PEX5 Binds the PTS1 Independently of Hsp70 and the Peroxin PEX12*

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Most peroxisomal enzymes are targeted to peroxisomes by virtue of a type-1 peroxisomal targeting signal (PTS1) at their extreme C terminus. PEX5 binds the PTS1 through its C-terminal 40-kDa tetrameric repeat domain and is essential for import of PTS1-containing proteins into peroxisomes. Here we examined the PTS1-binding activity of purified, recombinant, full-length PEX5 using a fluorescence anisotropy-based assay. Like its C-terminal fragment, full-length tetrameric PEX5 exhibits high intrinsic affinity for the PTS1, with a $K_d$ of 35 nM for the peptide lissamine-Tyr-Gln-Ser-Lys-Leu-COO$^-$.

The specificity of this interaction was demonstrated by the fact that PEX5 had no detectable affinity for a peptide in which the Lys was replaced with Glu, a substitution that inactivates PTS1 signals in vivo. Hsp70 has been found to regulate the affinity of PEX5 for a PTS1-containing protein, but we found that the kinetics of PEX5-PTS1 binding was unaffected by Hsp70, Hsp70 plus ATP, or Hsp70 plus ADP. In addition, we found that another protein known to interact with the PTS1-binding domain of PEX5, the PEX12 zinc RING domain, also had no discernable effect on PEX5-PTS1 binding kinetics. Taken together, these results suggest that the initial step in peroxisomal protein import, the recognition of enzymes by PEX5, is a relatively simple process and that Hsp70 most probably stimulates this process by catalyzing the folding of newly synthesized peroxisomal enzymes and/or enhancing the accessibility of their PTS1.

Peroxisomes lack nucleic acids and import all of their protein content (1). Peroxisomal matrix protein import is initiated when newly synthesized peroxisomal enzymes are bound by the peroxisomal matrix protein import receptors, PEX5 or PEX7 (2). These interactions usually require the presence of peroxisomal targeting signals (PTS)3 in the enzymes. Two classes of PTS are known, the PTS1 and PTS2, either of which is sufficient to direct proteins into the peroxisome lumen (3, 4). Most peroxisomal enzymes contain the PTS1, a 3-amino acid-long signal that functions only when present at the extreme C terminus of a protein. The canonical PTS1 is Ser-Lys-Leu-COO$^-$, though conservative variants of this motif can also direct proteins into the peroxisome. While the vast majority of peroxisomal enzymes use the PTS1, a few use the PTS2, a nonapeptide of the sequence RLX$_2$HL, or a conservative variant that is found at or near the N terminus of these proteins.

PEX5 binds the PTS1 and is thought to act as an import receptor, directing PTS1-containing proteins into the import pathway (5). PEX5 is a predominantly cytoplasmic, partly peroxisomal protein that appears to shuttle between these compartments as it mediates the import of PTS1-containing proteins (6, 7). The dynamic behavior of PEX5 suggests a model of peroxisomal matrix protein import that involves receptor-docking factors, translocation factors, and receptor-recycling factors. Several other lines of evidence support this model, including the identification of other peroxins (peroxisomal import factors) that have specific roles in receptor docking, enzyme translocation, and receptor recycling (8).

A clear understanding of the initial molecular events in peroxisomal matrix protein import is likely to reveal important features of subsequent steps in the process (9). Toward this end, we recently determined the structure of a 40-kDa, C-terminal fragment of PEX5 bound to a PTS1-containing peptide (10). PEX5 uses nearly its entire C-terminal half to bind the PTS1, with two triplets of the tetra-tripeptide repeat making all of the contacts with the PTS1. This structure, combined with our biochemical analysis of PEX5-PTS1 binding, established that this fragment of PEX5 acts as a monomer and binds the PTS1 peptide with a 1:1 stoichiometry, displays an apparent affinity ($K_d$) for the PTS1 peptide lissamine-Tyr-Gln-Ser-Lys-Leu-COO$^-$ of $≈-20$ nM, and binds the PTS1 in the absence of any cofactors (10).

Although the C-terminal half of PEX5 has intrinsic PTS1-binding activity, there is clearly a potential for accessory factors to promote or disrupt the PEX5-PTS1 interaction. For example, Harano et al. (12) reported that Hsp70 binds to PEX5 and enhances the PEX5-PTS1 interaction. Furthermore, PEX5 appears to act catalytically, which implies the existence of a PEX5-PTS1 dissociation event and factors that promote PEX5-PTS1 dissociation. PEX12 is an integral peroxisomal membrane protein that is essential for peroxisomal matrix protein import and is one of only two peroxins known to interact with the ligand-binding domain of PEX5 (13). The other is PEX8, a peroxisomal membrane protein that terminates with a PTS1 even though it does not use the PTS1 for targeting to peroxisomes (14–16). These properties suggest that PEX12 and PEX8 might also be regulators of PEX5-PTS1 binding. Although there are many possible mechanisms by which a regulator may alter the PEX5-PTS1 interaction, the simplest is that they bind to PEX5 and alter the affinity of PEX5 for the PTS1. Here we characterized the interaction between full-length PEX5 and

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* This work was supported by grants from the National Institutes of Health (to S. J. G. and J. M. B.). 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.

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2 G. Gatto, E. L. Maynard, A. L. Guerrio, B. V. Geisbrecht, S. J. Gould, and J. M. Berg, manuscript submitted.
the PTS1. Our results demonstrate that full-length, tetrameric PEX5 has high intrinsic affinity for the PTS1. In addition, we show that Hsp70 and PEX12 have no effect on PEX5-PTS1 binding.

EXPERIMENTAL PROCEDURES

Proteins—Human Hsp70 was purchased from StressGen. ATPase activity was determined by phosphate release and measured by a standard malachite green reaction (for protocol, see StressGen customer support). To generate human PEX5L containing an N-terminal hexa-histidine tag, the PEX5L open reading frame (6, 17) was amplified using oligonucleotides designed to place the sequence GTCGACC immediately preceding the start codon and the sequence GCGGCCGCG downstream of the stop codon. The resulting amplification product was cleaved with SalI and NotI and cloned into the plasmid pBEGO9A, also known as pT7 (18), a derivative of pET28a (Novagen). This plasmid was introduced into BL21(DE3) cells, and the resulting kanamycin-resistant strain was induced to express His–PEX5L by the addition of 0.5 mM isopropyl thiogalactoside at 25 °C. Following an overnight induction of the culture, the cells were incubated with lysosome (15 mg/ml for 15 min on ice) and then broken by sonication in lysis buffer, pH 7.8 (20 mM NaH2PO4-NaOH, 0.5 mM NaCl, 5 mM NaF, 5 mM benzamidine). The cell lysates were then clarified by centrifugation at 15,000 × g for 15 min and fractionated by nickel affinity chromatography as follows. A 100-ml lysate was poured over a 3-ml bed volume of nickel-nitrotriacetic acid Superflow-agarose (Qiagen) twice, washed in 50 ml of lysis buffer, pH 7.8, washed in 50 ml of lysis buffer, pH 6.0, and finally washed in 25 ml of lysis buffer, pH 6.0 containing 100 mM imidazole. Purified PEX5 was then eluted with 1 M imidazole in lysis buffer, pH 6.0. PEX5L was then concentrated by centrifugation in a Pall Macrosep 30K Omega filtration apparatus for 120 min at 5000 × g.

The plasmid encoding MBP-LacZ, pMAL-c2, is available from New England Biolabs. The plasmids encoding the maltose-binding protein (MBP) fusion with LacZ and PEX12 (MBP-LacZ and MBP-PEX12, respectively) have been previously described (13). To generate the MBP-PEX5 fusion, the full-length Pichia pastoris PEX5 open reading frame was amplified using oligonucleotides designed to place the sequence GTCGACC immediately preceding the start codon and the sequence GCGGCCGCG downstream of the stop codon. The resulting amplification product was cleaved with SalI and NotI and cloned into the plasmid pJM359, a derivative of pMAL-c2 that is also known as pMBP-c2. This plasmid places the SalI-NotI PEX5 fragment in frame with, and immediately downstream of, the tobacco etch virus protease recognition site, which lies downstream of the MBP open reading frame. The plasmids encoding MBP-LacZ, MBP-PEX12, and MBP-PEX5 were used to transform DH10B cells to ampicillin resistance, and the resulting strain was induced to express the MBP fusions by the addition of 0.5 mM isopropyl thiogalactoside followed by incubation of the culture at 25 °C overnight. The cells were then collected, incubated with lysosome, and broken by sonication in a buffer of 20 mM Tris, pH 7.5, 200 mM NaCl, 5 mM NaF, and 5 mM benzamidine. Purified PEX5 was then cleaved with SalI and NotI and cloned into the plasmid pBEGO9A, also known as pT7 (18), a derivative of pET28a (Novagen). This plasmid was introduced into BL21(DE3) cells, and the resulting kanamycin-resistant strain was induced to express His–PEX5L by the addition of 0.5 mM isopropyl thiogalactoside at 25 °C. Following an overnight induction of the culture, the cells were incubated with lysozyme (15 mg/ml for 15 min on ice) and then broken by sonication in lysis buffer, pH 7.8 (20 mM NaH2PO4-NaOH, 0.5 mM NaCl, 5 mM NaF, 5 mM benzamidine). The cell lysates were then clarified by centrifugation at 15,000 × g for 15 min and fractionated by nickel affinity chromatography as follows. A 100-ml lysate was poured over a 3-ml bed volume of nickel-nitrotriacetic acid Superflow-agarose (Qiagen) twice, washed in 50 ml of lysis buffer, pH 7.8, washed in 50 ml of lysis buffer, pH 6.0, and finally washed in 25 ml of lysis buffer, pH 6.0 containing 100 mM imidazole. Purified PEX5 was then eluted with 1 M imidazole in lysis buffer, pH 6.0. PEX5L was then concentrated by centrifugation in a Pall Macrosep 30K Omega filtration apparatus for 120 min at 5000 × g.

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Gel Filtration of PEX5—Purified recombinant PEX5L was separated on an S300 gel filtration (Amersham Biosciences) column at a flow rate of 0.8 ml/min at 4 °C in a solution of 20 mM Tris-HCl, pH 7.5, 200 mM NaCl, 5 mM NaF, and 5 mM benzamidine. Blue dextran (≥2 MDa), tetrameric β-galactosidase (465 kDa), and bovine serum albumin (67 kDa) were used to calibrate the column. Fractions were analyzed by SDS-PAGE and stained with Coomassie Blue to determine the approximate molecular weight of recombinant PEX5.

Protein Pull-down Experiments—Clariﬁed lysates from strains expressing MBP fusions were bound to an amylase resin and washed extensively in binding buffer (20 mM Tris, pH 7.5, 200 mM NaCl, 5 mM NaF, 10 mM 2-mercaptoethanol). The resulting protein-saturated resins were incubated with purified PEX5 and washed extensively. Samples were concentrated by binding buffer containing 10 mM maltose, separated by SDS-PAGE, and stained with Coomassie Blue.

PEX5-PTS1 Binding Assay—To examine the PEX5-PTS1 interaction in real time and in solution we employed a fluorescence anisotropy-based peptide-protein binding assay, essentially as described by Gatto et al. (10). To use fluorescence anisotropy for measuring PEX5-PTS1 binding events, we employed small peptides (YQSKL or YQSEL) with a fluorophore (lissamine) attached to their N terminus as described (9). Prior to anisotropy experiments, a 3.5-ml Spectrofil UV Quartz window fluorescence cuvette (Starna Cells, Inc.) containing a magnetic stir bar was incubated overnight at 25 °C in 320 μM gelatin in 10 mM HEPES, pH 7.5, and 100 mM NaCl to prevent protein or peptide from binding the walls of the cuvette. Peptide was added to a concentration of 200 nM, and stability of the fluorescence signal was determined by measuring the emission spectra of the fluorophore prior to and following the experiment. Increasing amounts of recombinant PEX5 were then titrated into the cuvette. At each protein concentration, the lissamine was excited with polarized light (Horiba Johin Yvon Spex Fluorolog-3 fluorometer), and the fluorescence anisotropy was measured by monitoring the lissamine emission intensity with the excitation and emission polarizers configured in the z-format. The excitation monochromator (at 568 nm) and the emission monochromator (at 588 nm) were set to a slit width of 2 and 4 nm, respectively. Each measurement was made with a 5-s integration time with at least 10 min of equilibration time between each addition of PEX5. A Neslab RTE-111 bath circulator was used to maintain constant temperature (25 °C). To test the effects of Hsp70 on PEX5-PTS1 binding, the buffer was supplemented with 5 mM MgSO4, human Hsp70 (15 ng/μl), ATP (10 mM), and ADP (10 mM). Concentrations of PEX5 used in the fluorescence anisotropy experiments are indicated in the figures. For the anisotropy experiment performed with PEX12, MBP-PEX12 was added at a 4-fold molar excess relative to the PEX5 concentration used throughout the titration.

Data Analysis—Determination of the binding affinity of PEX5 for the PTS1 requires that we first determine the fraction of peptide bound (f bound) for any given anisotropy value (r). Since a change in fluorescence intensity was typically observed upon ligand binding, this change must be accounted for in the final calculation. A correction factor, Q, represents the quantum yield ratio of the bound to the free form and is estimated by the ratio of the intensities of the bound to the free fluorophore. Thus using the following equation, we can determine the fraction bound.

\[
\frac{f_{\text{bound}}}{f_{\text{free}}} = \frac{r - r_{\text{free}}}{Q + (r - r_{\text{free}})}
\]

To calculate K_d, the data were fit using Kaleidagraph (Synergy Software).
RESULTS

Full-length PEX5 Binds the PTS1 Specifically and with High Affinity—For most peroxisomal proteins, the first step in import is their interaction with PEX5 (2). We previously characterized the interaction between a PTS1 peptide and the minimal domain of PEX5 that still retains specificity for the PTS1, a C-terminal, 40-KDa monomeric fragment of PEX5 (10). Full-length human PEX5 is nearly twice as long (6, 17) and assembles into tetramers (19), and there is evidence of interaction between the N-terminal and C-terminal halves of PEX5 (12). Thus, the PTS1 binding characteristics of full-length PEX5 may be more complex than the PTS1-binding properties of its C-terminal, monomeric, ligand-binding domain. We purified a recombinant form of full-length PEX5 containing an N-terminal hexahistidine tag from bacteria. For these studies we used PEX5L, one of two major PEX5 isoforms expressed in humans, which is 639 amino acids in length and can rescue all defects of PEX5-deficient human cells (17). Next, we characterized the interaction between tagged, full-length PEX5 and a fluoro-phore-containing, PTS1-containing peptide (lissamine-Tyr-Gln-Ser-Lys-Leu-COO\(^-\)) using the technique of fluorescence anisotropy (10). Plotting the amount of peptide bound against PEX5 concentration reveals a rectangular hyperbola showing that PEX5 displayed an apparent affinity (K\(_d\)) for the PTS1 of 35 nM, which is similar to the K\(_d\) reported for the interaction between the same peptide and the C-terminal ligand-binding domain of PEX5 (10). A nearly identical peptide carrying a very low efficiency PTS1 (lissamine-Tyr-Gln-Ser-Glu-Leu-COO\(^-\)) showed no affinity for PEX5 under identical conditions indicating that the PEX5/PTS1 binding is specific (Fig. 1A). Interestingly, purified PEX5 behaves as a tetramer in vitro. When purified PEX5L is separated by gel filtration chromatography, nearly half of the protein migrates with a size of ~300 kDa, as expected for a homotetramer of 70 kDa subunits, whereas the other half of the protein migrates in the void volume and probably represents aggregated forms of PEX5L that may not contribute to PTS1 binding (Fig. 1B).
The PEX5-PTS1 Interaction Is Not Affected by ATP or Hsp70—Harano et al. (12) reported that Hsp70 facilitated the binding of full-length PEX5 to an ~16-kDa PTS1-containing protein. Harano et al. also reported that Hsp70 bound to both PEX5 and the PTS1-containing protein. These results can be explained by two distinct hypotheses. One is that Hsp70 alters the affinity of PEX5 for the PTS1. The other is that Hsp70 alters the folding of the PTS1 protein and/or the presentation of its PTS1. To distinguish between these hypotheses we examined the effect of Hsp70 on the affinity of PEX5 for the PTS1. Using the same concentration of Hsp70 employed by Harano et al. (12), we found that Hsp70 had no effect on the PEX5-PTS1 interaction (Fig. 2A). The ability of Hsp70 to facilitate protein folding is dependent upon ATP, and the above experiment was performed in the presence of 10 mM ATP and 5 mM MgSO4. However, Harano et al. (12) reported that the presence of ATP reduced the stimulatory effect of Hsp70 on the interaction between PEX5 and the PTS1 protein used in their studies, leading them to conclude that Hsp70-ADP has a greater stimulatory effect on the PEX5-PTS1 interaction than Hsp70-ATP.

To determine whether Hsp70 might facilitate PEX5-PTS1 interactions in the presence of ADP, we compared the affinity of PEX5 for the PTS1 in the presence of Hsp70 and either ATP or ADP. No difference was observed (Fig. 2B) even though the Hsp70 used in the experiments retained significant ATPase activity (Fig. 2C).

Peroxisomal matrix protein import requires ATP (20), ATP depletion causes significant changes in PEX5 distribution within the cell (7), and ATP is an allosteric regulator of many proteins (21). To determine whether ATP itself might affect the PEX5-PTS1 interaction, we performed the PTS1 binding assay in the presence of ATP and found that it had no effect (Fig. 2D).

PEX12 Has No Effect on PEX5-PTS1 Binding—We previously established that PEX12 is an integral peroxisomal membrane protein required for peroxisomal matrix protein import (22) and that the C-terminal, a 10-kDa RING finger domain of PEX12, interacts with the ligand-binding domain of PEX5 (13). This interaction might reflect any number of possible roles for PEX12, but one distinct possibility is that PEX12 might alter the affinity of PEX5 for the PTS1. This is a particularly attractive hypothesis because a point mutation in this domain of PEX12 reduced its ability to bind PEX5 and resulted in a pronounced increase in the amount of intraperoxisomal PEX5 (13), which might result from a defect in PEX5-PTS1 dissociation.

To test the hypothesis that PEX12 might alter the affinity of PEX5 for the PTS1, we expressed a protein that contained the 10-kDa PEX5-binding domain of PEX12 fused to the C terminus of maltose-binding protein (MBP-PEX12). Following its purification, we used the fluorescence anisotropy assay to test whether MBP-PEX12 had any effect on PEX5-PTS1 interactions. PEX12 had no effect on PEX5-PTS1 interactions (Fig. 3A). This was not due to an inability of MBP-PEX12 to bind PEX5, since we were able to specifically pull down PEX5 with MBP-PEX12 but not with MBP-LacZ during amylose affinity chromatography (Fig. 3B).

PEX8 has yet to be identified in humans, precluding our ability to test whether it affects the interaction between human PEX5L and the PTS1. However, it has been identified in numerous yeast species, including P. pastoris (14–16). Therefore, we purified P. pastoris PEX5 and PEX8 proteins. As with human PEX5L, we expressed full-length P. pastoris PEX5 (5) with an N-terminal hexahistidine tag and purified the protein by nickel affinity chromatography. P. pastoris PEX8 was expressed as an MBP fusion and purified by amylose affinity chromatography.

As we found for human PEX5L, P. pastoris PEX5 bound the PTS1 as measured by fluorescence anisotropy indicating that
this is a general property of PEX5 proteins (Fig. 4). The apparent affinity of P. pastoris PEX5 for this PTS1 peptide was ~250 nM, approximately an order of magnitude higher than that for the human protein. Addition of a purified MBP-PEX8 fusion protein had no effect on PEX5-PTS1 binding (data not shown). However, we were unable to detect binding between P. pastoris PEX5 and PEX8, rendering that result irrelevant to the question of whether the binding of PEX8 to PEX5 affects the affinity of PEX5 for the PTS1.

DISCUSSION

Most peroxisomal matrix proteins contain the PTS1, are bound by PEX5 prior to their import, and are then released from PEX5 at some point during their translocation. It is therefore likely that peroxisomal protein import involves accessory factors that regulate the affinity of PEX5 for the PTS1. Furthermore, full-length PEX5 is a homooligomeric protein (19) that displays intramolecular interactions (12), making it possible that PEX5-PTS1 interactions are regulated, in part, by cooperativity in the binding reaction (12). The present study extends our understanding of PEX5-PTS1 interactions by providing a detailed molecular analysis of the interactions between full-length PEX5 proteins and PTS1-containing peptides. We have shown here that full-length forms of PEX5 from both humans and the yeast P. pastoris have intrinsic PTS1 binding activity. These results eliminate the possibility that accessory factors are required for PEX5-PTS1 binding. We have also established that full-length PEX5 binds PTS1 peptides specifically and with high affinity. Full-length PEX5 proteins bind the PTS1 peptide with an affinity similar to that of the isolated ligand-binding domain of human PEX5 (K<sub>d</sub> of ~35 nM). The actual avidity of PEX5 for PTS1-containing proteins is even higher since many peroxisomal enzymes are oligomeric and oligomerize prior to import (23–25), and multidentate interaction may occur between tetrameric PEX5 and folded, oligomeric PTS1-containing peptides. One disadvantage of studying the interactions between PEX5 and PTS1-containing peptides is that it eliminates potentially important PEX5-protein interactions that occur outside of the PTS1-binding pocket. However, this is also an advantage, since it allows a quantitative assessment of whether and how different factors affect the affinity of PEX5 for just the PTS1. The power of this approach is evident from our analysis and how different factors affect the affinity of PEX5 for the PTS1 because we were unable to generate a form of soluble, recombinant PEX8 that would bind to purified PEX5. Although the present study has failed to identify a regulator of PEX5-PTS1 binding, it will be interesting to see if genetic screens and selections might be sensitive to such an activity and help us identify the factors that participate in this critical step in peroxisomal matrix protein import.

Acknowledgments—We thank Gregory Gatto for the PTS1 peptide.

REFERENCES

1. Lazaro, P. B., and Fujiki, Y. (1985) Annu. Rev. Cell Biol. 1, 489–530
2. Sacksteder, K. A., and Gould, S. J. (2009) Annu. Rev. Genet. 43, 623–652
3. Gould, S. J., Keller, G. A., Bhatti, U., Wilkinson, J., and Subramani, S. (1989) J. Cell Biol. 108, 1657–1664
4. Swinkels, B. W., Gould, S. J., Bodnar, A. G., Rachubinski, R. A., and Subramani, S. (1991) EMBO J. 10, 3244–3252
5. McCollum, D., Monosov, E., and Subramani, S. (1993) J. Cell Biol. 121, 761–774
6. Dott, G., Braverman, N., Wong, C., Moser, A., Moser, H. W., Watkins, P., Valle, D., and Gould, S. J. (1995) Nat. Genet. 9, 115–124
7. Dott, G., and Gould, S. J. (1996) J. Cell Biol. 135, 1763–1774
8. Gould, S. J., and Valle, D. (2000) Trends Genet. 16, 340–344
9. Gould, S. J., and Collins, C. S. (2002) Nat. Rev. Mol. Cell Biol. 3, 382–389
10. Gatto, G. J., Jr., Geisbrecht, B. V., Gould, S. J., and Berg, J. M. (1999) Nat. Struct. Biol. 7, 1091–1095
11. Voss, G. K., Soukoupa, M., Hong, X., Erdmann, K. S., Kiel, J. A., Dott, G., Kunau, W. H., and Erdmann, R. (1999) Mol. Cell. Biol. 19, 2265–2277
12. Harano, T., Nave, S., Uezu, R., Shiranu, N., and Fujiki, Y. (2001) Biochem. J. 357, 157–163
13. Chang, C. C., Warren, D. S., Sacksteder, K. A., and Gould, S. J. (1999) J. Cell Biol. 147, 761–773
14. Waterham, H. R., Titerenko, V. I., Haima, P., Cregg, J. M., Harder, W., and Veenhuis, M. (1994) J. Cell Biol. 127, 737–749
15. Liu, H., Tan, X., Russell, K. A., Veenhuis, M., and Cregg, J. M. (1995) J. Biol. Chem. 270, 10940–10951
16. Rehling, P., Skaltsa-Rorowski, A., Girzalsky, W., Voorn-Brouwer, T., Franse, M. M., Distel, B., Veenhuis, M., Kunau, W. H., and Erdmann, R. (2000) J. Biol. Chem. 275, 3593–3602
17. Braverman, N., Dott, G., Gould, S. J., and Valle, D. (1998) Hum. Mol. Genet. 7, 1185–1205
18. Geisbrecht, B. V., Zhang, D., Schulz, H., and Gould, S. J. (2001) J. Cell Biol. 154, 21797–21803
19. Schliebs, W., Sadowski, J., Agianian, B., Dott, G., Herberg, F. W., and Kunau, W. H. (1999) J. Biol. Chem. 274, 5666–5673
20. Imanaka, T., Ito, T., and Lazaro, P. B. (1987) J. Cell Biol. 105, 2997–3004
21. Horovitz, A., Frimodt, Y., Kafri, G., and Yifrach, O. (2001) J. Struct. Biol. 135, 104–114
22. Chang, C. C., Lee, W. H., Moser, H., Valle, D., and Gould, S. J. (1997) Nat. Genet. 15, 385–388
23. McNew, J. A., and Goodman, J. M. (1994) J. Cell Biol. 127, 1245–1257
24. Glover, J. R., Andrews, D. W., and Rachubinski, R. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10541–10545
25. Walton, J. P., Hill, P., and Subramani, S. (1995) Mol. Cell. Biol. 6, 675–683
26. Otera, H., Harano, T., Hono, M., Ghaedi, K., Mukai, S., Tanaka, A., Kawai, A., Shiranu, N., and Fujiki, Y. (2000) J. Biol. Chem. 275, 21703–21714
27. Dott, G., Warren, D., Becker, E., Rehling, P., and Gould, S. J. (2001) J. Biol. Chem. 276, 41769–41781
28. Okamoto, K., Abe, I., and Fujiki, Y. (2000) J. Biol. Chem. 275, 25700–25710
29. Enomoto, Y., Kowast, L., Klein, A., Voorn-Brouwer, T., van den Berg, M., Metzger, B., America, T., Tabak, H., and Distel, B. (1996) Cell 85, 97–109
30. Erdmann, R., and Blobel, G. (1996) J. Cell Biol. 135, 111–121
31. Girzalsky, W., Rehling, P., Stein, K., Kipper, J., Blank, L., Kunau, W. H., and Erdmann, R. (1999) J. Cell Biol. 144, 1151–1162
32. Gould, S. J., Kalish, J. E., Morell, J. C., Bjorkman, J., Urquhart, A. J., and Crane, D. I. (1996) J. Cell Biol. 135, 85–95
33. Urquhart, A. J., Kennedy, D., Gould, S. J., and Crane, D. I. (2000) J. Cell Biol. 154, 21703–21714
34. Albertini, M., Rehling, P., Erdmann, R., Girzalsky, W., Kiel, J. A., Veenhuis, M., and Kunau, W. H. (1997) Cell 89, 83–92
35. Fransen, M., Terlecky, S. R., and Subramani, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8687–8692
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J. Biol. Chem. 2003, 278:7897-7901.
doi: 10.1074/jbc.M206651200 originally published online November 26, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M206651200

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