Changing Stereochemistry for a Metabolic Pathway in Vivo

EXPERIMENTS WITH THE PEROXISOMAL $\beta$-OXIDATION IN YEAST*

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(Received for publication, July 17, 1995, and in revised form, September 12, 1995)

The biosphere is inherently built of chiral molecules, and once their metabolism is established, the stereochemical course of the reactions involved is seen to remain highly conserved. However, by replacing the yeast peroxisomal multifunctional enzyme (MFE), which catalyzes the second and third reactions of $\beta$-oxidation of fatty acids via 3-hydroxyacyl-CoA intermediates, with rat peroxisomal MFE, which catalyzes the same reactions via L-3-hydroxy intermediates, it was possible to change the chiralities of the intermediates in a major metabolic pathway in vivo. Both stereochemical alternatives allowed the yeast cells to grow on oleic acid, implying that when the $\beta$-oxidation pathways evolved, the overall function was the determining factor for the acquisition of MFEs and not the stereospecificities of the reactions themselves.

Fatty acids are universal constituents of living cells, and they are used as essential components of biomembranes and as a store for combustion energy (1). Their degradative pathway ($\beta$-oxidation) was found to be confined to peroxisomes in all eukaryotic cells (2–4) and additionally to mitochondria in animal cells (5, 6). Until recently, the second and third reactions of $\beta$-oxidation, which are catalyzed by multifunctional enzymes (MFEs)† in extramitochondrial systems (7–9) and with very long-chain substrates also in mitochondria (10), were assumed to proceed in all organisms via L-3-hydroxyacyl-CoA esters when degrading fatty acids (11–13) and via D-3-hydroxy intermediates in the de novo synthesis of fatty acids (14–17). However, the observation that $\beta$-oxidation in the peroxisomes of Saccharomyces cerevisiae occurs via D-3-hydroxy metabolites (18) opened up a new perspective on the evolution of $\beta$-oxidation systems (Fig. 1). The peroxisomal MFEs of higher (mammals and plants) and lower eukaryotes (fungi) not only catalyze two reactions of opposite chiral specificity, but they also have different native molecular sizes and distinct amino acid sequences, indicating different phylogenetic origins (18, 19).

Using yeast cells carrying a deletion of the MFE gene (YFOX-2) (18), we studied whether the stereochemistry plays a role in the evolution of $\beta$-oxidation systems. As fungi possess only a peroxisomal $\beta$-oxidation (20, 21), this yeast mutant does not degrade fatty acids and is thus unable to grow on fatty acids as a carbon source. We replaced the yeast endogenous MFE, comprising 2-enoyl-CoA hydratase 2 and D-specific 3-hydroxyacyl-CoA dehydrogenase activities (18), with its peroxisomal counterpart in the rat, possessing enoyl-CoA hydratase 1 and L-specific 3-hydroxyacyl-CoA dehydrogenase activities (7, 22). This replacement resulted in functional complementation giving the YFOX-2 cells back their capability to grow on oleic acid.

EXPERIMENTAL PROCEDURES

Strains and Media—The S. cerevisiae strains and plasmids used are listed in Table I. Escherichia coli strain DH5α was used for all transformations and plasmid isolations.

Plasmid Construction—Into a Yep352 vector containing the catalase A sequence from S. cerevisiae (CTA1) (23) and modified as described by Kragler et al. (24), an additional unique restriction site was introduced by cutting with HindIII, dephosphorylating, and ligating again in the presence of a polynucleotide kinase-treated oligonucleotide representing the XhoI site (5'-AGCTTCTCGAGA-3'). The resulting plasmid pYE352-CTA1 contains the whole CTA1 gene with modifications allowing the replacement of the open reading frame by cutting with XbaI or Sad and HindIII or XhoI (Fig. 2A).

The coding region of cDNA for rat MFE (25) was obtained from total RNA isolated from clofibrate-treated rat liver by reverse transcription with moloney murine leukemia virus reverse transcriptase and amplification by polymerase chain reaction using rat MFE-specific primers and cloned into pUC18 vector using the Sure Clone cloning kit (Pharmacia, Uppsala, Sweden). This pUC18-rMFE was used as template for polymerase chain reaction with specific oligonucleotide primers containing unique restriction sites in their 5’ ends. The resulting rMFE cDNA had an extra Sad restriction site in the 5’ end and an XhoI cutting site in the 3’ end and was cloned in pUC18 (pUC18-rMFE(SacXho)) in order to allow the amplification of the fragment (Fig. 2B).

pYE352-CTA1 and pUC18-rMFE(SacXho) were digested with Sad and XhoI, and the fragments containing the rat MFE and pYE352 with catalase promoter were isolated from 0.7% agarose gel by Geniedee II kit (Bio 101 Inc., Vista, CA) and ligated overnight. The resulting construct contains the rat MFE open reading frame under the control of oleic acid-inducible catalase promoter, and the YFOX-2 cells were transformed by lithium acetate method (26). The transformants were selected on ura− plates and replica plated on oleic acid plates. Colonies growing on both plates were chosen for complementation studies. UTIL-7A, YFOX-2, and YFOX-2-CTA1 strains were used as controls.

Growth Conditions and Harvesting the Cells—Yeast cells were cultured at 30 °C in the following media: 1% yeast extract, 2% Bacto-peptone, 2% glucose (YPD); 2% glucose, 0.67% Bacto-yeast nitrogen base without amino acids, 0.2% dropout powder without uracil (27) (SC-ura); 0.1% dextrose, 0.5% yeast extract, 0.5% Bacto-peptone, 0.1% glucose, 0.5% Tween 40, 6.4 ml of vitamin solution/l iter, 60 ml of salt solution/l iter, pH 6.0 (oleic acid medium with uracil); and 0.1% glucose,
The activity catalyzing the hydration of trans-2-enoyl-CoA to 3-hydroxyacyl-CoA intermediates is called here 2-enoyl-CoA hydratase 1 (EC 2.1.2.17) (Hydratase 1) and that catalyzing the hydration of trans-2-enoyl-CoA to 3-hydroxyacyl-CoA intermediates is called 2-enoyl-CoA hydratase 2 (EC 2.1.2.17) (Hydratase 2). The dehydrogenation reactions are as follows: l-specific 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) (L-HADH) and d-specific 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.17) (D-HADH).

0.1% oleic acid, 0.5% Tween 40, 0.3% Bacto-yeast nitrogen base without amino acids, 0.2% dropout mix without uradil, 60 ml of salt solution/liter, 6.4 ml of vitamin solution/liter, pH 6.0 (oleic acid medium without uradil). Salt solution (as expressed per liter) was as follows: 125 g of (NH₄)₂SO₄, 25 g of KH₂PO₄, 12.5 g of MgSO₄·7H₂O, 2.5 g of NaCl, 2.5 g of CaCl₂, 10 mg of ZnSO₄·7H₂O, 1 mg of CuSO₄·5H₂O, 10 mg of MnSO₄·H₂O, 2.5 mg of KI, 8.3 mg of FeCl₃·6H₂O, 12.5 mg of H₂B₆O₉, 5.9 mg of Na₂MoO₄·H₂O. Vitamin solution (per liter) was as follows: 200 mg of thiamine, 200 mg of nicotinic acid, 200 mg of calcium pantothenate, 200 mg of pyridoxine hydrochloride, 100 mg of p-aminobenzoic acid, 100 mg of riboflavin, 1 mg of biotin, 1 mg of folic acid, 1 g of myo-inositol.

Single colonies from YPD or ura plates were grown overnight in YPD (UTL-7A) and in SC-ura (YFOX-2-rMFE). Lister batches of YPD medium (UTL-7A), SC-ura (YFOX-2-rMFE), oleic acid medium with uradil (UTL-7A), and oleic acid medium without uradil (YFOX-2-rMFE) were inoculated with overnight cultures to cell density of 1 x 10⁶ cells/ml and grown at 30 °C with shaking for 48 h. The cells were harvested by centrifuging (2000 x g for 10 min), washed twice with sterile distilled water, and centrifuged as above.

Northern Blot Analysis—The strains were grown in liquid cultures, and the REX total RNA isolation kit from USB (Cleveland, OH) was used for isolating total RNA from 0.5 g of yeast cells from each culture. 10-μg samples of each RNA were fractionated by electrophoresis in formaldehyde-agarose (0.8%) and blotted onto a nitrocellulose filter. The filter was hybridized with random prime labeled rat MFE probe. The filter was hybridized with random prime labeled rat MFE probe. The filter was hybridized with random prime labeled rat MFE probe. The filter was hybridized with random prime labeled rat MFE probe.

Enzyme Activity Assays and Studies on Stereospecificities of 2-Enoyl-CoA Hydratases and 3-Hydroxyacyl-CoA Dehydrogenases from Wild-type and Transformed Yeast Cells—2-Enoyl-CoA hydratase 1 activity was measured in the direction of hydration of trans-2-decenoyl-CoA to 3-hydroxydecanoyl-CoA. The incubation mixture consisted of 60 nmol of trans-2-decenoyl-CoA in 50 μM Tris/HCl, pH 9.0, 50 μM of bovine serum albumin, 50 μM of potassium chloride, 1 μM of NAD⁺, 5 μg of porcine 3-hydroxyacyl-CoA dehydrogenase (Sigma) in a volume of 1 ml at 23 °C. The reaction was started by adding a sample into the cuvette and monitored by following the NADH production spectrophotometrically. 2-Enoyl-CoA hydratase 2 activity was measured in the direction of hydration of trans-2-decenoyl-CoA to 3-hydroxydecanoyl-CoA under the conditions described above except that the L-3-hydroxyacyl-CoA dehydrogenase was replaced with 0.5 μmol x min⁻¹ of d-3-hydroxyacyl-CoA dehydrogenase activity (determined with d-3-hydroxydecanoyl-CoA).

For measuring the L-specific 3-hydroxyacyl-CoA dehydrogenase activity, 60 μmol of a racemic mixture of p-3-hydroxydecanoyl-CoA was incubated in the Tris/HCl bovine serum albumin/potassium chloride mixture (see above) containing 25 μM magnesium chloride, 1 μM sodium pyruvate, 10 μg of lactate dehydrogenase from rabbit muscle (Boehringer Mannheim) in the presence of recombinant d-specific 3-hydroxyacyl-CoA dehydrogenase. After the d-isomer was removed, the reaction was initiated by adding the sample. When assaying the d-specific 3-hydroxyacyl-CoA dehydrogenase, the d-isomer of the substrate was first removed from the reaction mixture with the l-specific 3-hydroxyacyl-CoA dehydrogenase followed by adding the sample. The oxidation of 3-hydroxydecanoyl-CoA was followed by monitoring the formation of magnesium-3-ketodecanoyl complex at 303 nm (18, 28, 29).

Recombinant d-specific 3-hydroxyacyl-CoA dehydrogenase was a truncated version of the peroxisomal MFE from Candida tropicalis, and it was produced as described by Hiltunen et al. (18). Stereospecificities of l- and d-hydroxyacyl-CoA dehydrogenases have been tested separately using synthesized l- and d-isomers of 3-hydroxyacyl-CoA, which were obtained as described by Mallia et al. (29).

Immunoelectron Microscopy—UTL-7A and FOX-2-rMFE cells were grown on oleic acid, and the pelleted cells were fixed in 8% paraformaldehyde, processed, and immunolabeled as described by Sormunen et al. (30). The rabbit anti-rMFE IgG bound on cell sections was localized with protein A-coupled gold labeling technique.

RESULTS

Growth of Yeast Cells on Different Media—All yeast strains tested (UTL-7A, YFOX-2, YFOX-2-CTA1, and YFOX-2-rMFE) were viable (Fig. 3) on glucose-rich YPD plates, whereas on oleic acid plates only YFOX-2-rMFE and wild-type UTL-7A cells were able to grow. In a control experiment on ura plates, only the strains transfomed with pYE352-rMFE or pYE352-CTA1 were viable (data not shown). Altogether, these results indicate that apparent complementation of fox2 mutation by the rat MFE encoded by the plasmid pYE352-rMFE had occurred.

Northern Blot and Immunoblot Analyses—To investigate further the expression of rat MFE in YFOX-2-rMFE, Northern blot analysis was carried out. The rat MFE cDNA probe hybridized only with the RNA isolated from YFOX-2-rMFE cells grown in oleic acid medium, the size of the signal (2.2 kb) corresponding well with the estimated size of the rat MFE RNA (25) (Fig. 4A). This signal was not seen in lane 3 of Fig. 4A (YFOX-2-rMFE grown in glucose medium), which shows that the CTA1 promoter is a strong glucose-repressible and oleic acid-inducible promoter, which is in agreement with earlier observations (31, 32).

In immunoblot analysis of soluble proteins from the four yeast strains using antibodies against rat MFE, a signal of 78 kDa was obtained only with proteins from YFOX-2-rMFE cells grown in oleic acid medium (Fig. 4B), which corresponds well with the signal obtained with the rat wild-type MFE purified from rat liver (22).

Enzyme Activity Assays—The yeast MFE and the rat peroxisomal MFE are known to catalyze the conversion of trans-2-enoyl-CoA to 3-ketoacyl-CoA via hydration and dehydrogenation steps, but the stereochemical courses of the reactions are different; in yeast the events occur via D-3-hydroxyacyl-CoA, whereas in rat they occur via the corresponding L-form (18, 28). This difference was used to design kinetic experiments to distinguish between the two enzymes. As shown in Fig. 5A, when the protein sample from oleic acid-grown UTL-7A cells was added to a racemic mixture of the D- and L-forms of 3-hydroxydecanoyl-CoA, only one half of the substrate was converted to 3-ketodecanoyl-CoA. The subsequent addition of L-specific 3-hydroxyacyl-CoA dehydrogenase from porcine heart resulted in consumption of the second half of the substrate. This indicated that the yeast extract used contained only d-specific hydroxyacyl-CoA dehydrogenase. This conclusion was further
confirmed by the observation that the addition of the recombinant d-3-hydroxyacyl-CoA dehydrogenase (18) did not result in any additional production of 3-ketoacyl-CoA ester. When the corresponding experiment was carried out with YFOX-2-rMFE rather than wild-type cells, the addition of the cell lysate again caused the removal of only half of the substrate (Fig. 5B), but this time separate additions of the auxiliary enzymes, L-specific and D-specific hydroxyacyl-CoA dehydrogenases, yielded opposite effects; the L-specific enzyme caused no reaction, whereas the D-specific one metabolized the remaining half of the substrate. These results clearly demonstrate that the transformed YFOX-2 strain was devoid of its endogenous d-3-hydroxyacyl-CoA dehydrogenase activity but possessed the expressed L-3-hydroxyacyl-CoA dehydrogenase of plasmid origin.

When soluble proteins were extracted from UTL-7A and YFOX-2-rMFE yeast strains grown on glucose and oleic acid media and the samples were tested for combined activity (i.e. capability to convert trans-2-decenoyl-CoA to 3-ketoacyl-CoA), activity was observed within the detection limits only in oleic acid-grown cells (Table II). When enzyme activities participating in 3-hydroxyacyl-CoA metabolism were studied in more detail, activities of 2-enoyl-CoA hydratase and D-3-hydroxyacyl-CoA dehydrogenase could be detected in extracts of UTL-7A cells grown on oleic acid.
peroxisomal MFE can be heterologously expressed in an active

2-enoyl-CoA hydratase 1 and L-3-hydroxyacyl-CoA dehydrogenase activities but were lacking D-3-hydroxyacyl-CoA dehydrogenase activity. Unexpectedly, about 42% of hydratase 2 activity was still detectable when compared with the wild-type cells. Because it has been shown earlier that the mRNA for FOX-2p (yeast peroxisomal MFE) is not produced in YFOX-2 cells, this result indicates that in addition to FOX-2p there exists another protein capable of catalyzing hydratase 2 reactions in yeast.

Interestingly, recent data have shown that in rat liver hydratase 2 activity can be found in two subcellular compartments: peroxisomes and microsomes (29).

Subcellular Localization—To study the subcellular location of rat MFE expressed in YFOX-2 cells, immunoelectron microscopy was carried out applying protein A colloid gold technique and using anti-rMFE antibodies. As shown in Fig. 6, most of the gold particles were found within peroxisomes in YFOX-2-rMFE cells grown on oleic acid in contrast to wild-type cells, for which no labeling was found with the same procedure. This implies that the targeting signal for rMFE is recognized by the yeast import mechanism.

**DISCUSSION**

Present work provides several lines of evidence that the rat peroxisomal MFE can be heterologously expressed in an active form in S. cerevisiae and that it complements the lack of the corresponding endogenous yeast peroxisomal MFE. (i) Both the mRNA and the rat protein were detected only in cells transformed with plasmid pYE352-rMFE. (ii) The transformants had gained 2-enoyl-CoA hydratase 1 and L-3-hydroxyacyl-CoA dehydrogenase activities, which were undetectable in wild-type yeast cells. (iii) Immunoelectron microscopy indicated that the rMFE was targeted into the peroxisomes. (iv) Finally, the expression rate of peroxisomal MFE allows the YFOX-2 mutant cells to utilize oleic acid as a carbon source.

The observed functional complementation, however, results in the change of stereochemistry of the second (hydration) and the third (oxidation) reactions of β-oxidation (Fig. 1), namely from D-3-hydroxyacyl-CoA-dependent pathway to L-3-hydroxyacyl-CoA intermediate-dependent one. Consequently, the results indicate that the functioning of the β-oxidation pathway in S. cerevisiae is independent of the stereochemistry of its second

**TABLE II**

| Activity | UTL-7A | FOX-2-rMFE |
|----------|--------|------------|
| Combined | 0.03 ± 0.01 | 0.11 ± 0.02 |
| H₂       | n.d.   | 2.45 ± 0.69 |
| D₁       | 0.97 ± 0.14 | 0.40 ± 0.10 |
| D₂       | n.d.   | 0.17 ± 0.04 |

**Fig. 3. Complementation of fox-2 mutant of S. cerevisiae by rat peroxisomal MFE.** The wild-type strain UTL-7A (1), fox-2 mutant devoid of yeast peroxisomal MFE (2), fox-2 mutant transformed with pYE352-CTA1 encoding catalase A (3), and fox-2 mutant strain transformed with pYE352-rMFE containing the cDNA of rat peroxisomal MFE under the control of catalase A promoter (4). The strains were grown on rich medium, YPD (A), and on a medium containing 0.1% oleic acid as a carbon source (B).

**Fig. 4. Northern and Western blot analyses of the yeast strains used cultivated in different media.** A, Northern blot. Lane 1, UTL-7A grown in YPD; lane 2, UTL-7A grown in oleic acid; lane 3, YFOX-2-rMFE grown in SC-ura; lane 4, FOX-2-rMFE grown in oleic acid medium. The sizes of yeast rRNAs are given on the left. B, Western blot analysis. The strains, media, and sample order are as in A. The purified wild-type rat peroxisomal MFE was used as sample in lane 5.

**Fig. 5. Stereospecificity of 3-hydroxyacyl-CoA dehydrogenase from wild-type and transformed yeast cells.** A shows the experiment carried out with YFOX-2-rMFE cells grown on oleic acid. B shows the experiment carried out with UTL-7A cells grown on oleic acid. The labeled arrows indicate the time of addition of 90 µg of soluble extract from UTL-7A (1), 10 µg of L-3-hydroxyacyl-CoA dehydrogenase (2), 2 µg of D-3-hydroxyacyl-CoA dehydrogenase (3), and 90 µg of soluble extract from YFOX-2-rMFE (4). In the assay 20 nm D,L-3-hydroxydecenoyl-CoA was used as substrate.
the many sequential reactions of their pathways (39, 40). It therefore seems surprising at first sight that a phylogenetically unrelated heterologous protein can efficiently replace the endogenous component of this metabolon. It cannot be excluded, however, that the two multifunctional proteins of rat and fungal peroxisomes may be related in terms of their three-dimensional structure. If this turns out to be true in the future, it will mean that in addition to functional convergence, structural convergence must also have occurred.

Acknowledgments—We thank A. Holappa, R. Pietilla, I. Vuoti, and U. Dorpmund for technical assistance.

REFERENCES

1. Goodridge, A. G. (1985) in Biochemistry of Lipids and Membranes (Vance, D. E. & Vance, J. E., eds) pp. 143–180, The Benjamin/Cummings Publishing Company, Menlo Park, California

2. Cooper, T. G. & Bevers, H. (1969) J. Biol. Chem. 244, 3514–3520

3. Lazarow, P. B. & de Duve, C. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 2043–2046

4. Tanaka, A., Osumi, M. & Fukui, S. (1982) Ann. N. Y. Acad. Sci. 386, 183–199

5. Kennedy, E. P. & Lehninger, A. L. (1949) J. Biol. Chem. 179, 957–972

6. Beattie, D. S. (1968) Biochem. Biophys. Res. Commun. 30, 57–62

7. Osumi, T. & Hashimoto, T. (1979) Biochem. Biophys. Res. Commun. 89, 580–584

8. Moreno de la Garza, M., Schultz-Borchard, U., Crabb, J. W. & Kunau, W.-H. (1985) Eur. J. Biochem. 148, 285–291

9. Behrends, W., Engeland, K. & Kindl, H. (1988) Arch. Biochem. Biophys. 263, 161–169

10. Uchida, Y., Izai, K., Orii, T. & Hashimoto, T. (1992) J. Biol. Chem. 267, 1034–1041

11. Lyfen, F. & Ochoa, S. (1953) Biochem. Biophys. Acta 12, 299–314

12. Green, D. E. (1954) Biochim. Biophys. Acta 1001, 109–120

13. Schulz, H. (1991) Biochim. Biophys. Acta 196, 257–258

14. Waki, S. J. (1961) J. Lipid Res. 2, 1–24

15. Lyfen, F. (1961) Fed. Proc. 20, 941–951

16. Vagelos, P. R., Majerus, P. W., Alberts, A. W., Larrabee, A. R. & Alilhaus, G. P. (1966) Fed. Proc. 25, 1485–1494

17. Waki, S. J. (1989) Biochemistry 28, 4523–4530

18. Hiltunen, J. K., Wenzel, B., Beyer, A., Erdmann, R., Fosså, A. & Kunau, W.-H. (1992) J. Biol. Chem. 267, 6646–6653

19. Nuttley, W. M., Alitchison, J. D. & Rachubinski, R. A (1988) Gene (Amst.) 69, 171–180

20. Veenhuis, M., Mateblowski, M., Kunau, W.-H. & Harder, W. (1987) Yeast 3, 77–84

21. Kunau, W.-H., Bühne, S., Moreno de la Garza, M., Klönka, C., Mateblowski, M., Schultz-Borchard, U. & Thieringer, R. (1988) Trends Biochem. Sci. 13, 418–420

22. Palosaari, P. M. & Hiltunen, J. K. (1990) J. Biol. Chem. 265, 2446–2449

23. Cohen, G. L., Balfe, A., Hoefler, J., Gaertner, J., Aikawa, J. & Chen, W. W. (1991) Biochem. Biophys. Res. Commun. 183, 163–168

24. Aubel, F. M., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., Struhl, K., Albright, L., Cooi, D. & Vorki, A. (1994) Current Protocols in Molecular Biology, pp. 13.1.2-13.1.3, John Wiley & Sons, Inc., New York

25. Osumi, T., Ishii, N., Hikijaka, M., Kamijo, K., Ozasa, H., Furuta, S., Miyazawa, S., Kondo, K., Irie, K., Kageman, H. & Hashimoto, T. (1985) J. Biol. Chem. 260, 10095–10101

26. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) J. Biol. Chem. 258, 163–168

27. Ausubel, F. M., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., Struhl, K., Albright, L., Cooi, D. & Vorki, A. (1984) The Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

28. Palosaari, M. P. & Hiltunen, J. K. (1990) J. Biol. Chem. 265, 2446–2449

29. Cohen, G. L., Balfe, A., Hoefler, J., Gaertner, J., Aikawa, J. & Chen, W. W. (1991) Biochem. Biophys. Res. Commun. 183, 163–168

30. Malila, L. H., Silvani, K. M., Mäkelä, M. J., Jalonen, J. E., Latipap, P. M., Kunau, W.-H. & Hiltunen, J. K. (1993) J. Biol. Chem. 268, 21578–21585

31. Sironmnen, R., Eskelinen, S. & Lehto, V.-P. (1993) Lab. Invest. 65, 652–662

32. Hoertner, H., Ammerer, G., Hatter, E., Hamilton, B., Rytkö, J., Bilinski, T. & Ruis, H. (1982) Eur. J. Biochem. 128, 179–184

33. Skoneczny, M., Chelstowska, A. & Rytkö, J. (1988) Eur. J. Biochem. 174, 297–302

34. DiRussa, C. C. (1990) J. Bacteriol. 172, 6459–6468

35. Nagahigashi, K. & Inukuchi, H. (1990) Nucl. Acids Res. 18, 4937

36. Yang, S.-Y. & Schulz, H. (1983) J. Biol. Chem. 258, 9780–9785

37. Kamijo, T., Aoyama, T., Miyazaki, J. & Hashimoto, T. (1993) J. Biol. Chem. 268, 26452–26460

38. Chen, G. L., Balfe, A., Erwa, W., Hoefler, J., Gaertner, J., Aikawa, J. & Chen, W. W. (1991) Biochem. Biophys. Res. Commun. 178, 1084–1093

39. Pressig-Muller, R., Guhnemann-Schafer, K. & Kindl, H. (1994) J. Biol. Chem. 269, 20475–20481

40. Serre, P. A. & Sumegi, B. (1994) Biocem. Soc. Trans. 22, 446–450

41. Nada, M. A., Rhead, W. J., Sprecher, H., Schulz, H. & Roe, C. R. (1995) J. Biol. Chem. 270, 530–535
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J. Biol. Chem. 1995, 270:27453-27457.
doi: 10.1074/jbc.270.46.27453

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