Research Focus

Special delivery from mitochondria to peroxisomes

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Inter-organellar communication and interactions are necessary and accepted consequences of the segregation of biochemical functions into subcellular organelles. Recently, Heidi McBride and her collaborators found a novel link between mitochondria and peroxisomes in their discovery of mitochondria-derived vesicles (MDVs), which appear to fuse with a fraction of pre-existing peroxisomes in mammalian cells. We discuss the potential role of this vesicle population in the context of pathways for the exchange of metabolites and/or macromolecules between these compartments.

Mitochondrial vesicles carrying specific cargo fuse with peroxisomes

Since the discovery of peroxisomes, there has been speculation about the origin of this compartment. The peroxisome is intimately involved in many lipid metabolic pathways, and its dysfunction causes many human peroxisome biogenesis disorders (PBDs). Since the discovery of peroxisomes, there has been speculation about the origin of this compartment. The current view is that peroxisomes arise both from the division of pre-existing peroxisomes and from the endoplasmic reticulum (ER) [1–5]. However, previous studies have invoked the mitochondrion [6], the ER [2–5,7] or endosymbionts [8] as sources of peroxisomes. This was based on the knowledge that various metabolites are shared among peroxisomes, mitochondria and the ER, and the observation that these organelles are usually in close proximity to each other or even in physical contact [9,10]. Some of the work in a recent paper by Neuspiel *et al.* [11] adds to this body of knowledge by providing strong support that previously uncharacterized mitochondria-derived vesicles (MDVs) account for a specific form of vesicular traffic between mitochondria and peroxisomes. We discuss the observations made by Neuspiel *et al.* [11] and provide our thoughts on the roles that such trafficking might play, drawing upon knowledge of similar transactions between other organelles.

Identification of mitochondria-derived vesicles

In a bioinformatics search for proteins influencing mitochondrial morphology, Neuspiel *et al.* [11] identified a candidate containing a conserved really interesting new gene (RING) domain, and they named this protein MAPL (mitochondria-anchored protein ligase) [11]. Overexpression of a MAPL–yellow fluorescent protein (YFP) fusion led to partial fragmentation of mitochondria provided that the RING-domain, a motif often found in zinc-binding, ubiquitin E3 ligases, remained intact. Mitochondrial fission, which is dependent on a dynamin-related or dynamin-like protein (DRP1/DLP1), did not occur in MAPL–YFP-overexpressing cells that were co-transfected with a dominant interfering mutant of DRP1, DRP1(K38E–cyan fluorescent protein [CFP]), suggesting that MAPL acts upstream of DRP1 during mitochondrial fission. In such cells expressing either wild type MAPL or its truncated version, which lacks the RING domain, confocal and electron microscopy revealed distinct MAPL-containing MDVs that were 70–100 nm in diameter and which were surrounded by either one or two membranes. In HeLa and COS7 cells, MDVs containing MAPL co-localized with a peroxisomal matrix marker, CFP–SKL (a green fluorescent protein [GFP] variant fused to a tripeptide, SKL, which is a peroxisomal targeting signal). However, fluorescence microscopy could not distinguish if both single and/or double membrane MDVs fused with peroxisomes. Additionally, the evidence for MDV–peroxisome fusion was limited to the use of fluorescence microscopy. Time-lapse microscopy showed that MDVs emanate in a DRP1-independent manner from the sides of mitochondrial tubules, where MAPL–CFP is enriched. After their formation, MDVs diffuse away from the mitochondrial body and fuse only with a minor subset (~10%) of peroxisomes.

Inter-organelle transactions

The authors then analyzed the membrane properties and cargo of the MDVs. MAPL-containing MDVs had no membrane potential ($\Delta\Psi$), and only 33–50% of these vesicles also contained a second mitochondrial marker from the matrix, inner membrane or intermembrane space. MDVs with selective cargo subsets were observed, with different MDV subpopulations containing either MAPL or the mitochondrial outer membrane protein, TOM20 (translocase in outer mitochondrial membrane 20). More importantly, TOM20-positive vesicles never co-localized with CFP–SKL, indicating that only the MAPL-containing MDVs fuse with peroxisomes. The physiological role of MAPL–MDVs remains unknown because silencing of MAPL does not interfere with the generation of TOM20-containing vesicles or with peroxisome morphology. Therefore, MAPL is not required for the generation of MDVs or for peroxisome fission, but is simply a marker for MDVs.

To put this discovery into context, it is relevant to note that mitochondria and peroxisomes share important biochemical pathways requiring inter-organelle exchanges. For example, similar enzymatic reactions, with enzymes encoded by specific genes, catalyze β-oxidation of fatty acids of different chain lengths in mammalian mitochondria and peroxisomes [12]. As part of the fatty acid β-oxidation cycle, acylcarnitine esters move from peroxisomes to mitochondria and, conversely, ATP produced in

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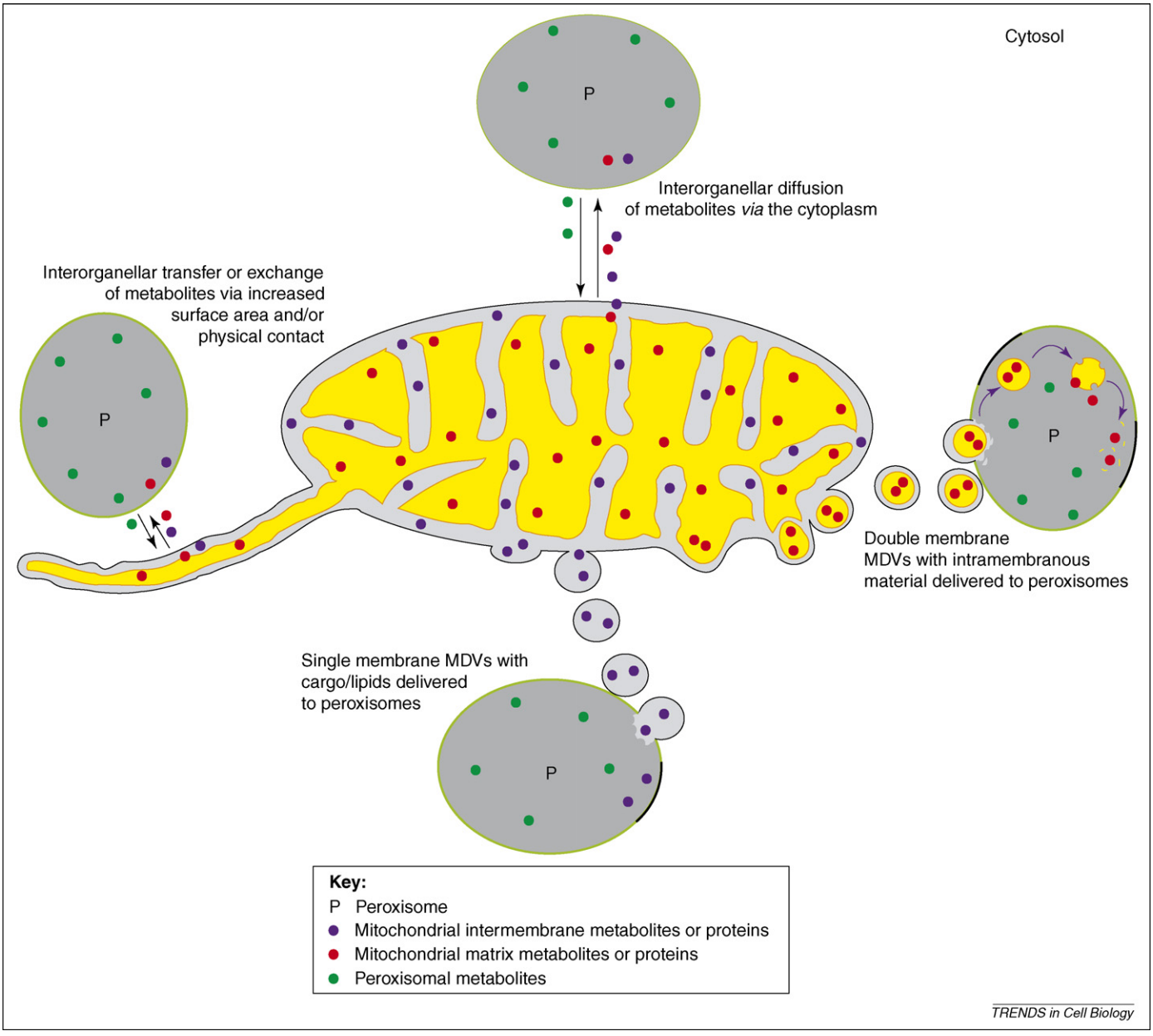

Figure 1. Schematic model of different pathways for the transfer of metabolites, proteins and/or lipids between mitochondria and peroxisomes. Metabolites can be exchanged between these compartments either through diffusion (top) or through mitochondrial protrusions. These processes are proposed to enhance such exchanges either by an increase in the surface area of the mitochondria or by physical contact between mitochondria and peroxisomes (left). Either single- (bottom) or double-membrane MDVs (right) would deliver mitochondrial contents to peroxisomes following fusion of these MDVs with the peroxisomal membrane.

mitochondria is transported into peroxisomes for the generation of fatty-acyl CoA esters of medium-chain fatty acids. Such transfer of metabolites or co-factors between compartments can occur by diffusion or through transporters without requiring physical contact or vesicle-mediated transport mechanisms (Figure 1). However, in addition to carrying metabolites, compartments may share membrane-embedded lipids, or even soluble and membrane proteins, and transferring these between compartments would be facilitated by physical contact or vesicle-mediated trafficking (Figure 1). For example, vesicle-mediated lipid and integral membrane protein transfer from the ER to pre-existing peroxisomes is thought to enable peroxisome growth [2,7,13,14]. If such pre-existing peroxisomes do not exist – this scenario can be created by preventing peroxisome inheritance from a mother to a daughter cell in yeast – the ER-derived pre-peroxisomal vesicles still mature into new peroxisomes, albeit more slowly [13].

Physiological function of MDVs

What role could MDVs have and why is it that only a subset of the peroxisomes co-localizes with MDV markers? Neuspiel *et al.* [11] consider some obvious possibilities. Peroxisomes and mitochondria share a subset of proteins required for organelle fission [15–18]. The tail-anchored membrane protein Fis1 exists with the same topology in both peroxisomal and mitochondrial membranes, and it recruits DRP1/DLP1 to these organelles to facilitate their fission [16,18]. It is unclear at present if Fis1 is independently sorted to peroxisomes and mitochondria. If, instead,

Fis1 was targeted only to the outer mitochondrial membrane, then MDVs would provide a mechanism to ferry Fis1 from mitochondria to peroxisomes. However, it seems unlikely that MDVs deliver Fis1 to peroxisomes, because all peroxisomes have Fis1, and recent unpublished data from H.K. Delille and M. Schrader indicate that targeting of mammalian Fis1 to peroxisomes and mitochondria are independent events. An alternative cargo destined for peroxisomes might be a recently described mitochondrial, tail-anchored protein, Mff (mitochondrial fission factor), which is required for both mitochondrial and peroxisomal fission [19]. Mff is predominantly located in the mitochondrial outer membrane with a substantial part exposed to the cytosol, but does not form a complex with Fis1, and it is also present, in small but significant levels, on peroxisomes. The morphology of mitochondria, in addition to that of peroxisomes, is affected by Mff small interfering RNA (siRNA) in a way that is indistinguishable from the effects of Drp1 or Fis1 siRNA. It remains to be tested whether MDVs provide a mechanism for peroxisomal delivery of Mff through mitochondria.

The experiments of Neuspiel *et al.* [11] show that MAPL knockdown by siRNA or the overexpression does not affect either MDV formation or peroxisome division and that MDV formation is also DRP1-independent. However, the more important experiment that cannot be done at present is to prevent MDV formation and to ask whether peroxisome division or morphology is affected.

Alternatively, MDVs might be involved in the delivery of specific membrane lipids by vesicular trafficking, and the authors consider cardiolipin as a possible candidate. However, the feasibility of this idea depends on whether the peroxisomal membrane has cardiolipin and whether there is sufficient cardiolipin in the mitochondrial outer membrane. Mammalian and some yeast peroxisomes have no detectable cardiolipin [20,21]. Peroxisomes of the yeast *Pichia pastoris* have cardiolipin [22], but it is unclear if this is the exception rather than the rule. Cardiolipin, a lipid found primarily in mitochondrial membranes, is synthesized from phosphatidyl–CMP and phosphatidylglycerol by cardiolipin synthase residing in the inner membrane [23]. After synthesis in the inner leaflet of the mitochondrial inner membrane, it is transferred to the outer leaflet of the mitochondrial inner membrane and remodeled by transacylation using other enzymes (e.g. tafazzin). Small quantities of cardiolipin also make it into the outer mitochondrial membrane, probably through contact sites between the inner and outer mitochondrial membranes. Additionally, cardiolipin delivery to the peroxisomal membrane is most easily envisioned if it is the single-membrane MDV that fuses with peroxisomes – a point that is still ambiguous.

Other possibilities could also be considered. Given that both single-membrane MDVs (derived from the mitochondrial outer membrane) and double-membrane MDVs (comprising the mitochondrial inner and outer membranes) are seen, their fusion with the single-membrane-enclosed peroxisomes would deliver, in addition to membrane-associated lipids and proteins, lumenal contents from the intermembrane space of mitochondria. In addition, fusion of the double-membrane MDVs with peroxisomes would deliver single-membrane-enclosed vesicles into the peroxisome lumen. If these vesicles were to rupture (an event perhaps catalyzed by the action of enzymes, such as lipases), then mitochondrial matrix contents would be delivered into peroxisomes. Indeed, intra-peroxisomal vesicles were described recently in a *Saccharomyces cerevisiae* mutant lacking a putative peroxisomal lipase, Lpx1; however, the fate and function, if any, of these vesicles is unknown [24]. All of this seems pretty complex and speculative, but there is a precedent for a similar type of transfer of GFP from the stroma of plastids, through stroma-filled tubules called stromules, from one plastid to another in plants [10,25]. In a similar manner to the double-membrane MDVs, these stromules contain both the outer and inner membrane of plastids surrounding the stroma. Tubular protuberances analogous to stromules have also been seen emanating from mitochondria (in which they are known as matrixules) and peroxisomes (peroxules) of plant leaf epidermal cells [10]. In fact, the vesicle formation from membranes of organelles of prokaryotic endosymbiont origin (e.g. mitochondria and chloroplasts) has parallels with the blebbing of vesicles from the outer membrane of bacteria [26,27]. Even free-streaming stromules that break off and move away from tubular extensions of chloroplasts have been observed by phase-contrast microscopy. This process is remarkably similar to the generation of MDVs from mitochondrial tubules [11].

Future directions

The studies of stromules might aid the design of experimental tests to determine the putative functions of MDVs. Stromule-mediated GFP transfer between plastids has been demonstrated convincingly [25]. It has been suggested that, in addition to facilitating the transfer of endogenous macromolecules (i.e. protein and nucleic acids), tubular extensions from subcellular organelles also greatly enhance the surface area of these organelles, thereby facilitating metabolite exchange between these organelles and the cytosol, or even between compartments. However, this explanation is not definitive. Stromule movement requires microfilaments, and the ATPase activity of myosin and actin, but it doesn't require microtubules, suggesting that stromules move along actin microflaments powered by the ATPase activity of myosin motors.

Additional studies focused on the nature of the MDVs, the lipid and proteomic profiling of MDVs, and the cargos they carry and deliver to peroxisomes, in addition to experiments that examine the physiological consequences of blocking MDV formation, will shed light on this interesting, but underappreciated, area of biology. Investigating the function and existence of MDVs in other models would also expedite the analysis of their role.

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