EXPERIMENTAL CELL RESEARCH 312 (2006) 1671–1684

available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/yexcr

Research Article

Peroxisome division is impaired in a CHO cell mutant with an inactivating point-mutation in dynamin-like protein 1 gene

Atsushi Tanaka[a], *Shinta Kobayashi*[b], *Yukio Fujiki*[a,b,c,*]

[a]*Department of Biology, Faculty of Sciences, Kyushu University Graduate School, Fukuoka 812-8581, Japan*
[b]*Department of Biology, Graduate School of Systems Life Sciences, Kyushu University, Fukuoka 812-8581, Japan*
[c]*SORST, JST, Kawaguchi, Saitama 332-0012, Japan*

ARTICLE INFORMATION

Article Chronology:
Received 1 November 2005
Revised version received
30 January 2006
Accepted 30 January 2006
Available online 9 March 2006

Keywords:
Peroxisome biogenesis
Morphogenesis
Dynamin-like protein
CHO cell mutant
Plasmalogen

ABSTRACT

We earlier isolated a Chinese hamster ovary cell line ZP121 showing morphologically abnormal, tubular peroxisomes, and apparent dysmorphogenesis of mitochondria. Here, we identified an inactivating point-mutation in dynamin-like protein 1 gene, *DLP1*, responsible for the phenotype of ZP121. One allele of *DLP1* possessed a point missense mutation resulting in G363D in the middle region of 699-amino-acid long DLP1, termed DLP1G363D, while the other allele was normal. DLP1G363D was apparently expressed at a higher level than DLP1. Abnormal morphogenesis of peroxisomes as well as mitochondria was restored when wild-type *DLP1* was transfected. The GTPase activity of DLP1G363D was barely detectable, indicating that the G363D mutation severely affected the GTPase activity. Moreover, a higher level of DLP1G363D expression in CHO-K1 cells reproduced the ZP121-type phenotype, hence indicating its dominant-negative activity to the wild-type DLP1, most likely by forming a heteromeric tetramer. The G363D mutation also gave rise to a temperature-sensitive phenotype showing normal morphogenesis of peroxisomes and mitochondria at 40°C. Microtubule organization was most likely involved in the elongation of peroxisomes. Furthermore, ZP121 was lowered in the level of phospholipids, plasmalogens, and phosphatidylethanolamine and was less sensitive to oxidative stresses. Thus, ZP121 is the first *dlp1* mutant in mammalian cells.

* *Corresponding author*. Department of Biology, Faculty of Sciences, Kyushu University Graduate School, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan. Fax: +81 92 642 4214.
E-mail address: yfujiscb@mbox.nc.kyushu-u.ac.jp (Y. Fujiki).

0014-4827/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2006.01.028

Abbreviations:
AOx, acyl-CoA oxidase
BS^3, bis(sulfosuccimidyl)-suberate
CHO, Chinese hamster ovary
DLP1, dynamin-like protein 1
EGFP, enhanced green
fluorescent protein
PNS, post-nuclear supernatant
PTS1 and PTS2, peroxisome targeting
signal types 1 and 2
ts, temperature-sensitive

Introduction

Peroxisomes are present in a wide variety of eukaryotic cells, from yeast to humans, and they function in various metabolic pathways including the β-oxidation of very long chain fatty acids and the synthesis of ether-lipids [1]. Peroxisomal proteins, both matrix and membrane proteins, are synthesized on free polyribosomes and transported to peroxisomes [2]. The functional significance of peroxisomes is highlighted by fetal human genetic peroxisome biogenesis disorders such as Zellweger syndrome, all of which are linked to a failure of peroxisome biogenesis. Over 30 protein factors, termed peroxins, essential for peroxisome assembly, have been cloned [3–7] by making use of peroxisome biogenesis-defective mutants of yeast and mammalian cells such as Chinese hamster ovary (CHO) cell mutants [3,8–10].

In the studies on mechanisms of peroxisome assembly, peroxisomal protein import processes are better understood, while morphogenesis of peroxisomes is little defined. In mammalian cells, the Pex11p family including Pex11pα [11–13], Pex11pβ [14–16], and Pex11pγ [13,17] has been reported to be involved in peroxisome morphogenesis. Pex11p proteins appear to be directly associated with the regulation in size and number of peroxisomes, more likely at the peroxisome division step.

A member of the large GTPase family, dynamin-like protein 1 (DLP1), also called DNM1 in yeast [18] and DVLP in humans [19], is required for the maintenance of mitochondrial morphology, especially for the membrane fission event [20]. DLP1 is localized to outer membrane of mitochondria, interacting with Fis1p [21,22] and Mdv1p [21], both involved in mitochondrial membrane fission. More recently by the methods of overexpression and RNA interference, DLP1 was also suggested to be requisite for the maintenance of peroxisome morphology, apparently in the membrane fission, in mammalian cells [23,24].

We earlier reported a CHO mutant ZP121, one with abnormal peroxisome morphology in the peroxisome-defective CHO mutant cell lines isolated by the 9-(1′-pyrene)nonanol (P9OH)/UV selection method [9]. In this report, we show that abnormal peroxisome morphology in ZP121 is caused by an inactivating, missense mutation in the middle region of DLP1. The impaired fission and/or fusion apparently affected peroxisome morphogenesis in ZP121, in a temperature-sensitive manner.

Materials and methods

Cell culture

CHO cell lines including wild-type CHO-K1, ZP121 [9], and *pex2* Z65 [25] were cultured at 37°C or 40°C, as described [26].

Cloning of Chinese hamster DLP1

A ^{32}P-labeled *NcoI–NheI* fragment from open reading frame of rat *DRP1* (nucleotide positions at 678–1470) [27] was used as a probe to screen 1 × 10^5 colonies of CHO-K1 cDNA library in *pSPORT* vector (Invitrogen Life Tech). One positive clone was identified and its both strands were sequenced using a Dye-terminator DNA sequencing kit (Perkin-Elmer).

DNAs and transfection

To attach Myc-epitope tag at N-terminus of *PEX11β* product, PCR was done using a forward primer PEX11β*Bam*HI+2Fw (5′-GCGCGGATCCTGGACGCC-3′) and a reverse primer PEX11β*Not*IRv (5′-ACGCGCGGCCGCTCAGGGCTT-3′), and with *pCMV-SPORT·HsPEX11β* [14] as a template. PCR product was cloned into *pcDNA3.1Zeo*$^+$*·Myc*$_6$– by replacing the *Bam*HI–*Not*I fragments of vectors. To attach Flag- and HA_2-epitope tags at N-terminus of *DLP1* protein, PCR was done using a forward primer DLP1*Bam*HI+2Fw (5′-ACGGATCCTGGAGGCGCTAATC-3′) and a reverse DLP1*Not*IRv (5′-ACACACACGCGGCCGCTCACCAAAGATGAGT-3′), and with *DLP1* cDNA as a template. PCR product was cloned into *pcDNA3.1Zeo*$^+$*·Flag*– and *pcDNA3.1Zeo*$^+$*·HA*$_2$– by replacing the *Bam*HI–*Not*I fragment. Transfection was done using Lipofectamine (Invitrogen Life Tech) according to the manufacture's instruction. DNA-transfected cells were cultured for 16 h to 3 days.

Mutation analysis of DLP1 from ZP121 cells

DLP1 cDNA from ZP121 was isolated by reverse transcription-PCR method described in [17], with a pair of primers, DLP1*Bam*HI+2Fw and DLP1*Not*IRv. Amplified DNA fragments were subcloned into *pGEM-Teasy* vector (Promega) and sequenced. Genomic DNA was isolated from ZP121 with a DNeasy Tissue kit (Qiagen). The zygosity of the *DLP1* allele was determined by PCR amplification between nucleotides at 843–1202 using the genomic DNA, forward