Rat Sertoli cell-specific regulation of the transferrin gene. F Guillou [1], E Schaeffer [2], D Part [2], S Suire [1], I Fontaine [1], M Zakin [2] *([1] INRA, PRMD, Unité "Biochimie Hormonale, Testicule et Spermatozoïde", URA CNRS 1291, 37380 Nouzilly; [2] Institut Pasteur, Unité "Expression des Gènes Eucaryotes", 75724 Paris Cedex 15, France)*

In the testis, Sertoli cells are responsible for creating a unique environment in which germinal cells divide and differentiate into spermatozoa. A number of Sertoli cell secretory products have been identified and their function determined; among them is the iron transport protein transferrin. It is an important marker of testis function since its synthesis and secretion are involved in the control of spermatogenesis. Our purpose is to elucidate the mechanisms controlling the transferrin gene expression in the Sertoli cells.

We have previously shown that the proximal promoter region (PR) of the human transferrin gene is sufficient to activate transcription in primary cultured Sertoli cells of rat testis (F Guillou *et al* (1991) *J Biol Chem* 266, 9876-9884). The relative importance of the PRI and PRII sequences has been tested by mutation of specific nucleotides within each sequence, followed by transient expression experiments in Sertoli cells, compared to hepatoma cells. The mutation of the PRI and PRII site inhibits transcription by 20% and 50% respectively in Sertoli cells, *versus* 85% and 70% in Hep 3B cells. This suggests that different DNA binding proteins interact with each site in testis compared to liver. This conclusion is further supported by methylation interference assays which show that testis and liver proteins interact in a different manner with the DNA sequence. The transcription factors present in testis or liver nuclear extracts have been further characterized by fractionation on a heparin–Sepharose column. The purified active fractions were tested in gel retardation assays with antibodies directed against various transcription factors. In liver, the PRI sequence is the binding site of the 2 proteins, HNF4 and COUP-TF; PRII is the binding site of the C/EBP transcription factors family. In testis, only COUP-TF interacts with PRI, since HNF4 is absent; the 2 proteins interacting with the PRII site do not belong to the C/EBP family and remain to be further characterized.

Transferrin secretion and transferrin mRNA increase when Sertoli cells are stimulated by follicle-stimulating hormone (FSH). By transfection experiments, we have identified a FSH response element *via* cAMP pathway in the -82, -52 pb region. The CRE element is absent in this region.

During post-natal testicular differentiation, there is a correlation between the increase in transferrin secretion and the initiation of spermatogenetic function. In Sertoli cell of 10-d-old rats, transferrin is secreted at a very low level; in 17-d-old rats, the level of secreted transferrin increases dramatically (30 times). However, in Sertoli cells of either 10-d-old or 17-d-old rats, the level of transferrin mRNA is identical. The post-transcriptional mechanism which regulates transferrin expression during testicular differentiation remains to be further characterized.

Expression of insulin-like growth factor (IGF) I and action of IGF I and II in the trout testis. F Le Gac, M Loir *(INRA, Laboratoire de Physiologie des Poissons, Campus de Beaulieu, 35042 Rennes Cedex, France)*

Salmon GH (sGH) binds specifically to trout testis membranes. *In vitro*, sGH modulates testicular steroidogenesis, while *in vivo*, GH and 17α20β-OH-P vary concomitantly (Le Gac *et al*, 1992 *Biol Reprod* 46, 949-957). Pickford *et al* (1972, *Biol Reprod* 7, 370-386) observed that *in vivo* treatment with b-GH stimulated spermatogonial proliferation in hypophysectomized killifish. It is not known whether GH acts directly or through IGF in steroidogenic cells and germ cells and until recently, no study dealt with possible secretion and function of IGF in the fish testis. We investigated these 2 points.

We found that trout testis RNAs hybridized with a coho salmon IGF-I DNA probe (fig 1). The size and/or relative intensity of the 2 main IGF-I transcripts observed on Northern blots of testicular RNA differed from those obtained with trout liver RNA. *In vivo* treatment with recombinant trout GH (Eurogentec) significantly increased IGF-I mRNA concentration in both the liver and the testis (fig 1).

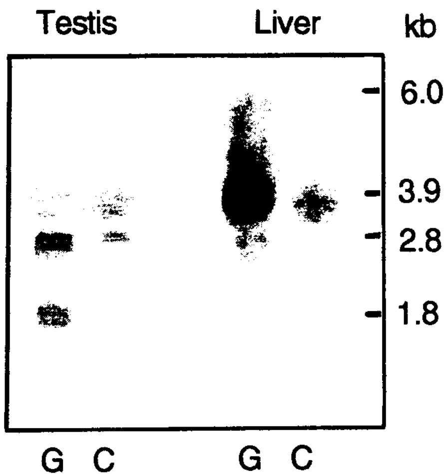

Fig 1. Hybridization of salmon IGF-I cDNA (pb388 PCR product from Duguay *et al; Mol Endocrinol*, in press) to a Northern blot of total RNA (20 μg) from rainbow trout testis and liver. Tissues were obtained 11 h after a single injection of trout growth hormone (G) or vehicle (C). On the right of the figure the size (in kb) of the different IGF-I transcripts found in salmon liver by Cao *et al* (1989; *Mol Endocrinol* 3, 2005-2010) and Sakamoto and Hirano (in press).

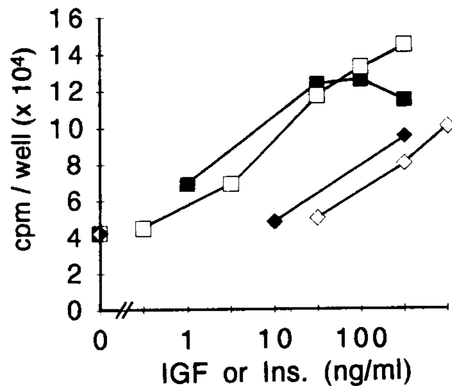

Fig 2. Effect of increasing concentrations of rhIGF I and II, s- and b-insulin on *in vitro* ^{3}H-T incorporation (incubation for 3 d; 8 μCi/ml ^{3}H-T and 3rd day) by trout germ cells (0.7 x 10^6 Go + Ci/well). Mean ± SD (*n* = 4). —■— IGF-I; —□— IGF-II; —◆— s-insulin; —◇— b-insulin.

Effects of recombinant human IGF-I and IGF-II (gift from Ciba-Geigy) and of salmon (gift from Dr E Plisetskaya) and bovine insulin on ^{3}H-thymidine (^{3}H-T) incorporation by spermatogonia (Go) and primary spermatocytes (Cl), cultured either in the absence or in the presence of Sertoli cells (SC), were examined *in vitro.* IGF-I (ED_{50} 3.5-12 ng/ml) and IGF-II (ED_{50} 27-38 ng/ml) stimulated ^{3}H-T incorporation by Go+Ci, in a dose-dependent manner (fig 2). A hundred- to 300-fold higher concentration of s- or b-insulin was required to produce an effect similar to that of IGF-I. In the presence of SC, the responsiveness of Go/Cl was decreased (ED_{50} 120 ng/ml). Combinations of various concentrations of IGF-I, IGF-II and insulin suggest that the effects of these molecules should be mediated through a unique IGF receptor.

Inhibition of production of maturation inducing steroid in rainbow trout granulosa cells: effect of oestradiol on gonadotropin stimulated 20 β-hydroxysteroid dehydrogenase activity. A Fostier, H Baek *(INRA, Laboratoire de Physiologie des Poissons, Campus de Beaulieu, 35042 Rennes Cedex, France)*

In most teleost species investigated, 17-hydroxy-20 β-dihydroprogesterone (17, 20-OHP) was the most effective maturation inducing steroid (MIS). Gonadotropin type 2 (GtH 2) is known to stimulate the 20 β-hydroxysteroid dehydrogenase activity (20βHSDH) in granulosa cells, the key enzyme converting 17-hydroxyprogesterone (17-OHP) into 17,20-OHP (Jalabert *et al,* 1991, *In: Vertebrate Endocrinology: Fundamental and Biomedical Implications.* Academic Press, NY, 4A, 23-90). High doses of oestradiol (E2), which is synthesized by granulosa cells during vitellogenesis, have been shown to depress 17,20-OHP production by ovarian follicles (Jalabert and Fostier, 1984;