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Regulation of peroxisome dynamics by phosphorylation☆

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ABSTRACT

Peroxisomes are highly dynamic organelles that can rapidly change in size, abundance, and protein content in response to alterations in nutritional and other environmental conditions. These dynamic changes in peroxisome features, referred to as peroxisome dynamics, rely on the coordinated action of several processes of peroxisome biogenesis. Revealing the regulatory mechanisms of peroxisome dynamics is an emerging theme in cell biology. These mechanisms are inevitably linked to and synchronized with the biogenesis and degradation of peroxisomes. To date, the key players and basic principles of virtually all steps in the peroxisomal life cycle are known, but regulatory mechanisms remained largely elusive. A number of recent studies put the spotlight on reversible protein phosphorylation for the control of peroxisome dynamics and highlighted peroxisomes as hubs for cellular signal integration and regulation. Here, we will present and discuss the results of several studies performed using yeast and mammalian cells that convey a sense of the impact protein phosphorylation may have on the modulation of peroxisome dynamics by regulating peroxisomal matrix and membrane protein import, proliferation, inheritance, and degradation. We further put forward the idea to make use of current data on phosphorylation sites of peroxisomal and peroxisome-associated proteins reported in advanced large-scale phosphoproteomic studies. This article is part of a special issue titled "Peroxisomes," edited by Ralf Erdmann.

1. Introduction

Peroxisomes are single membrane-bound metabolic organelles existing in virtually all eukaryotic cells. They are involved in a variety of essential catabolic and anabolic processes. Major functions are the degradation of fatty acids *via* β-oxidation and the detoxification of hydrogen peroxide, which is generated during various oxidative reactions carried out in peroxisomes. Further metabolic functions of mammalian peroxisomes are, among others, degradation of leukotrienes and prostaglandins, detoxification of glyoxylate as well as biosynthesis of bile acids and etherphospholipids (reviewed in [1]). In humans, a defect in peroxisome biogenesis or deficiency in the activity of a single peroxisomal enzyme or transporter ultimately leads to serious, often lethal diseases (e.g., Zellweger syndrome, X-linked adrenoleukodystrophy). The existence of many of such inherited peroxisomal disorders highlights the importance of peroxisomes for human health.

Aside from the diversity in metabolic functions in different organisms and cell types [2,3], peroxisomes are characterized by remarkable dynamics. Depending on the cellular requirements, peroxisomes may undergo drastic changes in size, shape, abundance, and protein composition. The dynamic adaptation in response to internal and external stimuli, but also maintenance of a certain steady-state level in peroxisome number and function, requires the concerted and tightly regulated action of diverse molecular mechanisms. These include signal perception and transduction, gene expression, trafficking of proteins and lipids to peroxisomes, import of peroxisomal membrane and matrix proteins, peroxisome proliferation, maintenance and inheritance, and the selective autophagic degradation of the organelles. A further process, which has been largely ignored so far, is the selective export of peroxisomal proteins [4]. Key players of most of these processes have been identified in recent years. However, while mechanisms of peroxisomal gene expression were more extensively studied, the regulation of and crosstalk between distinct processes that govern peroxisome dynamics remained largely unexplored.

In general, gene expression is tightly regulated and depends, for example, on the nature of nutrients available to the cell. For *Saccharomyces cerevisiae*, it is well established that the expression of many genes coding for peroxisomal proteins, predominantly metabolic

Abbreviations: CK, casein kinase; Cvt, cytoplasm-to-vacuole targeting; DRPs, dynamin-related proteins; ER, endoplasmic reticulum; EPCONS, ER-to-peroxisome contact sites; mPTS, membrane peroxisomal targeting signal; MS, mass spectrometry; mTOR, mammalian target of rapamycin; PAS, pre-autophagosomal structure; PMP, peroxisomal membrane protein; PTM, posttranslational modification; PTS, peroxisomal targeting signal; SGD, *Saccharomyces* Genome Database; TOM, translocase of the outer mitochondrial membrane; TSC, tuberous sclerosis complex.

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enzymes but also some proteins required for peroxisome biogenesis (collectively termed "peroxins"), are repressed in the presence of glucose and specifically induced when fatty acids such as oleic acid are provided as sole carbon source (for a review, see [5]). This ultimately leads to an increase in peroxisome number and size [6]. Aside from the control on the level of gene expression, virtually all cellular processes in eukaryotes are further regulated posttranslationally. Compared to transcriptional mechanisms, modification on the level of proteins enables the cells to respond in a faster, more dynamic, and generally reversible way to changes in environmental or developmental conditions. Protein phosphorylation, i.e., the reversible addition of covalently bound phosphate groups mainly to serine (S), threonine (T), and tyrosine (Y) residues, is one of the most prominent types of posttranslational modifications (PTMs) [7] and arguably the best studied to date. Phosphorylation and dephosphorylation events are catalyzed by protein kinases and protein phosphatases, respectively. The human kinome comprises 518 putative protein kinase genes [8]; in yeast, the existence of more than 110 was proposed [9].

The introduction of a negatively charged hydrophilic phosphate group at a specific amino acid residue within a polypeptide chain often leads to changes in a protein's properties, including conformational changes and the creation of protein interaction domains. Reversible protein phosphorylation is known to promote protein–protein interactions, to induce changes in the subcellular location or stability of proteins, and/or to modulate catalytic activity [7,10]. Approximately 30% of all proteins in the human and 75% of the proteins in the yeast proteome have been reported to be phosphorylated [11,12], underscoring the high relevance of this PTM in eukaryotic biology.

In this review, we summarize and discuss the current knowledge about phosphorylation-dependent processes related to peroxisomes in yeast and mammalian cells, highlighting the impact of protein phosphorylation on peroxisome dynamics. For further details on basic principles governing peroxisome biogenesis, matrix protein import, fission, inheritance, and degradation, we refer the reader to other reviews [3, 13–21].

2. Protein phosphorylation associated with the peroxisomal life cycle

In *S. cerevisiae*, the impact of individual kinases and phosphatases for nutrition-dependent repression and the induction of gene expression, for peroxisome formation, and for the regulation of peroxisome number and size was recently assessed by the analysis of 249 kinase and phosphatase deletion mutants [22]. This study provided strong evidence that phosphorylation is an important element for the regulation of peroxisome biogenesis. In a further global study, Aitchison and co-workers analyzed differences in the phosphoproteome of cells grown under peroxisome-inducing *versus* peroxisome-repressing conditions, which resulted in the identification of a set of differentially phosphorylated proteins [23]. However, the phosphorylation of peroxisomal proteins was not reported therein.

Current state-of-the-art phosphoproteomic methodologies allow for the identification of thousands of phosphorylation sites in a single study [24–26] leading to a wealth of information about protein phosphorylation from yeast to man. Of note, these data are made available to the scientific community by storing them in public databases such as UniProtKB [27], PhosphoSitePlus [28], PhosphoGRID [29], or the *Saccharomyces* Genome Database (SGD; [30]. Impressively, more than 125,000 distinct phosphorylation sites are currently listed in different databases [31]. In order to delineate the current phosphorylation landscape of proteins directly acting in peroxisome biogenic and degradation processes, we extracted information about phosphorylation sites from the SGD for *S. cerevisiae* and from the UniProtKB for human and mouse (Table 1). Certainly, these data are far from reflecting the true picture of the phosphorylation status of peroxisomal proteins. Nevertheless, they clearly demonstrate that numerous peroxisomal proteins are phosphorylated *in vivo*. Interestingly, phosphoproteins appear to be involved in all peroxisome-related cellular processes, as depicted in Fig. 1 for *S. cerevisiae*.

To date, the biological significance for most of the phosphorylation sites listed in Table 1 is still unknown. However, given the frequency of occurrence in peroxisomal proteins, it is reasonable to predict that reversible protein phosphorylation is of high importance for the regulation of diverse aspects of peroxisome biology—it is time to unravel the enigma.

3. Potential role of protein phosphorylation in peroxisomal protein import

For the import of peroxisomal membrane proteins (PMPs), two different pathways have been described (for details, refer to [3,15,16]). Class I PMPs are recognized *via* an internal membrane peroxisomal targeting signal (mPTS) by the cytosolic receptor protein and chaperone Pex19p. Docking of the Pex19p-cargo complex to the peroxisomal membrane then occurs *via* Pex3p (and Pex16 in mammalian cells) followed by insertion of the cargo into the peroxisomal membrane [16,32,33] by a so far ill-defined mechanism. Class II PMPs (e.g., Pex3p, Pex16p, and Pex22p [34–36]) migrate to peroxisomes *via* the endoplasmic reticulum (ER) and are targeted to peroxisomes *via* ER-derived vesicles, a process that requires the secretory proteins Sec20p, Sec39p, and Dsl1p [37].

Peroxisomal matrix proteins use a different import machinery (reviewed in [3,14,16]). With a few exceptions, they carry a C-terminal PTS1 or an N-terminal PTS2 sequence, which are recognized by their respective cytosolic receptors Pex5p and Pex7p. The Pex7p-dependent PTS2 import in *S. cerevisiae* further requires the co-receptors Pex18p or Pex21p [38]—a situation unique for this species. Following recognition, the receptor–cargo complex is targeted to the docking complex (Pex14p/Pex13p/Pex17p in *S. cerevisiae* [39]) at the peroxisomal membrane. For the PTS1 import pathway, it has been shown that Pex5p and Pex14p, a central component of the docking complex, form a large and highly dynamic pore [40]. This dynamic pore facilitates the translocation of the cargo protein into the lumen of the peroxisome. For PTS2 import, a ternary import complex, consisting of cargo, Pex7p, and Pex18p/21p, assembles hierarchically [41]. In further steps, the PTS1 receptor Pexp5 and the PTS2 co-receptor Pex18p are either mono- or polyubiquitinated, thereby marked for recycling or proteasomal degradation, and released from the membrane in an ATP-dependent process [42–44].

Considering the high complexity of peroxisomal membrane and matrix protein import pathways, coordination and a tight control of distinct steps are certainly required to facilitate smooth operation and dynamic adaptation to varying cellular conditions. Remarkably, large-scale phosphoproteomic data from *S. cerevisiae* and mammalian cells indicate that most of the key proteins involved in peroxisomal membrane and matrix protein import exist in a phosphorylated form *in vivo* (Table 1). Although experimental evidence is missing so far, it is tempting to speculate that – besides ubiquitination – reversible phosphorylation of peroxisomal and non-peroxisomal proteins mediating peroxisomal protein import is part of the underlying regulatory mechanisms. This may allow for the direct and rapid modulation of peroxisome functions at the level of protein import in response to different stimuli such as changes in nutritional conditions, thereby placing them into larger intracellular signaling networks. Interestingly, in yeast, the phosphorylation-dependent regulation of organellar protein import has been reported before. The phosphorylation of Sec63p by casein kinase (CK) 2 has been shown to stimulate the assembly of the ER translocon [45]. In addition, import of nuclear-encoded proteins into mitochondria through the translocase of the outer mitochondrial membrane complex has been demonstrated to be controlled by site-specific phosphorylation, which is mediated by several cytosolic kinases [46–49].

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