The peroxisome: orchestrating important developmental decisions from inside the cell

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The peroxisome has long been known for its role in lipid metabolism and hydrogen peroxide detoxification. However, growing evidence supports the view that this organelle can also function both as an intracellular signaling compartment and as an organizing platform that orchestrates certain developmental decisions from inside the cell. This review highlights various strategies that peroxisomes employ to regulate the processes of development, differentiation, and morphogenesis and critically evaluates several molecular mechanisms by which peroxisomes promote these processes.

Recent studies have uncovered new and unexpected roles for organelles in cell proliferation, differentiation, death, and survival. Organelles generate and distribute signaling molecules and morphogens (González-Gaitán, 2003; Newmeyer and Ferguson-Miller, 2003; Scorrano et al., 2003), assemble active signaling protein complexes on their surface and transport them to specific subcellular locations (Sorkin and von Zastrow, 2002; Hancock, 2003), and serve as organizing platforms for the development of some viral and bacterial pathogens (Desjardins, 2003). A growing body of evidence supports the view that the peroxisome, an organelle known for its role in lipid metabolism and hydrogen peroxide detoxification (Titorenko and Rachubinski, 2001), is actively involved in orchestrating important developmental decisions from inside the cell. In humans, defects either in the biogenesis of the peroxisome or in its metabolic functions result in developmental disorders known as the peroxisomal disorders. These disorders are divided into two groups, the peroxisome biogenesis disorders (PBDs) and the peroxisome single enzyme disorders. The PBDs are due to autosomal recessive mutations in any of 12 *PEX* genes encoding proteins called peroxins (Gould et al., 2001; Matsumoto et al., 2003). A prominent

feature of the Zellweger spectrum of the PBDs is a global developmental delay caused by the incomplete migration and differentiation of neuroblasts during psychomotor development, defects in the development of central white matter, and post-developmental enhanced apoptosis of neurons (Powers and Moser, 1998). Another distinct form of PBD, rhizomelic chondrodysplasia punctata type 1, is characterized by abnormal psychomotor development and mental retardation (Gould et al., 2001). Due to the extreme complexity of the human organism and its limited accessibility to genetic and biochemical analyses, the use of genetically and biochemically manipulable organisms with sequenced genomes as model systems with which to study the role of peroxisomes in development becomes increasingly important. Recent studies with such model systems have revealed various strategies and identified several mechanisms through which peroxisomes organize the processes of development, differentiation, and morphogenesis in evolutionarily distant organisms (Fig. 1).

The peroxisome is an intracellular signaling compartment that promotes developmental decisions

The rate and efficiency of lipid metabolism in mammalian peroxisomes define the steady-state levels of several signaling lipids, including retinoic acid, phytanic acid, and long-chain fatty acids, outside the peroxisome (Desvergne and Wahli, 1999). Targeted to the nucleus by their binding proteins (Tan et al., 2002), these signaling lipids bind and activate ligand-inducible transcription factors, the retinoic acid receptors (RARs) and the peroxisome proliferator-activated receptors (PPARs), which belong to the superfamily of nuclear hormone receptors (Kersten et al., 2000). Only when stimulated by signaling lipids will RARs and PPARs activate the transcription of numerous genes whose protein products are essential for the development of the embryo and for the differentiation of adipose, skin, brain, and placental tissues in humans and other mammals (Kersten et al., 2000; Di-Poi et al., 2002; Michalik et al., 2002; Fig. 1

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Figure 1. **Strategies and molecular mechanisms for the involvement of peroxisomes in development, differentiation and morphogenesis.** See text for details. The peroxisome and the nucleus are colored blue and green, respectively. FA-CoA, acyl-CoA esters of fatty acids; HDAC1, histone deacetylase 1; PPAR, peroxisome proliferator-activated receptors; RAR, retinoic acid receptor; including hydrogen peroxide, superoxide radicals, and nitric oxide; VLCFA, very long-chain fatty acids.
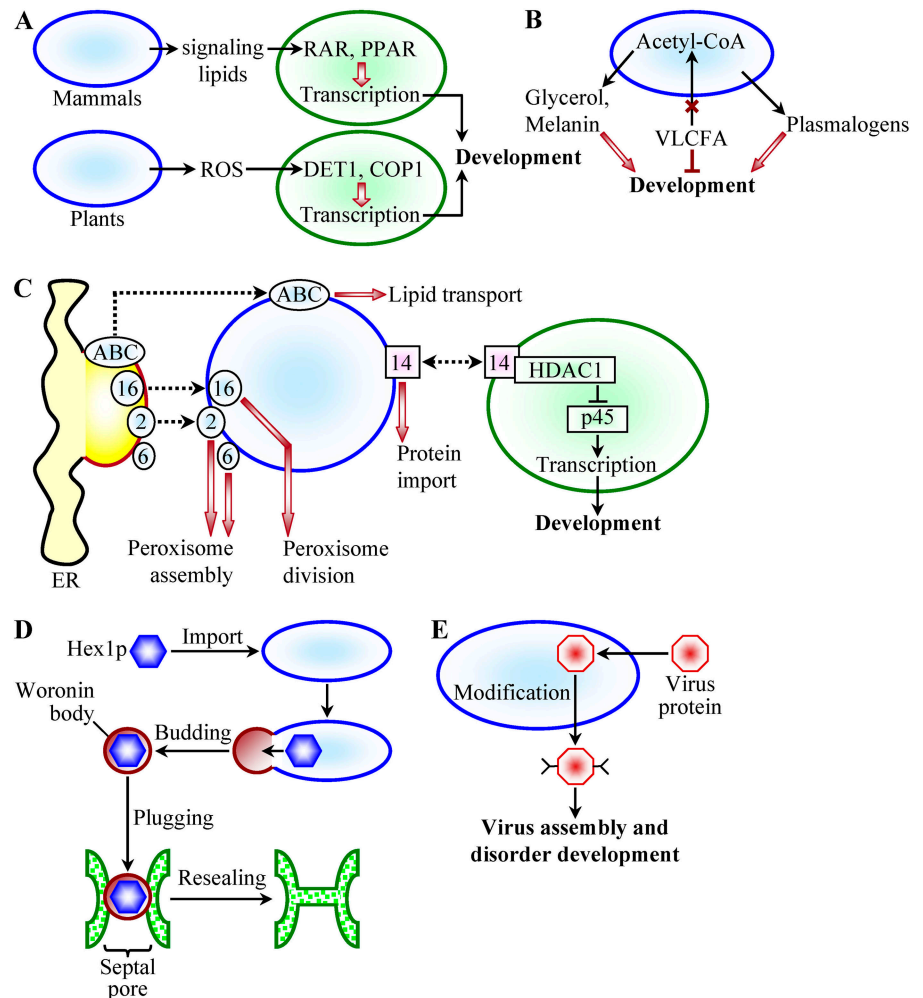
A). Peroxisomes in plants produce and release reactive oxygen species (ROS), namely hydrogen peroxide and superoxide radicals, and nitric oxide (NO) (Corpas et al., 2001). These signaling molecules are potent cellular messengers that function in intra- and intercellular signaling and regulate the transcription of peroxisome- and stress-related genes (Lopez-Huertas et al., 2000; Desikan et al., 2001). Recent data from *Arabidopsis thaliana* have suggested two targets for ROS and NO in the nucleus, the DET1 and COP1 proteins (Hu et al., 2002). These nuclear proteins are global repressors of a distinct developmental program called photomorphogenesis. They negatively regulate the transcription of hundreds of light-responsive genes involved not only in various peroxisomal functions but also in light signaling, photosynthesis, and the stress response (Hu et al., 2002; Ma et al., 2002). The DET1- and COP1-regulated transcription of these photomorphogenesis-related genes is orchestrated by signaling lipids, including ROS and NO, that are generated and released to the cytosol by plant peroxisomes (Hu et al., 2002; Ma et al., 2002; Fig. 1 A).

The peroxisome compartmentalizes metabolic pathways essential for development and differentiation

Peroxisomes carry out the initial steps of a limited set of biosynthetic and degradative pathways for a group of compounds that play pivotal roles in developmental and differen-

tiation programs (Fig. 1 B). In mammalian cells, the first two steps of the biosynthesis of plasmalogens, which are especially abundant in nervous tissue and central white matter, occur in peroxisomes (Gould et al., 2001). Depletion of plasmalogens due to peroxisome dysfunction causes many of the features of PBD pathogenesis, including the incomplete migration and differentiation of neuroblasts and defects in the development of central white matter (Gould et al., 2001). Moreover, plasmalogen biosynthesis in peroxisomes plays a crucial role in the post-embryonic development of the nematode *Caenorhabditis elegans*, an invertebrate model for the human PBDs (Motley et al., 2000; Petriv et al., 2002).

In phytopathogenic fungi, acetyl-CoA derived from peroxisomal fatty acid β-oxidation is used for the biosynthesis of melanin and glycerol (Thines et al., 2000; Kimura et al., 2001), which play pivotal roles in a distinct developmental program, the differentiation of asexual spores called conidia into an infectious structure, the appressorium (Kimura et al., 2001). Appressoria eventually penetrate the host plant, where they form infectious hyphae that invade the plant tissue. The inability to assemble peroxisomes due to a lack of the peroxisome-associated peroxin Pex6p, and the resultant deficiency in acetyl-CoA for the biosynthesis of melanin and glycerol, hamper phytopathogenicity (Kimura et al., 2001).

Impaired peroxisomal oxidation or transport of fatty acids, including very long-chain fatty acids, phytanic acid, and

pristanic acid, in Zellweger patients leads to an accumulation of these toxic compounds outside the peroxisome and results in global developmental delay and severe neurological dysfunction (Gould et al., 2001). If not oxidized in peroxisomes, these abnormal fatty acids are incorporated into the membranes of neurons, perturbing their microenvironment and causing defects in their migration and differentiation, along with other developmental defects characteristic of PBD pathogenesis (Powers and Moser, 1998).

It appears that only a limited set of metabolic pathways and metabolite transporters operating in peroxisomes, rather than all the basic metabolic functions of these organelles, is required for certain developmental programs. Using RNA-mediated interference for the post-transcriptional silencing of numerous nematode genes controlling a wide range of peroxisome-related functions, Petriv et al. (2002) demonstrated that major metabolic pathways in the peroxisome, such as the β-oxidation of various fatty acids, which supplies acetyl-CoA for energy production in mitochondria (Hettema and Tabak, 2000), are not required for post-embryonic development of the nematode. On the other hand, a defect in the peroxisomal transport of acyl-CoA esters of fatty acids, which results in their accumulation outside the peroxisome, and a deficiency in plasmalogen biosynthesis cause an arrest at the first larval stage of development (Petriv et al., 2002). Notably, in addition to post-embryonic development of the nematode, several other developmental programs in yeast (Titorenko et al., 1997), filamentous fungi (Jedd and Chua, 2000), plants (Footitt et al., 2002), and humans (Gavva et al., 2002) do not rely on the energy provided by the mitochondrial oxidation of acetyl-CoA derived from the peroxisomal β-oxidation of fatty acids.

Peroxisomal proteins possess dual subcellular localization and function

A few peroxisomal proteins possess a dual subcellular localization. Although proteins belonging to this distinct group have initially been recognized for their essential roles in peroxisome biogenesis and function, they have turned out to be also required for certain developmental, differentiation, and morphogenetic programs (Fig. 1 C). Recent studies have provided strong evidence that the developmental role of these bifunctional peroxisomal proteins with dual subcellular localization is independent of metabolic pathways operating in peroxisomes. In particular, while the peroxisome-associated pools of these proteins operate in peroxisome biogenesis and function, their pools in other subcellular organelles promote certain developmental decisions regardless of the metabolic state of peroxisomes (Titorenko et al., 1997; Titorenko and Rachubinski, 1998).

The human peroxin Pex14p is an integral peroxisomal membrane protein that functions as the initial docking site for cargo-laden cytosolic shuttling receptors of peroxisomal matrix proteins (Titorenko and Rachubinski, 2001). Human Pex14p is also a nuclear protein (Gavva et al., 2002). Targeted to the nucleus by its nuclear localization signal, human Pex14p interacts specifically with the p45 subunit of a DNA binding transcription factor, NF-E2. NF-E2 is a transcriptional regulator of erythroid and megakaryotic genes in pluripotential hematopoietic stem cells of the blood and lymphoid systems (Gavva et al., 2002). In the nucleus, a complex formed between Pex14p and a histone deacetylase is proposed to act as a corepressor of the NF-E2–mediated transcription of these genes (Gavva et al., 2002). The involvement of a nuclear pool of human Pex14p in regulating gene transcription is still under debate.

The essential roles of three peroxisome-bound peroxins of the yeast *Yarrowia lipolytica*, Pex2p, Pex6p, and Pex16p, in peroxisome biogenesis are well established (Titorenko and Rachubinski, 2001). *Y. lipolytica* Pex2p and Pex16p are initially sorted to the ER and are then delivered in a Pex6p-dependent manner from the ER to peroxisomes via ER-derived vesicles (Titorenko et al., 1997; Titorenko and Rachubinski, 1998). Whereas the major portion of Pex2p, Pex6p, and Pex16p resides in peroxisomes, a minor fraction is ER associated (Titorenko and Rachubinski, 1998). While the peroxisome-bound pools of Pex2p, Pex6p, and Pex16p operate in peroxisome assembly and division, their ER-bound pools orchestrate a specific cell polarization and differentiation program, the dimorphic transition from a round yeast form to a filamentous (mycelial) form, by promoting the delivery of mycelium-specific proteins from the ER to the cell envelope (Titorenko et al., 1997; Titorenko and Rachubinski, 1998). The role for at least one peroxisome- and ER-localized bifunctional protein, Pex16p, in development and differentiation is evolutionarily conserved among yeasts and plants. The *Arabidopsis* orthologue of *Y. lipolytica* Pex16p, the SSE1 protein, is required not only for peroxisome assembly but also for the biogenesis of protein and lipid bodies, a cellular differentiation program for energy storage in maturing seeds (Lin et al., 1999). The biogenesis of protein and lipid bodies depends on the ER and involves the sorting of oleosins, the most abundant protein components of oil bodies, via the ER (Sarmiento et al., 1997). Importantly, Pex16p complements developmental defects in a cross-species fashion, as the *Arabidopsis* SSE1 protein reestablishes the dimorphic transition in the *Y. lipolytica pex16* mutant deficient in the delivery of mycelium-specific proteins from the ER to the tip of the growing filament (Lin et al., 1999).

Another bifunctional peroxisomal membrane protein of plants, CTS, is not only involved in the transport of acyl-CoA esters of fatty acids (FA-CoAs) into the peroxisome but is also essential for the transition from dormancy to germination, an important step in the embryonic development of *Arabidopsis* (Footitt et al., 2002). Seeds carrying *cts* mutations do not germinate and exhibit a "forever dormant" phenotype. Importantly, the inability of *cts* mutants to metabolize lipid body–derived FA-CoAs cannot account for the "forever dormant" phenotype, suggesting that the functions of CTS in the peroxisomal transport of FA-CoAs and in embryo development are different (Footitt et al., 2002). CTS is an orthologue of the human peroxisomal protein ALDP, a member of the ATP-binding cassette (ABC) transporter family. Because the binding of other known ABC transporters to certain passive ion channels modulates their development-related activities (Gaedeke et al., 2001), it has been proposed that the activity of CTS in embryo dormancy may involve its interaction with such channels (Footitt et al., 2002). It remains to be established what subcellular compartment serves as an organizing platform for the involve-

ment of CTS in development. It is noteworthy that a human peroxisomal ABC transporter is present in a specialized subdomain of the ER from which peroxisomes form (Geuze et al., 2003). It is tempting to speculate that the ER-associated form of CTS promotes embryo development, while its peroxisomal form functions in the transport of FA-CoAs into this organelle. Experimental confirmation of this scenario awaits the detailed characterization of the protein composition and developmental dynamics of the peroxisomal ER subdomain that has been suggested to serve as a template for peroxisome formation (Titorenko and Rachubinski, 2001; Geuze et al., 2003).

The peroxisome is a template for the formation of a distinct subcellular compartment essential for fungal development

The peroxisome provides a template for the formation of a specialized subcellular compartment essential for a distinct morphogenetic program in the filamentous fungus *Neurospora crassa* (Fig. 1 D). Germination of conidia and mycelium formation in *N. crassa* induce the synthesis of the Hex1p protein and promote its targeting to the peroxisome (Jedd and Chua, 2000). Crystallization of Hex1p in the peroxisome initiates budding of a distinct peroxisomal vesicle, the Woronin body, which, in contrast to the general peroxisome population, does not function in fatty acid β-oxidation (Jedd and Chua, 2000). The Woronin body is essential for a multistep process in cell morphogenesis initiated by physical damage to hyphae. To prevent the loss of cytosol and subcellular organelles from damaged hyphae, the Hex1p-containing Woronin body initially plugs the most proximal septal pore. This initiates a cascade of events that leads ultimately to resealing of the plasma membrane at the plugged pore and resumption of polarized growth from a position adjacent to the sealed pore. During this multistep morphogenetic process, either the Woronin body itself or Woronin body–derived exocytic vesicles may fuse with the plasma membrane (Jedd and Chua, 2000).

The peroxisome is an intracellular platform for the development of some viral pathogens

The life cycles of two human viruses, human immunodeficiency virus (HIV) and rotavirus (RV), include the sorting of some of their proteins to the peroxisome (Fig. 1 E). In the cytosol, the HIV Nef protein, which lacks a peroxisomal targeting signal (PTS), forms a complex with a PTS-containing peroxisomal matrix protein, thioesterase II, and is imported into the peroxisome by a "piggy-back" mechanism (Cohen et al., 2000). Remarkably, the peroxisomal sorting of HIV Nef is mandatory for the life cycle of the HIV virus and is essential for the development and manifestation of AIDS (Cohen et al., 2000). The virion surface protein VP4 of another deadly human virus, RV, is targeted to the peroxisome by its own PTS (Mohan et al., 2002). Because lipids are required for the infectivity of HIV and RV, specifically for myristylation of Nef and VP4, it has been suggested that lipid modification of these viral proteins inside the peroxisome is an essential step in the development of both viruses (Cohen et al., 2000).

Summary and perspectives

An important conceptual advance in our understanding of the functioning of peroxisomes in cells is that, in addition to its well known roles in lipid metabolism and hydrogen peroxide detoxification, the peroxisome can function as a signaling compartment and an organizing platform for the orchestration of certain developmental decisions from inside the cell. Much progress has recently been made in defining various strategies and molecular mechanisms for the integration of peroxisomes into the processes of development, differentiation, and morphogenesis. Future work will aim at understanding how defects in peroxisome biogenesis and function at the organellar level are translated into defects in development and differentiation at the cellular and organismal levels. The challenge remains to define how the peroxisome communicates with other organelles in order to promote certain developmental decisions. This knowledge will ultimately provide greater insight into the mechanisms responsible for the global developmental delay and severe neurological dysfunction characteristic of the human peroxisomal disorders.

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