Induction of the major integral membrane protein of mouse liver peroxisomes by peroxisome proliferators

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Peroxisome proliferators are known to increase the volume of the peroxisomal compartment in rodent liver. We have examined the induction of the major integral membrane protein of mouse liver peroxisomes (PMP68) by a number of these agents, and compared this with their effect on the peroxisomal bifunctional protein (PBP), an enzyme of the β -oxidation pathway which is located in the peroxisome matrix. Dietary clofibrate, di-2-(ethylhexyl)phthalate and Wy-14,643, three structurally unrelated proliferators, all increased the mRNA and protein content of PMP68 approx. 2-fold, whereas PBP was induced 8–13-fold. The kinetics and sequence of induction of PMP68 and PBP following a single dose of Wy-14,643 were compared and shown to be similar, and the effects were reversible. Another proliferator, BM 15766, caused maximal induction of PMP68 but only a low induction of PBP; further PBP induction was achieved by the administration of BM 15766 in combination with Wy-14,643. Similarly, BM 15766 and Wy-14,643 increased transcription of the PMP68 gene *in vitro*, whereas PBP gene transcription was increased by Wy-14,643 but not by BM 15766. Thus peroxisome proliferators enhance the expression of the genes for both the membrane protein PMP68 and the matrix protein PBP, but the regulation of this expression appears to be mediated by different mechanisms.

INTRODUCTION

A number of agents have been shown to induce rapid proliferation of the peroxisomal compartment within rodent liver [1–5]. Such agents, termed peroxisome proliferators, increase the content of peroxisomes in the liver at least 5-fold, to the extent that this organelle may account for 10% or more of total hepatocyte protein [6,7]. These agents also selectively induce the synthesis of specific peroxisomal proteins, in particular the enzymes of the fatty acid β -oxidation pathway, whereas the synthesis of other peroxisomal proteins, such as the normally abundant matrix protein catalase, is relatively unaffected [7–9].

Reddy *et al.* [10] have attributed the increase in the levels of the β -oxidation enzymes following treatment with proliferators to a co-ordinated induction of gene transcription. These enzymes are imported into the matrix compartment of peroxisomes [11]. In contrast, information regarding the effect of proliferators on proteins located in the membrane of peroxisomes is limited, even though proliferation of these organelles necessarily involves new membrane synthesis [11].

The major integral membrane protein of mouse liver peroxisomes has a subunit M_r of approx. 68000, and has been designated PMP68 [6,12]. The function of this protein is unknown, but it may be expected to have an involvement in metabolite/protein binding and/or transport, and a major structural role. The content of PMP68 in the liver is also increased following treatment of mice with proliferators, although its specific content in the peroxisomal membrane appears to be unchanged [6]. These observations supposedly relate to the requirement for new peroxisomal membrane, and may indicate a mechanism of genetic regulation of this protein which is similar to that of the β -oxidation enzymes.

Kamijo *et al.* [13] have recently reported the characterization of a major protein of rat liver peroxisomal membranes which is induced by peroxisome proliferators. In this paper, we report that this rat peroxisomal membrane protein is similar to PMP68

of mouse liver. In order to examine the relationship between the genetic regulation of membrane and matrix proteins, we have made use of specific antibodies and cDNA probes for this peroxisomal membrane protein and for the peroxisomal matrix proteins [catalase and the bifunctional protein (PBP)] to examine changes to the levels of these proteins and their mRNAs following induction of peroxisome proliferation in mouse liver.

MATERIALS AND METHODS

Materials

Mature female mice (Quackenbush strain) were obtained from the Central Animal Breeding House, Moggill, Brisbane, Australia. Clofibrate (*p*-chlorophenoxyisobutyric acid, ethyl ester; Atromid-S) was a gift from I.C.I. Australia; Wy-14,643 {4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio}acetic acid} was from Wyeth-Ayerst Research, Princeton, NJ, U.S.A.; BM 15766 {4-(2-[1-(4-chlorocinnamyl)piperazin-4-yl]ethyl)benzoic acid} was from Boehringer-Mannheim. DEHP [di-2-(ethylhexyl)phthalate] was obtained from Fluka AG, Buchs, Switzerland.

A partial cDNA for mouse liver catalase, pmCAT-34 [14], was kindly donated by Dr. G. Bewley (North Carolina State University, Raleigh, NC, U.S.A.), and cDNAs for rat liver PBP (pMJ26) [15] and rat liver peroxisomal PMP70 (cPM201) [13] were gifts from Professor T. Hashimoto and Professor T. Osumi (Shinshu University, Shinshu, Japan). A cDNA for baboon liver alcohol dehydrogenase [16] was provided by Dr. I. Beacham, Griffith University. [32 P]dATP and [32 P]UTP were obtained from Amersham. The nick-translation kit was from Boehringer-Mannheim. ProBlott membrane was from Applied Biosystems. Horseradish peroxidase-labelled goat anti-(rabbit IgG) was from Bio-Rad Laboratories, Richmond, CA, U.S.A. All other reagents were of analytical/reagent grade or molecular biology grade.

Treatment of animals

Mice weighing about 40 g had access to laboratory chow

Abbreviations used: PBP, peroxisomal bifunctional protein (enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase); PMP68, peroxisomal integral membrane protein of subunit mass 68 kDa; DEHP, di-2-(ethylhexyl)phthalate; PBS, phosphate-buffered saline.

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pellets and water *ad libitum*. For diet administration, drugs were dissolved in ethanol and added to chow pellets, and the ethanol evaporated. The concentrations of individual drugs in the chow and the periods of administration were: clofibrate, 0.35% (w/w) for 10 days; Wy-14,643, 0.2% (w/w) for 7 days; DEHP, 2% (w/w) for 14 days [6]. Intraperitoneal administration of Wy-14,643 was as a single dose (75 mg/kg body wt.) of the drug suspended in dimethyl sulphoxide. Control animals received dimethyl sulphoxide alone. BM 15766 was suspended in methylcellulose and administered as a daily intragastric dose of 75 mg/kg body wt. for 7 days [17]. Control animals received methylcellulose alone.

Preparation of liver homogenates

Livers were excised from mice and 0.5 g portions were finely minced using a tissue chopper and then homogenized vigorously in 1.5 ml of cold 0.25 M-sucrose using a Potter-Elvehjem homogenizer. The homogenates were mixed with 0.5 vol. of a 3 × concentrated SDS/PAGE sample buffer, heated immediately in a boiling water bath for 5 min and stored at -30°C .

SDS/PAGE and immunoblotting

SDS/PAGE in 7% or 7–15% gradient gels and electrophoretic transfer of proteins to nitrocellulose were carried out as described previously [12]. Nitrocellulose membranes were blocked overnight at 4°C using 10 mM-phosphate-buffered 0.15 M-saline, pH 7.4 (PBS), containing 0.05% Tween-20 and 5% low-fat dried milk, then washed extensively in PBS/0.05% Tween-20. The membranes were incubated in antiserum (anti-PBP, 1:6000 dilution in PBS/Tween; anti-PMP68, 1:6000 dilution) or purified antibody (anti-catalase, 1:20000) for 1 h at room temperature and then washed as above. The washed nitrocellulose membranes were incubated in horseradish peroxidase-labelled anti-(rabbit immunoglobulin) (1:10000 dilution) for 1 h at room temperature, followed by extensive washing in PBS/Tween. Peroxidase activity was detected using the ECL detection system (Amersham), followed by autoradiography for 10–30 s.

Antisera

Antisera to mouse PBP and PMP68 have been characterized previously [12,18]. Catalase was purified from mouse liver [19], and antibodies to this protein were raised in rabbits and affinity-purified as described by Lazarow & de Duve [20].

N-Terminal peptide sequencing

Peroxisomes were isolated from mouse liver as described previously [21], and their membranes were isolated by carbonate extraction [22]. Membrane proteins were resolved by SDS/PAGE (7% gels) and then electrophoretically transferred to polybrene-coated glass-fibre sheets [23]. The protein band corresponding to PMP68 was excised and the N-terminal amino acid sequence was determined by automated Edman degradation on a gas-phase sequencer (Applied Biosystems Inc.; model 470A). Purified mouse PBP was partially digested with *Staphylococcus aureus* V8 proteinase [18], and carboxymethylated, and the polypeptides were resolved by SDS/PAGE and transferred to a ProBlott membrane. Selected polypeptide bands were excised for sequencing, as above.

Isolation of RNA

Total RNA was isolated from mouse liver using a procedure developed by Trezise [24]. Livers were excised from mice and homogenized, using an Ultra-Turrax homogenizer for 30 s, in 8 vol. of a 1:1 mixture of homogenizing buffer [20 mM-Tris/HCl/10 mM-NaCl/3 mM-magnesium acetate/5% (w/v)

sucrose, pH 8.6] and buffer (20 mM-Tris/HCl/10 mM-NaCl, pH 8.6)-saturated phenol. The homogenate was then extracted twice with buffer-saturated phenol and once with phenol/chloroform/isoamyl alcohol (25:24:1, by vol.). To the extracted solution was added 0.1 vol. of 3 M-sodium acetate, pH 5.2, and 2 vol. of ethanol, and the mixture was incubated overnight at -70°C . The precipitated nucleic acids were collected by centrifugation at 27000 g for 40 min and resuspended in 10 mM-Tris/HCl/100 mM-NaCl, pH 8.6. RNA was precipitated at -20°C over 2 h following addition of LiCl to a final concentration of 2 M, collected by centrifugation as above, and resuspended in 10 mM-Tris/HCl/100 mM-NaCl, pH 8.6. The RNA solution was extracted four times with buffer-saturated phenol and once with phenol/chloroform/isoamyl alcohol (25:24:1, by vol.). The RNA was then precipitated by adding 0.1 vol. of 3 M-sodium acetate, pH 5.2, and 2 vol. of ethanol, and stored at -70°C .

Northern blot analysis

Formaldehyde/agarose gel electrophoresis of RNA and transfer of RNA to nitrocellulose were performed according to Seed [25]. Dot-blotting of RNA to nitrocellulose was carried out using a Hybri Dot manifold (BRL). Radioactive ^{32}P -labelled cDNA probes were prepared from recombinant plasmid DNA by nick-translation [26]. Hybridization of nitrocellulose-immobilized RNA with radioactive cDNA probes was carried out using a modification of the method of Seed [25]. The prehybridization solution contained 0.2% polyvinylpyrrolidone, 0.2% BSA, 0.2% Ficoll 400, 1 M-NaCl, 0.1% sodium pyrophosphate, 10% (w/v) dextran sulphate, 100 μg of herring sperm DNA/ml, 100 μg of tRNA/ml and 50 mM-Tris/HCl, pH 7.5. Hybridization was carried out at 65°C overnight and the nitrocellulose filter was then washed twice for 30 min at 65°C in 75 mM-NaCl/7.5 mM-sodium citrate/0.1% SDS, and autoradiographed.

Isolation of nuclei and measurement of RNA transcription

Liver nuclei were isolated as described by Lamers *et al.* [27], but omitting Triton X-405 from the homogenizing medium. Nuclear run-off assays and isolation of labelled nuclear RNA were also as described [27]. Hybridization of labelled RNA to plasmid DNA was carried out as described by Nieto *et al.* [28], except that filter hybridization was for 72 h at 42°C .

Densitometric scanning

Quantification of protein and RNA blots was by densitometric scanning on an LKB Ultrosan XL Laser Densitometer.

Statistical analysis

Results are expressed as means $\pm$ S.E.M. for the numbers of animals specified. Statistical significance of differences was assessed by Student's *t* test.

RESULTS

Sequence analysis of PMP68 and PBP

The N-terminal sequence for PMP68 determined by Edman degradation was Asp(Ala)-Xaa-Phe-Ser-Lys-Tyr-Leu-Thr-Ala-Arg-Asn-Thr?-Ser-Leu-Ala-Gly(Ser)-Ala-Asn(Ala)-Phe-. This sequence is in close agreement with the sequence reported for the corresponding protein, PMP70, from rat liver [13] and with the amino acid sequence predicted for PMP70 from the nucleotide sequence of a cDNA encoding this protein [13]. In addition, DNA sequence analysis of a 1.1 kb cDNA for PMP68 yielded 94.6% nucleotide similarity (97.9% amino acid similarity) with the C-terminal sequence of rat PMP70 (results not shown).

Fig. 1. Steady-state levels of protein and mRNA for peroxisomal proteins in mice following dietary treatment with peroxisome proliferators

Mice were fed a diet containing one of the following peroxisome proliferators; Con, control; Clo, clofibrate; D, DEHP; Wy, Wy-14,643. Protein (a) and mRNA (b) contents in liver were determined for PMP68, PBP and catalase (CAT). Proteins in liver homogenates (100 μ g of protein) were separated by SDS/PAGE and immunoblotted. The values shown represent protein levels relative to the controls, as determined by densitometric scanning. mRNA levels were determined by Northern blot analysis of 40 μ g of total liver RNA. These analyses used the same animals as in (a). mRNAs were estimated at the sizes indicated by comparison with RNA size markers. The values shown represent mRNA levels relative to the controls as determined by densitometric scanning, and normalized to alcohol dehydrogenase mRNA levels.

For PBP, sequence analysis of a V8-proteinase-generated peptide of this protein purified from mouse liver yielded the sequence Phe-Gly-Phe-Lys-Met-Gly-Pro-Phe-Arg-Val-Ser-Asp-Leu-Ala-, which has close sequence similarity with residues 511–524 of the PBP from rat liver, differing only by way of a Lys substitution for Arg at residue 514 [15]. Results of hybrid-selected mRNA translation experiments were also consistent with the protein similarities determined above (results not shown). Altogether these data have been taken as a validation of the use of cDNA probes of these rat proteins in the Northern analysis of mouse liver mRNA levels (see below).

Effects of peroxisome proliferators on the steady-state mRNA and protein levels of PMP68, PBP and catalase

Western and Northern blot analyses were adopted to determine changes caused by peroxisome proliferators to liver protein and mRNA levels for the membrane protein PMP68 and the matrix proteins catalase and PBP. Protein levels for PMP68 were increased 1.73-, 1.79- and 1.80-fold over controls following treatment of animals with clofibrate, DEHP and Wy-14,643 respectively (Fig. 1a). The increases in PBP protein levels were

more marked, being greater than 12-fold following Wy-14,643 feeding. Catalase levels were minimally affected.

The content of mRNAs for these proteins was measured by means of Northern analysis, using as probes cDNA clones for rat PMP70 (cPM201), rat PBP (pMJ26) and mouse liver catalase (pmCAT-34). As shown in Fig. 1(b), the corresponding mouse liver mRNAs were estimated by comparison with RNA size markers to be: PMP68, 3.5 kb; PBP, 3.2 kb; catalase, 2.4 kb. The patterns of increase of the mRNA levels by the proliferators were similar to those seen for the corresponding protein levels, although the mRNA/protein ratio was higher for PMP68 than for PBP. Alcohol dehydrogenase mRNA levels were similarly quantified to allow normalization of total RNA loading on gels.

Induction of peroxisomal proteins following a single dose of Wy-14,643

A single intraperitoneal dose of Wy-14,643 (75 mg/kg body wt) was administered to mice. The animals were killed after specified times and the livers removed for determination of protein and mRNA levels, as before. mRNA and protein levels for both PMP68 and PBP increased rapidly within 8 h of Wy-14,643

Fig. 2. Kinetics of induction of peroxisomal proteins by Wy-14,643

mRNA (a) and protein (b) levels were quantified for PMP68 (●) and PBP (▲) in livers of mice administered a single intraperitoneal dose of Wy-14,643 and killed at the times indicated. Analysis was made of 40 μ g of total liver RNA for Northern blots, and of 100 μ g of liver protein for immunoblots. Data were obtained by scanning densitometry of autoradiograms. mRNA levels have been normalized to the levels of alcohol dehydrogenase mRNA. Data are presented as a percentage of maximum levels, having set the level at zero time at 0%.

administration, with the protein levels mirroring the mRNA changes (Fig. 2). The sequence of induction of PMP68 was similar to that for PBP, in that the rates of increase in mRNA

Table 2. Effect of peroxisome proliferators on PMP68 gene transcription *in vitro*

Nuclei isolated from livers of mice fed peroxisome proliferators for 7 days were used for *in vitro* run-off experiments. The 32 P-labelled nuclear RNA (2×10^6 c.p.m.) was hybridized to filter-immobilized plasmids containing cDNAs for PMP68 or PBP, and quantified by scanning densitometry of autoradiograms. For details, see the Materials and methods section. Values have been corrected for non-specific plasmid hybridization, and are means $\pm$ S.E.M. of three experiments. Statistically significant effects of densitometric integrals over control values are indicated by: * $P < 0.05$; ** $P < 0.01$.

Treatment	Increase over control (fold)	
	PMP68	PBP
Control	1.00 $\pm$ 0.04	1.00 $\pm$ 0.05
Wy-14,643	1.74 $\pm$ 0.27*	2.57 $\pm$ 0.25**
BM 15766	2.33 $\pm$ 0.28**	1.03 $\pm$ 0.21

and protein for both were similar, and peak levels occurred for both between 1 and 2 days. The effects caused by Wy-14,643 were reversible, with levels falling away to control levels within 4 days in the case of the mRNAs, and more slowly in the case of protein. Catalase mRNA and protein levels were minimally affected over these same periods (results not shown).

Peroxisome proliferation by BM 15766

Administration of another peroxisome proliferator, BM 15766 [17], for 7 days resulted in a 1.6-fold increase in PMP68 protein levels in the liver (Table 1). A comparable level of PMP68 protein was reached within 16 h of intraperitoneal administration of Wy-14,643. This 16 h time point was chosen to allow for possible further increases in PMP68 levels (cf. Fig. 2). Intraperitoneal administration of Wy-14,643 in combination with the BM 15766 treatment did not result in a further elevation of PMP68 levels ($P > 0.05$). The comparative mRNA levels for PMP68 mirrored the changes in protein levels (Table 1).

The level of PBP protein in liver, on the other hand, was elevated by BM 15766 treatment to only a fraction of the level observed following intraperitoneal Wy-14,643 treatment (Table 1). Wy-14,643 administration in combination with BM 15766 feeding produced no further elevation of PBP mRNA and protein levels over those observed with Wy-14,643 treatment alone.

Table 1. Induction of peroxisomal proteins by BM 15766

mRNA and protein levels were quantified for PMP68 and PBP in livers of mice administered BM 15766, either alone or in combination with Wy-14,643. BM 15766 was administered for 7 days. Wy-14,643 was administered as a single intraperitoneal dose and animals were killed 16 h later. Data were obtained by scanning densitometry of Northern blots (40 μ g of RNA) and immunoblots (100 μ g of protein). Data are presented as means $\pm$ S.E.M. for the numbers of experiments shown in parentheses. Statistically significant effects of densitometric integrals (Student's *t* test) over controls are given by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and over BM 15766 values by: † $P < 0.001$.

Treatment	Increase over control (fold)			
	PMP68		PBP	
	mRNA (<i>n</i> = 3–4)	Protein (<i>n</i> = 5)	mRNA (<i>n</i> = 3–4)	Protein (<i>n</i> = 5)
Control	1.00 $\pm$ 0.17	1.00 $\pm$ 0.13	1.00 $\pm$ 0.17	1.00 $\pm$ 0.08
BM 15766	3.16 $\pm$ 1.09*	1.59 $\pm$ 0.23*	1.92 $\pm$ 0.51	2.64 $\pm$ 0.24***
Wy-14,643	4.45 $\pm$ 1.41*	1.67 $\pm$ 0.29*	7.86 $\pm$ 4.12*	4.93 $\pm$ 0.39***†
BM 15766 + Wy-14,643	5.06 $\pm$ 0.99**	1.85 $\pm$ 0.26**	8.37 $\pm$ 4.26*	4.61 $\pm$ 0.45***†

Catalase mRNA and protein levels were unchanged by any of the treatments described (results not shown).

Effect of peroxisome proliferators on the rate of transcription of the PMP68 gene

Nuclear run-off experiments were carried out to determine the effect of peroxisome proliferators on the rate of transcription of the PMP68 gene, compared with their effect on PBP gene expression. As shown in Table 2, the rate of transcription of the PMP68 gene *in vitro* was increased 1.74-fold and 2.33-fold by Wy-14,643 and BM 15766 respectively. PBP gene transcription was more than doubled by Wy-14,643 treatment, but was not affected by BM 15766 treatment. These changes in the rates of gene transcription *in vitro* for PMP68 and PBP reflect the changes in mRNA levels determined by Northern blotting, though not giving such large differences as those observed by this latter procedure (see Table 1).

DISCUSSION

Dietary treatment of mice with the structurally unrelated peroxisome proliferators clofibrate, DEHP and Wy-14,643 resulted in marked increases in the liver mRNA and protein levels of PBP. This was consistent with the known effect of these agents in inducing peroxisomal β -oxidation [7–9]. The membrane protein PMP68 was likewise induced by these agents, but not to the extent seen with PMP. With regard to the process of peroxisome proliferation, these data reflect a modest expansion of the peroxisomal compartment and an enrichment of this compartment with peroxisomal β -oxidation enzymes.

An examination of the kinetics of induction of PMP68 and PBP following a single administration of Wy-14,643 provides a means of delineating the sequence of biogenesis of the peroxisomal membrane and matrix compartments. The results obtained do not lend support to the proposal that membrane formation precedes matrix protein import, but instead are more consistent with a model in which these processes occur concurrently. The rate and extent of these changes in PBP were found to be similar to those established previously by Reddy *et al.* [10] for the induction of both PBP and fatty acyl-CoA oxidase of rat liver. These changes were shown by these authors to be due to increased rates of gene transcription, and were interpreted as being selective for the enzymes of the fatty acid β -oxidation system. The effect of peroxisome proliferators on the rate of transcription of the gene for the membrane protein PMP68, as established in the present work, now extends this selective gene response to include this major membrane protein as well. It is of interest in this regard that the experiments reported here on gene transcription *in vitro* indicated that the increases in the transcription of the PMP68 and PBP genes were less than the corresponding increases in steady-state levels of liver mRNA determined by Northern blot analysis. The reason for this discrepancy is not immediately apparent; however the previous data by Reddy *et al.* [10], describing a close relationship between changes in mRNA levels and gene transcription for PBP in livers of rats fed peroxisome proliferators, would tend to suggest an underestimation of changes to transcription rates in the present experiments.

The co-ordination of regulation of PMP68 and PBP, as seen in the Wy-14,643 experiments, does not completely concur, however, with the data obtained from the BM 15766 experiments. The effect of treatment with this agent differs from that with Wy-14,643 (and, by inference, with clofibrate and DEHP): whereas PMP68 is induced by BM 15766 to the level seen with Wy-14,643 treatment, PMP is only elevated to a fraction of the level achieved following Wy-14,643 administration. Furthermore this

'deficit' in the level of induction of the bifunctional protein is obviated by Wy-14,643 administration in combination with the BM 15766 treatment. Thus the regulation of the expression of the genes for PMP68 and PBP can be distinguished by the action of BM 15766.

A proposal has been put forward that peroxisome proliferator action is mediated by receptor proteins [29,30], and may be similar to the mechanism observed for steroid hormones [30]. Two different receptor proteins have been identified, and for each receptor the affinity of individual peroxisome proliferators is different [29,30]. In the light of these previous findings, the present data may be construed as evidence for the existence of different receptor proteins affecting the PMP68 and the PBP genes, with these receptors exhibiting differential affinities for proliferators, and in particular for BM 15766 and Wy-14,643.

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REFERENCES

- Hess, R., Staubli, W. & Riess, W. (1965) *Nature* (London) **208**, 856–858
- Leighton, F., Coloma, L. & Koenig, C. (1975) *J. Cell Biol.* **67**, 281–309
- Reddy, J. K. & Krishnakanth, T. P. (1975) *Science* **190**, 787–789
- Moody, D. E. & Reddy, J. K. (1978) *Am. J. Pathol.* **90**, 435–445
- Ganning, A. E., Brunk, U. & Dallner, G. (1983) *Biochim. Biophys. Acta* **763**, 72–82
- Crane, D. I., Chen, N. & Masters, C. J. (1988) *Mol. Cell. Biochem.* **81**, 29–36
- Reddy, J. K., Lalwani, N. D., Qureshi, S. A., Reddy, M. K. & Moehle, C. M. (1984) *Am. J. Pathol.* **114**, 171–183
- Lazarow, P. B. & de Duve, C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2043–2046
- Furuta, S., Miyazawa, S. & Hashimoto, T. (1982) *J. Biochem. (Tokyo)* **92**, 319–326
- Reddy, J. K., Goel, S. K., Nemali, M. R., Carrino, J. J., Laffler, T. G., Reddy, M. K., Sperbeck, S. J., Osumi, T., Hashimoto, T., Lalwani, N. D. & Rao, M. S. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1747–1751
- Lazarow, P. B. & Fujiki, Y. (1985) *Annu. Rev. Cell Biol.* **1**, 489–530
- Chen, N., Crane, D. I. & Masters, C. J. (1988) *Biochim. Biophys. Acta* **945**, 135–144
- Kamijo, K., Taketani, S., Yokota, S., Osumi, T. & Hashimoto, T. (1990) *J. Biol. Chem.* **265**, 4534–4540
- Shaffer, J. B., Sutton, R. B. & Bewley, G. C. (1987) *J. Biol. Chem.* **262**, 12908–12911
- Osumi, T., Ishii, N., Hijikata, M., Kamijo, K., Ozasa, H., Furuta, S., Miyazawa, S., Kondo, K., Inoue, K., Kagamiyama, H. & Hashimoto, T. (1985) *J. Biol. Chem.* **260**, 8905–8910
- Trezise, A. E. O., Godfrey, E. A., Holmes, R. S. & Beacham, I. R. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5454–5458
- Baumgart, E., Volkl, A., Pill, J. & Fahimi, H. D. (1990) *FEBS Lett.* **264**, 5–9
- Crane, D. I., Chen, N. & Masters, C. J. (1989) *Biochem. Biophys. Res. Commun.* **160**, 503–508
- Pegg, M., Crane, D. & Masters, C. (1989) *Mol. Cell. Biochem.* **86**, 77–85
- Lazarow, P. B. & de Duve, C. (1973) *J. Cell Biol.* **59**, 507–524
- Crane, D. I., Hemsley, A. C. & Masters, C. J. (1985) *Anal. Biochem.* **148**, 436–445
- Fujiki, Y., Fowler, S., Shio, H., Hubbard, A. L. & Lazarow, P. B. (1982) *J. Cell Biol.* **93**, 103–110
- Vandekerckhove, J., Bauw, G., Puype, M., Van Damme, J. & Van Montagu, M. (1985) *Eur. J. Biochem.* **152**, 9–19
- Trezise, A. E. O. (1989) Ph.D. Thesis, Griffith University
- Seed, B. (1979) in *Genetic Engineering: Principles and Methods* (Setlow, J. K. & Hallander, A., eds.), vol. 4, pp. 91–102, Plenum Press, New York

26. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251
27. Lamers, W. H., Hanson, R. W. & Meisner, H. M. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5137–5141
28. Nieto, A., Mira, E. & Castano, J. G. (1990) *Biochem. J.* **267**, 317–323
29. Lalwani, N. D., Alvares, K., Reddy, M. K., Reddy, M. N., Parikh, I. & Reddy, J. K. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5242–5246
30. Issemann, I. & Green, S. (1990) *Nature (London)* **347**, 645–650

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