysomes. Thus, the curvature-stabilizing reticulons seem to cooperate with membrane-bound polysomes and associated membrane proteins to form segregated SER and RER domains that are functionally specialized in lipid synthesis and protein translocation, respectively.

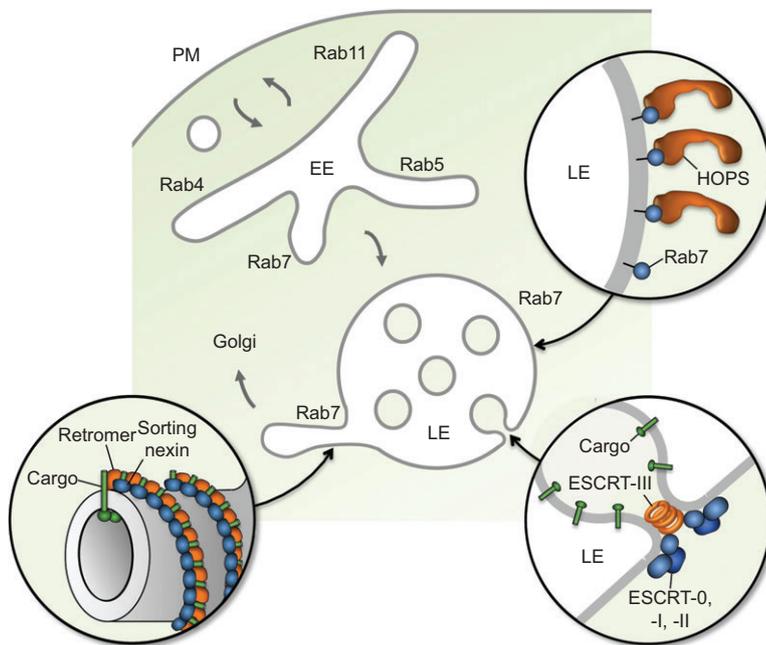
## The endosomal membrane as a paradigm of a dynamic membrane microcompartment

As discussed above, membranes can be laterally subdivided into several functional zones. Here, we will use the endosome as an example to illustrate how protein-protein and protein-lipid crosstalk can mediate subcompartmentation on one membrane.

Endosomes form, as a consequence of vesicular traffic, from the plasma membrane and the Golgi, which merge at the early endosome. Early endosomes further mature into late endosomes but also need to recycle receptors back to the plasma membrane. It was thus no surprise that endosomes have distinct zones that are positive for Rab-GTPases involved in early endosome function (Rab5), receptor recycling back to the plasma membrane (Rab11), and late endosome function (Rab7) (Figure 2) (Sonnichsen et al., 2000; de Renzis et al., 2002; Rink et al., 2005). Rab GTPases are switch-like proteins that require guanine nucleotide exchange via a GEF, bind in their active GTP-form to effectors, and require a GTPase-activating protein (GAP) for efficient nucleotide hydrolysis (Barr and Lambright, 2010; Itzen and Goody, 2011). Rab binding to effectors is important for membrane organization and fusion.

Even though the exact organization of such zones is not yet known, the lateral segregation of membrane domains on endosomes might follow similar principles as described above for other membrane domains and will involve Rab-specific binding of effectors as well as the generation of specific phospholipids like phosphoinositide-3-phosphate (PI-3-P), which serves as a second recognition signal along the endocytic pathway (Huotari and Helenius, 2011). Some proteins like mammalian rabenosyn bind, for instance, both Rab4 and Rab5 and could bridge different membrane domains (de Renzis et al., 2002). Likewise, the Rab5 guanine nucleotide exchange factor Rabex5 binds to rabaptin 5, which interacts with the activated Rab5 (Horiuchi et al., 1997). This combination of GEF and effector might stabilize such membrane domains. The better an effector binds, the more extensive the domain may be, thus separating fusion from recycling zones. If similar principles apply for the other Rab zones on endosomes, the separation into several membrane microcompartments would depend on the relative levels of activated Rab on the endosomal membrane.

Besides the formation of distinct domains, endosomes also have additional mechanisms to generate microcompartments that are specific for receptor downregulation, recycling, and finally fusion. When early endosomes mature, three membrane-active complexes act on



**Figure 2** Subcompartmentation of endosomes.

Early endosomes (EE) can contain multiple Rab-GTPase-positive domains. Rab7-positive late endosomes (LE) have several microcompartments, required for recycling via retromer (A), intraluminal sorting via ESCRTs (B), and fusion via Rab7-HOPS (C). See text for details.

endosomes likely in a coordinated manner: (i) receptors and other membrane proteins that are destined for down-regulation will be sorted into intraluminal vesicles via the ESCRT complexes (Henne et al., 2011); (ii) recycling of cargo receptors that deliver hydrolases to the vacuole occurs via the retromer complex (Bonifacino and Hurley, 2008); and (iii) finally, fusion of mature late endosomes occurs via Rab7, its HOPS effector complex, and the endolysosomal SNAREs (Figure 2) (Epp et al., 2011). It is expected that the first two machineries act in parallel and could separate the cargo into microdomains on membranes via their respective binding affinities. Both complexes may be considered both as scaffolds and active constituents that define their microcompartment. It is expected that both ESCRT and retromer should have finished their sorting events before an endosome can fuse with the lysosome. Indeed, mature endosomes are filled with vesicles and lack retromer cargo (Russell and Odorizzi, 2012), which was sorted into tubular carriers beforehand (Cullen, 2008). Interestingly, retromer also binds Rab7, which is the receptor for the HOPS fusion complex and requires this Rab for its recruitment to the endosomal membrane (Rojas et al., 2008; Seaman et al., 2009). One possible scenario would be that retromer function would need to be finished first before Rab7 becomes available for HOPS and thus acts in fusion. Two studies support this idea. First, overexpressed yeast Rab7 (Ypt7) causes a premature fusion with vacuoles and delivery of cargo receptors to the vacuole surface (Balderhaar et al., 2010). Second, mutants in retromer that bind efficiently to Rab7 interfere with vacuole morphology, likely due to a prolonged interaction of retromer with Rab7 (Liu et al., 2012).

The Rab7 binding to two distinct machineries that need to be coordinated at the late endosome leaves the question on how Rab7 activation is coordinated. If active Rab7 becomes available, it should first bind retromer, and not the fusion machinery. The GEF for Rab7 is the Mon1-Ccz1 complex (Nordmann et al., 2010), which likely has a similar function in *Caenorhabditis elegans* and humans (Kinchen and Ravichandran, 2010; Poteryaev et al., 2010). It has been shown that Rab5 and Rab7 are present in a consecutive order on the same membrane, in agreement with a maturation of the early to the late endosome (Rink et al., 2005; Poteryaev et al., 2010; Huotari and Helenius, 2011). It is thus plausible that Rab5 is involved in the recruitment of the Rab7 GEF, which has been suggested by competition assays (Poteryaev et al., 2010) (Figure 2).

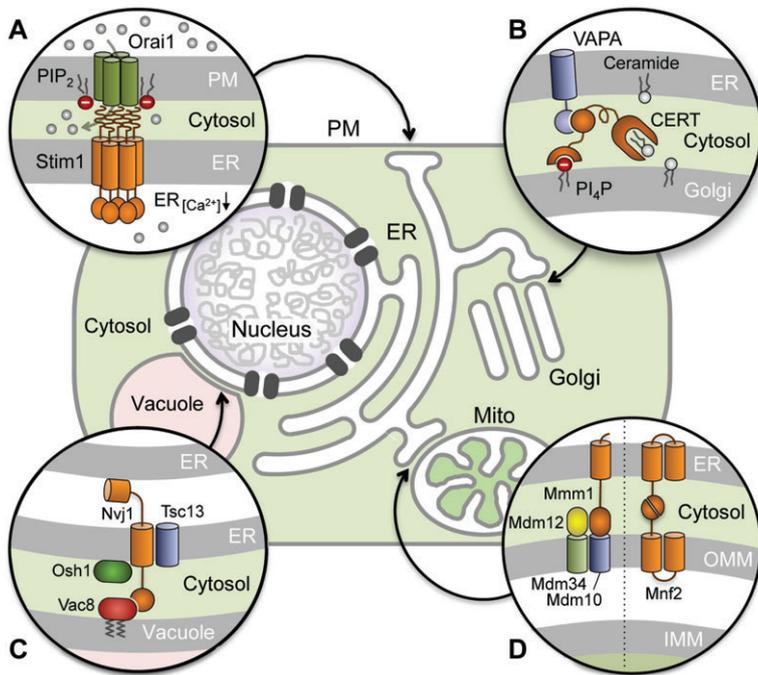
This complicated, but incomplete description of the crosstalk during endosomal maturation indicates how several microcompartments may form in a consecutive order on a single endosome and could contribute to its

alteration. Taking into account that endosomes and lysosomes also participate in multiple signaling and sensing events (Gould and Lippincott-Schwartz, 2009; Sancak et al., 2010), involving distinct protein complexes on each organelle, it becomes obvious that these multiple microcompartments along the endocytic pathway will be highly cooperative and regulated.

## Organellar contact zones as novel microcompartments

The subdivision of eukaryotic cells in distinct membrane-bound organelles creates a need for inter-organelle exchange of information and metabolites. One way in which organelles communicate and coordinate cellular activities is through the membrane contact sites (MCS), regions where the membranes of two organelles come within 10–30 nm of one another (Elbaz and Schuldiner, 2011; Toulmay and Prinz, 2011). The MCSs are laterally differentiated subcompartments that contain the molecular tethers, by which two opposing organelles are physically interconnected as well as proteins that are specialized to mediate the inter-organelle transfer of metabolic cues and small molecules, such as ions and lipids (Figure 3).

MCSs were first described between the ER and the mitochondria, where they are implicated in the exchange of  $\text{Ca}^{2+}$  and lipids (Rizzuto et al., 1998). Mitochondrial uptake of  $\text{Ca}^{2+}$  released from the ER is important for the tuning of the mitochondrial activity through the activation of  $\text{Ca}^{2+}$ -sensitive matrix dehydrogenases and transporters, whereas  $\text{Ca}^{2+}$ -overload can cause permeabilization of the outer mitochondrial membrane and trigger cell death (Rizzuto, 2006). The finding that tightening the physical link between the ER and the mitochondria with synthetic tethers makes the mitochondria susceptible to  $\text{Ca}^{2+}$  overload and cells prone to apoptosis (Csordás et al., 2006) points at a critical role of ER-mitochondria MCSs in  $\text{Ca}^{2+}$  signaling. In mammalian cells, a chaperone-mediated interaction between the mitochondrial voltage-dependent anion channel VDAC1 and the ER-resident  $\text{Ca}^{2+}$  channel inositol trisphosphate receptor IP3R (Szabadkai et al., 2006) as well as the homotypic interaction between the ER and the mitochondria-associated pools of mitofusin 2 (MFN) (de Brito and Scorrano, 2008) contribute to the physical link between the two organelles (Figure 3D, right). However, the removal of IP3R and MFN2 in each case has only a minor effect on the ER-mitochondria MCS formation, suggesting that these proteins have redundant functions in tethering or play an indirect role in this process.



**Figure 3** Membrane contact sites as novel microcompartments.

The endoplasmic reticulum (ER) forms membrane contact sites (MCS) with multiple organelles, including the plasma membrane (A), Golgi complex (B), vacuole (C) and mitochondria (D). See text for details.

Besides providing a major store of  $\text{Ca}^{2+}$ , the ER also synthesizes the bulk of the membrane lipids in a cell. Because the mitochondria are not connected by vesicular trafficking pathways, they must import the lipid components of their inner and outer membranes by a non-vesicular mechanism from the ER. Cell fractionation experiments showed the co-sedimentation of mitochondria with a subdomain of the ER enriched in phospholipid biosynthetic enzymes, referred to as the mitochondria-associated membranes (MAM) (Rusiñol et al., 1994). The observation that newly synthesized phosphatidylserine specifically accumulates in the MAM fraction when blocking its translocation to the mitochondria argues that MCSs are important for lipids to traverse from the ER to the mitochondria (Daum and Vance, 1997). Consistent with this idea, mutants lacking the components of a protein complex that provides a physical tether between the ER and the mitochondria in yeast, the ERMES complex, show a reduced exchange of phospholipids between the two compartments (Kornmann et al., 2009). ERMES contains one ER protein (Mmm1), two outer mitochondrial membrane proteins (Mdm10, Mdm34), and one cytosolic protein (Mdm12; Figure 3D, left). Whether ERMES has a direct role in shuttling lipids between the ER and the mitochondria or merely facilitates the recruitment of soluble lipid transfer proteins at the ER-mitochondria MCSs is unclear.

The MCSs also form between the the ER and the plasma membrane (PM), although their structural components are still largely unknown. In muscle cells, ER-PM contact sites are needed for  $\text{Ca}^{2+}$  exchange between these organelles and for excitation-contraction coupling. Upon ER  $\text{Ca}^{2+}$  depletion, the ER  $\text{Ca}^{2+}$  sensor STIM1 and the PM-resident  $\text{Ca}^{2+}$ -channel ORAI1 interact, promoting the formation of new ER-PM contact sites and resulting in a  $\text{Ca}^{2+}$  influx across the PM to allow the replenishment of the  $\text{Ca}^{2+}$  reservoir in the ER (Figure 3A) (Wu et al., 2006). The ER-PM contact sites are also believed to play a critical role in the non-vesicular exchange of phospholipids and sterols between the ER and PM (Milligan et al., 1997; Schulz et al., 2009).

Several cytosolic proteins have been implicated in the non-vesicular lipid transfer between the ER and the other organelles. The ceramide transfer protein CERT extracts newly synthesized ceramide from the ER and carries it to the Golgi complex for conversion into sphingomyelin (Hanada et al., 2003). CERT contains a pleckstrin homology (PH) domain that binds to the Golgi, a FFAT motif that binds to the ER, and a START domain that binds ceramide. Consequently, CERT might bind ER and Golgi membranes simultaneously at MCSs, allowing the START domain to mediate rapid lipid transfer (Figure 3B). The MCSs between the ER and the Golgi have been observed by electron microscopy (Ladinsky et al., 1999). A similar arrangement

has been reported for the oxysterol-binding proteins Osh2 and Osh3, which contain FFAT and PH domains required for targeting them to ER-PM contact sites (Toulmay and Prinz, 2011). Osh proteins have previously been implicated in non-vesicular sterol trafficking between the ER and the PM, but recent work suggests an additional role of these proteins as phosphatidylinositol-4-phosphate (PI-4-P) sensors that modulate PI-4-P levels in the PM by regulating the ER-resident PI-4-P phosphatase Sac1 at the ER-PM contact sites (Stefan et al., 2011). A recent study of Drin, Antonny, and coworkers demonstrated that Osh4 binds sterols and PI-4-P in the same binding site and may, thus, generate a sterol gradient between the ER and the Golgi (de Saint-Jean et al., 2011). Such a behavior may apply to all sterol-binding proteins, which may use PIPs for their specific targeting and sterol delivery. A structurally related protein, ORP1L, is believed to act as a sterol sensor that controls MCS formation between the ER and the endosomes (Rocha et al., 2009).

The MCSs also participate in cellular processes beyond the interorganellar exchange of small molecules and metabolic cues. In yeast, contact sites between the nucleus and vacuole, termed nucleus-vacuole junctions (NVJ), form by the association of the vacuolar protein Vac8 and the ER-resident protein Nvj1 (Figure 3C). NVJ formation is necessary for piecemeal microautophagy of the nucleus (PMN), which can be induced upon carbon or nitrogen starvation (Roberts et al., 2003). The sterol-binding protein Osh1 and fatty acid elongase Tsc13 localize to the NVJ. The recent finding that sterol and sphingolipid biosynthetic mutants are defective in PMN suggests that a specific lipid environment at the NVJ is required to recruit proteins required for PMN (Dawaliby and Mayer, 2010). Whereas the molecular architecture and cellular functions of MCSs have only recently begun to emerge, it is clear that these suborganellar structures are highly dynamic and subject to tight regulation, involving extensive crosstalk between proteins and lipids. Whether they also include key scaffolding proteins or are defined by the interaction of the constituents of either the participating membrane remains an open question as only a few have been described in detail, and none has been reconstituted.

## Summary, perspectives, and challenges

The spatial organization of cells is of profound importance for their vital functions. Spatial organization is achieved through membrane-mediated compartmentalization of

cells into subcellular organelles. These organelles, in turn, are subdivided into distinct microcompartments that coexist within the same membrane or lumen, with each microcompartment having a specialized function. This is apparent when discussing the subdivision of the mitochondrial inner membrane, the membrane domains on the endosomes, or the contact zones between the organelles. This concept has several advantages. It provides a common denominator to describe the subcompartments in membranes, within organelles, or within the cytosol. It is clearly separated from organellar compartments and also excludes single protein-protein interactions. The emerging concept of ‘cellular microcompartments’ thus provides an additional level of sophistication, by which cells can coordinate the mind-blowing array of chemical reactions that define the living state.

Understanding how microcompartments form and execute their specialized functions poses a major problem that will confront cell biologists for many years to come. One of the challenges is that microcompartments exist over a wide range of spatial and temporal scales, with dimensions often below the resolution of conventional light microscopes. This dynamic behavior also urges a need to study the microcompartments under a wide variety of conditions to fully grasp their function and regulation. The microcompartments in the membranes have particular challenges, as their formation relies on a complex, often poorly understood interplay between proteins and lipids. We expect that the principles of membrane-bound microcompartments will follow similar rules, which may be determined by key proteins that may sculpt the membrane or undergo limited polymerization on the membranes. Likewise, the participation of the key lipids may be critical for their formation, e.g., sterols. It will thus be critical to understand the composition, dynamics, and turnover of the key constituents to unravel the common principles. Likewise, non-membrane-associated compartments will likely take advantage of the binding platforms, which could be scaffolding proteins like the cytoskeleton.

Given the current progress in high-speed and super-resolution microscopy (see Hensel et al. unpublished), combined with ongoing developments in the emerging fields of chemical and systems biology, we anticipate that the next few years will see a revolution in our perception of microcompartments as suborganellar functional units in cells.

**Acknowledgments:** The authors thank Helmut Wiczorek for discussions and suggestions. Their research is funded by the DFG (Sonderforschungsbereich 944, Project 11 and 14).

Received July 30, 2012; accepted September 28, 2012

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