Peroxisomal Degradation of trans-Undecenoic Fatty Acids in the Yeast Saccharomyces cerevisiae

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**Degradation of trans-unsaturated fatty acids was studied in the yeast Saccharomyces cerevisiae. Propagation of yeast cells on trans-9 elaidic acid medium resulted in transcriptional up-regulation of the SPS19 gene, whose promoter contains an oleate response element. This up-regulation depended on the Pip2p-Oaf1p transcription factor and was accompanied by induction of import-competent peroxisomes. Utilization of trans fatty acids as a single carbon and energy source was evaluated by monitoring the formation of clear zones around cell growth on turbid media containing fatty acids dispersed with Tween 80. For metabolizing odd-numbered trans double bonds, cells required the β-oxidation auxiliary enzyme Δ^2Δ^-enoyl-CoA isomerase Eci1p. Metabolism of the corresponding even-numbered double bonds proceeded in the absence of Sps19p (2,4-dienoyl-CoA reductase) and Dei1p (Δ^2Δ^-2,4-dienoyl-CoA isomerase). trans-2,trans-4-Dienoyl-CoAs could enter β-oxidation directly via Fox2p (2-enoyl-CoA hydratase 2 and D-specific 3-hydroxyacyl-CoA dehydrogenase) without the involvement of Sps19p, whereas trans-2,cis-4-dienoyl-CoAs could not. This reductase-independent metabolism of trans-2,trans-4-dienoyl-CoAs resembled the situation postulated for mammalian mitochondria in which oleic acid is degraded through a di-isomerase-dependent pathway. In this hypothetical process, trans-2,trans-4-dienoyl-CoA metabolites are generated by Δ^2Δ^-enoyl-CoA isomerase and Δ^2Δ^-2,4-dienoyl-CoA isomerase and are degraded by 2-enoyl-CoA hydratase 1 in the absence of 2,4-dienoyl-CoA reductase. Growth of a yeast fox2spsl9A mutant in which Fox2p was exchanged with rat peroxisomal multifunctional enzyme type 1 on trans-9,trans-12 linolealaidic acid medium gave credence to this theory. We propose an amendment to the current scheme of the carbon flux through β-oxidation taking into account the dispensability of β-oxidation auxiliary enzymes for metabolizing trans double bonds at even-numbered positions.

Double bonds in naturally occurring unsaturated fatty acids are mostly in the cis configuration. However, certain foods such as beef, milk, and margarine, also contain fatty acids in the trans configuration due to their enzymatic synthesis in ruminants by gastrointestinal microorganisms or their chemical synthesis during partial hydrogenation (hardening) of fats and vegetable oils (1). Diets high in trans-unsaturated fatty acids have previously been shown in healthy humans to lead to increased levels of serum lipoproteins of the type that can increase the risk of coronary heart disease (2). For this reason, it is important to study the metabolism of trans fatty acids.

Previous work using rat hepatocytes demonstrated that trans-unsaturated fatty acids are oxidized preferentially in peroxisomes (3). Peroxisomes are single membrane-limited organelles that occur in all eukaryotic cells examined. In the yeast Saccharomyces cerevisiae peroxisomes represent the sole site for fatty acid β-oxidation (4). The yeast β-oxidation process is catalyzed by the enzymes acyl-CoA oxidase (Pox1p), 2-enoyl-CoA hydratase 2, D-specific 3-hydroxyacyl-CoA dehydrogenase (Fox2p; multi-functional enzyme type 2; MFE^1 type 2), and 3-ketoacyl-CoA thiolase (Pot1p/Fox3p) as shown in Scheme 1A (5–8). To break down odd-numbered cis double bonds in unsaturated fatty acids such as oleic acid (cis-C^16:1,9), yeast cells rely on peroxisomal Eci1p (9, 10) representing the auxiliary enzyme Δ^2Δ^-enoyl-CoA isomerase (3,2-isomerase; Scheme 1B). Eci1p is also crucial for metabolizing even-numbered cis double bonds in fatty acids such as petroselinic acid (cis-C^16:1,9) in a process that additionally requires Sps19p (11), which corresponds to the auxiliary enzyme 2,4-dienoyl-CoA reductase (2,4-reductase; Scheme 1C).

In the present work S. cerevisiae was examined to determine whether it could serve as a model system for studying the degradation of trans-unsaturated fatty acids. The experimental advantages of using yeast for this study include the availability of mutant cells blocked at various steps in the β-oxidation process and the possibility of replacing the requisite genes affected in these mutants using the corresponding rat cDNAs. Yeast cells were examined for growth on trans-unsaturated fatty acids, and the requirement for β-oxidation auxiliary enzymes for breaking down trans double bonds was determined. In addition, the effect of expressing rat monofunctional ECI and peroxisomal MFE type 1 was monitored in yeast mutants lacking Eci1p or both Fox2p and Sps19p that were grown on trans-unsaturated fatty acids.

**EXPERIMENTAL PROCEDURES**

Strains—Escherichia coli strain DH10B was used for all plasmid amplifications and isolations. The S. cerevisiae strains used here were derived from BJ1991 (12), NKYS57, or UTL-7fox2 and are listed in Table I. Construction of strains BJ1991eci1Δ (9), NKYS57eci1Δ (9), yAG760 (9), yAG856 (9), yAG826 (9), yAG827 (9), BJ1991spsl9A (11),...
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yAG257 (11), yAG259 (11), yAG456 (11), BJ1991dci11Δ (13), BJ1991pex6Δ (14), yAG485 (14), yAG554 (15), and BJ1991pex2Δsof1Δ (16) has been described. Strain BJ1991dci11spso19Δ (yAG935) was constructed by disrupting the SPS19 locus in the BJ1991dci11Δ strain after transformation with an sps19Δ::LEU2 fragment generated by digesting plasmid pAG129 (11) with ScaI and StuI. Yeast colonies that had been rendered prototrophic for leucine were examined for utilization of oleic acid (cis-C_{18:1(9)}) and petroselinic acid (trans-C_{18:1(9)}) medium consisted of 0.67% (w/v) yeast nitrogen base with amino acids added, 0.1% (w/v) yeast extract and 2% (w/v) glucose was added, and cells were grown as described (11). Liquid oleic acid or ethanol media consisted of YP to which 0.2% (w/v) oleic acid and 0.02% (w/v) Tween 80 Tween 80 adjusted to pH 7.0 with NaOH or 2% (v/v) ethanol were added. Plates used to assess utilization of fatty acids were prepared as described (11) and contained either 0.125% (w/v) oleic acid (cis-C_{18:1(9)}), 0.125% (w/v) oleic acid and 2% (v/v) ethanol, 0.125% (w/v) linoleic acid (cis,cis-C_{18:2(9,12)}), or linolelaidic acid (trans,trans-C_{18:2(9,12)}) and 0.5% (w/v) Tween 80. For assays using culture drops, cells were grown in rich-glucose medium (YP and 2% (w/v) D-glucose) overnight to late log phase or in liquid elaidic acid medium, serially diluted, and applied to solid media. Preparation of protein extracts and measurement of β-galactosidase activity were described previously (21). Electrophoretic mobility shift assays were performed with crude protein extracts as described (21). The FOX3 oleic acid-response element (ORE) was excised from plasmid pSKF0304 (22) after digestion with XhoI and EcoRI and 2.5% (w/v) agarose gel electrophoresis. The DNA fragment was purified using QIAEX II (Qiagen Inc., Valencia, CA), labeled with [α-32P]dATP using Klenow enzyme, and purified again with QIAEX II before incubation with protein extracts. Fluorescence microscopy of living yeast cells expressing green fluorescent protein was done according to published methods (9).

RESULTS

Tween 80 as a Dispersing Agent in Trans Fatty Acid Media—

The methods employed here to address the issue of how yeast cells degrade trans fatty acids include the use of wild-type yeast cells and otherwise isogenic pex5Δ or pex6Δ mutants that are unable to degrade fatty acids due to defective peroxisomes. Yeast is routinely examined for utilization of fatty acids as a single carbon and energy source, but these must be dispersed in media using agents such as Tween 80 (polyoxyethylensorbitan monoleate; Sigma). Since yeast might hydrolyze Tween 80 to extract and 2% (w/v) meat peptone to which 0.05% (w/v) n-glucose was added, and cells were grown as described (11). Liquid oleic acid or ethanol media consisted of YP to which 0.2% (w/v) oleic acid and 0.02% (w/v) Tween 80 Tween 80 adjusted to pH 7.0 with NaOH or 2% (v/v) ethanol were added. Plates used to assess utilization of fatty acids were prepared as described (11) and contained either 0.125% (w/v) oleic acid (cis-C_{18:1(9)}), 0.125% (w/v) oleic acid and 2% (v/v) ethanol, 0.125% (w/v) linoleic acid (cis,cis-C_{18:2(9,12)}), or linolelaidic acid (trans,trans-C_{18:2(9,12)}) and 0.5% (w/v) Tween 80. For assays using culture drops, cells were grown in rich-glucose medium (YP and 2% (w/v) D-glucose) overnight to late log phase or in liquid elaidic acid medium, serially diluted, and applied to solid media. Preparation of protein extracts and measurement of β-galactosidase activity were described previously (21). Electrophoretic mobility shift assays were performed with crude protein extracts as described (21). The FOX3 oleic acid-response element (ORE) was excised from plasmid pSKF0304 (22) after digestion with XhoI and EcoRI and 2.5% (w/v) agarose gel electrophoresis. The DNA fragment was purified using QIAEX II (Qiagen Inc., Valencia, CA), labeled with [α-32P]dATP using Klenow enzyme, and purified again with QIAEX II before incubation with protein extracts. Fluorescence microscopy of living yeast cells expressing green fluorescent protein was done according to published methods (9).

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release oleic acid, this could provide abundant cis fatty acids in the medium, thereby complicating the interpretation of the growth data. To underscore the appropriateness of using Tween 80 for dispersing the fatty acids used in this work, drops of serially diluted yeast cultures were applied on solid media containing either 0.125% (w/v) oleic acid dispersed with 0.5% (w/v) Tween 80 or the latter two combined with 2% (v/v) ethanol (Fig. 1).

The results showed that on the opaque medium consisting of oleic acid/Tween 80 (upper panel; Fig. 1) the wild-type strain produced the characteristic clear zone, whereas the two pex mutants failed to do so. The clear zone produced by the wild type is indicative of utilization of the fatty acid as a sole carbon and energy source. On the other hand, all three strains produced clear zones on medium containing oleic acid/Tween 80 plus ethanol (lower panel; Fig. 1). This demonstrated that in the presence of alternative carbon sources such as ethanol that do not require peroxisomes for their degradation, mutant yeast cells could internalize the fatty acids present in the medium.

However, Tween 80-dispersed fatty acids alone could not be internalized by pex mutants, and therefore, unlike ethanol, Tween 80 combined with fatty acids did not represent a good alternative carbon source. This plate assay therefore served to underscore the difference between β-oxidized fatty acids and those internalized into cells for assimilation into membranes or lipid droplets.

The Response of S. cerevisiae Cells to Elaidic Acid Depends on the Pip2p Transcription Factor—S. cerevisiae cells grown on cis-unsaturated fatty acids such as oleic acid as the sole carbon source dramatically increase their peroxisomal compartment (23). This expansion is tightly associated with an induced synthesis of peroxisomal matrix enzymes (21). From the outset of this study, it was not clear whether yeast could also utilize trans-unsaturated fatty acids as a single carbon and energy source. The following two sections are concerned with whether (i) trans-unsaturated fatty acids mediate transcriptional up-regulation of genes encoding β-oxidation enzymes, and (ii) growth on these fatty acids requires an expandable peroxisomal compartment.

To determine whether trans-unsaturated fatty acids emulate the transcriptional effect seen using the corresponding cis isomers, levels of transcription of the oleic acid-responsive gene SPS19 were monitored in cells containing an SPS19-lacZ reporter gene. Wild-type cells (yAG456) as well as a pip2Δ (21) mutant strain (yAG485) in which the response to oleic acid is abolished (11) were grown to late log phase on rich-glucose (YP

### Table II

| Fatty acid                 | Description                        |
|---------------------------|------------------------------------|
| Oleic acid                | cis double bond at position Δ⁹      |
| Elaidic acid              | trans double bond at position Δ⁵    |
| Linoleic acid             | cis double bonds at positions Δ⁵ and Δ¹² |
| Linolelaidic acid         | trans double bonds at positions Δ⁵ and Δ¹² |

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Oae1p transcription factor (16, 21, 26, 27) to the ORE in their promoters. To underscore that transcriptional induction under elaidic acid-medium conditions was coincidental with the formation of a complex between Pip2p-Oae1p and OREs, an electrophoretic mobility shift assay was performed using the FOX3 ORE. Soluble protein extracts were obtained from wild-type cells grown in media containing either Tween 80 or elaidic acid/Tween 80. Extracts were incubated with labeled DNA, and soluble and bound DNA were resolved on a 5% (w/v) polyacrylamide gel (Fig. 2). The results showed that the intensity of the Pip2p-Oae1p complex formed under Tween 80 medium conditions (_lane 2_; Fig. 2) was significantly reduced compared with that generated using the corresponding elaidic acid/Tween 80 extract (_lane 3_; Fig. 2). Hence, despite consisting of a fatty acid derivative, Tween 80 alone represented a poor carbon source for inducing the formation of a Pip2p-Oae1p complex with FOX3 ORE. The situation seen here with respect to the difference in Pip2p-Oae1p complex intensities between elaidic acid/Tween 80- and Tween 80-derived protein extracts closely resembled that known to occur when comparing protein extracts from oleic acid–grown cells with those grown on ethanol (21).

**Yeast Growth on Elaidic Acid Medium Requires Functional Peroxisomes—** A milestone in the sequence of events leading to the ability of yeast to utilize fatty acids is the expansion of the peroxisomal compartment (23), which represents the sole site of β-oxidation in this organism (4). The state of this compartment could be studied under _trans_ fatty acid medium conditions, using a peroxisomally targeted green fluorescent protein (GFP-Eci1p). Expression of this reporter in wild-type cells resulted in punctate fluorescence, whereas in _pex6Δ_ or _pex8Δ_ cells devoid of functional peroxisomes, the reporter protein remained cytosolic (9, 10).

To view yeast peroxisomes under _trans_-unsaturated fatty acid-medium conditions, a wild-type strain expressing the GFP reporter was propagated in liquid elaidic acid medium. As a control, GFP-expressing _pex6Δ_ cells devoid of normal peroxisomes (29) were also examined. The results demonstrated that like the situation with oleic acid, under elaidic acid-medium conditions, cells exhibited a fluorescence pattern that consisted of closely bunched points (Fig. 3). On the other hand, the punctate pattern was significantly less dense under both Tween 80- and ethanol-medium conditions (Fig. 3). Expression of the reporter protein in _pex6Δ_ cells resulted in diffuse fluorescence (Fig. 3). Hence, unlike the situation with Tween 80–grown cells, under elaidic acid-medium conditions, GFP-Eci1p was compartmentalized into peroxisomes that were both numerous and bunched.

Finally, to determine whether yeast cells could actually grow...
on trans-unsaturated fatty acids and whether this growth depended on peroxisomes, wild-type cells were compared with otherwise isogenic pex5Δ and pex6Δ mutants lacking a functional peroxisomal compartment (30) for growth on elaidic acid medium. Late log phase cells were transferred to elaidic acid medium at an A600 of 0.2, and cell growth was monitored by a vital count after 4 days of incubation. The results demonstrated that on elaidic acid/Tween 80 medium conditions wild-type cells gave rise to cell culture concentrations that were at least 4-fold more dense than those produced by the two pex mutants used (Table IV). On Tween 80, no difference was detected between the three strains. To reiterate the difference in the number of wild-type cells compared with the pex6Δ mutant after growth on the two media, 2.5-ml culture aliquots from the serial dilutions used for the vital counts were applied to His2 glucose medium (Fig. 4). The results demonstrated an order of magnitude more wild-type cells than pex6Δ cells devoid of functional peroxisomes after propagation on elaidic acid/Tween 80 medium. This growth difference was not manifested after propagation in medium containing Tween 80 alone (Fig. 4). This indicated that, like the situation with other fatty acid media, growth on elaidic acid medium was facilitated by functional peroxisomes.

**A Sole β-Oxidation Auxiliary Enzyme, Eci1p, Is Required for S. cerevisiae Cells to Metabolize Trans-unsaturated Fatty Acids with Double Bonds at Odd-numbered Positions**—The only double bond that can be degraded by the classical β-oxidation process is at the Δ2-position and in the trans configuration (Scheme 1). Hence, removal of other double bonds necessitates β-oxidation auxiliary enzymes (31). For this reason, yeast cells lacking the auxiliary enzyme Sps19p are unable to degrade cis-unsaturated fatty acids with double bonds at even-numbered positions (11). In addition, cells devoid of the auxiliary enzyme Eci1p, which is essential for repositioning cis double bonds from the Δ3 to the Δ2 positions, are blocked in the breakdown process of cis-unsaturated fatty acids with double bonds at any position (9, 10). DCII-deleted yeast cells (32) were also unable to degrade cis-unsaturated fatty acids with double bonds at any position.

**TABLE IV**

Yeast growth on elaidic acid/Tween 80 or Tween 80 media

| Medium          | Elaidic acid/Tween 80 | Tween 80 |
|-----------------|-----------------------|----------|
| BJ1991 wild type| 4.4 x 10^9            | 1.0 x 10^8 |
| BJ1991 pex5Δ    | 0.8 x 10^7            | 0.9 x 10^8 |
| BJ1991 pex6Δ    | 1.0 x 10^7            | 1.1 x 10^8 |

* cells/ml culture.
devoid of Δ3,5,Δ2,4-dienoyl-CoA isomerase (di-isomerase) are not known to be blocked at any step of β-oxidation of cis-unsaturated fatty acids (13, 33, 34).

To determine whether repositioning of trans double bonds at odd- or even-numbered positions other than Δ12 also requires β-oxidation auxiliary enzymes, wild-type and mutant yeast cells were streaked on trans-9,trans-12 linolelaidic acid medium (upper panel; Fig. 5). As a control, cells were also applied to medium containing the corresponding cis,cis isomer linoleic acid (lower panel; Fig. 5). The results demonstrated that eci1Δ mutant cells did not form clear zones in either media (Fig. 5). This indicated that Eci1p was critical at least for metabolizing odd-numbered trans double bonds originally at position 9 in the fatty acid substrate and, therefore, represents a physiological Δ3-cis/trans-Δ2-trans-enoyl-CoA isomerase.

On the other hand, the results in Fig. 5 demonstrated that sps19Δ cells, which failed to form a clear zone in the control cis-9,cis-12 linoleic acid medium due to their inability to metabolize the Δ12 cis double bond, formed a broad clear zone in the corresponding trans-9,trans-12 linolelaidic acid medium. This showed that although Sps19p was essential for transforming trans-2,cis-4-dienoyl-CoAs to trans-3 metabolites suitable for acting as substrate for Eci1p (Scheme 1C), metabolism of trans-2,trans-4-dienoyl-CoAs did not depend on this enzyme. This implied that either an additional, novel, peroxisomal 2,4-reductase specific for trans-2,trans-4-dienoyl-CoAs exists or that even-numbered trans metabolites act directly as substrate for the second step of classical β-oxidation represented by Fox2p.

The demonstration in Fig. 5 that dci1Δ cells formed well defined clear zones in both media supports our earlier studies reporting the dispensability of Dci1p for utilizing fatty acids (13). Consonant with this observation, the sps19Δdci1Δ double mutant in which the putative reduction pathway for metabolizing odd-numbered cis double bonds is completely blocked also gave rise to a broad clear zone in the trans-9,trans-12 linolelaidic acid medium (upper panel; Fig. 5). The absence of a corresponding clear zone around the double mutant in the cis-9,cis-12 linoleic acid medium (lower panel; Fig. 5) was due to the missing Sps19p required for metabolizing the double bond originally at position Δ12. Hence, the salient points derived from the experiments depicted in Fig. 5 were as follows. (i) Yeast Eci1p acted on trans-3-enoyl-CoA metabolites to support degradation of unsaturated fatty acids with odd-numbered trans double bonds, and (ii) to degrade even-numbered trans double bonds, yeast cells did not rely on Eci1p, Sps19p, or Dci1p.

Expression of Rat Monofunctional 3,2-Isomerase or Rat Multifunctional Enzyme Type 1 in eci1Δ Mutant Cells Restores Utilization of Trans-unsaturated Fatty Acids—The results depicted in Fig. 5 demonstrated that eci1Δ cells did not utilize...
trans-unsaturated fatty acids such as linoleic acid. Hence, these mutants could serve as a test organism for examining whether, in addition to accepting cis-3 metabolites, mammalian Eci1p analogs could also accept trans-3 metabolites as a physiological substrate. Previous work using cis-ununsaturated fatty acids has shown that yeast Eci1p can be functionally replaced by the two rat proteins peroxisomal MFE type 1 and monofunctional ECI (15). Therefore, to determine the effect on mutant eci1Δ cells expressing the respective heterologous enzymes, transformants were streaked on trans-9,12-linoleic acid media.

The results demonstrated that the rat proteins had efficiently substituted for yeast Eci1p under both medium conditions, since their expression in the mutant strain gave rise to clear zones that were indistinguishable from those produced by the self-complemented cells. Control eci1Δ mutants harboring the plasmid vector did not form a clear zone in either medium (Fig. 6). Therefore, rat peroxisomal MFE type 1 and monofunctional ECI represented proteins with Δ5-cis/trans-Δ5-trans-enoyl-CoA isomerase activities.

**Rat Peroxisomal MFE Type 1 Accepts trans-2,trans-4 Metabolites as Substrate in Vivo**—Current understanding of β-oxidation stipulates that the second reaction step represented by trans-2-enoyl-CoA hydratase cannot proceed if a double bond is present at the Δ4 position (Scheme 1C). The results in Fig. 5 (upper panel) demonstrated that for utilizing trans-2,trans-4-dienoyl-CoA intermediates formed during the metabolism of the trans-double bond originally at the Δ12 position, Sp19p (and therefore also Eci1p) were dispensable. This raised the issue of whether yeast Fox2p could accept trans,trans intermediates as substrates. Such intermediates have been previously shown to be degraded by trans-2-enoyl-CoA hydratase in the absence of 2,4-reductase (35). However, these experiments were conducted using an in vitro mammalian β-oxidation system reconstituted from mitochondrial enzymes. Hence, to examine whether 2,4-reductase might be dispensable for degrading such trans,trans metabolites in vivo, a system based on the mammalian enzyme representing peroxisomal trans-2-enoyl-CoA hydratase was used.

The rat protein corresponding to this enzyme activity, peroxisomal MFE type 1, has previously been shown to substitute for yeast Fox2p during utilization of oleic acid (17). To determine whether an exchange between yeast Fox2p and rat peroxisomal MFE type 1 would also function in a strain additionally devoid of Sp19p, a fox2sp19Δ double mutant was generated and transformed with the plasmid expressing this rat protein. As a control, these mutant cells were transformed with the native Fox2p. The strains were streaked on trans-9,12-linoleic acid and cis-9,cis-12 linoleic acid media.
Degradation of trans-Unsaturated Fatty Acids

We demonstrated here for the first time that S. cerevisiae cells utilize trans double bonds at even-numbered positions in the absence of the known \( \beta \)-oxidation auxiliary enzymes. The S. cerevisiae genome does not contain any novel peroxisomal proteins with obvious homologies to 2,4-reductases or 3,2-isomerases that could have potentially substituted for Sps1p or Eci1p. In light of these new findings, we propose an amendment to the accepted scheme of the carbon flux through \( \beta \)-oxidation (Scheme 2). In contrast to the dispensability of auxiliary enzymes for metabolizing even-numbered trans double bonds, yeast cells engaged the same \( \beta \)-oxidation auxiliary enzyme Eci1p used for breaking down the corresponding cis isomers (Scheme 1C). Both rat 3,2-isomerases, peroxisomal MFE type 1 and monofunctional ECI, were shown here to be able to execute the requisite \( \Delta^3 \) to \( \Delta^2 \) repositioning of trans double bonds for ensuring the carbon flux through \( \beta \)-oxidation. Due to the dual distribution of ECI (15), at least in rodents this faculty is extended to both the peroxisomal as well as the mitochondrial \( \beta \)-oxidation compartments.

Like the situation in yeast in which Fox2p accepts trans-2, trans-4-dienoyl-CoAs as substrate, the second step of mammalian \( \beta \)-oxidation executed by rat peroxisomal MFE type 1 also accepted this intermediate without requiring the repositioning of the double bonds by Sps1p. This is the first in vivo demonstration of the final step in the di-isomerase-dependent route for degrading fatty acids (28). In this di-isomerase-dependent route, which represents an alternative to the reductional pathway, 3,2-isomerase and di-isomerase are sufficient to establish the carbon flux, since the combined enzyme activities result in the production of trans-2, trans-4-dienoyl-CoA intermediates that feed directly into \( \beta \)-oxidation.

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