Loss of Compartmentalization Causes Misregulation of Lysine Biosynthesis in Peroxisome-Deficient Yeast Cells

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To characterize the metabolic role of peroxisomes in yeast cells under physiological conditions, we performed a comprehensive meta-analysis of published microarray data. Previous studies of yeast peroxisomes have mainly been focused on the function of peroxisomes under extreme conditions, such as growth on oleate or methanol as the sole carbon source, and may therefore not be representative of the normal physiological role of yeast peroxisomes. Surprisingly, our analysis of the microarray data reveals that the only pathway responding to peroxisome deficiency in mid-log phase is lysine biosynthesis, whereas classical peroxisomal pathways such as beta-oxidation are unaffected. We show that the upregulation of lysine biosynthesis genes in peroxisome-deficient yeasts shares many characteristics with the physiological response to lysine starvation. We provide data that suggest that this is the result of a “pathological” stimulation of the Lys14p transcriptional activator by the pathway intermediate aminoadipate semialdehyde. Mistargeting of the peroxisomal lysine pathway to the cytosol increases the active concentration of aminoadipate semialdehyde, which is no longer contained in the peroxisome and can now activate Lys14p at much lower levels than in wild-type yeasts. This is the first well-documented example of pathway misregulation in response to peroxisome deficiency and will be useful in understanding the phenotypic details of human peroxisome-deficient patients (Zellweger syndrome).

Peroxisomes are single-membrane-bounded organelles present in most eukaryotic cells, with the exception of Plasmodium, Giardia, Trichomonas, Entamoeba, and related species. Peroxisomes typically contain the enzymes of fatty acid beta-oxidation and catalase, which converts the hydrogen peroxide formed by fatty acyl-coenzyme A (CoA) oxidase to water and oxygen. Several other oxidases, e.g., monoamine oxidase, urate oxidase, hydroxycacid oxidase, methanol oxidase, and acetyl-spermidine oxidase, are also present in peroxisomes of some species and benefit from the same detoxification mechanism. In addition to these oxidative reactions, peroxisomes may contain many other biosynthetic pathways. Enzymes of these supplemental pathways are usually absent or localized in non-peroxisomal compartments in most eukaryotes, and their peroxisomal localization is a specialization of relatively few species. Examples of facultative peroxisomal pathways are glycolysis and purine salvage (in the peroxisomes of kinetoplastids, such as Trypanosoma and Leishmania [23]), isoprenoid biosynthesis (in vertebrates [1]), ether phospholipid biosynthesis (in kinetoplastids and animals [15, 33]), and the glyoxylate cycle, which channels the products of beta-oxidation into gluconeogenesis in plants, yeasts, and Caenorhabditis elegans (7).

Peroxisome-deficient mutants of most organisms are viable (with the possible exception of kinetoplastids), but a lack of functional peroxisomes can lead to severe pathologies in multicellular organisms (3, 5, 8, 16, 20, 24). In all organisms examined, the most severely affected metabolic pathway is the beta-oxidation of fatty acids, but in human patients bile acid production and cholesterol biosynthesis also seem to be impaired. Affected human patients show a characteristic complex of symptoms, including metabolic deficiencies, hepatomegaly, facial malformations, and severe neurological disturbances (Zellweger syndrome [24]).

Peroxisome biogenesis and physiology has been examined in detail in several yeast species (Pichia, Yarrowia, Hansenula, and Saccharomyces spp. [27, 28, 31]). Most of these studies were, however, focused on the function of peroxisomes under extreme conditions, such as growth on oleate or methanol as the sole carbon source, and may therefore not be representative of the normal physiological role of yeast peroxisomes. In order to characterize the function of peroxisomes in Saccharomyces cerevisiae, we performed a meta-analysis of two large-scale microarray experiments (13, 18). Our analysis demonstrates that the only pathway affected by peroxisome deficiency in mid-log phase on complete medium is lysine biosynthesis. Furthermore, growth rate determinations show that the upregulation of lysine biosynthesis after peroxisome disruption is not due to a severe deficiency of the enzymes but is most likely the result of the mislocalized production of the transcriptional modulator aminoadipate semialdehyde.

MATERIALS AND METHODS

Microarray analysis. The microarray analysis is based on two large published datasets (Gasch et al. [13] and Hughes et al. [18]). The data were obtained from these authors and then analyzed by using customized Perl scripts to identify coregulated genes (these programs are available from R. Breitling upon request).

Data set 1: PEX12 disruption. PEX12 disruption was described previously (18). Mutant (pes12::SanR–MTA1/mta1::hox3-1/– hox2-0/– mta15-0/– una3-0/– hox2-0/– hox2-0/–) and wild-type (BY4743, MATA1/MTA1/mta1::hox3-1/– hox2-0/– una3-0/– mta15-0/– una3-0/– hox2-0/– hox2-0/–) cells were grown in synthetic complete (SC) medium with 2% glucose at 30°C overnight to mid-log phase. They were diluted to 0.4 to...
1 million cells/ml and grown an additional 5 to 7 h to reach 4 to 10 million cells/ml).

Data set 2: Stress response. The stress response was evaluated as described previously (13). Wild-type (DBY7286: *ura3-52 GAL2*) cells or variants thereof were grown in rich medium (yeast extract-peptone-dextrose) at 30°C to early log phase (optical density /H11002.75 to 0.4); a time zero reference sample was then collected. The cells then were grown for some time under one of several stress conditions (e.g., heat shock, temperature shift, osmolarity, hydrogen peroxide, dithiothreitol, nitrogen depletion, and stationary phase). During this time data were collected over the course of 2 to 3 h in most cases. A total of 142 samples was analyzed.

Two-dimensional visualization. The distance between gene expression profiles was calculated from the Pearson correlation coefficient (PCC), with distance calculated as follows: (1 − PCC)/2. For visualization, the resulting n × n matrix describing the pairwise distances between n genes was projected into a plane. This was done by a modified multidimensional scaling, determining the two-dimensional arrangement that minimizes the discrepancy between actual and displayed distances.

Nearest-neighbor analysis. Because of the nonnormality of the correlation coefficients, a close distance in the two-dimensional representation described above does not necessarily indicate whether two genes are highly correlated or not. This problem is aggravated by the fact that only a selection of genes is included in the pictures. To get a more reliable impression of the significance of the clustering, we determined the nearest neighbors of each gene, i.e., the genes among the complete data set that show the highest correlation coefficient to a given gene. If gene A is among the ten nearest neighbors of gene B in the complete data set, the two genes are connected by a line in the two-dimensional representations. In this way, significant coregulated clusters are easily identified.

Yeast experiments. Diploid gene-deleted yeast strains in the BY4743 background were obtained from Research Genetics. They were maintained in yeast extract-peptone-dextrose medium plus 200 μg of Geneticin/ml. Growth experiments were performed in SC medium with or without lysine. A yeast strain disrupted in the hydroxymethyl-glutaryl-CoA reductase (HMGI) gene, which is unrelated to lysine biosynthesis and peroxisomal metabolism, served as a reference.

Complete genomes. Genomic sequences for the white-rot fungus *Phanerochaete chrysosporium* were obtained from the Department of Energy Joint Genome Institute website (http://www.jgi.doe.gov). All other sequences are available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Gene expression changes in peroxisome-deficient yeasts. To characterize the function of yeast peroxisomes under physiological conditions, we first analyzed the transcriptional response of a *PEX12*-disrupted yeast strain (data provided by Hughes et al. [18]). Pex12p is a central member of the peroxisomal import machinery and the disruption of Pex12p leads to a complete loss of matrix protein import and absence of functional peroxisomes (2). Mutations of the human homologue lead to Zellweger syndrome (complementation group 3 [6]). Figure 1 shows that the expression of most of the more than
6,000 yeast genes present on the microarray does not change in response to peroxisome disruption. PEX12 expression is undetectable, as expected. Only a very small fraction of genes are highly upregulated (Table 1): 11 genes show upregulation by more than a factor of 2 (11/6,172 = 0.18%). Of these, five are involved in lysine biosynthesis (LYS9, LYS20, LYS4, LYS1, and LYS12). Of the six other highly upregulated genes, two (SNZ1/SNO1) encode a complex that is involved in pyridoxal phosphate biosynthesis, i.e., production of an essential cofactor of lysine biosynthesis, and one (YBR147W) shows similarity to a nitrogen-starvation sensor of Schizosaccharomyces pombe, and two (CTP1 and YJL200C) are supposed to convert citrate and might also interact with homocitrate, the first product of the lysine biosynthesis pathway. The eleventh gene (YDL183C) was previously uncharacterized. Two further lysine biosynthesis genes (LYS21 and LYS2) are upregulated by factors of 1.64 and 1.57, respectively. Downregulation by a factor of 2 or more was observed in only a few genes coding for unrelated proteins (heat shock protein HSP12, inositol-3-phosphate synthase INO1, zinc uptake transporter ZRT1, and the glucose transporter HXT10). Taken together, this expression pattern indicates the activation of a specific lysine starvation response in the peroxisome-deficient yeasts. This is especially surprising since the PEX12-deficient yeasts were grown in SC medium, containing sufficient amounts of lysine. In contrast to the activation of lysine biosynthesis, no significant upregulation of classical peroxisomal pathways was observed (e.g., catalase CTA1 [upregulated by a factor of 1.14], multifunctional beta-oxidation enzyme FOX2 [upregulated by a factor of 1.01], and glyoxylate cycle isocitrate lyase ICL1 [upregulated by a factor of 1.06]). Since in wild-type cells these pathways are predominantly activated in stationary phase (data not shown), a lack of upregulation in PEX12-disrupted yeasts in mid-log phase might be expected.

Phylogenetic profile of peroxisomal targeting signals in lysine biosynthesis genes. The specific upregulation of lysine biosynthesis gene expression in a peroxisome-deficient yeast strain is a strong indication that lysine biosynthesis is at least partially regulated in yeast. The phylogenetic profile of peroxisomal targeting signals in lysine biosynthesis enzymes is shown in Fig. 2. The phylogenetic relationship between the yeast species is indicated on the right (the tree is based on data in Heckman et al. [17]). Mya, million years ago.

![Phylogenetic profile of peroxisomal targeting signals in lysine biosynthesis enzymes](image-url)

**TABLE 1.** Genes that are upregulated by a factor of 2 or more in response to a disruption of the PEX12 gene<sup>a</sup>

| Gene   | ORF      | Extent of upregulation (factor) | Function                                                   | PTS   | Lysine-auxotroph? |
|--------|----------|---------------------------------|-----------------------------------------------------------|-------|------------------|
| LYS9   | YNR050C  | 5.06                            | Saccharopine dehydrogenase                                | -SLx5HP- | Yes              |
| LYS20  | YDL182W  | 3.96                            | Homocitrate synthase                                      | Yes    |                  |
| CTP1   | YBR291C  | 3.39                            | Citrate transporter (mitochondrial)                       | Partially† |                 |
| LYS4   | YDR234W  | 2.99                            | Homoaconitase                                             | Yes    |                  |
| SNO1   | YMR095C  | 2.83                            | Stationary-phase induced gene                             | No     |                  |
| YIL200C| YIL200C  | 2.74                            | Aconitase homologue                                       | Partially† |                 |
| LYS1   | YIR034C  | 2.56                            | Saccharopine dehydrogenase                                | -SRL* | Yes              |
| YIL12  | YIL094C  | 2.47                            | Homoisocitrate dehydrogenase                              | -SRL  | Yes              |
| YBR147W| YBR147W  | 2.45                            | STMI-homologue, 7-TM nitrogen starvation sensor            | No     |                  |
| SNZ1   | YMR096W  | 2.26                            | Stationary-phase induced gene                             | No     |                  |
| YDL183C| YDR183C  | 2.07                            | Uncharacterized                                           | No     |                  |
| LYS21  | YDL131W  | 1.64                            | Homocitrate synthase                                      | Yes    |                  |
| LYS2   | YBR115C  | 1.57                            | Aminoadipate semialdehyde dehydrogenase                   | -KLx5HL- | Yes              |
| LYS5   | YGL154C  | 1.17                            | Lys2p-phosphopantetheinyl transferase                     | Yes    |                  |
| LYS21  | YDL131W  | 1.64                            | Homocitrate synthase                                      | Yes    |                  |
| LYS2   | YBR115C  | 1.57                            | Aminoadipate semialdehyde dehydrogenase                   | Yes    |                  |
| LYS5   | YGL154C  | 1.17                            | Lys2p-phosphopantetheinyl transferase                     | Yes    |                  |
| LYS21  | YDL131W  | 1.64                            | Homocitrate synthase                                      | Yes    |                  |
| LYS2   | YBR115C  | 1.57                            | Aminoadipate semialdehyde dehydrogenase                   | Yes    |                  |
| LYS5   | YGL154C  | 1.17                            | Lys2p-phosphopantetheinyl transferase                     | Yes    |                  |

* For reference, three other genes involved in lysine biosynthesis are shown at the bottom of the table. Functional properties according to the Saccharomyces Genome Database (http://genome-www.stanford.edu/Saccharomyces). PTS, predicted peroxisomal targeting signal. Peroxisomal targeting has been shown by GFP fusion (14) and is indicated by an asterisk. The doubling time is increased by ca. 150% in YIL200C-disrupted and 10% in CTP1-disrupted yeast in lysine-deficient medium and is indicated by a dagger.

* ORF, open reading frame.
partly a peroxisomal pathway. Indeed, Geraghty et al. (14) has shown that two enzymes of the pathway (Lys4p and Lys1p) contain C-terminal peroxisomal targeting signals (PTS-1) and are targeted to peroxisomes. We identified an additional putative PTS-1 (serine-arginine-lysine-COOH) in Lys12p. In addition, possible PTS-2-like sequences are present in the N-terminal sequence of Lys9p and Lys2p (Table 1). To determine whether peroxisomal localization is a general feature of the lysine biosynthetic pathway, we identified the homologues of Saccharomyces -Lys1p, -Lys4p, and -Lys12p in the complete genomic sequences of four other yeast species (Fig. 2). In none of these species are the C-terminal targeting signals conserved, even though the sequences -IKRL and -IKRI in Lys12p of Aspergillus fumigatus and Phanerochaete chrysosporium might function as degenerate signals (25). This is in marked contrast to the case of classical peroxisomal proteins, such as multifunctional beta-oxidation protein (Fox2p), 2,4-dienoyl-CoA reductase (Sps19p), or catalase (Cta1p), and indicates that the peroxisomal localization of lysine biosynthesis is a rather recent specialization of baker’s yeast. Just like the other supplemental peroxisomal pathways described above, lysine biosynthesis does not require an oxidase reaction. One reason for its localization in peroxisomes may be facilitated metabolic channeling; another might be the reduction of cytosolic aminoadipate semialdehyde, a pathway intermediate that is toxic at a high concentration (32). This analysis does not imply that the whole pathway is peroxisomal. Peroxisomal targeting signals are conspicuously absent from Lys5p (which could be piggy-backing into peroxisomes with its dimerization partner Lys2p [12]) and from the amino acid aminotransferases (Aro8p and Aro9p) that are proposed to act in the lysine biosynthesis pathway (19). A cytosolic detour is also observed for the peroxisomal glyoxylate cycle in yeasts and plants, where the central aconitase reaction takes place in the cytosol.

**Growth on lysine-deficient medium.** Given the role of peroxisomes in lysine biosynthesis, we predicted that peroxisome-deficient yeasts would show a severe defect of lysine biosynthesis, when grown in the absence of lysine. However, when we grew PEX5-, PEX7-, and PEX12-disrupted yeast strains—which are deficient in PTS-1, PTS-2, and both peroxisomal import pathways, respectively—in lysine-deficient SC medium, we observed no significant changes in doubling times com-

![FIG. 3. Two-dimensional visualization of similarities of expression profiles in wild-type cells under environmental stress. Genes are arranged in the plane in such a way that genes with highly correlating expression patterns are shown closest together. The diameter of the circle corresponds to the maximal distance of (anticorrelated) genes. The data were extracted from Gasch et al. (13). Red, beta-oxidation genes; blue, lysine biosynthesis genes; green, peroxisome biogenesis genes.](image-url)
pared to a HMG1-disrupted strain, which should show a wild-type response. Growth was retarded by at most 10% in PEX5- and PEX12-deficient strains and was equal to wild-type growth in the PEX7 mutant. This indicates that even if substrate dilution and/or enzyme instability may affect the mislocalized pathway, lysine biosynthesis is still effectively carried out in the peroxisome-deficient yeasts.

We also show that a yeast strain carrying a targeted disruption of the YJL200C gene shows severely retarded growth on lysine-deficient medium (doubling time ca. 200 min, compared to 80 min on lysine-containing medium). Yjl200cp is predicted to be a citrate aconitase isozyme but might also be a homocitrate aconitase acting in concert with Lys4p. A slight, but consistent, lysine dependence is also seen in a CTP1-deficient yeast strain (doubling time increased ca. 10% in the absence of lysine). Ctp1p is a mitochondrial citrate transporter and might be involved in the transport of homocitrate produced by nuclear homocitrate synthase (Lys20p/Lys21p) into the peroxisome for further conversion by homocitrate aconitase (Lys4p). These data again confirm the lysine specificity of the transcriptional response seen in the peroxisome-disrupted yeast.

Regulation of peroxisome biogenesis and peroxisomal pathways in wild-type yeast. To compare the transcriptional response observed in the PEX12-disrupted yeast to the wild-type situation, we analyzed the most comprehensive microarray data set examining wild-type gene expression under a variety of physiological stress conditions (13). Figure 3 shows in a condensed way the similarity of the expression patterns of peroxin genes under these conditions compared to one another, as well as compared to genes of beta-oxidation and lysine biosynthesis. It can be seen that peroxin genes do not form a tight cluster, but their expression profiles are only weakly correlated, i.e., scattered over a large part of the plane in a two-dimensional visualization. This may be due to the fact that most peroxins are stable proteins and are needed only at relatively low levels, so that regulatory responses may be spurious. This is corroborated by the fact that the most typical expression pattern of all peroxin genes is shown by PEX18, which has the highest average correlation with other peroxins (average correlation coefficient = 0.456, compared to an average of 0.275 for other peroxins). Pex18p is an unstable protein that is constitutively degraded (26), so its expression pattern should most accurately reflect the demands of peroxisome biogenesis.

Figure 4 shows a more detailed analysis of the coregulation of peroxisome biogenesis and beta-oxidation. The distances and arrangement of the genes are the same as in Fig. 3, i.e., genes that have highly correlated expression patterns are shown close to one another, but only beta-oxidation genes and closely coregulated genes are shown. Genes that are among each other’s 10 nearest neighbors in the complete data set are connected by lines. It can be seen that beta-oxidation genes have highly coregulated expression patterns, i.e., they are connected by a dense network of mutual next-neighbor relationships. The topography of this network has no relationship to the topography of the biosynthetic pathway, e.g., genes for the beta-oxidation of unsaturated fatty acids (EC12, SPS19, EC11, and IDP3) are not preferentially coregulated. This indicates that the genes of beta-oxidation form a single regulatory module. They also show a close relationship to peroxisome biogenesis: 100% of the beta-oxidation genes include at least one peroxin gene among their 10 nearest neighbors, and 10 of 11 genes have an expression pattern that is closely similar to that of PEX18, which (as discussed above) is the most typical peroxin with regard to its expression pattern.

In Fig. 5 the same analysis is shown for the lysine biosynthetic pathway. Again, the genes form a tightly coregulated
cluster. This cluster contains all of the lysine biosynthesis genes that are upregulated in the PEX12-disrupted yeasts, including *YJL200C* and *CTP1*, which were previously not implied in lysine biosynthesis. On the other hand, it excludes those lysine biosynthesis genes that were not significantly changed in the PEX12-disrupted yeasts (e.g., *PEX5* and oxoadipate aminotransferase genes). This indicates that a common mechanism is responsible for the upregulation seen in the peroxisome-deficient yeast and in wild-type yeast under physiological conditions. In fact, the lysine biosynthetic cluster is even tighter than that of beta-oxidation, as can be seen from the fact that many genes are each other’s nearest neighbors in the complete data set, i.e., no other nonlysine biosynthesis gene shows a more similar expression pattern (indicated by bold lines in Fig. 5).

The four genes that are upregulated in PEX12-deficient yeasts but are not directly involved in lysine biosynthesis form two coregulated pairs (*SNZ1/SNO1* and *YDL183C/YBR147W*), thus confirming that these genes are also specifically upregulated in response to peroxisome deficiency because, if their upregulation were nonspecific, one would not expect concerted upregulation of both genes of a coregulated pair. Somewhat surprisingly, the expression patterns of all genes that are upregulated in the PEX12-disrupted yeast strain do not show any special affinity to peroxisome biogenesis.

The similarity between the upregulation observed in the peroxisome-deficient yeast strain and the wild-type yeast is also confirmed by the comparison to the response of yeast cells to lysine starvation (Fig. 6). Both the extent of upregulation (two- to fivefold) and the pattern of upregulation (highest for *LYS9* and lowest for *LYS2*) are strikingly similar to the changes of enzyme activity seen in wild-type yeast that are transferred from lysine-containing to lysine-deficient medium (29). Because Lys2p produces the toxic intermediate aminoadipate semialdehyde, which is converted further by Lys9p, this regulatory pattern guarantees that the concentration of the toxic compound is kept at the lowest possible levels even if the pathway is fully activated.

**Regulation of lysine biosynthesis in other gene-deleted yeast strains.** To further characterize the regulatory mechanisms that may be responsible for the upregulation of lysine biosyn-

![Fig. 5. Nearest-neighbor relationships of lysine biosynthesis genes superimposed on a two-dimensional visualization of expression profile similarities (compare Fig. 3). For details, see legend to Fig. 4 and text. Red, beta-oxidation genes; blue, lysine biosynthesis genes; green, peroxisome biogenesis genes.](image)

![Fig. 6. PEX12 disruption and lysine starvation. The upregulation of enzyme activity in response to lysine starvation (29) is shown in comparison to the upregulation of gene expression in response to a disruption of PEX12 (18).](image)
thesis genes in the PEX12-deficient yeasts, we searched the data provided by Hughes et al. (18) for other gene-deleted yeast strains that show a similar response. Indeed, several yeast strains disrupted in single genes show a concerted upregulation of all or most of the lysine biosynthesis genes (Table 2). However, in each of these cases, hundreds of other genes show an equally high upregulation. Furthermore, no common denominator is detectable among the disrupted genes that cause this upregulation, indicating that it represents a relatively unspecific response to disturbances in cell cycle (SWI4 and ASE1) or cellular metabolism (ERG3, ERG28, and RPL27A). On the other hand, two yeast strains disrupted in GCN4 and CKB2, respectively, show a concerted downregulation of lysine biosynthesis genes. Both of these genes are involved in the “general control” of amino acid biosynthesis, the pathway that normally upregulates gene expression in response to amino acid starvation (11, 22, 30). These findings confirm and extend earlier observations made by Urrestarazu et al. (29) that lysine biosynthesis is under general control. These data, however, cannot explain the specific upregulation of lysine biosynthesis in the PEX12-disrupted yeasts.

**Promoter analysis of lysine biosynthesis genes.** Analysis of the promoter regions of all genes that are upregulated in the PEX12-disrupted yeasts revealed the presence of predicted Lys14p binding sites (TCCRNYGGA, allowing one mismatch [4]) in all promoters, within 1,000 bp of the start codon (Fig. 7). A Lys14p binding site is conspicuously absent in the promoters of **LYS5**, **LYS20**, and **LYS14** itself, which are not, or only weakly, upregulated in the peroxisome-deficient yeasts, and in the case of **LYS5** and **LYS14** show no coregulation with other lysine genes.

![FIG. 7](image_url)  
**FIG. 7.** Gene structure and promoters of genes that are upregulated in PEX12-disrupted yeasts. Predicted Lys14p binding sites in the promoter regions are indicated by circles. Four of the genes form tail-to-tail clusters (**YDL183C/LYS20** and **SNZ1/SNO1**).
biosynthesis gene in wild-type yeasts. Lys14p is a transcriptional activator specific for the lysine biosynthesis pathway (9). Its regulation is unusual in that it is activated by the pathway intermediate aminoadipate semialdehyde, and apparent repression of Lys14p by lysine is caused indirectly by inhibition of the committed step catalyzed by the two homocitrate synthase enzymes (Lys20p and Lys21p), which are localized in the nucleus (10). This obviously suggests a mechanism for the upregulation of lysine biosynthesis genes in the presence of lysine: mistargeting of the pathway enzymes to the cytosol. Mistargeting of the pathway to the cytosol can inappropriately activate the starvation response even in the presence of exogenous lysine. To the best of our knowledge, this is the first well-documented example of pathway misregulation in response to peroxisome disruption. This observation is of great relevance for the interpretation of physiological findings in human Zellweger patients, in whom cytosolic accumulation of metabolic intermediates may also interfere with otherwise normal pathways.

The present study also documents the usefulness of public microarray data for the examination of specific cell biological questions. It allowed us to conclusively demonstrate the important role of peroxisomes for lysine biosynthesis and at the same time identified a possible “pathogenic” mechanism for the upregulation of lysine biosynthesis in peroxisome-deficient yeasts. “Wet” experiments were used to confirm the specificity of the observed effects. Although the specificity observed here will most likely remain an exceptional case even for yeast microarrays, the continuous accumulation of new expression datasets holds great potential for further studies.

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