Molecular basis of non-responsiveness to peroxisome proliferators: the guinea-pig PPAR α is functional and mediates peroxisome proliferator-induced hypolipidaemia

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The guinea pig does not undergo peroxisome proliferation in response to peroxisome proliferators, in contrast with other rodents. To understand the molecular basis of this phenotype, the peroxisome proliferator activated receptor α (PPAR α) from guinea-pig liver was cloned; it encodes a protein of 467 amino acid residues that is similar to rodent and human PPAR α . The guinea-pig PPAR α showed a high substitution rate: maximum likelihood analysis was consistent with rodent monophyly, but could not exclude rodent polyphyly ($P \approx 0.06$). The guinea-pig PPAR α cDNA was expressed in 293 cells and mediated the induction of the luciferase reporter gene by the peroxisome

proliferator, Wy-14,643, dependent on the presence of a peroxisome proliferator response element. Moreover the PPAR α RNA and protein were expressed in guinea-pig liver, although at lower levels than in a species which is responsive to peroxisome proliferators, the mouse. To determine whether the guinea-pig PPAR α mediated any physiological effects, guinea pigs were exposed to two selective PPAR α agonists, Wy-14,643 and methylclofenapate; both compounds induced hypolipidaemia. Thus the guinea pig is a useful model for human responses to peroxisome proliferators.

INTRODUCTION

Peroxisome proliferators are structurally diverse chemicals that induce hypolipidaemia and a variety of effects in rodent liver, including growth, the proliferation of peroxisomes, and a co-ordinated induction of enzymes of fatty acid metabolism [1,2]: long-term administration leads to non-genotoxic carcinogenesis [3]. The peroxisome proliferator activated receptor α (PPAR α) binds peroxisome proliferators [4,5] and is required for the induction of peroxisome proliferation in knockout mice [6]; it is thought that PPAR α mediates the peroxisome proliferation response [7–9].

Species differences in peroxisome proliferation in response to peroxisome proliferators are poorly understood. Mice and rats are extremely responsive, and guinea pigs and humans are either non-responsive or markedly less responsive [10,11] to peroxisome proliferators. These differences are not due to pharmacokinetics [12] and have been reproduced in cultured hepatocytes (see, for example, [13]). The human PPAR α is known to respond to peroxisome proliferators as well as the mouse receptor [14,15]. It has been proposed that the non-responsive nature of some species to peroxisome proliferators is due to fundamental deficiencies in the PPAR α [16]. This hypothesis has implications for the assessment of human hazard from peroxisome proliferators, and for understanding how humans respond to dietary fat. However, there is as yet no molecular mechanism to explain why some species (human, guinea pig) are non-responsive to peroxisome proliferators, whereas rats and mice respond.

We have therefore investigated the molecular basis of non-responsiveness in a model species, the guinea pig. Our studies reveal that the guinea pig has a PPAR α ; we report studies on the cloning, functional analysis and physiological role of this gene.

MATERIALS AND METHODS

Animals

Dunkin–Hartley guinea pigs (400 g) and C57Bl/6 mice were obtained from Harlan-Olac, and fed with standard laboratory chow *ad libitum*. Guinea pigs were dosed intraperitoneally with 1 ml/kg corn oil, with or without 50 mg/kg Wy-14,643 or methylclofenapate, and killed 24 h later by terminal exsanguination under anaesthesia.

Cloning of guinea-pig PPAR α cDNA

RNA was prepared from guinea-pig liver as described [17], poly(A)⁺ RNA was prepared and reverse transcription was performed as described [18]. PCR reactions used 500 ng of first-strand liver cDNA as a template, KlenTaq DNA polymerase and oligonucleotides GPIGP3 (5'-CTCAGTACATGTCCTGTAGAT-3') and GPIGP4 (5'-TACGGAGTTCACGCATGTGAA-GGCTGCAAGGGCTTCTT-3'), corresponding to residues 463–468 and 114–126 of the mouse PPAR α respectively, to generate a 1056 bp clone after amplification (94 °C for 1 min, 56 °C for 1 min, 72 °C for 2 min; 25 cycles). 5'-Rapid amplification of

Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; PPAR α , peroxisome proliferator activated receptor α ; PPRE, peroxisome proliferator response element; RACE, rapid amplification of cDNA ends.

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cDNA ends (RACE) used the Gibco 5'-RACE kit, with the oligonucleotides GPIGP6 primer, 5'-GCCCTTTGCAGCCTT-CACATGCGTGAAGTCC-3' (residues 126–115) and GPIGP7 primer 5'-GATCTTGCAGCTGCGGTACATTTGTCG-3' (residues 146–136). The sequence of residues 114–126 was confirmed by PCR across this region with PCR primers GPIGP7 and GPIGP14 (5'-CTTGGAGGCCGAGGACCTGGAG-3') on first-strand cDNA. PCR products were cloned into pGEM-T (Promega). A full-length cDNA clone was generated by overlap PCR with the 1056 bp clone and the 5'-RACE product (94 °C for 1 min, 57 °C for 1 min, 72 °C for 2.5 min; 10 cycles), followed by 25 cycles with oligos GPIGP16 (5'-GGACTGGCTCCTCCCC-GCGGACATGGTGG-3') and GPIGP6 (5'-GCCCTTTGCAG-CCTTCACATGCGTGAAGTCC-3'); the 1.4 kb product was cloned into pGEM-T. This insert was cloned as a *SacI*–*NotI* full-length coding fragment into pBK-CMV (Stratagene) to yield pBK-CMV.gPPAR. All sequences were confirmed by obtaining a double-stranded sequence of at least three independent sub-clones to verify sequence identity. Sequence reactions were performed on Quiagen plasmid DNA preparations and run on an ABI 373 fluorescent sequencer.

Sequence analysis

Overlapping clone sequences were aligned with the GCG [19] and STADEN [20] software packages. The deduced protein sequence of the guinea-pig PPAR α was initially aligned with the mouse (GenBank accession number X57638), rat (M88592), human (S74349) and *Xenopus* (M84161) PPAR α , and the mouse PPAR β (U10375) and PPAR γ (U10374) with CLUSTALW1.6 [21], and refined with SAGA [22]; the alignment was displayed with GENEDOC (K. B. Nicholas and H. B. Nicholas, Jr. <http://www.cris.com/~ketchup/genedoc.shtml>). Maximum likelihood analysis of the aligned peptide sequences utilized PROTML [23] and PUZZLE 4 [24], compiled to run on OS/2 using the GNU C compiler; tree output was displayed with TREEVIEW.

Western blotting and SDS/PAGE

Guinea-pig and mouse liver nuclear extracts were prepared essentially as described [9]; protein content was normalized by Bradford assay (Bio-Rad). Protein samples were separated by SDS/PAGE, transferred to Millipore Immobilon P and detected with the enhanced chemiluminescence detection system (Amersham) [9]. The primary antibody was raised against mouse PPAR α [9,25].

RNase protection assays

RNA was normalized by absorbance at 260 nm; this was confirmed by denaturing agarose-gel electrophoresis to reveal the rRNA bands. RNase protection assays on 30 mg of RNA were as described previously [1,9], with *AseI* digestion of the murine PPAR α to yield a 193 bp protected fragment. The guinea-pig PPAR α cDNA was digested with *PvuII* to give a 97 bp protected fragment. Dried gels were quantified by phosphor-imaging on a Bio-Rad GS-250 imager.

Reporter gene analysis

A total of 293 cells (from ATCC, ECACC no. 85120602) were seeded (5×10^5 per 25 cm³ flask) in Dulbecco's modified Eagle's medium (DMEM) containing 10% (w/v) BSA, and cultured for 3 days in air/CO₂ (19:1). Cells were washed in DMEM then incubated for 5 h with 2.4 ml of DMEM and 600 μ l of trans-

fection reagent, consisting of 300 μ l containing 10 μ g of DNA, and 300 μ l of DMEM containing 5 μ g of *N*-[1-(2,3-dioleoyloxy)-propyl]-*N,N,N*-triethylammonium (DOTMA)/dioleoylphosphatidylethanolamine (DOPE) (1:1, w/w) [26] that had been freshly mixed. The DNA contained 10 ng of the *Renilla* luciferase transfection control, pRL-CMV (Promega), 50 ng of pBK-CMV vector (with or without various inserts), 100 ng of the beetle luciferase reporter plasmid, p(ACO)2.GL3-promoter (Promega), and was made up to 10 μ g with pGEM7. The reporter construct, p(ACO)2.GL3, contained two copies of the acyl-CoA oxidase PPRE constructed by inserting the oligonucleotide 5'-CCTTTC-CCGAACGTGACCTTTGTCTCTGGTCCCCTTTTGCTA-3' [27] and its complement into the *SacI* and *BglII* sites of pGL3. Cells were cultured overnight in DMEM and then dosed for 24 h with test chemicals in DMSO vehicle. Cells were harvested and dual luciferase assays were performed exactly as described in the Promega dual luciferase assay kit. The activity of the beetle luciferase was normalized to the *Renilla* internal transfection control. Statistical analysis was performed with Student's *t* test.

Lipid measurement

Blood was collected in EDTA tubes; plasma was analysed for triacylglycerol and cholesterol with a kit from Trace Scientific Ltd. (Melbourne, Australia). Plasma lipid levels were compared between the control and two treatment groups by one-way analysis of variance with Instat; positive results were further compared with the Dunnett multiple comparisons test.

RESULTS

Cloning of the guinea-pig PPAR α cDNA

cDNA clones encompassing the full-length guinea-pig PPAR α were obtained by reverse transcriptase-mediated PCR with primers based on residues 462–468 and 114–126 of the mouse PPAR α ; the 5' end was obtained by 5'-RACE; at least three independent PCR clones were analysed to exclude the possibility of PCR amplification artifacts. The sequence of residues 462–468 is derived from the PCR oligonucleotides and has not been independently verified. The methionine residue denoted as 1 has a strong Kozak consensus sequence [28], but we did not detect an in-frame stop codon upstream of this position. The clones span an open reading frame encoding a polypeptide of 467 residues, with a predicted molecular mass of 52889 Da and a pI of 6.4 (Figure 1, upper panel). Translation of the guinea-pig PPAR α *in vitro* in an reticulocyte lysate demonstrated that the protein migrated at approx. 52 kDa (results not shown), in agreement with the predicted molecular mass.

The deduced amino acid sequence of the guinea-pig clone shows 100% identity with the mouse and human in the DNA-binding domain (residues 102–166), 93 and 91% identity in the ligand-binding domain, and 78 and 85% identity in the A/B-domain respectively, thereby identifying the protein as a PPAR α . The PPAR α sequence from two responsive species (mouse and rat), a non-responsive species (human), and *Xenopus* were aligned (Figure 1, upper panel). There were 30 positions at which guinea pig differed from human, mouse and rat, and 22 where human and guinea pig differed from mouse and rat, including several non-conservative mutations. Mouse PPAR β and PPAR γ were added to the alignment as outgroups, and the data were analysed with the maximum likelihood method, which is robust against variation in substitution rates. As shown in Figure 1 (lower panel), the guinea-pig PPAR α is evolving rapidly, in common with several other guinea-pig genes [29], and the most likely tree places the guinea pig between human and rodent, with a bootstrap

Figure 1 cDNA cloning of guinea-pig PPAR α

Upper panel: the deduced amino acid sequences of the guinea-pig (gppar), human (hppar), mouse (mppar), rat (rppar) and *Xenopus* (xppar) PPAR α genes were aligned and displayed with Genedoc, as described in the Materials and methods section. A dot indicates a conserved residue and a dash a missing residue; the amino acid is shown where the sequence differs from the guinea pig. Residues differing in the mouse and rat from the human and guinea pig are highlighted by a grey background; residues differing in the guinea pig from the human, mouse and rat are highlighted by a black background. Lower panel: phylogenetic analysis of PPAR α genes. The guinea-pig, mouse, human, rat and *Xenopus* PPAR α were aligned with mouse PPAR β and PPAR γ , and a typical output from PUZZLE4 with JTT matrix is displayed with TREEVIEW. The scale reflects the rate of evolutionary change. All the branches are strongly supported ($P > 0.89$).

probability of approx. 0.93; however, it was notable that the most likely remaining trees, with a combined bootstrap probability of approx. 0.07, excluded a monophyletic association of guinea pig with mouse and rat.

Table 1 Guinea-pig PPAR α is activated by peroxisome proliferators

A total of 293 cells were transfected with 10 ng of pRL-CMV, 100 ng of p(ACO)2.GL3 and 50 ng pBK-CMV vector (Vector) or pBK-CMV.gPPAR (gPPAR), then treated for 24 h with DMSO or 100 μ M Wy-14,643. Samples were analysed for *Renilla* and beetle luciferase, and results are normalized to the *Renilla* luciferase. Values are means $\pm$ S.D. for six independent experiments; * $P < 0.05$. Each set of experiments was repeated on at least two occasions.

Transfecting agent	Luciferase activity ratio (beetle: <i>Renilla</i>)	
	DMSO	Wy-14,643
Vector	0.032 $\pm$ 0.003	0.049 $\pm$ 0.004*
gPPAR	0.134 $\pm$ 0.1	0.313 $\pm$ 0.037*

Functional analysis of guinea-pig PPAR α

The full-length cDNA was inserted into the mammalian expression vector pBK-CMV and transfected into 293 cells. In the absence of guinea-pig PPAR α , there was minimal transcription of luciferase from p(ACO)2.GL3, and a small (1.4-fold) but significant induction of luciferase after the addition of 100 μ M Wy-14,643 (Table 1). The guinea-pig PPAR α activated transcription in the absence of peroxisome proliferator from a reporter plasmid containing a dimer of the acyl-CoA oxidase peroxisome proliferator response element (PPRE) upstream of luciferase, in agreement with previous studies on the constitutive activation of human and mouse PPAR α [14,15,30,31]. Addition of the peroxisome proliferator, Wy-14,643 (100 μ M) caused a significant induction over control levels ($P < 0.001$). Induction of the luciferase reporter gene was absolutely dependent on the presence of the PPRE in the reporter construct (results not shown), and the human PPAR α produced a similar magnitude of response (between 1.2-fold and 3-fold; results not shown).

Expression of guinea-pig PPAR α

The hepatic expression of guinea-pig PPAR α RNA was examined in comparison with the levels in mouse liver. As shown in Figure 2 (top panel), the guinea-pig PPAR α was detected in guinea-pig liver RNA, but was present at much lower levels than in mouse (approx. one-tenth on a molar basis). Given the specificity of the RNase protection assay (see, for example, [32]) and the high divergence of the PPAR family in the ligand-binding domain, it is likely that the protection assay is absolutely specific for the PPAR α species in the guinea pig. There was no evidence for diurnal variation in levels of this RNA in the guinea pig (results not shown). An antibody raised against recombinant mouse PPAR α [9,25] was characterized to be specific for the α isoform, as opposed to PPAR β or PPAR γ (Figure 2, middle panel). Western blotting of guinea-pig and mouse liver nuclear extracts revealed the presence of a band of approx. 52 kDa in mouse liver (Figure 2, bottom panel), and also revealed the presence of a band of similar mobility in guinea-pig liver that was less intense. Thus this immunoreactive band demonstrates the presence of guinea-pig PPAR α protein.

Effect of peroxisome proliferators on blood lipids

The presence of functional PPAR α raised the possibility that it might mediate physiological effects. To test this, guinea pigs were

Figure 2 Expression of guinea-pig PPAR α in liver

Top panel: RNase protection analysis of PPAR α in liver. Guinea pigs (GP) and mice (M) were killed at midday, and liver RNA was isolated from each of three animals. Mouse and guinea-pig probes were prepared with [^{32} P]CTP of identical specific radioactivities, and protections were exposed on the same piece of film or phosphorimager. Y represents a protection with yeast tRNA; and the arrow represents the position of the protected fragment. Calculations to determine the relative amounts of guinea-pig and mouse RNA species permitted normalization for the lengths of the probes. Middle panel: specificity of antibody against mouse PPAR α . The indicated quantities of mouse (m) PPAR α , PPAR β (PPAR δ) and PPAR γ were subjected to SDS/PAGE, then to Western blotting with an antibody to PPAR α , followed by chemiluminescence detection. Bottom panel: Western blot of nuclear extract of 10 μ g of protein from mouse (M) or guinea-pig (GP) liver, detected with an antibody to PPAR α . Guinea pigs were killed at 06:00 h, mice at 00:00 h, and nuclear extracts prepared as described in the Materials and methods section from each of three animals; Rec indicates recombinant mouse PPAR α [9].

treated with vehicle control, Wy-14,643 or methylclofenapate, and blood lipids were assayed at 24 h. As shown in Table 2, the two peroxisome proliferators had no significant effect on plasma

Table 2 Effect of peroxisome proliferators on blood lipid levels in guinea pigs

Groups of six guinea pigs were dosed with 25 mg/kg Wy-14,643, methylclofenapate (MCP) or corn oil vehicle, and blood samples were taken by terminal exsanguination 24 h later. Total triacylglycerols and cholesterol were measured as described in the Materials and methods section. Values are means $\pm$ S.D. * $P < 0.05$.

Treatment	Triacylglycerols (mM)	Cholesterol (mM)
Corn oil	0.90 $\pm$ 0.16*	1.1 $\pm$ 0.23
MCP	0.60 $\pm$ 0.18*	1.02 $\pm$ 0.19
Wy-14,643	0.56 $\pm$ 0.19*	0.93 $\pm$ 0.24

cholesterol but induced a significant decrease in plasma triacylglyceride levels ($P < 0.05$).

DISCUSSION

The molecular basis whereby rat and mouse readily undergo peroxisome proliferation after exposure to peroxisome proliferators, but humans and guinea pigs do not, is of critical importance to the assessment of hazard of peroxisome proliferators to humans [16]. We now provide results to elucidate the role of the PPAR α in species differences in physiology by demonstrating that the guinea pig has a functional PPAR α that is expressed in liver. The guinea-pig PPAR α is highly similar to the mouse and human PPAR α genes; however, the rapid evolution of this gene is surprising, including multiple non-conservative substitutions, and the loss of a lysine residue at position 447. This high rate of substitution in the guinea pig has been observed for several genes involved in lipid metabolism [29]; however, the monophyletic placement of the guinea pig with rodents remains controversial [33,34].

The guinea-pig PPAR α mediates transcriptional activation through the PPRE of the acyl-CoA oxidase enhancer [27] (Table 1). This activation via a PPRE is consistent with the conservation of the DNA-binding domain in the PPAR α family, including the unusual arrangement of cysteine residues in the DNA-binding domain that is believed to interact with the extended recognition site of the PPARs [8]. The PPAR α mediated transcriptional activation in the absence of exogenous peroxisome proliferator [7,8,14,15,30], which is thought to be due to the presence of endogenous activators [31]. However, addition of the potent peroxisome proliferator Wy-14,643 (100 μ M) led to a significant induction in transcription of the luciferase reporter gene, which was dependent on the presence of a PPRE. This demonstrates that the guinea-pig PPAR α is capable of responding to peroxisome proliferators. The magnitude of the response seen with the guinea-pig PPAR α was similar to that seen with the human PPAR α (results not shown), which in turn has been shown to be similar to that in mouse [14,15].

It has been well characterized that the guinea pig fails to undergo hepatic peroxisome proliferation after treatment with peroxisome proliferators [10–13]. We therefore investigated whether the non-responsive phenotype is due to a lack of expression of guinea-pig PPAR α in liver. Figure 2 shows that the guinea-pig PPAR α is indeed expressed in liver, both as an RNA species and a protein. RNase protection assays were normalized to total RNA rather than to a specific housekeeping gene, because the levels of expression of a specific housekeeping gene cannot be assumed to be identical between species. Although Figure 2 (bottom panel) shows a smaller amount of immuno-

reactive protein in guinea-pig liver than in mouse liver, it is necessary to compare the immunoreactivities of purified mouse and guinea-pig PPAR α before making a definitive statement on the relative abundances of the protein in the two species. However, the level of RNA in guinea-pig liver is clearly smaller than that in mouse liver. A preliminary account of studies on PPAR α in human liver suggests that there might also be low levels of this RNA in humans [35]. It is an axiom of receptor pharmacology that the amount of receptor can alter the effect of a ligand; it might be that there is a critical level of PPAR α needed to sustain peroxisome proliferation in hepatic tissue, and that the guinea pig does not have sufficient PPAR α . It is clear that there is no induction of CYP4A13 [11] or peroxisome proliferation [12,13] by peroxisome proliferators in guinea pig.

The administration of fats in the diet leads to changes in lipid metabolism in the guinea pig [36]: PPAR α is known to be activated by lipids [4,5]; PPAR α might mediate these effects. We therefore investigated whether peroxisome proliferators could induce hypolipidaemia in this species. Table 2 demonstrates that the two structurally dissimilar peroxisome proliferators induce a lowering of plasma triacylglycerols. It is known that Wy-14,643 and methylclofenapate are selective activators of PPAR α , as opposed to PPAR β and PPAR γ [4,5,37]; it is therefore likely that the hypolipidaemia is mediated through PPAR α . Indeed, while this paper was in review, it was demonstrated that peroxisome proliferators induce hypolipidaemia in mice through the PPAR α [38]. This gives rise to the surprising finding that the level of PPAR α in guinea-pig liver might be insufficient to mediate peroxisome proliferation, yet the guinea pig still undergoes peroxisome proliferator-mediated hypolipidaemia. We propose that the hypolipidaemia induced by peroxisome proliferators is mediated primarily through an extrahepatic effect.

The guinea pig models the human response to peroxisome proliferators, where other rodents differ fundamentally in their regulation of hepatic lipid metabolism. The mechanisms whereby peroxisome proliferators induce hypolipidaemia are still relatively unclear; the guinea pig offers a model system for understanding the induction of hypolipidaemia and responsiveness to peroxisome proliferators in humans.

While this paper was in review, we became aware of an independent database entry for the guinea-pig PPAR α (CPAJ-222), and a report demonstrating that PPAR α is expressed at low levels in human liver [39].

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REFERENCES

- Bell, D. R., Bars, R. G., Gibson, G. G. and Elcombe, C. R. (1991) *Biochem. J.* **275**, 247–252
- Lock, E. A., Mitchell, A. M. and Elcombe, C. R. (1989) *Annu. Rev. Pharmacol. Toxicol.* **29**, 145–163
- Tucker, M. J. and Orton, T. C. (1995) *Comparative Toxicology of Hypolipidaemic Fibrates*, Taylor and Francis, London
- Kliewer, S. A., Sundseth, S. S., Jones, S. A., Brown, P. J., Wisely, G. B., Koble, C. S., Devchand, P., Wahli, W., Willson, T. M., Lenhard, J. M. and Lehmann, J. M. (1997) *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4318–4323
- Forman, B. M., Chen, J. and Evans, R. M. (1997) *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4312–4317
- Lee, S. S.-T., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L., Fernandez-Salguero, P. M., Westphal, H. and Gonzalez, F. J. (1995) *Mol. Cell. Biol.* **15**, 3012–3022
- Palmer, C. N. A., Hsu, M.-H., Muerhoff, A. S., Griffin, K. J. and Johnson, E. F. (1994) *J. Biol. Chem.* **269**, 18083–18089
- Palmer, C. N. A., Hsu, M. H., Griffin, K. J. and Johnson, E. F. (1995) *J. Biol. Chem.* **270**, 16114–16121
- Jones, P. S., Savory, R., Barratt, P., Bell, A. R., Gray, T. J. B., Jenkins, N. A., Gilbert, D. J., Copeland, N. G. and Bell, D. R. (1995) *Eur. J. Biochem.* **233**, 219–226
- Lake, B. G., Evans, J. G., Gray, T. J. B., Korosi, S. A. and North, C. J. (1989) *Toxicol. Appl. Pharmacol.* **99**, 148–160
- Bell, D. R., Plant, N. J., Na, L., Rider, C. G., Brown, S., Ateitalla, I., Acharya, S. K., Davies, M. H., Elias, E., Jenkins, N. A. et al. (1993) *Biochem. J.* **294**, 173–180
- Pacot, C., Petit, M., Rollin, M., Behechti, N., Moisan, M., Deslex, P., Althoff, J., Lhuguenot, J. C. and Latruffe, N. (1996) *Arch. Biochem. Biophys.* **327**, 181–188
- Elcombe, C. R. and Mitchell, A. M. (1986) *Environ. Health Perspect.* **70**, 211–219
- Mukherjee, R., Jow, L., Noonan, D. and McDonnell, D. P. (1994) *J. Steroid Biochem. Mol. Biol.* **51**, 157–166
- Sher, T., Yi, H.-F., McBride, O. W. and Gonzalez, F. J. (1993) *Biochemistry* **32**, 5598–5604
- Ashby, J., Brady, A., Elcombe, C. R., Elliott, B. M., Ishmael, J., Odum, J., Tugwood, J. D., Kettle, S. and Purchase, I. F. H. (1994) *Human Exp. Toxicol.* **13** (suppl. 2), S1–S117
- Cathala, G., Savouret, F., Mendez, B., West, B. D., Karin, M., Martial, J. A. and Baxter, J. D. (1983) *DNA* **2**, 329–335
- Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Wisconsin Package Version 9.0, Genetics Computer Group, Madison, WI
- Bonfield, J. K., Smith, K. F. and Staden, R. (1995) *Nucleic Acids Res.* **23**, 4992–4999
- Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994) *Nucleic Acids Res.* **22**, 4673–4680
- Notredame, C. and Higgins, D. G. (1996) *Nucleic Acids Res.* **24**, 1515–1524
- Adachi, J. and Hasegawa, M. (1996) *J. Mol. Evol.* **42**, 459–468
- Strimmer, K. and von Haeseler, A. (1996) *Mol. Biol. Evol.* **13**, 964–969
- Savory, R. L. (1996) Ph.D. Thesis, University of Nottingham
- Felgner, P. L. and Ringold, G. M. (1989) *Nature (London)* **337**, 387–388
- Osumi, T., Wen, J.-K. and Hashimoto, T. (1992) *Biochem. Biophys. Res. Commun.* **175**, 866–871
- Kozak, M. (1994) *Biochimie* **76**, 815–821
- Semenkovich, C. F., Chen, S.-H., Wims, M., Luo, C.-C., Li, W.-H. and Chan, L. (1989) *J. Lipid Res.* **30**, 423–431
- Aldridge, T. C., Tugwood, J. D. and Green, S. (1995) *Biochem. J.* **306**, 473–479
- Hsu, M.-H., Palmer, C. N. A., Griffin, K. J. and Johnson, E. F. (1995) *Mol. Pharmacol.* **48**, 559–567
- Bars, R. G., Bell, D. R., Elcombe, C. R., Oinonen, T., Jalava, T. and Lindros, K. L. (1992) *Biochem. J.* **282**, 635–638
- D'Erchia, A. M., Gissi, C., Pesole, G., Saccone, C. and Arnason, U. (1996) *Nature (London)* **381**, 597–600
- Cao, Y., Okada, N. and Hasegawa, M. (1997) *Mol. Biol. Evol.* **14**, 461–464
- Tugwood, J. D., Aldridge, T. C., Lambe, K. G., Macdonald, N. and Woodyatt, N. J. (1996) *Ann. N. Y. Acad. Sci.* **804**, 252–265
- Lin, E. C. K., Fernandez, M. L., Tosca, M. A. and McNamara, D. J. (1994) *J. Lipid Res.* **35**, 446–457
- Kliewer, S. A., Forman, B. M., Blumberg, B., Ong, E. S., Borgmeyer, U., Mangelsdorf, D. J., Umehono, K. and Evans, R. M. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7355–7359
- Peters, J. M., Hennuyer, N., Staels, B., Fruchart, J. C., Fievet, C., Gonzalez, F. J. and Auwerx, J. (1997) *J. Biol. Chem.* **272**, 27307–27312
- Palmer, C. N. A., Hsu, M. H., Griffin, K. J., Raucy, J. L. and Johnson, E. F. (1998) *Mol. Pharmacol.* **53**, 14–22