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Review

Peroxisomes as a cellular source of reactive nitrogen species signal molecules

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ABSTRACT

Peroxisomes are single membrane-bounded subcellular organelles with an essentially oxidative type of metabolism and are probably the major sites of intracellular H_2O_2 production. These organelles also generate superoxide radicals ($O_2^{\cdot-}$) and besides catalase they have a complex battery of antioxidative enzymes. In recent years the existence of L-arginine-dependent nitric oxide synthase (NOS) activity and the generation of the reactive nitrogen species (RNS) nitric oxide (NO) have been demonstrated in plant peroxisomes. The inter-cellular and intracellular NO carrier *S*-nitrosoglutathione (GSNO) can be generated inside peroxisomes and the presence of this RNS has been demonstrated in peroxisomes from several plant species. This review analyzes the available evidence concerning the properties of the NOS activity and the generation of the RNS messengers NO and GSNO in peroxisomes in the context of the cellular function of these organelles as a source of RNS signaling molecules. The important physiological functions displayed by NO and other RNS in intra- and inter-cellular communication in different organisms indicate that more attention should be payed to the RNS signaling function of peroxisomes in human, animal and fungal cells, where it is very likely that similar mechanisms to those found in plant peroxisomes are also operative.

Introduction

Peroxisomes are subcellular organelles bounded by a single membrane and devoid of DNA that contain as basic enzymatic constituents catalase and hydrogen peroxide (H_2O_2)-producing flavin oxidases, and occur in almost all eukaryotic cells [1–3]. At the beginning of the 1960s, when these organelles were first isolated and characterized from mammalian tissues [4] it was thought that their main function was the removal by catalase of toxic hydrogen peroxide generated in the peroxisomal respiratory pathway by different oxidases. However, in recent years it has become increasingly clear that peroxisomes are involved in a range of important cellular functions in almost all eukaryotic cells [3,5–12]. Table 1 shows different functions that have been described so far for peroxisomes in plant cells.

The peroxisome of plant cells is a highly dynamic compartment that is dependent upon the actin cytoskeleton, not microtubules, for its subcellular distribution and movements [32,33]. In plants, there are several types of peroxisomes which are specialized in certain metabolic functions. Glyoxysomes are specialized peroxisomes, occurring in the storage tissue of oilseeds, that contain the fatty acid β-oxidation and glyoxylate cycle enzymes to convert the seed reserve lipids into sugars which are used for germination and plant growth [1,3,16]. Leaf peroxisomes are specialized peroxisomes present in photosynthetic tissues that carry out the major reactions of photorespiration [13,14]. Another type of specialized peroxisome is the root-nodule peroxisome from certain tropical legumes, in which the synthesis of allantoin, the major metabolite for nitrogen transport within these plants, is carried out [17]. However, since the differences among these types of peroxisomes are not very large, in order to avoid confusion it is recommended to use the general term "peroxisome" to describe all these types of specialized peroxisomes [16].

Evidence for the existence of regulatory proteins in peroxisomes, like heat shock proteins, kinases, and phosphatases, is emerging [27,34,35]. In recent years, a proteomic burst has taken place in peroxisome biology [36,37]. Screening the Arabidopsis (*Arabidopsis thaliana*) genome has led to the identification of about 280 genes that encode proteins containing putative PTS-1 (220) and PTS-2 (60) peptides [34,35,38–40]. Peroxisomal targeting signals (PTS)[1] are located at either the C (PTS-1) or the N protein termi-

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[1] *Abbreviations used:* PTS, peroxisomal targeting signals; PPAR, peroxisome proliferator-activated receptor; SODs, superoxide dismutases; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; ICDH, isocitrate dehydrogenase; NO·, nitric oxide; NOS, nitric oxide synthase; BH_4, tetrahydrobiopterin; GDC, glycine decarboxylase complex; RNS, reactive nitrogen species; EPR, electron paramagnetic resonance; DAF-2 DA, 4,5-diaminofluorescein diacetate; NOA, nitric oxide analyzer; AG, aminoguanidine; iNOS, inducible nitric oxide synthase; CM, carboxymethoxylamine; AAN, aminoacetonitrile; CLSM, confocal laser scanning microscopy; nNOS, neuronal nitric oxide synthase; GSNO, *S*-nitrosoglutathione; Prx, peroxiredoxin; XOD, xanthine oxidase; PCD, programmed cell death; XOR, xanthine oxidoreductase; MDAR, monodehydroascorbate reductase.

Table 1
Functions described for peroxisomes in plant cells.

Function	References
Photorespiration	[13,14]
β-Oxidation of fatty acids	[3,15]
Glyoxylate cycle	[3,16]
Metabolism of ureides	[3,17]
Purine catabolism	[18]
Polyamine catabolism	[19,20]
Sulfur metabolism	[21–23]
Metabolism of reactive oxygen and nitrogen species (ROS and RNS)	[7,9,24]
Photomorphogenesis	[25,26]
Biosynthesis of phytohormones (auxin, jasmonic acid, salicylic acid)	[15,27]
Leaf senescence	[28]
Defense against pathogens	[27,29–31]

nus (PTS-2). PTS-2 correspond to polypeptides that are proteolytically cleaved upon its entrance into the organelle [27]. These *in silico* analyses indicate that although only a few dozen proteins have been functionally characterized as peroxisomal proteins, the total number of proteins in plant peroxisomes may be more than 300 [40,41]. In plants, like in humans and yeasts, a public data base (AraPerox) has been created on the analysis of gene sequences, where the information on subcellular targeting prediction, homology, and *in silico* expression analysis of *Arabidopsis* proteins from plant peroxisomes are compiled [35]. In addition, a dynamic database dedicated to plant organelle research has been developed to try to understand how organelle dynamics regulate the integrated functions of plants' responses to environmental signals [42].

A characteristic property of peroxisomes is their oxidative type of metabolism and metabolic plasticity, because their enzymatic content can vary depending on the organism, cell/tissue-type and environmental conditions [1–3]. An example of the inducible nature of peroxisomal metabolism is the light-induced transition of glyoxysomes, the specialized peroxisomes of oilseeds, to leaf-type peroxisomes [1,43]. During the senescence of leaves the reverse process is observed, whereby leaf peroxisomes are converted into glyoxysomes [28,44,45]. These metabolic transitions are also observed in plants under conditions of abiotic stress by cadmium; that is, this heavy-metal stress causes leaf peroxisomes to adopt a glyoxysome-type metabolism [46].

Peroxisome proliferation and the induction of some peroxisomal β-oxidation enzymes was first documented in the livers of rats exposed to a variety of xenobiotics and subsequently found in other mammalian species [2,47,48]. In plants, the cellular population of peroxisomes can proliferate during senescence [28,49], under different stress conditions produced by xenobiotics [50–53], ozone [54], cadmium [55] and H_2O_2 [56], and light can also induce the proliferation of these organelles [26]. Peroxisome proliferator-activated receptor (PPAR), the transcription factor involved in peroxisomal proliferation and induction of peroxisomal fatty acid β-oxidation in animal tissues, was recently expressed in tobacco plants. In the transgenic plants obtained, PPARα from frog (*Xenopus laevis*) was functional and its expression in tobacco led to changes in general lipid metabolism and induced the proliferation of peroxisomes, as reported in animal tissues [52].

In plant cells, as in most eukaryotic organisms, peroxisomes are probably the major sites of intracellular H_2O_2 production, as a result of their essentially oxidative type of metabolism. During the last decade, it has been demonstrated that peroxisomes, like mitochondria and chloroplasts, also produce superoxide radicals ($O_2^{\cdot-}$) as a consequence of their normal metabolism. In peroxisomes the existence of, at least, two sites of superoxide generation has been demonstrated: one in the organelle matrix, the generating system being xanthine oxidase, and another site in the peroxisomal membranes dependent on NAD(P)H [7,57–60]. On the other hand, the presence of different proteases in peroxisomes has been demonstrated and peroxisomal endoproteases could take part in a regulated modification of proteins which do not necessarily imply their complete degradation [61,62].

In peroxisomal membranes, three integral polypeptides with molecular masses of 18, 29, and 32 kDa have been shown to generate $O_2^{\cdot-}$ radicals [7] and they have been characterized [63,64]. Besides catalase, a complex battery of antioxidative systems have been demonstrated in plant peroxisomes, including different superoxide dismutases (SODs) [65–67], the four enzymes of the antioxidative ascorbate–glutathione cycle as well as the antioxidants glutathione and ascorbate, and three NADP-dependent dehydrogenases [7,68–70]. The recycling of NADPH from $NADP^+$ can be carried out in peroxisomes by three dehydrogenases: glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), and isocitrate dehydrogenase (ICDH) [69,71,72]. In the last decade, evidence has accumulated suggesting that peroxisomes have a ROS-mediated cellular function in leaf senescence and in stress situations induced by xenobiotics and heavy metals [9,23,28,73].

The gaseous free radical nitric oxide ($NO^{\cdot}$) is a widespread intracellular and inter-cellular messenger with a broad spectrum of regulatory functions in many physiological processes of animal and plant systems [74–85]. The use of NO by higher plants was first reported in 1960 [86] much earlier than in animals, and the NO emission from plants was first observed in 1975, in soybean plants treated with herbicides [87]. In recent years, NO was reported to be involved in many key physiological processes of plants, such as ethylene emission [88], response to drought [89], disease resistance [90–95], growth and cell proliferation [96], maturation and senescence [97], apoptosis/programmed cell death [95,98–101], and stomatal closure [79,102,103].

On the other hand, the application of exogenous NO to plants has been used as a tool to study how this molecule affects some physiological processes, such as inhibition of certain enzyme activities [95,104], cell wall lignification [105], the alternative oxidase pathway [106], cell death [107–109], accumulation of ferritin [110], wound signaling [111], and root organogenesis [112].

In animal systems most of the NO produced is due to the enzyme nitric oxide synthase (NOS; EC 1.14.13.39) [74,76]. This enzyme catalyzes the oxygen- and NADPH-dependent oxidation of L-arginine to NO and citrulline in a complex reaction requiring FAD, FMN, tetrahydrobiopterin (BH_4), calcium and calmodulin [113,114] (Fig. 1).

NO can be produced in plants by non-enzymatic and enzymatic systems [80,115,116]. Nitrate reductase is a well-established enzymatic generator of NO in plants [117–120]. Other enzymes that have been shown to produce NO are a plasma membrane-bound enzyme of tobacco roots, nitrite–NO oxidoreductase [121,122], and several nitric oxide synthase-like activities [80,82,116,123], although there are other potential enzymatic sources of $NO^{\cdot}$ in plants [80,124]. Since 1996 there has been an increasing number of reports showing the presence of a nitric oxide synthase activity in plants similar to mammalian NOS. A variant of the P protein of the mitochondrial glycine decarboxylase complex (GDC) [125] and the protein AtNOS1 codified by an *Arabidopsis* "NOS gene"

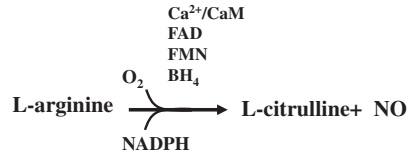

Fig. 1. Scheme of the nitric oxide synthase reaction.