Analysis of a salmon prolactin promoter in transgenic rainbow trout

Claire Amoros, Y. Le Dréan, Daniel Chourrout, C.L. Hew, Patrick Prunet

18th Conference of European Comparative Endocrinologists from Molecular to Integrative Biology

September 10th-14th, 1996
Palais des Congrès
Rouen - France

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the oogenesis from the oogonia to the mature eggs, based on differences of staining, size and on nucleus and cytoplasm structure, as viewed through the ligth microscope. Three of these stages correspond to the first or previtellogenic phase and the other three to the second or vitellogenic phase. Several types of atresic eggs are distinguished which probably represent different phases in the process of atresia. The testicular tissue belongs to the continuous or unrescticted spermatogonial type (as classified by Grier, 1981), and forms a complex network of seminiferous tubules.

CONTROL OF GENE EXPRESSION

P148 BONE GLA PROTEIN IN FISH : MOLECULAR CLONING AND DEVELOPMENTAL APPEARANCE IN EARLY LARVAL STAGES OF THE SEA BREAM *SPARUS AURATA*

J.I. Pinto, D. Power and M.L. Cancela

Universidade do Algarve/UCTRA, 8000 Faro, Portugal.

Bone Gla Protein (BGP) is a bone-specific protein isolated at the protein level from a variety of species from fish to mammals but its gene structure and control of expression have been determined exclusively in mammals. Our objectives were to clone the BGP cDNA and study its developmental appearance in a non-mammalian vertebrate. Sea bream larvae 12 to 90 days old were collected and development of cartilage and bone structures was followed by histological techniques. Calcification was found to be complete by 60 days. Total RNA from fully calcified larvae was reverse transcribed and then amplified by PCR using a 3' oligo dT primer and a degenerated 5' primer designed according to aminoacid 23 to 30 of the sequence of the mature seabream BGP protein previously purified (1). The 325 bp cDNA thus obtained was sequenced revealing an open reading frame corresponding to the aminoacid sequence previously obtained for the seabream BGP in this region of the protein and extending 252 bp of 3' non-coding region from the stop codon to the insertion of the poly(A) tail. The presence of BGP mRNA was followed throughout the early stages of development of seabream by in situ hybridization using an antisense RNA probe. Detection of specific hybridization paralleled appearance of the first calcified structures : the mandibulary region, the skin (due to the presence of calcified scales) and sites of fin insertion. Unexpectedly, specific BGP labeling was also seen in non calcified structures, particularly in brain and gill bars, suggesting that BGP is present in other than calcified structures in fish. Alternatively, it could indicate the presence of other protein(s) of the same gene family with distinct patterns of expression. This possibility is currently being investigated. (1) M.L. Cancela, M.K. Williamson, P.A. Price. Int. J Peptide Protein Research, 46 :419, 1995.

P149 ANALYSIS OF A SALMON PROLACTIN PROMOTER IN TRANSGENIC RAINBOW TROUT

C. Amoros (1), Y. Le Drean (3), D. Chourrout (2), C.L. Hew (3) and P. Prunet (1)

(1) Laboratoire de Physiologie des Poissons, Rennes ; (2) Laboratoire de Génétique des Poisons, INRA, Jouy-en-Josas, France ; (3) Research Institute, Hospital for Sick Children, and Department of Clinical Biochemistry, University of Toronto, Canada.

A gene encoding for the chinook salmon prolactin has been recently isolated and fully sequenced by Xiong et al. (1992). The purpose of this study was to demonstrate the cell specific expression driven by cis-regulatory elements of the salmon PRL gene in transgenic rainbow trout. For that purpose, stable lines of transgenic fish having integrated the foreign gene were produced. Fish harbored a fusion gene containing 2.4 Kb of the 5' flanking sequence (Xiong et al., 1992) and the reporter gene, lac Z encoding β-galactosidase protein.

This construct was retained in approximatively 30 % of 9 months-old fish derived from micro-injected eggs and induced a very low expression : analysis of β-galactosidase activity showed that only a few PRL cells expressed the reporter gene in two fish among 28 positives. However these first results agreed with a cell specific expression. In order to exclude the problem linked to mosaic integration of the transgene, we produced 6 F_1 families from wild-type females mated with F_0 transgenic males. The percentage of inheritance varies between families (17.5-28 %). Interestingly, β-gal activity was detected in pituitary gland from different animals with a variable intensity, but only in PRL cell. Southern blot analysis indicate that multiple copies, often organized in concatemers, were integrated into the genomes. Fifteen different patterns have now been identified. For each of them we produce the F_2 generation. In some of them, we confirmed stable inheritance of the transgene and specific expression of β-Galactosidase in PRL cells. A comparative analysis of β-gal expression for each of these integration pattern in our different F_2 families is currently been performed.

P150 *IN VITRO* STUDY OF THE TSH SUBUNIT mRNA REGULATION IN PRIMARY CULTURE OF PITUITARY CELLS OF THE EUROPEAN EEL, *ANGUILLA ANGUILLA*

M. Schmitz, B. Pradet-Balade, N. Lebelle, S. Dufour and B. Querat

Laboratoire de Physiologie Générale et Comparée, MNHN, URA 90 CNRS, 7 rue Cuvier, 75231 Paris Cedex 05, France.

Recent *in vivo* studies demonstrated that both T_3 and T_4 down regulate TSH subunit mRNAs in the European eel at the silver stage. To study the direct action of the thyroid hormones (TH) at the pituitary level we inititiated *in vitro* experiments on pituitary cells in primary culture.

Dot-blot analysis showed a 2.5 fold increase in TSH β mRNA in control cells between the first and 14th day of culture, which probably reflects the abolition of in vivo inhibition either by circulating TH or by negative regulation exerted by the hypothalamus. Addition of T_3 or T_4 to the culture medium decreased TSH β mRNA of about 70 % compared to day 14 control cells, indicating that both TH are able to down regulate TSH β mRNA expression through direct action at the pituitary level.

To increase the sensitivity of the detection and quantification of the TSH mRNA we developed RNAse protection assays (RPA) for the α and TSH β subunit mRNA. These RPAs are used to further characterize the negative feed-back of TH on TSH mRNA expression as well as investigate the role of brain neuromediators in the regulation of TSH mRNA.

P151 COMPARISON OF THE BIOLOGICAL POTENCIES OF THE TWO TILAPIA PROLACTINS ON tiPRL RECEPTOR TRANSFECTED IN HUMAN FIBROBLASTES (293) CELL LINE

F. Sohm (1), A. Pezet (2), O. Sandra (3), M. Edery (2), P. Prunet (3), P. Kelly (2), B. Demeneix (1) and A. de Luze (1)

(1) Laboratoire Physiologie, URA 90 CNRS, MNHN, Paris. (3) Laboratoire physiologie des poissons, INRA Rennes, (2) Laboratoire d'Endocrinologie Moléculaire, U344, INSERM, Paris, France.

Tilapia has served as an important model system for understanding PRL functions in teleosts. Two forms of prolactin (tiPRL$_I$ and tiPRL$_{II}$) with only 69 % sequence identity have been shown to be produced by the pituitary prolactin cell, tiPRL$_I$ being more similar to other fish prolactins. Although the two PRLs have been shown to have different potencies in various fonctions in tilapia, only one form of receptor have been cloned and detected in the various tissues in this species. The biological significance of the two PRLs being unknown, further analysis at the molecular level on signal transduction pathways was undertaken. Because the PRL receptor in fish as well as in mammals belongs to the cytokine receptor family, it was of interest to determine if the transduction pathway already described using PRLR in mammals also apply to fish RPRL. tiRPRL was cotransfected in 293 cells with the STATS responsive region (tK-LHRR-luc). All biological activities were normalized against constitutive cotransfected β-gal activities. A chimeric short form of tiPRL receptor was constructed and used as a negative control. Recombinant tiPRLs (tiPRL$_I$ and tiPRL$_{II}$) were a gift of Dr F. Rentier (Leuwen). Comparaison of the potencies of the two forms of tiPRL and oPRL showed good agreement between results obtained by radioreceptor assays and biological activities : oPRL = tiPRL-I > tiPRL-II.