Expression of PEX11β Mediates Peroxisome Proliferation in the Absence of Extracellular Stimuli*

(Received for publication, May 27, 1998, and in revised form, August 4, 1998)

Michael Schrader‡, Bernadette E. Reuber§, James C. Morrell§, Gerardo Jimenez-Sanchez¶, Cassandra Obie‡, Tina A. Stroh‡, David Vallee‡‡, Trina A. Schroer‡, and Stephen J. Gould‡‡‡

From the ‡Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218 and the Departments of §Biomolecular Chemistry and Cell Biology and Anatomy, and ¶Pediatrics and Molecular Biology and Genetics, and the **Howard Hughes Medical Institutes, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Mammalian cells typically contain hundreds of peroxisomes but can increase peroxisome abundance further in response to extracellular stimuli. We report here the identification and characterization of two novel human peroxisomal membrane proteins, PEX11α and PEX11β. Overexpression of the human PEX11β gene alone was sufficient to induce peroxisome proliferation, demonstrating that proliferation can occur in the absence of extracellular stimuli and may be mediated by a single gene. Time course studies indicated that PEX11β induces peroxisome proliferation through a multistep process involving peroxisome elongation and segregation of PEX11β from other peroxisomal membrane proteins, followed by peroxisome division. Overexpression of PEX11α also induced peroxisome proliferation but at a much lower frequency than PEX11β in our experimental system. The patterns of PEX11α and PEX11β expression were examined in the rat, the animal in which peroxisome proliferation has been examined most extensively. Levels of PEX11β mRNA were similar in all tissues examined and were unaffected by peroxisome-proliferating agents. Conversely, PEX11α mRNA levels varied widely among different tissues, were highest in tissues that are sensitive to peroxisome-proliferating agents, and were induced more than 10-fold in response to the peroxisome proliferators clofibrate and di(2-ethylhexyl) phthalate. Taken together, these data implicate PEX11β in the constitutive control of peroxisome abundance and suggest that PEX11α may regulate peroxisome abundance in response to extracellular stimuli.

Peroxisomes are ubiquitous components of eukaryotic cells, absent only from mature erythrocytes and certain primitive unicellular eukaryotes. One of the more intriguing aspects of peroxisome biogenesis is how cells control the abundance of this organelle. Mammalian cells contain hundreds of peroxisomes under normal growth conditions, suggesting that there are constitutive mechanisms for raising peroxisome abundance above one per cell. In addition, peroxisome abundance may change in response to extracellular stimuli, indicating the existence of a signal transduction pathway that exerts additional control over peroxisome abundance. Inducers of peroxisome proliferation include both hypolipidemic drugs (e.g. clofibrate) and plasticizing agents (e.g. di(2-ethylhexyl) phthalate (DEHP)), which act through PPARα, the α isoform of the peroxisome proliferator-activated receptor (1–3). PPARα is a member of the nuclear hormone receptor superfamily and functions as a heterodimer with retinoid X receptor (RXR), another nuclear hormone receptor. The activated PPARα-RXR heterodimer binds peroxisome proliferator-responsive elements (PPREs) and mediates transcriptional activation of a large array of PPRE-containing genes in a drug-dependent manner (4). However, the pathway between altered gene expression and peroxisome proliferation remains to be elucidated.

Peroxisome proliferation has also been observed in lower eukaryotes. In the yeast Saccharomyces cerevisiae, fatty acid oxidation is an exclusively peroxisomal process. Exposure to fatty acids, particularly oleic acid, leads to an increase in peroxisome abundance from 1–2/cell to 10–20/cell (5). This example of peroxisome proliferation is also associated with dramatic changes in gene expression and requires the transcription factors PIP2 (6) and OAF1 (7, 8). Together, these two proteins bind oleate-response elements within transcriptional control regions of responsive genes and are required for both the transcriptional response to oleic acid and the proliferation of peroxisomes. Of the many genes known to be induced by oleic acid, PEX11 is the only one (other than PIP2 itself) that is required for the normal peroxisome proliferation response: pex11 mutants accumulate only 4–5 very large peroxisomes when incubated in oleic acid (5, 9). Furthermore, overexpression of PEX11 can enhance fatty acid-induced peroxisome proliferation. These data demonstrate a role for PEX11 in the regulation of peroxisome abundance. However, there is no evidence that overexpression of ScPEX11 alone, in the absence of extracellular stimuli, can mediate peroxisome proliferation. Here we report the identification and characterization of two mammalian PEX11 genes that can induce peroxisome abundance in the absence of extracellular stimuli.

EXPERIMENTAL PROCEDURES

Plasmide—Plasmids corresponding to apparent full-length cDNAs for human PEX11α (GenBank accession number R18258, EST cDNA clone number 30793) and human PEX11β (GenBank accession number AA227332, EST cDNA clone number 663688) were obtained from Genome Systems (St. Louis). The cDNA inserts in these plasmids were sequenced in their entirety on both strands and the sequences of human

* This work was supported by Grant DK45787 from the National Institutes of Health (to S. J. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡‡ Investigator of the Howard Hughes Medical Institutes.

1 The abbreviations used are: DEHP, di(2-ethylhexyl) phthalate; EST, expressed sequence tag(s); DPBS, Dulbecco’s modified phosphate-buffered saline; PMP, peroxisomal membrane protein.
**FIG. 1.** Alignment of the deduced protein sequences of human PEX11α (PEX11a), human PEX11β (PEX11b), and ScPEX11. Sequences were aligned by the clustal method using DNASTAR software.

**FIG. 2.** Panel A, alignment of the human and mouse PEX11α (PEX11a) protein sequences. Panel B, alignment of the human and mouse PEX11β (PEX11b) protein sequences. Sequences were aligned by the clustal method using DNASTAR software.

**PEX11α** (accession number AF093668 and PEX11β (accession number AF093670) are available from GenBank. The PEX11α expression vector, pBER22, was created by excising the PEX11α cDNA from clone 30793 by digestion with HindIII (partial) and NotI and inserting it between the HindIII and NotI sites of the mammalian expression vector pcDNA3 (Invitrogen, San Diego), downstream of the cytomegalovirus promoter. The PEX11β expression vector was created by excising the PEX11β cDNA from clone 663888 by digestion with EcoRI and XhoI and inserting it between the EcoRI and XhoI sites of pcDNA3. The COOH-terminally tagged versions of PEX11α and PEX11β were generated by polymerase chain reaction using gene-specific oligonucleotides designed to amplify the appropriate open reading frame with the addition of the sequence 5′-GGTACC-3′ immediately preceding the ATG of the open reading frame and with the sequence 5′-ACCATGGCGGAGCAGAAGCTGATCTCCGAGGAGG-3′ in place of the stop codon. Each polymerase chain reaction product was cleaved with HindIII and cloned between the EcoRI and XhoI sites of the mammalian expression vector pcDNA3-Nmyc (a modified version of pcDNA3 that carries HindIII and NotI sites of pcDNA3). The COOH-terminally myc-tagged versions of PEX11α and PEX11β each open reading frame was amplified using appropriate gene-specific primers designed to replace the start codon with the sequence 5′-ACCATGGCGGAGCAGAAGCTGATCTCCGAGGAGG-3′ in place of the stop codon.

**Transfections, Immunofluorescence, and Antibodies—**HepG2 cells and 5756T human fibroblast cells were cultured under standard conditions (11). Transfections were performed by electroporation (12). Indirect immunofluorescence was performed on cells grown on glass coverslips. 2 days after transfection, cells were fixed by incubation in 3% formaldehyde in Dulbecco’s modified phosphate-buffered saline, pH 7.5 (DPBS, Life Technologies, Inc.), for 20 min and then washed twice in DPBS. Next, cell membranes were permeabilized by incubation in either 0.1% Triton X-100 and DPBS for 5 min (standard permeabilization conditions) or in 25 μg/ml digitonin and DPBS for 5 min (differential permeabilization conditions). Cells were then washed twice in DPBS and incubated with primary antibodies diluted in DPBS containing 0.1% bovine serum albumin (1:1,000 for the anti-PMP70 antiserum, 1:1 for the anti-myc hybridoma tissue culture supernatant, and 1:300 for the anti-SKL antibodies). After 10 washes with DPBS, cells were incubated with fluorescently labeled secondary antibodies diluted in DPBS (fluorescein or Texas Red), washed an additional 10 times, mounted, and viewed using an Olympus fluorescence microscope. To ensure the integrity of the peroxisome membrane in all differential permeabilization experiments, equivalent cell samples were stained with anti-SKL antibodies, which detect multiple peroxisomal matrix proteins (13). Data from differential permeabilization experiments were used only if these antibodies failed to detect peroxisomes in cells permeabilized with digitonin but did detect peroxisomes in matched samples permeabilized with Triton X-100. Antiserum directed against PMP70 was a generous gift from Dr. Suresh Subramani (UCSD, La Jolla, CA). Anti-myc antibody was obtained from the tissue culture supernatant of the hybridoma cell line 1-9E10 (14). Sheep anti-catalase antibodies were obtained from The Binding Site (San Diego). Secondary antibodies specific
for rabbit, sheep, or mouse antibodies were obtained from standard commercial sources.

Northern Blots—To assess the regulation of gene expression by peroxisome-proliferating agents in rat liver, rats were fed either standard chow or chow supplemented with DEHP (2% w/w in rat chow) for 7 days or clofibrate (0.5% w/w in rat chow) for 2 weeks (15). Total RNA was isolated from livers of control, DEHP-, and clofibrate-fed rats and analyzed by Northern blot. The rat multitissue Northern blot (2 µg poly(A) plus mRNA/lane) was obtained from CLONTECH (Palo Alto, CA). Probes were generated by random primed labeling of gene-specific cDNA fragments, and hybridizations were carried out using standard protocols (16). Detection was by exposure to XAR-5 film (Kodak) or a Fuji PhosphorImager.

RESULTS

Identification of PEX11α and PEX11β—We used the BLAST algorithm to scan the data base of expressed sequence tags (dbEST) for human cDNAs capable of encoding proteins similar to ScPEX11. Several overlapping ESTs from a single human gene were identified, and the longest cDNA for this gene was obtained and sequenced. The product of this human homolog was more similar to ScPEX11 than to any other S. cerevisiae protein. However, its closest relative was the product of a second human gene, also represented by several ESTs. We obtained and sequenced cDNAs for this second human homolog as well. These two human genes were designated PEX11α and PEX11β based on the similarity of their products to yeast PEX11 (Fig. 1). The presence of two human PEX11 genes was unexpected because all previously characterized PEX genes are present in a single copy in both yeast and humans. This result led us to examine the S. cerevisiae genome for any proteins similar to ScPEX11, but none was detected by the BLAST algorithm. The presence of two PEX11 genes was not unique to humans: PEX11α and PEX11β genes were also identified in mouse (Fig. 2). Although PEX11α and PEX11β are the mammalian proteins with greatest similarity to yeast PEX11, there is only ~20% amino acid identity between the mammalian proteins and their yeast homolog. Furthermore, these human proteins differ from ScPEX11 in that they contain a strongly predicted membrane-spanning domain at their COOH termini (residues 220–239 of PEX11α and 230–255 of PEX11β) and a second membrane-spanning domain located about 100 amino acids from their NH2 termini (residues 94–114 of PEX11α and 94–113 of PEX11β). The transmembrane segment prediction algorithm (TM predict; http://ulrec3.unil.ch/software/TMPRED_form.html) also suggested that PEX11α and PEX11β would be oriented with their termini extending into the cytoplasm. In contrast, ScPEX11 is not predicted to have a membrane-spanning segment and does not behave as an integral peroxisomal membrane protein (PMP) (5).

Overexpression of PEX11β Induces Peroxisome Proliferation—Although the sequence similarities between yeast PEX11 and human PEX11α and PEX11β were relatively mild, they did suggest that these proteins may play some role in the control of peroxisome abundance. We explored this possibility by testing whether overexpression of PEX11α or PEX11β alone could stimulate peroxisome proliferation. Human cells were transfected with plasmids designed to express either PEX11α or
PEX11b. 2 days after transfection, peroxisome abundance was assessed by indirect immunofluorescence using antibodies specific for PMP70, a ubiquitous PMP (17). Peroxisome abundance was increased in cells transfected with the PEX11b expression vector (Fig. 3). The proportion of cells displaying increased peroxisome abundance in transfected cell populations corresponded roughly to the transfection efficiency in each instance, approximately 30–50% in different trials and cell lines. In contrast to these results, peroxisome proliferation was detected in less than 1% of the cells transfected with the PEX11a expression vector (data not shown).

Peroxisomes can be quite heterogeneous in composition. Therefore, we tested whether expression of PEX11b also induced the proliferation of catalase-containing peroxisomes. Cells were transfected as above but processed for double indirect immunofluorescence using anti-catalase and anti-PMP70 antibodies. If there is any heterogeneity in the composition of these peroxisomes it is not apparent in the distribution of these proteins: both PMP70 and catalase were detected in almost all peroxisomes in cells that had undergone PEX11b-mediated peroxisome proliferation (Fig. 4).

Peroxisome proliferation is normally mediated by extracellular stimuli. To test whether the changes in peroxisome abundance observed above were limited to those cells that overexpressed PEX11a or PEX11b, we repeated these transfection experiments with plasmids designed to express myc epitope-tagged derivatives of PEX11a and PEX11b. Expression of a COOH-terminally myc-tagged version of PEX11b, PEX11bmyc, revealed that 1) PEX11bmyc induced peroxisome proliferation in almost all expressing cells (Fig. 5, A and B), 2) peroxisome proliferation was detected only in those cells that expressed PEX11bmyc, 3) the degree of peroxisome proliferation correlated roughly with the extent of PEX11bmyc expression, and 4) PEX11bmyc colocalized with PMP70, demonstrating that it is a peroxisomal protein. We also examined the activity and subcellular distribution of an NH2-terminally tagged mycPEX11b protein. This protein also induced peroxisome proliferation in a cell-limited fashion and colocalized with PMP70 (Fig. 5, C and D).

During the course of these experiments we made the rather curious observation that PEX11bmyc and mycPEX11b were detected only rarely (and weakly) in cells that had been fixed and then permeabilized with Triton X-100 (0.1% for 5 min, our standard permeabilization protocol) but were detected readily in 30% of the same transfected cell population when the cells were fixed and then permeabilized with digitonin (25 μg/ml for 5 min), as apparent from Fig. 5. Normally, these two permeabilization techniques allow one to determine whether a protein is intraperoxisomal (if detected in cells permeabilized with Triton X-100 but not in cells permeabilized with digitonin) or a peroxisomal membrane protein with at least some epitopes exposed to the cytoplasm (if detected in cells permeabilized with either agent). The ability to detect PEX11bmyc and mycPEX11b under digitonin permeabilization conditions suggests that PEX11b is a peroxisomal membrane protein with its termini exposed to the cytoplasm and provides experimental sup-
port for the predicted topology of PEX11β (see above). However, we have no explanation for why we were unable to detect the expression of PEX11βmyc or mycPEX11β in cells that were permeabilized with Triton X-100.

We also examined the activity and distribution of tagged derivatives of PEX11α. Human fibroblasts were transfected with plasmids designed to express NH₂- and COOH-terminally tagged versions of PEX11α and were processed for indirect immunofluorescence 2 days later. Both PEX11α proteins were expressed efficiently in human cells and were detected in approximately 30% of the transfected cell population. However, peroxisome proliferation was detected in less than 5% of the cells that expressed these proteins (data not shown). This result contrasted sharply with those obtained for PEX11β, a protein that induced peroxisome proliferation in nearly all cells in which it was expressed (see above). Nevertheless, those rare cells that responded to the myc-tagged forms of PEX11α displayed an increase in peroxisome abundance (Fig. 6, A–D) similar to that induced by PEX11β. Both tagged forms of PEX11α also colocalized with PMP70 under differential permeabilization conditions, data that support the hypothesis that PEX11α extends its termini into the cytoplasm. It should also be noted that the tagged versions of PEX11α could be detected easily in cells permeabilized with Triton X-100, suggesting that the inability to detect the myc-tagged versions of PEX11β under these conditions reflected a specific property of PEX11β.

PEX11β Drives Peroxisome Proliferation in a Multistep Process—To shed some light on the process of peroxisome proliferation that is mediated by PEX11β, we examined peroxisomes at various times after transfection with the PEX11βmyc expression vector. At the earliest time point, just 6 h after transfection, a dramatic change in peroxisome shape was apparent: the peroxisomes in cells expressing PEX11βmyc displayed an elongated, tubular morphology (Fig. 7, A and B). Quantitation of peroxisome morphology in cells transfected with PEX11βmyc or vector alone demonstrated that this shift in peroxisome morphology from vesicular structures to tubules was significant (Fig. 7C). Peroxisome tubules declined in abundance over the following 3 days and were replaced by more numerous, smaller vesicles, as shown earlier (Figs. 3 and 4). Interestingly, the peroxisome tubules that were detected at early stages of proliferation displayed a significant spatial heterogeneity in the distribution of PMPs, with PEX11βmyc and PMP70 segregating to discrete bands along the peroxisome (Fig. 8). Many of these peroxisomes contained alternating bands of PEX11βmyc and PMP70, with PMP70 often concentrated at peroxisome termini.

Peroxisome-proliferating Agents Induce PEX11α but Not PEX11β—The above data demonstrated that overexpression of PEX11β alone can drive the proliferation of peroxisomes. The activity of this gene led us to speculate that changes in its expression might be responsible for drug-induced peroxisome proliferation in mammalian systems. This possibility was tested in rat liver, the best model system for studying drug-induced peroxisome proliferation. RNA was extracted from livers of rats that had been fed control diets or diets supplemented with either of the peroxisome proliferators, clofibrate or DEHP. Message RNA abundance was inferred from Northern blot experiments using the murine PEX11α and PEX11β cDNAs as probes. The PEX11α transcript was induced more than 10-fold
by either DEHP or clofibrate, whereas the PEX11β mRNA was unaffected by these drugs (Fig. 9). These results indicate that peroxisome-proliferating agents may act via PEX11α but are unlikely to act through PEX11β, at least at the transcriptional level. It should also be noted that we have yet to establish directly whether the products of the rat PEX11α and PEX11β genes are involved in peroxisome proliferation.

To characterize further the expression of the PEX11α and PEX11β genes, we next performed Northern analysis on mRNAs from multiple rat tissues. These experiments revealed that the abundance of PEX11α mRNA varied significantly among different tissues, with highest levels in kidney, significant expression in liver, lung, brain and testis, and very low levels in heart, spleen and skeletal muscle (Fig. 10A). In contrast, PEX11β was expressed at roughly equivalent levels in all of these tissues (Fig. 10B). Similar loading and transfer of mRNA samples were confirmed by examination of actin mRNA abundance (Fig. 10C). Given that the specific activities of the PEX11α and PEX11β probes were similar and that the above exposures were for identical lengths of time, we infer that PEX11β is expressed at higher levels than PEX11α in many tissues. There are approximately three times as many expressed sequence tags for PEX11β in the data base of expressed tags as there are for PEX11α, a result that is consistent with this hypothesis.

**DISCUSSION**

Under normal conditions, peroxisome proliferation is induced by extracellular signals and associated with altered expression of a wide array of genes. As a result, it has been difficult to determine whether increases in peroxisome abundance are mediated by altered expression of just a single gene or a set of multiple genes. Previous studies have identified several genes, including PEX10 (18) and PEX11 (5, 9), which can augment proliferation when overexpressed in the context of a peroxisome proliferation event. However, in no instance has overexpression of these genes been shown to induce peroxisome proliferation in the absence of appropriate extracellular stimuli. Our observation that overexpression of human PEX11β alone can efficiently induce peroxisome proliferation demonstrates that altered expression of a single gene can indeed mediate peroxisome proliferation, even in the absence of extracellular stimuli. In contrast to our results with human PEX11β, peroxisome proliferation was detected in less than 5% of the cells that overexpressed PEX11α. However, the difference in peroxisome proliferation-promoting activity which we observed may reflect a limitation of our experimental system rather than a significant biochemical difference between these proteins.

The fact that myc-tagged versions of PEX11β retained peroxisome proliferation-promoting activity allowed us to follow the effect of PEX11β expression on peroxisome morphology and abundance over time as well as to gain some insight into the subcellular distribution of PEX11β itself. Tagged versions of PEX11β behave as peroxisomal membrane proteins and can be

---

**FIG. 7.** PEX11βmyc induces peroxisome elongation before proliferation. HepG2 cells were transfected with the PEX11βmyc expression vector and seeded onto glass coverslips. 6 h after transfection the cells were processed for double indirect immunofluorescence using antibodies specific for PMP70 (panel A) and PEX11βmyc under differential permeabilization conditions (panel B). Bar = 2 μm. Panel C, peroxisome morphology at various times after transfection with the PEX11βmyc expression vector (open squares) or with vector alone (solid circles). The percentage of peroxisomes with the elongated, tubular morphology is presented on the x axis, and the time after transfection is presented on the y axis. The slight increase in the abundance of peroxisome tubules observed in the control population was expected because earlier studies have established that trypsinization alone stimulates the formation of peroxisome tubules and mild peroxisome proliferation (19–21).
detected in peroxisomes just 6 h after transfection. The initial consequence of PEX11β overexpression is the conversion of peroxisomes from spherical vesicles into elongated tubules. This process is also associated with the generation of subdomains in the peroxisome membrane, some of which are enriched for PEX11β, whereas others are enriched for PMP70. The formation of peroxisome tubules was followed by the appearance of numerous, small, vesicular peroxisomes, a conversion that was almost complete by 72 h after transfection. These observations suggest a precursor-product relationship between the tubules that are formed early after transfection and the more numerous vesicles detected at later time points. It should be noted that this general model for peroxisome division has been proposed to explain the proliferation of peroxisomes which is induced by the passaging of HepG2 cells (19–21). Additional time course experiments, ultrastructural studies, and real time analysis of peroxisome proliferation will be required to test this model rigorously as well as to determine whether the type of peroxisome division we observed occurs by peroxisome fission or vesicle budding.

Although it is important to elucidate the morphological steps in peroxisome division, a molecular analysis of PEX11β activity is also required. PEX11β does not share any sequence similarity or motif with any proteins other than PEX11 homologs from

---

**FIG. 8. Peroxisome elongation coincides with the segregation of PEX11βmyc from PMP70.** HepG2 cells were transfected with the PEX11βmyc expression vector and processed for indirect immunofluorescence 12 h after transfection using antibodies specific for PMP70 (detected with a rhodamine-labeled secondary antibody) and PEX11βmyc (detected with a fluorescein-labeled secondary antibody). Arrows point to peroxisome tubules with multiple, alternating bands of PEX11βmyc and PMP70. Arrowheads point to peroxisomes with globular termini, which always stain preferentially with anti-PMP70 antibodies. A portion of an untransfected cell is visible on the left edge of the image and lacks peroxisome tubules. Bar = 10 μm.

**Figs. 9. Effect of peroxisome-proliferating agents on PEX11α and PEX11β expression.** 10 μg of total rat liver RNA from animals fed a control chow diet (lanes 1 and 4), a diet supplemented with DEHP (lanes 2 and 5), and a diet supplemented with clofibrate (lanes 3 and 6) was separated by denaturing agarose gel electrophoresis, transferred to nylon membranes, and probed with a radioactively labeled fragment of the PEX11α cDNA to detect the 2.4-kilobase PEX11α mRNA (upper panel, lanes 1–3) and the PEX11β cDNA to detect the 2-kilobase PEX11β mRNA (upper panel, lanes 4–6). The abundance of 28 S rRNA in each lane is shown separately in the lower panels.
PEX11α and PEX11β Induce Peroxisome Proliferation

other species. Thus, there is no clear picture of how PEX11β may mediate peroxisome proliferation. Interestingly, rat PEX11α, which shares the same topology in the peroxisome membrane as human PEX11α and PEX11β, recruits ARF and COPI to peroxisome membranes (22). ARF and COPI recruitment appears to occur at the dilysine motif, KXXKKCOOH, which is present at the COOH terminus of PEX11α. These and other observations have led Passreiter et al. (22) to propose that PEX11α may mediate a coat-dependent budding of peroxisomes from pre-existing peroxisomes. Human and mouse PEX11α share the same COOH-terminal tail as rat PEX11α, suggesting that they may function in a similar manner. However, both human and mouse PEX11β lack the dilysine motif at their COOH terminus and instead terminate in the sequence RXXKKCOOH. It is interesting to note that the dilysine motif is also absent from yeast PEX11.

The existence of two mammalian PEX11 genes alone indicated that these genes may have distinct roles. The data of this report and of Passreiter et al. (22) provide additional support for this hypothesis. PEX11α appears to interact with ARF/COPI and stimulate vesicle budding from peroxisomes (22). It also displays tissue-specific and drug-induced changes in expression but exhibits relatively weak peroxisome proliferation-promoting activity in our experimental system. Conversely, PEX11β lacks the sequence motifs common to ARF/COPI-binding proteins. Furthermore, PEX11β is expressed at similar levels in all tissues examined, is unresponsive to peroxisome proliferator drugs, and exhibits robust peroxisome-proliferating activity in our experimental system. These observations are consistent with a model in which PEX11β controls constitutive peroxisome division and PEX11α regulates peroxisome abundance in response to dietary, hormonal, or other stimuli. However, additional experiments will be required to test this hypothesis directly.

REFERENCES

1. Issemann, I., and Green, S. (1990) Nature 347, 645–650
2. Kliever, S. A., Sundetse, S. S., Jones, S. A., Brown, P. J., Wisely, G. B., Kohle, C. S., Devechand, P., Wahi, W., Wilson, T. M., Lenhard, J. M., and Lehmann, J. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4318–4323
3. Forman, B. M., Chen, J., and Evans, R. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4312–4317
4. Reddy, J. K., and Chu, R. (1996) Ann. N. Y. Acad. Sci. 804, 176–201
5. Marshall, P., Krkimovich, Y., Lark, R., Dyer, J., Veenehs, M., and Goodman, J. (1995) J. Cell Biol. 129, 345–355
6. Rottensteiner, H., Kal, A. J., Filigita, M., Binder, M., Hamilton, B., Tabak, H. F., and Ruis, H. (1996) EMBO J. 15, 2924–2934
7. Rottensteiner, H., Kal, A. J., Hamilton, B., Ruis, H., and Tabak, H. F. (1997) Eur. J. Biochem. 247, 776–783
8. Karpichev, I. V., Luo, Y., Mariane, R. C., and Small, G. M. (1997) Mol. Cell. Biol. 17, 69–80
9. Erdmann, R., and Blobel, G. (1995) J. Cell Biol. 128, 599–523
10. Yahraus, T., Braverman, N., Dott, G., Kalisky, J. E., Morrell, J. C., Moser, H. W., Valle, D., and Gould, S. J. (1997) Cell 87–96
11. Slawekki, M., Dott, G., Steinberg, S., Moser, A. B., Moser, H. W., and Gould, S. J. (1995) J. Cell Sci. 108, 1817–1829
12. Chang, C.-C., Lee, W.-H., Moser, H. W., Valle, D., and Gould, S. J. (1997) Nat. Genet. 15, 385–388
13. Gould, S. J., Krisans, S., Keller, G. A., and Subramani, S. (1990) J. Cell Biol. 110, 27–34
14. Evan, G. E., Lewis, G. K., Ramsay, G., and Bishop, J. M. (1985) Mol. Cell. Biol. 5, 3610–3616
15. FitzPatrick, D. R., Germain-Lee, E., and Valle, D. (1995) Genomics 27, 457–466
16. Sambrook, J., Fritsch, E., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Kamijo, K., Taketani, S., Yokota, S., Osumi, T., and Hashimoto, T. (1990) J. Cell Biol. 69, 804–817
18. Tan, X., Waterham, H. R., Veenhuis, M., and Creeg, J. M. (1995) EMBO J. 15, 2924–2934
19. Schrader, M., Baumgart, E., Volkl, A., and Fahimi, H. D. (1994) Eur. J. Cell Biol. 64, 281–294
20. Schrader, M., Burkhardt, J. K., Baumgart, E., Luers, G., Spring, H., Volkl, A., and Fahimi, H. D. (1996) Eur. J. Cell Biol. 69, 34–35
21. Schrader, M., Kriegstein, K., and Fahimi, H. D. (1998) Eur. J. Cell Biol. 75, 87–96
22. Passreiter, M., Anton, M., Lay, D., Frank, R., Harter, C., Wieland, F. T., Gorgas, K., and Just, W. W. (1998) J. Cell Biol. 141, 373–383