Int. J. Dev. Biol. 40: 507-514 (1996)

Original Article

Thyroid hormone regulation of germ cell-specific EF-1α expression during metamorphosis of *Xenopus laevis*

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ABSTRACT ***In situ*** **hybridization was used to follow the distribution of the mRNAs encoding the somatic form of elongation factor 1α (EF-1αS) and the germinal counterparts of this factor, thesaurin a and EF-1αO, throughout metamorphosis in the gonads of *Xenopus laevis* tadpoles. EF-1αS mRNA is detected before metamorphosis in both the somatic and germ cells of the gonads. In contrast, thesaurin a and EF-1αO mRNAs are first detected in spermatogonia and oogonia at stages 60-62, corresponding to the climax of metamorphosis and to the peak of circulating thyroid hormone. To determine whether thyroid hormone, the instigator of metamorphosis, is involved in regulating the expression of the germinal gene EF-1αO, *Xenopus* XTC cells were transfected with an EF-1αO promoter sequence inserted in front of the luciferase reporter gene. Addition of T3 to the cell culture medium induced a dose-dependent increase in transcription from the EF-1αO promoter. This effect was enhanced when the construct was cotransfected with an expression vector for a *Xenopus* thyroid hormone receptor. Our data show that germ cells switch from a somatic to a germ-cell specific mode of expression during metamorphosis. Furthermore, this switch appears to be induced by thyroid hormone.**

KEY WORDS: *metamorphosis, thyroid hormone, germ cells, gene expression, Xenopus laevis*

Introduction

Previtellogenic oocytes of *Xenopus laevis* contain three major kinds of nucleoprotein particles involved in the long term storage of RNA: the mRNP, the 7S and 42S particles. mRNP particles store messenger RNA, 7S particles contain 5S RNA while 42S particles store both transfer RNA and 5S RNA (Darnbrough and Ford, 1981; Denis and le Maire, 1983). The protein components of the *X. laevis* nucleoprotein particles have been purified and characterized as belonging to families of transcription or translation factors (Mazabraud *et al.*, 1992). A first family includes TFIIIA (the protein component of 7S particles), and thesaurin b (the minor protein of 42S particles, also called 42Sp43). TFIIIA and thesaurin b have nine Zn-finger domains (Miller *et al.*, 1985; Joho *et al.*, 1990). Both proteins bind 5S RNA (Picard and Wegnez, 1979; Joho *et al.*, 1990). However, only TFIIIA binds DNA and is active in oocytes as a transcription factor specific for the 5S RNA genes (Honda and Roeder, 1980; Pelham and Brown, 1980; Joho *et al.*, 1990).

While thesaurin b can be considered as a transcription-related factor because of its homology to TFIIIA, thesaurin a, the major protein component of 42S particles (also called 42Sp50), is a translation factor. Thesaurin a belongs to the EF-1α family of elongation factors (Mattaj *et al.*, 1987; Viel *et al.*, 1987; Djé *et al.*, 1990). Besides thesaurin a, two members of this family are known in *X. laevis*: EF-1αS, which performs the elongation function in somatic cells (Krieg *et al.*, 1989), and EF-1αO which is active in oocytes (Djé *et al.*, 1990; Morales *et al.*, 1991; Viel *et al.*, 1991). EF-1αO and thesaurin a differ in their amino acid sequence (Djé *et al.*, 1990) and in their intracellular location since in previtellogenic oocytes, EF-1αO is concentrated in the Balbiany body, whereas thesaurin a is evenly distributed in the oocyte cytoplasm (Viel *et al.*, 1990). However, both proteins bind and deliver aminoacyl tRNA to the A site of the ribosomes (Morales *et al.*, 1991; Viel *et al.*, 1991). The genes encoding EF-1αO and both thesaurins are active not only in female germ cells but also in male germ cells (Abdallah *et al.*, 1991a,b). In previtellogenic oocytes, the function of the thesaurins a and b is to

Abbreviations used in this paper: CAT, chloramphenicol acetyl transferase; EF-1α, elongation factor-1α; EF-1αO and EF-1αS, oocyte-type and somatic-type elongation factor-1α, respectively; LUC, luciferase; PEI, polyethyleneimine; SFM, serum free medium; Tha, thesaurin a; T3, 3,3',5 triiodothyronine.

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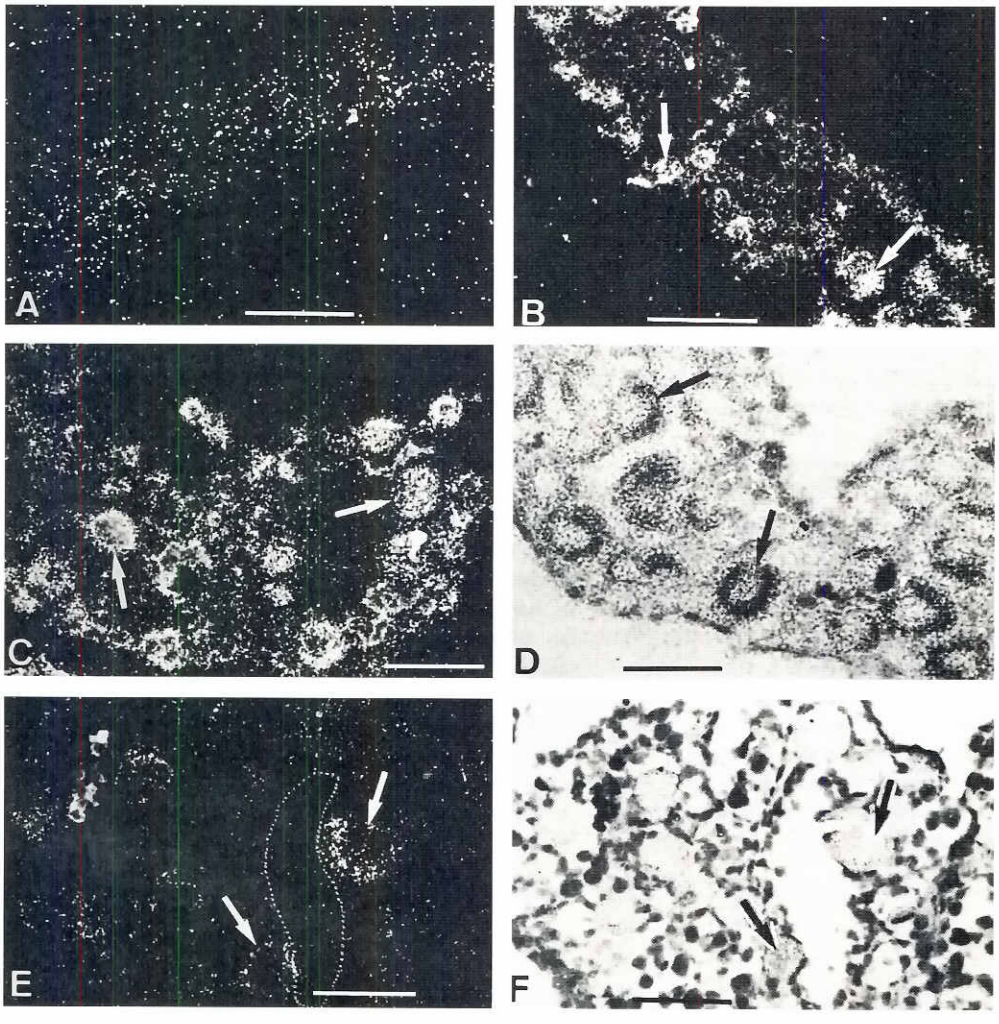

Fig. 1. *In situ* hybridization of an EF-1αS probe to sections of tadpole gonads. *Section of stage 55 gonad (premetamorphosis) hybridized with sense* **(A)** *or anti-sense probe* **(B).** *Gonia (arrows) are distinctly labeled. Section of a testis* **(C)** *and of an ovary* **(D)** *at stage 62 (metamorphosis) hybridized with anti-sense probe.* **(E and F)** *Sections of stage 66 ovary hybridized with anti-sense probe. The hybridization signal in germ cells (arrows) drops noticeably between stage 55 and stage 66. A-C and E, dark-field illumination. D and F, bright-field illumination. Scale bars, 50 μm.*

permit accumulation and storage of tRNA and 5S RNA over several months (Picard *et al.*, 1980; Le Maire and Denis, 1983). However, there is no function known for these proteins in male germ cells. In these latter cells, the thesaurins do not associate to form storage particles (Abdallah *et al.*, 1991b). Given the importance of the storage function of thesaurins in oocytes, we thought that their presence in male germ cells could simply be due to the simultaneous activation of common transcriptional and translational mechanisms in male and female germ cells.

The question therefore arose as to the developmental regulation of these different elongation factors in different cell types. To address this problem, we used *in situ* hybridization to follow the distribution of the mRNAs encoding EF-1αS, thesaurin a and EF-1αO in developing gonads. The two latter mRNAs first became detectable by this method in male and female germ cells at metamorphosis, around stage 60 (Nieuwkoop and Faber, 1956) when thyroid hormone peaks (Leloup and Buscaglia, 1977).

As the thyroid hormone, T3 (3, 5, 3' triiodothyronine), is known to affect the expression of many genes at metamorphosis (Shi and Brown, 1990, 1993), we tested the possible effect of T3 on EF-1αO gene expression using an established *Xenopus laevis* cell line (XTC2). We observed a significant increase in the T3-dependent transcription from the EF1αO promoter but not from the EF-1αS promoter. Taken together, our results suggest that T3 regulates the expression of germ cell specific elongation factors during metamorphosis.

Results

In situ hybridization was used to follow expression of the three EF-1α genes during metamorphosis of *Xenopus* tadpoles. We used specific EF-1αO or EF-1αS sequences to avoid problems arising from possible heterologous hybridization. Figures 1, 2, and 3 show typical examples of *in situ* hybridization for EF-1αS (a 124 pb probe), EF-1α0 (a 72 pb probe) and thesaurin a (whole cDNA probe), respectively, on gonads of pre-metamorphosing and metamorphosing tadpoles. Quantification of the results from all the *in situ* hybridizations is given in Figure 4, where stages 55 to 57, 60 to 62 and 62 to 64 have been pooled. It is known from previous studies that EF-1αS mRNA is abundant in embryos starting from midblastula transition and in all somatic tissues of the adult (Krieg *et al.*, 1989; Djé *et al.*, 1990). It appears from this study that in embryos this mRNA is not restricted to somatic cells but is also present in the germ cells of the gonad prior to metamorphosis (Fig. 1). After stages 60-62 which correspond to the

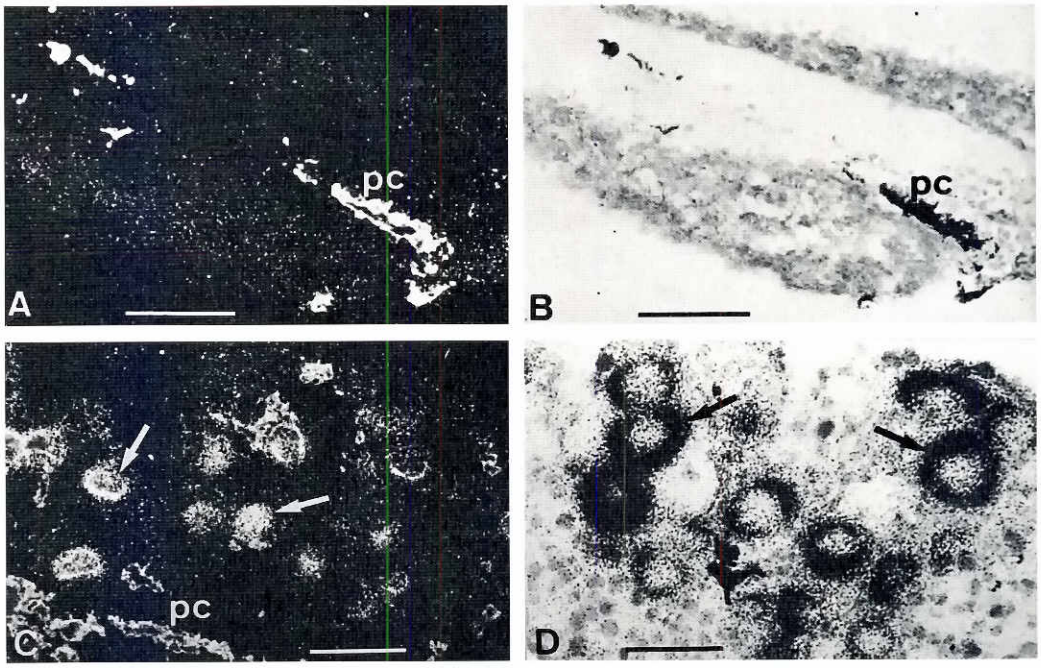

Fig. 2. *In situ* hybridization of an EF-1αO probe to sections of tadpole gonads. (A and B) *Sections of stage 55 gonad hybridized with anti-sense probe. No significant labeling can be observed. The spots marked pc are due to pigment cells. Section of stage 62 testis* **(C)** *and of stage 66 ovary* **(D)** *hybridized with anti-sense probe. Spermatogonia and oogonia (arrows) are heavily labeled. A and C, dark-field illumination. B and D, bright-field illumination. Scale bars, 50 μm.*

climax of metamorphosis, EF-1αS mRNA concentration falls to a low level (Figs. 1 and 4).

In contrast to its somatic counterpart, EF-1αO mRNA cannot be detected in embryos (Djé *et al.*, 1990). We show here that gonads of pro-metamorphosing tadpoles (around stage 55) contain very little, if any, EF-1-αO mRNA (Figs. 2 and 4) but at climax, expression reaches very high levels (Figs. 2 and 4). Taken together, the *in situ* hybridization results show that soon after the beginning of metamorphosis, EF-1αO and thesaurin a mRNA rapidly accumulate in parallel in male and female germ cells.

Thyroid hormone regulation of germ cell-specific EF-1α expression

Since EF-1α germ cell-specific genes begin to be expressed at metamorphosis when T3 levels peak (Leloup and Buscaglia, 1977), we thought that T3 might be responsible for their activation. We therefore decided to test the effect of T3 hormone on the expression of the EF-1αO, EF-1αS and thesaurin genes using the established *Xenopus* cell line XTC2. Such lines have been proposed as useful models for the study of gene expression under hormonal control (Kanamori and Brown, 1993). First, we examined whether the mRNA encoding the elongation factors studied were present and regulated in this cell line, then we performed transfection experiments to analyze transcriptional regulation.

As shown in Figure 5, EF-1αO and thesaurin a genes are expressed in the XTC2 cell line grown in 10% fetal calf serum, but in the absence of added T3. Addition of T3 in the cell culture medium increased 2-fold the amounts of EF-1αO or thesaurin a mRNAs (Fig. 5). A similar result was also obtained with the *Xenopus borealis* XB693T cell line using homologous *(X. borealis)* probes for EF-1αO (data not shown). T3 acts on gene transcription by binding to its nuclear receptors (TRs) that recognize specific sequences (TRE) in the promoter of the target gene (Brooks *et al.*, 1989; Yaoita *et al.*, 1990; Fondell *et al.*, 1993; Desvergne, 1994; Elieceiri and Brown, 1994; Glass, 1994). We determined the relative amount of the TRß messenger RNA in these cells treated or not with T3. As for EF-1αO, TRß messenger RNA increased with T3 treatment (Fig. 5).

To analyze whether the positive effects of T3 on EF-1αO mRNA accumulation were exerted at the transcriptional level, we transfected a pEF-1αO-Luc reporter construct into XTC-2 cells. This construct carries a 3 kb long DNA of the 5' flanking sequence of EF-1αO gene inserted in front of the luciferase reporter gene (see Materials and Methods). The dose response of the EF-1αO promoter to 48 h treatment with increasing concentrations of T3 is shown in Figure 6. A basal level of luciferase expression is observed in the absence of exogenous T3 and this expression is increased significantly by the addition of physiological concentrations of T3. The maximum was seen in the presence of 10 nM T3, which increased expression three times over basal levels.

This T3-dependent transcription from the EF-1αO promoter was increased by co-expression of a TR. As shown in Figure 7, expression of TRα increased transcription three-times over the levels seen in cells cotransfected with the EF-1αO promoter construct and a control plasmid. T3 treatment did not modify transcription from the EF-1αS promoter (Fig. 7), nor from the pGL2 control vector (data not shown). However, T3 did increase transcription from a palindromic T3 response element, T3REtkLuc, a gift from Dr. C.K. Glass (Fig. 7).

Discussion

Expression of elongation factors in embryonic gonads of Xenopus

Our aim in these experiments was to follow the expression of somatic and germinal-specific elongation factors in the gonads of *Xenopus* tadpoles before and during metamorphosis. Using *in situ* hybridization, we show that somatic and germinal factors

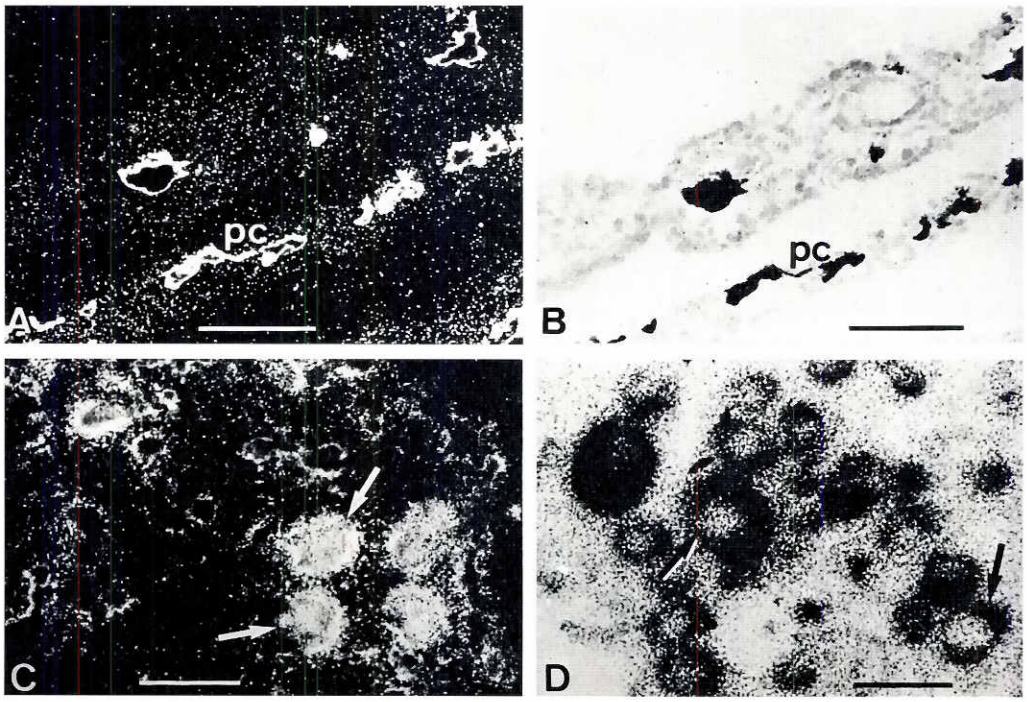

Fig. 3. *In situ* hybridization of a thesaurin a probe to sections of tadpole gonads. (A and B) *Section of stage 55 gonad hybridized with anti-sense probe. No specific labeling can be observed.* **(C)** *Section of stage 62 testis hybridized with anti-sense probe.* **(D)** *Section of stage 66 ovary hybridized with anti-sense probe. Spermatogonia and oogonia (arrows) are heavily labeled. A and C, dark-field illumination. B and D, bright-field illumination. Scale bars, 50 μm.*

have contrasting expression profiles. On the one hand, we show that EF1αS is expressed at high levels in the gonads, as in other tissues (Krieg *et al.*, 1989; Djé *et al.*, 1990), before metamorphosis, i.e. at stages 55-57. During metamorphosis mRNA levels in the gonads decrease significantly. Then in post-metamorphic animals, expression of EF1αS is no longer detected in the germ cells of the gonads (Abdallah *et al.*, 1991b). On the other hand, expression of the germ cell-specific genes, EF1αO and thesaurin a, is undetectable in the gonads of stage 55-57 tadpoles, but expression increases dramatically with the onset of metamorphosis when T3 levels peak (Leloup and Buscaglia, 1977).

The early expression of EF1α-S in germ cells provides a plausible explanation for the existence of retropseudogenes of the EF1α-S type in *X. laevis* chromosomes (Abdallah *et al.*, 1991b). Such genes are thought be created by reverse transcription of processed mRNA, followed by integration of the copies into chromosomal DNA. The latter event must occur in germ cells, otherwise the integrated copies are not transmitted to the following generations (Vanin, 1985; Weiner *et al.*, 1986). Thus, when we first found EF1α-S retropseudogenes in *X. laevis*, we were unable to explain their origin as the EF1α-S genes are not expressed in the germ cell of adult *Xenopus* (Abdallah *et al.*, 1991a,b). The present data showing the abundance of EF-1αS mRNA in embryonic germ cells suggest that EF-1αS pseudogenes could have been created in embryonic germ cells in ancestors of present day *Xenopus*.

Expression of EF-1α genes in Xenopus cell lines

Expression of the germ cell-specific gene EF-1αO in various *Xenopus* cell lines (results not shown, except for the XTC2 cell line) was unexpected. As far as the somatic counterparts are concerned, it is known that tumoral or immortalized cell lines express high levels of EF-1αS (Sanders *et al.*, 1992; Tatsuka *et al.*, 1992). Some authors have proposed that this high expression could result in an increase in lifespan (Webster, 1985; Cavallius *et al.*, 1986; Shepherd *et al.*, 1989) but this hypothesis has not been confirmed (Shikama *et al.*, 1994). We too have carried out a number of experiments in heterologous systems that have failed to show any role of EF1α-O in immortalization and oncogenesis.

One explanation for the expression of EF1α-O in the cell lines studied is that expression would be induced by the T3 present in the serum necessary for maintaining the cultures. Indeed, the results shown here (Figs. 6 and 7) clearly indicate that T3 hormone does regulate positively EF-1αO, thesaurin a and TRβ expression, while not affecting EF-1αS expression. This corroborates other data showing that thyroid receptors are either present or inducible by T3 hormone in different cell lines (Kanamori and Brown, 1992; Machuca and Tata, 1992). The increase of TRβ mRNA that we observed was comparable to the levels observed by Machucha *et al.* (1995) with standard cell culture medium. In conditions where the basal activity was reduced (by overexpression of unliganded TRα or TRβ or depleting the fetal calf serum), a much more impressive increase of TRβ mRNA was observed when T3 was added (Kanamori and Brown, 1992, 1993; Machuca and Tata, 1992; Machuca *et al.*, 1995). Under the conditions that we used, addition of T3 (10^{-7} M) induced increases in EF1αO and thesaurin a mRNAs concentrations that were in the same order of magnitude as those seen for TRβ mRNA.

Transcriptional activation of EF-1αO gene by T3

We next chose to analyze whether T3 exerts its effect on EF1αO and thesaurin a genes at the transcriptional level. As we had already isolated a large 5' sequence of the EF1αO gene, we inserted this in front of the luciferase reporter gene so as to follow the effects of T3 on the transcription of this gene. We found

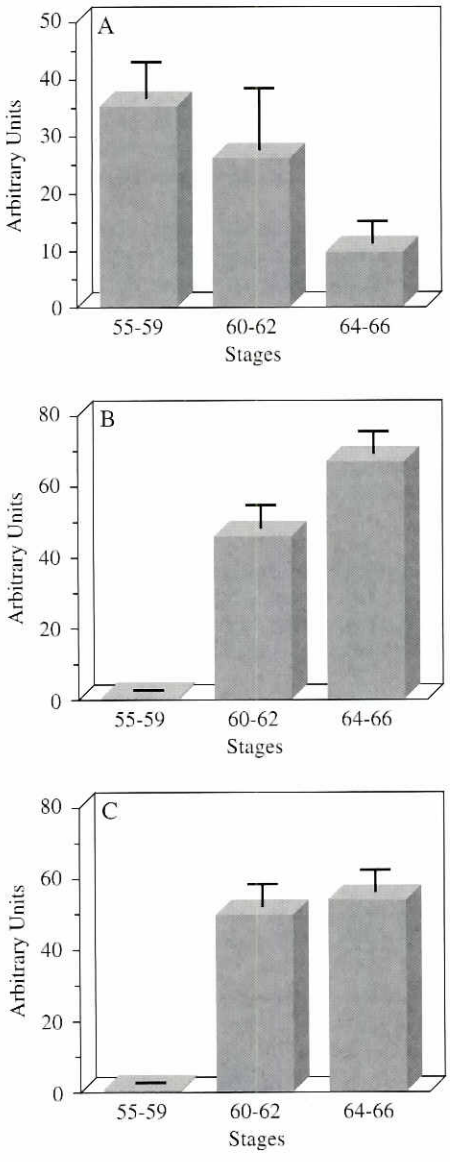

Fig. 4. Histogram showing the relative abundance of EF-1αS (A), EF-1αO (B) and thesaurin a (C) mRNAs *in male and female germ cells at three developmental stages: 55 (pre-metamorphosis), 60 (climax) and 66 (post-metamorphosis). The results from in situ hybridization (Figs. 1-3) are expressed in arbitrary units as the grain density of the cell sections. Each column represents the mean ±SEM of about 40 measurements (randomly selected) carried out on male and female germ cells. Vertical bars indicate SEM. The difference in probe lengths used to detect EF-1αS, EF-1αO and thesaurin a mRNA prevent comparison of the column heights for the different mRNAs. Each mRNA must be considered separately during development.*

that T3 increased transcription in a dose-dependent manner. The concentration of T3 giving half maximal response (ED50) was 10^{-9} M (Fig. 6), corresponding to the minimum concentration necessary to induce the synthesis of TRβ mRNA in XTC2 cells (Machuca and Tata, 1992). The effect of T3 is necessarily dependent on the concentrations of available T3 receptors in the cell. In *Xenopus*, as in mammals, two receptors, TRα and TRβ, have been characterized (Yaoita *et al.*, 1990) but the precise contribution of each of them to transcription activation is still poorly understood. In the XTC2 cells, it is known that TRβ is highly induced with T3, whereas with TRα induction is less marked. However, TRα is expressed at a higher overall level both before and after T3 treatment (Kanamori and Brown, 1992; Machuca and Tata, 1992). We therefore cotransfected TRα to examine its effect on the level of EF1αO transcription. Cotransfection of an expression vector for *Xenopus* TRα indeed increased three-fold the transcription induced by physiological concentrations (10^{-9} M) of the hormone (Fig. 7).

Possible TRE in the 5' sequence of germ cell specific EF-1α genes

The finding that T3 exerts a transcription control on EF1αO expression led us to investigate whether consensus TREs could be found in the EF1αO 5' sequence. We also examined the 5' sequence of the thesaurin gene using data kindly made available to us by Keith Joho (LSU Medical Center, New Orleans, USA). Looking for the consensus sequence: (A/G)GGTCA N..N TGACCT (Glass *et al.*, 1988; Umesono *et al.*, 1988, 1991; Naar *et al.*, 1991; Glass, 1994; Ranjan *et al.*, 1994; Machuca *et al.*, 1995), and using the "bestfit" program based on the method of Waterman (1994) to make alignments, we found the potential TRE or retinoic acid receptor (RAR) sequence GAGTCA GT TGACTT at 858 bases upstream of the ATG translation initiation site. On the thesaurin a promoter gene, we found the palindromic sequence GGGGTCA T TGACCCC located 880 bases upstream of the ATG resembling a 9-cis retinoic acid receptor (RXR), and the sequence AGGTGA GCTC AGGACT resembling the myosin heavy chain thyroid hormone receptor response element AGGTGA CAGG AGGACA (Glass *et al.*, 1988). Thus,

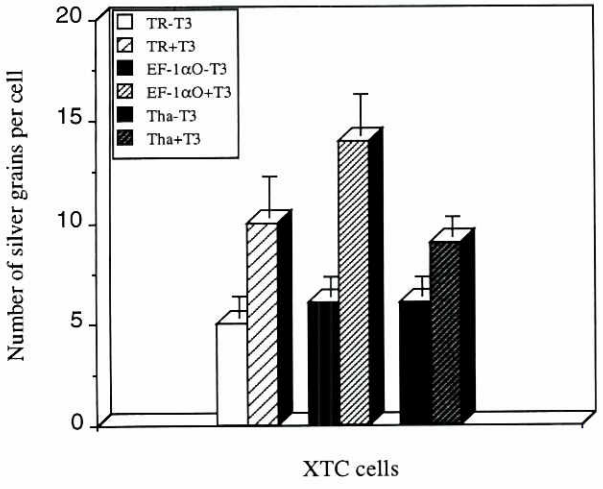

Fig. 5. Histogram showing the relative abundance of TRß (TR), EF-1αO and thesaurin a (Tha) mRNAs in the XTC2 *Xenopus laevis* cell line *in the absence (-T3) or the presence (+T3) of 10^{-7} M exogenous T3 in the medium during 48 h. The results were obtained by in situ hybridization using homologous antisense probes after subtraction of values obtained with control sense probes. Each column represents the mean of several measurements (>50 cells for each probe). Vertical bars indicate SEM. As in Figure 4, the difference in probe lengths used to detect EF-1αO, thesaurin a and TR mRNA prohibit comparison of the column heights for the different mRNAs, although TR and thesaurin a probes are similar (2302bp and 2178bp).*

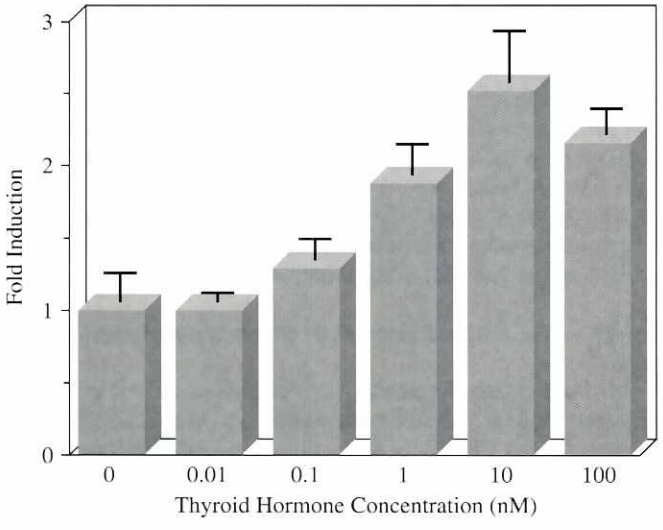

Fig. 6. Transcription from the EF-1αO promoter transfected into XTC-2 cells line is T3 dependent. *Results are expressed as the fold induction of Luc activity obtained with increasing doses of T_3 (0.01 to 100 nM) to the activity in the absence of T3, which was arbitrarily set to 1. Proteins were measured 48 h after transfection of pEF1αO-LUC in cultures exposed to increasing doses of T_3 (0.01 to 100 nM). The means ±SEM of four separate determinations for each condition are given. A representative experiment is shown, the experiment was repeated three times obtaining similar results each time.*

potential T3 regulatory elements are present in the promoters of both genes shown to be T3 responsive in these experiments.

In conclusion our data show that germ cells switch from a somatic to a germ-cell specific mode of EFα expression during metamorphosis. One key player in this gene switching is thyroid hormone. This is shown by the fact that the gene encoding the germ cell-specific elongation factor, EF-1αO, is transcriptionally activated by T3 in the transient transfection assays. In these assays no reciprocal repression of EF-1αS by T3 was seen, despite the fact that expression of this somatic gene was depressed in the germ cells of the intact tadpole undergoing metamorphosis. The cause of the repression of EF-1αS revealed by *in situ* hybridization in germ cells during metamorphosis remains to be investigated.

Materials and Methods

Plasmids

In a search for the EF1αO gene, we isolated a 12kb DNA clone from a λEMBL4 *Xenopus laevis* genomic library. This clone is composed of about 4kb of the EF1αO gene (up to position 3253 downstream of the ATG) and a noncoding sequence 5' of the gene. The pEF1αO-LUC plasmid was constructed by subcloning a 3kb fragment from this 5' flanking sequence of the EF-1αO gene extending from 35 bases ahead of the ATG translation start into pBluescript (Stratagene) between the *EcoRV* and *PstI* sites and then recloning it between the *KpnI* and *SacI* sites of pGL2 basic (Promega). This creates a vector carrying the luciferase structural gene linked at its 5' end to approximately 3kb of 5'-flanking sequence of the *Xenopus* EF-1αO gene. To check the functionality of the promoter sequence we injected a pEF-1αO-CAT construct (see below) into *Xenopus* oocytes. We found high levels of expression from this 3kb 5' sequence in stage IV and V oocytes, confirming the functional nature of the EF-1αO promoter we had isolated.

The pEF1αS-LUC plasmid was constructed starting from a 4.5kb fragment of *Xenopus* EF-1αS promoter. The EF-ßgal plasmid (a gift from Paul Krieg, University of Texas at Austin, Texas, USA) containing the EF-1αS promoter upstream of the lacZ coding sequence, was first cut by XbaI and sealed to eliminate the lacZ coding region. The plasmid was then linearized by BglII and partially cut by HindIII. The 4.5kb fragment containing the EF1αS promoter was then subcloned into BglII, HindIII cut pGL2 basic (Promega).

pCAT-control: this plasmid (Promega) constitutively expresses Chloramphenicol Acetyl Transferase under the SV40 promoter.

pGL2-control: this plasmid (Promega) expresses the luciferase gene under SV40 promoter.

xTRα (pSVLXenTRαHE): this vector, a gift of R.W. Old (University of Warwick, Coventry, UK) is a pSVL (Pharmacia) derived expression plasmid in which the *Xenopus* thyroid hormone receptor (TRα) cDNA is driven by the SV40 late promoter.

The pEF1αO-CAT plasmid was constructed by subcloning the same 3kb fragment of pEF1αO-LUC plasmid into pEMBL9-CAT plasmid (a gift from D. Rüngger, University of Geneva, Switzerland).

Animals

Tadpoles were staged according to the normal table of Nieuwkoop and Faber (1956). The sexual differentiation of the gonads begins at stage 52. Metamorphosis starts at stage 57, culminates at stages 60-62 (climax) and is completed by stage 66. About 20 animals were analyzed for each stage.

In situ hybridization

For *in situ* hybridization, typical stages 55-57 (premetamorphosis), 60-62 (climax) and 64-66 (end of metamorphosis) were used. Embryonic gonads (1-2 mm long and 50-100 µm thick) were dissected out from anesthetized animals together with the kidneys, fixed and processed as described previously (Hourdry *et al.*, 1988).

Sections of 7 µm (about 40 per stage) were hybridized with [^{35}S]-labeled antisense RNA probes (10^6 cpm per slide) prepared by transcription of *Xenopus laevis* EF-1αS cDNA (a 124bp sequence-specific

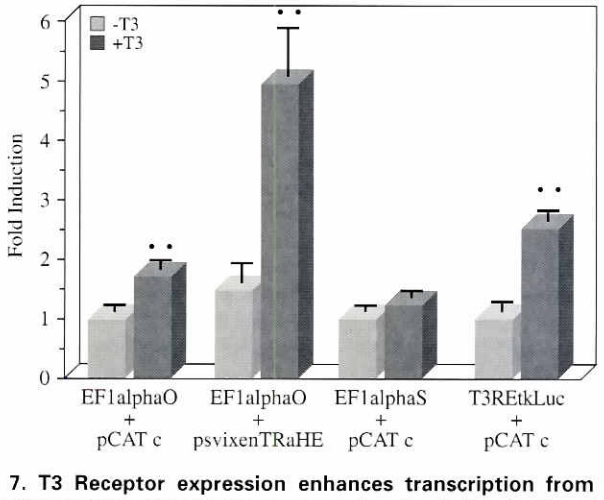

Fig. 7. T3 Receptor expression enhances transcription from the EF1αO promoter. *Results are expressed as the fold induction of Luc activity obtained in the presence of TH relative to the activity in the absence of TH, which was arbitrarily set to 1. Proteins were measured 48 h after cotransfection of pEF1αO-Luc with pCAT-control or with xTRα in the presence (+) or in the absence (-) of T_3 (1 nM). Controls included EF-1αS-Luc + pCAT and a TRE-Luc with the same control plasmid. The means ±SEM of four separate determinations for each condition are given. A representative experiment is shown, the experiment was repeated three times obtaining similar results each time.*

DNA fragment between positions 1434 and 1558 near the 3' end of the cDNA, (Krieg *et al.*, 1989), *Xenopus laevis* EF-1αO cDNA (a 72bp DNA fragment between positions 1421 and 1493 at the 3' end of the cDNA, a sequence not found in EF-1αS, (Djé *et al.*, 1990), *Xenopus laevis* thesaurin a (complete cDNA of 2178bp), *Xenopus borealis* EF-1αO (whole cDNA of 1436bp) and *Xenopus laevis* TRα (complete cDNA of 2302bp). Sense probes were also prepared and used as controls to evaluate background levels. Hybridization was performed as previously described (Hourdry *et al.*, 1988). After hybridization, the slides were washed and dipped in Kodak NTB2 emulsion and exposed for 8-12 days at 4°C.

Quantification of results

The grain density on the autoradiograms was measured by computerized video-microscopy using a digital image processor (Optilab program from Graftek running on a Macintosh II). The microscopic image is first digitalized and saved as a disk file in the best conditions of illumination and contrast. The image is then analyzed on the saved file. Selected regions corresponding to single cells are delimited under visual control. For each cell a histogram is constructed which represents the darkening level with respect to a reference scale. All values were corrected for background. The difference in probe lengths and hence varying specific activities could lead to difficulties in quantitatively comparing results between gene expressions. For this reason we only made comparisons for a same probe at different developmental stages.

Cell culture

The XTC2 cell line, derived from *Xenopus laevis* tadpole carcasses (Pudney *et al.*, 1973), was a gift of J-C Boucaut (Université Paris VI). Cells were maintained in Leibovitz medium (ATGC, Paris, France) (61% vol/vol with H_2O) containing 10% fetal bovine serum (GIBCO/BRL), streptomycin (50 u/ml), penicillin (50 u/ml) and kanamycin (50 u/ml) at 23°C. Cells were treated with different doses of T_3 (Sigma, France) in 68% Leibovitz serum free medium (SFM).

Cell transfections

A novel procedure, [Boussif, 1995 #487], was used for transfection. DNA (2 μg of pEF1αO-LUC) was complexed using 9 equivalents of polyethylenimine (PEI), to one equivalent of DNA (PEI to DNA ratio was calculated on the basis of PEI nitrogen over DNA phosphate). DNA and PEI were mixed in 0.015 M NaCl, then diluted into an appropriate volume of SFM. The mixture was added directly to the cells, which were briefly centrifuged in the dishes and incubated together. In some experiments cells were cotransfected with 0.5 μg of pCAT-control or 0.5 μg of xTRα. After 1 h the transfection medium was replaced with fresh SFM. The transfected cells were treated with different doses of T_3 between 0.01 and 100 nM and 48 h allowed for expression of the transgenes. Cells were then lysed and extracts used for luciferase assay.

Protein and luciferase enzyme assays

Protein assays were performed using the Bio-Rad Dye reagent kit. Luciferase activity was measured according to the standard protocol (Luciferase assay system, Promega) in a single-well Tropix™ luminometer (ILA911, MGM Instruments, Hassden, CT, USA) calibrated against a tritium standard. Light emitted from 100 μl of the culture extracts was measured over 10 sec.

Acknowledgments

We thank Dr. G. Jouanin (Service d'Imagerie Scientifique, Université de Paris XI) and Dr. N. Touzet (Laboratoire d'Embryologie Moléculaire et Expérimentale, Université de Paris XI) for their help, J-C. Boucaut for the Xenopus cell line, C.K. Glass, P.A. Krieg, R.W. Old and D. Rüngger for the plasmids and K.E. Joho and P.A. Krieg for kindly communicating their results prior to publication. This work was supported by grants from "l'Institut National de la Santé et de la Recherche médicale (grant n°920706)" and The "Association pour la Recherche contre le Cancer" (to B.D.).

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Received: December 1995
Accepted for publication: January 1996