

Prolactin inhibits auto- and cross-induction of thyroid hormone and estrogen receptor and vitellogenin genes in adult *Xenopus* (Amphibia) hepatocytes

Elida M.L. Rabelo¹ and Jamshed R. Tata²

ABSTRACT

It is well known that virtually every tissue of the amphibian larvae is highly sensitive to the mutually antagonistic actions of thyroid hormone (TH) and prolactin (PRL), but it is not known if adult amphibian tissues respond similarly to these two hormones. We have previously shown that very low doses of triiodothyronine (T₃) rapidly and strongly potentiate the activation of silent vitellogenin (Vit) genes by estrogen (E₂) and the autoinduction of estrogen receptor (ER) transcripts in primary cultures of adult *Xenopus* hepatocytes. This response to T₃ is accompanied by the upregulation of thyroid hormone receptor β (TR β) mRNA. Using Northern blot and RNase protection assays, we now show that ovine PRL added for 12 h along with 2×10^{-9} M T₃ will completely prevent potentiation of E₂ induction of Vit mRNA in primary cultures of adult *Xenopus* hepatocytes. PRL also abolished the auto-upregulation of TR β mRNA and the cross-activation of autoinduction of ER mRNA. Thus, we show for the first time that the anti-TH action of PRL that is manifested in *Xenopus* tadpole tissues during metamorphosis is retained in adult liver, and suggest that the mutually antagonistic actions of the two hormones may be brought about by similar molecular mechanisms in larval and adult amphibian tissues.

INTRODUCTION

Whereas virtually every tissue of amphibian larvae is a target for thyroid hormones (TH), there is no evidence that these hormones have any effect on adult amphibian tissues. However, we were able to demonstrate, in a previous work, that very low doses of triiodothyronine (T₃) strongly potentiated vitellogenin (Vit) gene activation by estrogen (E₂) in primary cultures of adult *Xenopus* hepatocytes (Rabelo and Tata, 1993). This unequivocal response of adult cells to T₃ was later found to be reproducible in *Xenopus* tadpole liver (Rabelo *et al.*, 1994).

One major characteristic of amphibian metamorphosis is inhibition or retardation of natural or TH-induced development by homologous or heterologous preparations of prolactin (PRL) (Beckingham Smith and Tata, 1976; Gilbert and Frieden, 1981; White and Nicoll, 1981; Kikuyama *et al.*, 1993; Tata, 1993). This "juvenilizing" action of prolactin has proved to be useful in analyzing some of the mechanisms underlying both morphogenesis and extensive cell death that occur during metamorphosis. However, until now the ability of this hormone to block thyroid hormone action has only been established in larval amphibian tissues during postembryonic development, and it is not known if similar interaction between the two hormones occurs in adult tissues. In view of our previous observation of the rapid potentiation by T₃ of E₂-induced vitellogenin gene activation in adult *Xenopus* hepatocytes (Rabelo and Tata, 1993), we decided to explore the

¹ Departamento de Parasitologia, ICB, UFMG, Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brasil. Send correspondence to E.M.L.R.

² Laboratory of Developmental Biochemistry, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

possibility of a PRL- T_3 interaction in adult amphibian tissue.

The present study reports that the anti-TH action of PRL exhibited during late larval development is retained in adult *Xenopus* liver. By using primary cell cultures of adult male *Xenopus* liver, we confirmed that low doses of T_3 (2×10^{-9} M) strongly and rapidly potentiated silent vitellogenin gene activation by estrogen while, at the same time, induced transcripts of its own receptor TR β and enhanced autoinduction of an estrogen receptor. When together with T_3 and E_2 in the culture medium, prolactin abolished T_3 -enhanced accumulation of all the above transcripts. Thus, we show, for the first time, that an adult amphibian tissue is responsive to both prolactin and thyroid hormone. It is most likely that adult tissues follow the same mechanism as larval tissues.

MATERIAL AND METHODS

3,3',5-Triiodo-L-thyronine Na salt was synthesized at the National Institute for Medical Research (London, UK), Estradiol-17 β was purchased from Sigma Chemical Co. (Poole, UK), and ovine prolactin was a gift from the National Hormone and Pituitary Program, National Institutes of Health (Rockville, MD). For labelling complementary DNA (cDNA) and RNA (cRNA) probes, [α - 32 P]dCTP (3000 Ci/mmol) and [α - 32 P]UTP (450 Ci/mmol), respectively, were purchased from Amersham International (Amersham, UK). All other materials were of analytical grade and purchased from Sigma Chemical Co. (Poole, UK).

Primary cell cultures

Adult male *Xenopus* were purchased from Blade Biological (Cowden, Kent, UK) and maintained in our amphibian facility, as described earlier (Kawahara *et al.*, 1991). Primary liver cultures were prepared following standard laboratory procedures (Perlman *et al.*, 1984), where the cells were maintained in culture for 64 h before hormonal manipulation. Each culture dish had cells from the equivalent of ~800 mg of livers pooled from 8 male frogs. T_3 and PRL were added to the cell cultures in different sequences 12 h before E_2 . After a further 12 h in culture, hepatocytes were washed, and RNA was extracted.

RNA analysis

Total RNA was extracted from batches of 2 dishes of hepatocytes following the guanidinium

isothiocyanate procedure (Chomczynski and Sacchi, 1987). After its purity was checked by gel electrophoresis and spectroscopy, RNA was analyzed either by Northern blot or RNase protection assay. Vitellogenin mRNA was measured by Northern blot with 32 P-labelled *Xenopus* vitellogenin B1 cDNA (Baker and Tata, 1990). The blots were also probed with a 32 P-labelled *Xenopus* actin cDNA as loading controls. TR β and ER mRNAs were detected and estimated by RNase protection assay with 32 P-labelled riboprobes. For this purpose a 325-nt TR β fragment (Kawahara *et al.*, 1991) and an ER cRNA derived from a 167-nt *Bgl*III-*Hind*III cDNA fragment (Weiler *et al.*, 1987) were used as 32 P-labelled cRNA probes. A 120-nt *Xenopus* 5S RNA cRNA was used as a loading control for RNase protection assay. Autoradiograms of Northern blot and protection assays were scanned and quantified in a Molecular Dynamics Imagequant.

The signal from each sample was normalized to actin mRNA or 5S RNA and expressed as arbitrary units.

RESULTS AND DISCUSSION

Silent vitellogenin gene activation in male *Xenopus* hepatocytes by exogenous estrogen added to primary cell cultures has allowed a more precise analysis of gene expression regulation by steroid hormones than is possible in intact animals (Wangh and Schneider, 1982; Perlman *et al.*, 1984; Shapiro *et al.*, 1989; Tata, 1991). Recently, we have shown that T_3 added only a few hours before E_2 strongly potentiates vitellogenin gene activation (Rabelo and Tata, 1993). Both these hormonal effects have been reproduced and the results are showed in Figure 1.

Exposure of male hepatocytes to 2×10^{-9} M T_3 for 12 h prior to addition of 10^{-8} M E_2 for 12 h resulted in strong Vit mRNA accumulation (lanes 2 and 3). Autoradiogram scanning showed that the increase was 4-fold. Pre-exposure of cell to PRL (0.5 IU/ml culture media) before T_3 and E_2 or together with T_3 abolished or substantially diminished the T_3 effect. (Figure 1, lanes 6 and 8). In data not shown, 0.2 IU PRL had the same inhibiting effect as 0.5 IU PRL. It acted much the same way as shown earlier for organ cultures of *Xenopus* tadpole tails (Baker and Tata, 1992). However, PRL did not inhibit the T_3 effect in adult liver primary cell cultures, if it was not maintained throughout the duration of the experiment, i.e., included with E_2 during the last 12 h of the culture period (Figure 1, lanes 5 and 7). This finding may either reflect PRL instability in our cultures, or an acceleration of Vit mRNA breakdown

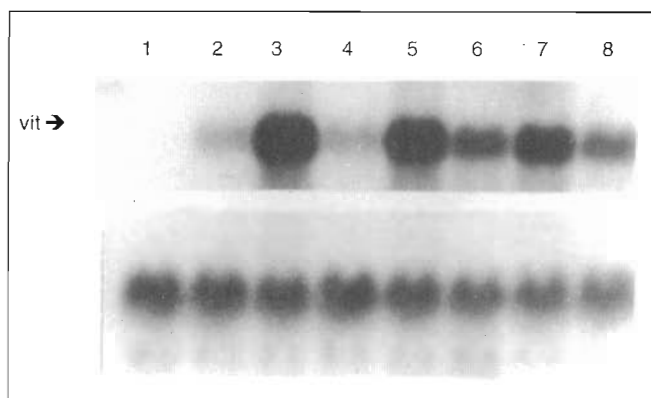


Figure 1 - Prolactin inhibits the potentiation by T₃ of E₂ induced vitellogenin mRNA in primary cultures of adult *Xenopus* hepatocytes. Vitellogenin genes were activated by the addition of 10⁻⁸ M E₂ to the cells for the last 12 h of incubation (lanes 2-8), following different pre-treatments for 12 h with 2 × 10⁻⁹ M T₃ and 0.5 IU/ml PRL alone or together (lanes 3-8), as indicated below. At the end of the culture period, total RNA was extracted from the hepatocytes and the amount of Vit mRNA accumulated was determined with a Northern blot, using as probes a *Xenopus* vitellogenin B1 cDNA, and a *Xenopus* cytoplasmic actin cDNA as loading control. The autoradiogram of the filter blot is shown here. Lane 1: Control (no hormonal treatment); lane 2: E₂ alone for the last 12 h. Pre-treatment before adding E₂: lane 3: 2 × 10⁻⁹ M T₃ for 12 h only; lane 4: PRL only for 12 h; lane 5: PRL for 12 h, then PRL + T₃ for 12 h; lane 6: PRL for 12 h, then PRL + T₃ for 12 h and PRL with E₂ for the last 12 h. Lane 7: PRL + T₃ together for 12 h; lane 8: PRL + T₃ for 12 h, then PRL only with E₂ for the last 12 h.

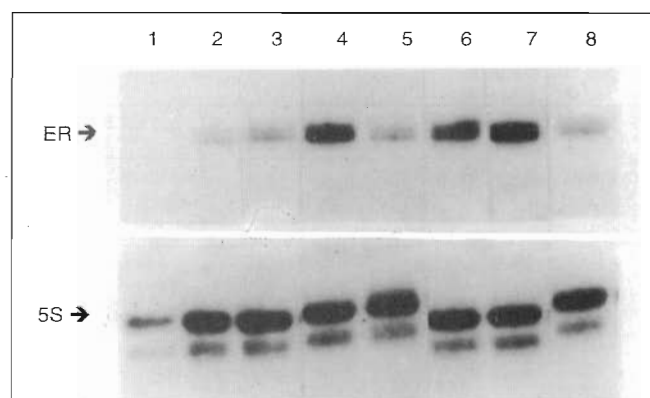


Figure 2 - PRL inhibition of T₃ potentiation of ER mRNA auto-induction in primary cultures of adult *Xenopus* hepatocytes. Liver cells were incubated for the last 12 h with 10⁻⁸ M E₂ (lanes 3-8) following pre-treatment with 2 × 10⁻⁹ M or 10⁻⁷ M T₃ and 0.5 IU/ml PRL in different combinations (lanes 4-8). Total RNA was extracted and the amount of ER mRNA determined by RNase protection assays with a 167-nt *Xenopus* ER cRNA probe and a 384-nt *Xenopus* 5S RNA cRNA as a loading control. Major protected bands corresponding to ER mRNA and 5S RNA are indicated by arrowheads. Lane 1: tRNA; lane 2: control (no hormone added); lane 3: E₂ only for the last 12 h; lane 4: pre-treated with 2 × 10⁻⁹ M T₃ for 12 h before E₂ for another 12 h; lane 5: PRL + 2 × 10⁻⁹ M T₃ for 12 h before PRL + E₂ for the next 12 h; lane 6: 10⁻⁷ M T₃ for 12 h followed by E₂ for 12 h; lane 7: PRL + 10⁻⁷ M T₃ for 12 h followed by PRL + E₂ for 12 h; lane 8: PRL + E₂ for 12 h.

caused by PRL. The latter is improbable because PRL did not significantly diminish the modest amount of Vit mRNA in hepatocytes treated for 12 h with E₂ without being pre-treated with T₃ (Figure 1, lane 4). Thus, the most noticeable action of PRL is directed against the potentiation by T₃ of E₂ action and not against the induction of Vit genes by E₂ (the genes remain silent when T₃ is added without E₂ (Rabelo and Tata, 1993).

Hitherto, PRL action has only been demonstrated in amphibian larval stages when undergoing TH-dependent metamorphosis (Gilbert and Frieden, 1981; Kikuyama *et al.*, 1993; Tata, 1993). We have previously found in *Xenopus* tadpoles that in inhibiting T₃-induced metamorphosis, PRL abolished the auto-induction of TR (Baker and Tata, 1992). In view of the above results concerning T₃-antagonist effect of PRL in adult *Xenopus* hepatocytes, we wondered if PRL has the same effect on adult tissues regarding nuclear receptor mRNA induction as it does in tadpoles, i.e., if the PRL effect on Vit mRNA would also be accompanied by the inhibition of auto- and cross-induction of ER and TR by E₂ and T₃. The RNase protection assay (Figure 2, lanes 2 and 3) confirms the ability of E₂ to upregulate its own receptor transcripts in primary cultures of male *Xenopus* hepatocytes. Pre-treatment of the cells for 12 h with 2 × 10⁻⁹ M or 10⁻⁷ M T₃ potentiates ER mRNA autoinduction (compare lanes 4 and 6 with lane 3). Prolactin inhibited potentiation by 2 × 10⁻⁹ M T₃ of estrogen action (lane 5).

Quantitatively, the 4-fold potentiation by 2 × 10⁻⁹ M T₃ of ER autoinduction was completely eliminated through continuous PRL presence (Table I). PRL, without T₃ pre-treatment, did not inhibit the autoinduction of ER (Figure 2, lane 8). Interestingly, raising T₃ concentration to 10⁻⁷ M overcame inhibition by PRL (lanes 6 and 7). Since the cellular response to PRL remains largely unknown (Kelly, 1990; Kelly *et al.*, 1991), it is difficult to explain the dose-related competition between this hormone acting via membrane receptors and T₃ which acts via nuclear receptors (Chin, 1991; Chatterjee and Tata, 1992). On the other hand, this phenomenon may explain why rising levels of thyroid hormones overcome the antimetamorphic action of prolactin during natural metamorphosis (Kikuyama *et al.*, 1993). As for the accumulation of ER mRNA, PRL had to be added together with E₂ in order to observe this effect. PRL inhibition of T₃ cross-induction of ER is relatively significant in the context of *de novo* activation of Vit genes in male *Xenopus* liver by E₂. Earlier studies of the close association between the upregulation of functional ER and transcription *in vivo* of Vit genes had suggested that the basal level of the receptor was not sufficient to activate Vit genes, but that additional ER was necessary for the induction of Vit mRNA (Perlman *et al.*, 1984; Shapiro *et al.*, 1989; Tata *et al.*, 1993). Our present data are fully compatible with this suggestion.

Table I - Prolactin abolishes both TR β mRNA autoinduction and T₃ enhancement of ER autoinduction in primary cultures of adult *Xenopus* hepatocytes.

Treatment	TR β mRNA		ER RNA	
	Arbitrary units	Fold induction	Arbitrary units	Fold induction
Control	32	-	51	-
T ₃	153	4.8	63	1.2
E ₂	46	1.4	170	3.3
T ₃ /E ₂	138	4.3	680	13.4
T ₃ + PRL/E ₂ + PRL	10	-	79	1.5

Where indicated, the hepatocytes were exposed to 2×10^{-9} M T₃ with or without 0.5 IU/ml PRL for 12 h prior to the addition of 10^{-8} M E₂. After a further 12 h of incubation, total RNA was extracted and the relative concentration of TR β and ER mRNAs measured by scanning autoradiograms obtained from RNase protection assays. All other details found in Figures 2, 3 and in the text.

There is now increasing evidence from several laboratories that many receptors, and their transcripts, of the steroid/thyroid hormone/retinoic acid receptor family are autoinduced by their own ligands, particularly in a developmental context (Tata *et al.*, 1993). Previous studies from our laboratory (Baker and Tata, 1992; Rabelo and Tata, 1993) have focused on the autoinduction of *Xenopus* TR α and β mRNA in larval tissues and in primary cultures of adult male *Xenopus* hepatocytes. Since the autoinduction of TR β mRNA is more pronounced than that of α isoform in the above tissues, we restricted the present study to measure only the steady-state levels of TR β mRNA as a function of the treatment of primary hepatocyte cultures with different combinations of the three hormones. As shown in Figure 3, T₃ produced a strong upregulation of TR β mRNA (compare lanes 3 and 5 with 1 and 2), confirming an earlier observation (Rabelo and Tata, 1993). E₂ did not alter the amount of TR β mRNA nor the extent of its autoinduction (compare lanes 1, 2, 3 and 5). However, if PRL was added in the same sequence as in the experiments shown in Figures 1 and 2, TR β mRNA was not upregulated by T₃ (lane 4). The magnitude of auto- or cross-induction (or de-induction) of ER and TR β transcripts is depicted in Table I. This shows that the 4-fold induction of TR β mRNA by T₃, irrespective of whether or not E₂ was added, was almost completely eliminated by PRL. When the numbers in Table I are compared with the autoradiographic signals for Vit gene activation in Figure 1, it is obvious that the latter is closely associated with the auto- and cross-regulation of TR and ER gene regulation.

The present results are particularly significant when considering T₃ and PRL action in larval and adult amphibia. Until very recently, both hormones have

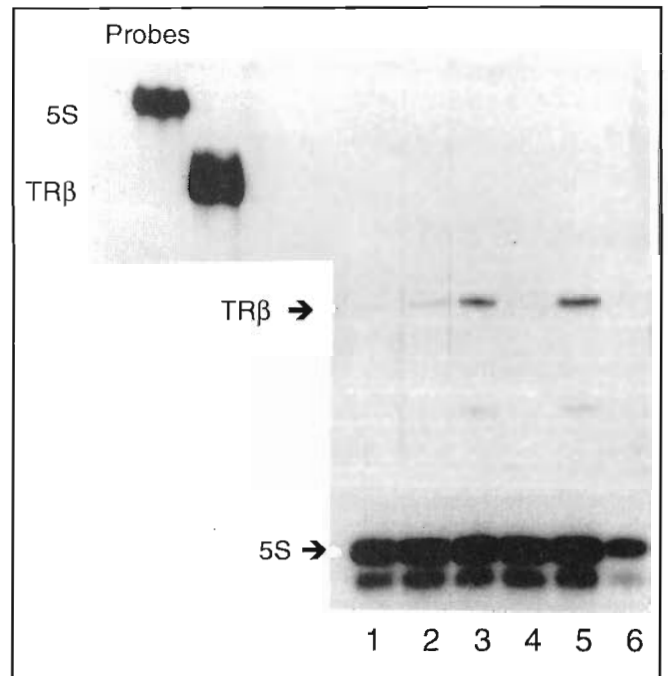


Figure 3 - RNase protection assay showing that PRL prevents the autoinduction of TR β mRNA in primary cultures of adult *Xenopus* hepatocytes. The cells were incubated for the last 12 h with 10^{-8} M E₂ (lanes 2-4) or 2×10^{-9} M T₃ (lane 5) preceded or not by pre-treatment for 12 h with 2×10^{-9} M T₃ and 0.5 IU/ml PRL. Total RNA was extracted and probed with *Xenopus* TR β cRNA, and a 5S cRNA as loading control. The cRNA probes and the protected bands of TR β mRNA and 5S RNA (arrowheads) are marked on the autoradiogram. Lane 1: Control (no hormone added); lane 2: E₂ alone for the last 12 h; lane 3: pre-treated with T₃ for 12 h before incubation with E₂; lane 4: PRL + T₃ for 12 h followed by PRL + E₂ for 12 h; lane 5: T₃ alone for the last 12 h; lane 6: tRNA control.

been recognized almost exclusively for the important role they play in amphibian metamorphosis, particularly in anurans (Beckingham Smith and Tata, 1976; Gilbert and Frieden, 1981; Kikuyama *et al.*, 1993; Tata, 1993). However, there was no convincing evidence that low doses of TH had any effect on adult amphibian tissues nor that PRL would suppress its action. Studies on the effects of these hormones directly on primary cultures of adult hepatocytes allow one to show that the expression of three different genes, namely Vit, TR β and ER, responds the same way in adult and larval livers. Previously, Wangh found in long-term maintenance of primary cultures of adult *Xenopus* hepatocytes that 2-week exposure to T₃ enhanced Vit induction by E₂ (Wangh, 1982; Wangh and Schneider, 1982). What is so striking about our present results and previous findings (Rabelo and Tata, 1993) is the rapidity and extent of potentiation of E₂ action by T₃. It is difficult to say whether or not the acute and long-term responses to T₃ are brought about by the same mechanisms, so it would be of considerable interest to determine what effect PRL would have on long-term cultures and in adult *Xenopus* *in vivo*.

Finally, our results highlight the general precept of endocrinology and cellular signalling that hormonal interplay is particularly important for the regulation of developmental processes and specific gene expression (Gorbman and Bern, 1962; Gilbert and Frieden, 1981; Tata, 1984; Baulieu and Kelly, 1990). More specifically, they reinforce the participation of thyroid hormone in the multihormonal networks that regulate diverse physiological processes (Pitt-Rivers and Tata, 1959; Gorbman and Bern, 1962; Oppenheimer and Samuels, 1983; Chin, 1991; Tata, 1993). Prolactin is also known to similarly participate in many developmental processes, particularly lactation and reproductive functions, via mechanisms that are not fully understood (White and Nicoll, 1981; Kelly, 1990). What our present data do establish is that now one has to seriously consider the possibility that both thyroid hormone and prolactin may play a role in adult amphibian life as, say, in reproductive functions. They also offer a novel approach to the analysis of how these important hormones, which have been put to diverse uses through evolution, exert their action as participants in hormonal networks that control morphogenesis and cell death.

ACKNOWLEDGMENTS

We wish to thank Mrs. Betty Baker for help with many aspects of experimental work. E.M.L.R. was the recipient of a CAPES predoctoral fellowship from the Brazilian Government during the execution of this work.

RESUMO

É bem estabelecido que virtualmente todos os tecidos das larvas de anfíbios são altamente sensíveis à ação mutuamente antagonista dos hormônios tireoideanos (TH) e prolactina (PRL). Porém, não é sabido se os tecidos do anfíbio adulto respondem de uma forma semelhante a estes hormônios. Em um trabalho anterior, nós demonstramos que baixas doses de triiodotironina (T₃) potenciam rapidamente e com alta intensidade a ativação pelo estrogênio (E₂) de genes silenciosos da vitelogenina (Vit), bem como a autoindução de transcritos de receptores para estrogênio (ER) em culturas primárias de hepatócitos de *Xenopus* adultos. Esta resposta para o T₃ é acompanhada pela ativação da transcrição do mRNA de receptores β para o hormônio tireoideano (TRβ). Através das técnicas de Northern blotting e "RNase protection assay", nós agora estamos mostrando que prolactina ovina adicionada por 12 h, juntamente com 2 × 10⁻⁹ M de T₃, em culturas primárias de hepatócitos adultos de *Xenopus* bloqueia completamente a ação de T₃ na potenciação da indução de mRNA da Vit pelo hormônio E₂. PRL também aboliu a autoregulação positiva do mRNA para TRβ e a

ativação cruzada da autoindução do mRNA do ER. Assim, nós estamos mostrando pela primeira vez que a ação anti-TH da PRL, manifestada em girinos de *Xenopus* durante a metamorfose, é mantida no fígado adulto, sugerindo que esta ação mutuamente antagonista dos dois hormônios pode ser desempenhada por mecanismos moleculares semelhantes nos tecidos de ambos os estágios do anfíbio.

REFERENCES

- Baker, B.S. and Tata, J.R.** (1990). Accumulation of proto-oncogene *c-erb-a* related transcripts during *Xenopus* development: association with early acquisition of response to thyroid hormone and estrogen. *EMBO J.* 9: 879-885.
- Baker, B.S. and Tata, J.R.** (1992). Prolactin prevents the auto-induction of thyroid hormone receptor mRNAs during amphibian metamorphosis. *Dev. Biol.* 149: 463-467.
- Baulieu, E.-E. and Kelly, P.A.** (Eds.) (1990). *Hormones: From Molecules to Disease*. Hermann, Paris.
- Beckingham Smith, K. and Tata, J.R.** (1976). Amphibian metamorphosis. In: *Developmental Biology of Plants and Animals* (Graham, C.F. and Wareing, P.F., eds.). Blackwell, Oxford, pp. 232-245.
- Chatterjee, V.K.K. and Tata, J.R.** (1992). Thyroid hormone receptors and their role in development. *Cancer Surv.* 14: 147-167.
- Chin, W.W.** (1991). Nuclear thyroid hormone receptors. In: *Nuclear Hormone Receptors* (Parker, M., ed.). Academic Press, London, pp. 79-102.
- Chomczynski, P. and Sacchi, P.** (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156-159.
- Gilbert, L.I. and Frieden, E.** (Eds.) (1981). *Metamorphosis: A Problem in Developmental Biology*. Plenum Press, New York.
- Gorbman, A. and Bern, A.** (1962). *A Textbook of Comparative Endocrinology*. John Wiley, New York.
- Kawahara, A., Baker, B.S. and Tata, J.R.** (1991). Developmental and regional expression of thyroid hormone receptor genes during *Xenopus* metamorphosis. *Development* 112: 933-943.
- Kelly, P.A.** (1990). Growth hormone and prolactin. In: *Hormones: From Molecules to Disease* (Baulieu, E.-E. and Kelly, P.A., eds.). Hermann, Paris, pp. 191-217.
- Kelly, P.A., Djiane, J., Postel-Vinay, M.-C. and Edery, M.** (1991). The prolactin/growth hormone family. *Endocr. Rev.* 12: 235-251.
- Kikuyama, S., Kawamura, K., Tanaka, S. and Yamamoto, K.** (1993). Aspects of amphibian metamorphosis: hormonal control. *Int. Rev. Cytol.* 145: 105-148.
- Oppenheimer, J.H. and Samuels, H.H.** (Eds.) (1983). *Molecular Basis of Thyroid Hormone Action*. Academic Press, New York.
- Pelzman, A.J., Wolffe, A.P., Champion, J. and Tata, J.R.** (1984). Regulation by estrogen receptor of vitellogenin

- gene transcription in *Xenopus* hepatocyte cultures. *Mol. Cell. Endocrinol.* 38: 151-161.
- Pitt-Rivers, R. and Tata, J.R.** (1959). *The Thyroid Hormones*. Pergamon Press, London.
- Rabelo, E.M.L. and Tata, J.R.** (1993). Thyroid hormone potentiates estrogen activation of vitellogenin genes and autoinduction of estrogen receptor in adult *Xenopus* hepatocytes. *Mol. Cell. Endocrinol.* 96: 37-44.
- Rabelo, E.M.L., Baker, B.S. and Tata, J.R.** (1994). Interplay between thyroid hormone and estrogen in modulating expression of their receptor and vitellogenin genes during *Xenopus* metamorphosis. *Mech. Dev.* 45: 49-57.
- Shapiro, D.J., Barton, M.C., McKearin, D.M., Chang, T.-C., Lew, D., Blume, J., Nielsen, D.A. and Gould, L.** (1989). Oestrogen regulation of gene transcriptions and mRNA stability. *Recent Prog. Horm. Res.* 45: 29-58.
- Tata, J.R.** (1984). The action of growth and developmental hormones. In: *Biological Regulation and Development* (Goldberger, R.F. and Yamamoto, K.R., eds.). Plenum Publishing, New York, Vol. 3B, pp. 1-58.
- Tata, J.R.** (1991). Hormonal and developmental regulation of *Xenopus* estrogen receptor and egg protein gene expression. In: *The New Biology of Steroid Hormones* (Hochberg, R.B. and Naftolin, F., eds.). Serono Symposium Publications, Raven Press, New York, Vol. 74, pp. 213-225.
- Tata, J.R.** (1993). Gene expression during metamorphosis: an ideal model for post-embryonic development. *BioEssays* 15: 239-248.
- Tata, J.R., Baker, B.S., Machuca, I., Rabelo, E.M.L. and Yamauchi, K.** (1993). Autoinduction of nuclear receptor genes and its significance. *J. Steroid Biochem. Mol. Biol.* 46: 105-119.
- Wangh, I.J.** (1982). Glucocorticoids act together with estrogens and thyroid hormones in regulating the synthesis and secretion of *Xenopus* vitellogenin, serum albumin, and fibrogen. *Dev. Biol.* 89: 294-298.
- Wangh, L.J. and Schneider, W.** (1982). Thyroid hormones are corequisites for estradiol-17 β *in vitro* induction of *Xenopus* vitellogenin synthesis and secretion. *Dev. Biol.* 89: 287-293.
- Weiler, I.J., Lew, D. and Shapiro, D.J.** (1987). The *Xenopus laevis* estrogen receptor: Sequence homology with human and avian receptors and identification of multiple estrogen receptor messenger ribonucleic acids. *Mol. Endocrinol.* 1: 355-362.
- White, B.A. and Nicoll, C.S.** (1981). Hormonal control of amphibian metamorphosis. In: *Metamorphosis. A Problem in Developmental Biology* (Gilbert, L.I. and Frieden, E., eds.). Plenum Press, New York, pp. 363-396.

(Received June 3, 1997)