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SHORT COMMUNICATION

Differential Expression and Cellular Localization of Activin and Inhibin mRNA in the Rainbow Trout Ovary and Testis

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An inhibin cDNA from rainbow trout consisted of 1305 bp, which coded for 352 amino acid residues. The deduced amino acid sequence of mature inhibin was 50 to 60% identical to mammalian sequences. Distribution of inhibin **α** and activin **β** A and **β** B in different ovarian and testis compartments was studied in rainbow trout by *in situ* hybridization with complementary RNA probes. In testis tissue, inhibin **α** and activin **β** A and **β** B were expressed only in the testicular interstitia between the seminal lobules, where Sertoli cells and Leydig cells are distributed. The localizations and intensities of the reactions were constant throughout the maturation of the testis. Within ovarian tissue, the theca cell layers of follicles showed strong reactions of Dig-labeled antisense mRNA probes hybridizing against inhibin **α** and activin **β** A and **β** B in all samples over the same sampling period. In regressing oocytes, a positive reaction was observed in the granular cell layer of the follicles.

Key Words: rainbow trout; activin; inhibin; cDNA; *in situ* hybridization.

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INTRODUCTION

Three activin subforms have been purified from porcine ovarian follicular fluid resulting from combinations of two β A chains (activin A) (Vale *et al.*, 1986), two β B chains (activin B) (Nakamura *et al.*, 1992), two β B chains (activin B [2]), or one β A and β B chain (activin AB) (Ling *et al.*, 1986). Two forms of inhibin have been found in follicular fluid. Inhibins are composed of an inhibin α subunit linked to either an activin β A subunit (inhibin A) or an activin β B subunit (inhibin B) (Vale *et al.*, 1988). Activin was originally discovered by its ability to stimulate the secretion of follicle-stimulating hormone (FSH) from cultured anterior pituitary cells (Ling *et al.*, 1986; Vale *et al.*, 1986). By contrast, inhibin suppresses pituitary FSH secretion (Ying, 1988).

Immunohistochemical studies have localized all three subunits within the granulosa layer of developing follicles in normal human (Rabinovici *et al.*, 1992, Yamoto *et al.*, 1992; Jaatinen *et al.*, 1994) and have localized the inhibin α subunit within the granulosa–luteal cells of the corpus luteum (Smith *et al.*, 1991). In theca cells, however, no staining for any of the three subunits was observed (Yamoto *et al.*, 1992), although expression of inhibin α and activin β A mRNA was

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detected (Jaatinen *et al.*, 1994). Human granulosa cells have been shown to express inhibin α and activin β A and β B mRNAs, and theca cells have been shown to express inhibin α and activin β B mRNAs.

Inhibin and activin are also biochemically related proteins thought to play an important role in paracrine regulation of testicular function (Mather *et al.*, 1992). mRNAs of all three subunits, inhibin α and activin β A and β B, have been demonstrated in the testes of embryonic, prepubertal, and adult rats either through measurements of mRNA levels in testicular homogenates or through *in situ* hybridization (Bhasin *et al.*, 1989; De Jong, 1988; Feng *et al.*, 1989; Kaipia *et al.*, 1991, 1992; Keinan *et al.*, 1989; Krummen *et al.*, 1989; Roberts *et al.*, 1991). The localization and distribution of inhibin and activin in the ovary and testis of mammals have been studied. Previously, we cloned and characterized the cDNA for the β A and β B subunits of rainbow trout activin (Tada *et al.*, 1998). However, little information is available regarding the localization and distribution of inhibin and activin of fish.

In the current study, we describe the cellular localization of inhibin and activin subunit expression in rainbow trout ovary and testis, as determined by *in situ* hybridization.

MATERIALS AND METHODS

Experimental Fish and Gonad Samples

Rainbow trout (*Oncorhynchus mykiss*), kept in the Oizumi experimental station of the Tokyo University of Fisheries, were used. In this study, 3 1-year-old males (mean body wt 265 g) and 3 2-year-old females (mean body wt 990 g) were sampled every month during the maturation of testis and ovary from March to December 1996. Their testes and ovaries were fixed in phosphate-buffered formalin (pH 7.2) at 4° overnight and embedded in paraffin. Thin sections (5 μm) were prepared and then subjected to Hematoxylin and Eosin (H–E) stain and *in situ* hybridization. For the *in situ* hybridization, the sections were stored at 4° until the commencement of *in situ* procedures.

```
        10         20         30         40         50         60
ccatgcagactggtccttctaccgtcctctcctgtgccctgttactcctgtggatccaga
   M  Q  T  G  P  S  T  V  L  S  C  A  L  L  L  L  W  I  Q  T
        70         80         90        100        110        120
ccctgacccaggcctgccaggggacgagctgcctcgtgacgtggtgttggactggttca
   L  T  Q  A  C  Q  G  D  E  L  P  R  D  V  V  L  D  W  F  K
       130        140        150        160        170        180
aacagcgcctcctggatgggttagggctggagcagcccccgagcccagccaccaggcccc
   Q  R  L  L  D  G  L  G  L  E  Q  P  P  S  P  A  T  R  P  L
       190        200        210        220        230        240
tgacgggggcagagagagagcagaggcagggcgaggtcaccggagatccaccagggtag
   T  G  G  R  E  R  A  E  A  G  R  G  H  R  R  S  T  R  V  G
       250        260        270        280        290        300
ggaggacagcctgggcacaagaccacaggaggcatcaccaggagagccatgagcaagtca
   R  T  A  W  A  Q  D  H  R  R  H  H  Q  E  S  H  E  Q  V  I
       310        320        330        340        350        360
tcctcttccccagctctgactctacctgtgatagctcagactccccatcagagagagcga
   L  F  P  S  S  D  S  T  C  D  S  S  D  S  P  S  E  R  A  T
       370        380        390        400        410        420
ccagccacttcacctactacttccaatcctccctcgacaaccaggagtctgccattactt
   S  H  F  T  Y  Y  F  Q  S  S  L  D  N  Q  E  S  A  I  T  S
       430        440        450        460        470        480
cagccaatttctggttctatgccggcgaaggggccagcaggaacatcacccctctcttcc
   A  N  F  W  F  Y  A  G  E  G  A  S  R  N  I  T  P  L  F  L
       490        500        510        520        530        540
tcctcacttcagaccagcagctcctccaggtggcagagtttccggccaagaccaccgctg
   L  T  S  D  Q  Q  L  L  Q  V  A  E  F  P  A  K  T  T  A  D
       550        560        570        580        590        600
atggctggaccacctaccacttcgagcaccacctcctcagcgccctgacccaaggcccct
   G  W  T  T  Y  H  F  E  H  H  L  L  S  A  L  T  Q  G  P  F
        610        620        630        640        650        660
tcgtactccaggtgcgctgccccgcctgcgaatgccacgctaacgaagccgacaaaatgc
   V  L  Q  V  R  C  P  A  C  E  C  H  A  N  E  A  D  K  M  P
       670        680        690        700        710        720
cattcctccacctgcacacccgacctcacggcccagaccgctccccacggcgagcggccg
   F  L  H  L  H  T  R  P  H  G  P  D  R  S  P  R  R  A  A  A
       730        740        750        760        770        780
ccaccattccctggttcccatcgtccatcgacctcctgatgcggccatcgcagcagaagc
   T  I  P  W  F  P  S  S  I  D  L  L  M  R  P  S  Q  Q  K  P
       790        800        810        820        830        840
cagagtacagtgactgtcagcgggagatgatcaacatctcgttccaggagctgggctggg
   E  Y  S  D  C  Q  R  E  M  I  N  I  S  F  Q  E  L  G  W  D
       850        860        870        880        890        900
acaactggatcgttcacccgagttctttcatcttctactactgtcatggcacctgctcgg
   N  W  I  V  H  P  S  S  F  I  F  Y  Y  C  H  G  T  C  S  A
       910        920        930        940        950        960
ctttggaccgcaccactgctattctggggatcaaacagtgctgcgcccgggtccctggga
   L  D  R  T  T  A  I  L  G  I  K  Q  C  C  A  R  V  P  G  T
       970        980        990       1000       1010       1020
ccatgaggtcactacggtttaccactacgtctgacggagggtactcctttaaatacgaga
   M  R  S  L  R  F  T  T  T  S  D  G  G  Y  S  F  K  Y  E  T
      1030       1040       1050       1060       1070       1080
cccttcccaacatcataccagaggagtgcacctgtatctaggcctaaaacctctgagatg
   L  P  N  I  I  P  E  E  C  T  C  I
      1090       1100       1110       1120       1130       1140
ggctccgtttttccaagtaagattaatagttatgctaggtgtaatctaggccagggcaac
      1150       1160       1170       1180       1190       1200
tgtcggagaaaacctaggctagacctataactgataagcctgtatttgtcatgcaccaag
      1210       1220       1230       1240       1250       1260
attatagagcaaaataaagtttgttattgatacagctacacaagatgcattttttaatcaa
      1270       1280       1290       1300
aataatttaagaaactgaaaaaaaaaaaaaaaaaaa
```

FIG. 1. Nucleotide and amino acid sequences of the rainbow trout inhibin α chain.

Cloning of Inhibin cDNA

A rainbow trout ovary cDNA library was constructed using a cDNA synthesis kit (Amersham–Pharmacia, United States) with an oligo dT primer. The cDNA library was constructed in λ ZAPII vectors according to the instructions of the manufacturer (Stratagene, United States).

Two PCR primers were used to amplify a cDNA corresponding to part of the inhibin gene. A primer set for the conserved region of inhibin and the 3′-cDNA specific primer were 5′-GGNTGGGAYMRNTTGATHGT-3′ and 5′-TGGAAGAATTCGCGGCCGCAG-3′, respectively.

```
inh-RT         1 MQTGPSTVLSCA---------LLLLWIQTLTQACQGDELPRDVVLDWFKQRLLDGLG-LE   50
inh-chicken    1 .----------LLLLHLLPAV---.PASA.G-S.T.AGAD.QL.--LA.V.ARV-.EH.S   43
inh-human      1 ----MV-----LHL-L----LF...TP.GGH-S...L..A.EL.--LA.V.A-LF.DA.G   42
inh-bovine     1 ---MW------LQL-L----L..-.AP.GGH-G.H.L..D.EL.--LA.V.A-LF.DA.G   41
inh-mouse      1 .VSQR.-----L-L-LL---L-.-.TLRDVD-S...P..V.EL.--LA.VKA-LF.DA.G   44
                 .          . . .    . . *  .    .*.* .. *..*  ..*... ..*..*.

inh-RT        51 QPP--------SPATRPLTGGRERAEAGRGHRRSTRVGRTAWAQDHRRHHQ----ESHEQ   98
inh-chicken   44 P-.-----AMQE.QKD-V---------R.V...DVLED----G----GATE-EQEDT-S.   77
inh-human     43 P-.AVTREGG-D--PG-V---------R.LP..HAL-.----GFT..G-SEPEEE.DVS.   82
inh-bovine    42 P-.PVTGEGG-D--PG-V---------R.L...HAV-.----GFMR.G-SEPEDQD-VS.   80
inh-mouse     45 P-.AMDGEGG-D--PG-I---------R.LP..HAV-.----GFM..T-SEPEEED-VS.   83
                 . *    ... .  .. .          .*..**..  .    .. ... .... .. ..*

inh-RT        99 VILFPSSDSTCDSSDSPSE-------RATSHFTYYFQSSLDNQESAITSANFWFYA----  147
inh-chicken   78 ......T.VP.EPT-Q.D--KLLE-E--EGI...L..P.AHALSRTL...QL...S----  127
inh-human     83 A....AT.AS.--E.KSAARG-LAQEAEEGL.R.M.RP.QHTRSRQV...QL..HTGLDR  139
inh-bovine    81 A....AAGAS.--G.E.DA----G-EAEEGL...V..P.QHTRSRQV...QL..HTGLDR  133
inh-mouse     84 A....ATGA..--E.Q.AARG-LAQEAEEGL...V.RP.QHIRSHQV...QL..HTGLGR  140
                 .****.... *    . . .    .  ......*.* *..*.. .....***..**.... .

inh-RT       148 GEG-ASRNIT--PLF--L--LTSDQQLLQVAEFPAKTTADGWTTYHFEHHLLSALTQGPF  200
inh-chicken  128 .PS-.RP.HSA-.AVLT---.SPQGRV-P.VATASR.PE-H..VFD.GPDA.PQ.A.-.L  179
inh-human    140 Q-.T.AS-NSSE.-LLG.LA.SPGGPV-A.PMSLGHAPP-H.AVL.LATSA..L..H-.V  193
inh-bovine   134 Q-ET.AA-NSSE.-LLG.LV...GGPM-P.PMSLGQAPP-R.AVL.LATSAFPL..H-.V  187
inh-mouse    141 K-ST.AA-NSSA.-LLD.LV.S.GGPM-A.PVSLVQGPP-R.AVL.LAASAFPL..H-.I  194
                     .*.  ... * .. .. *.....   *. ..   ..  *...... .....*.. *

inh-RT       201 V-LQVRCPACECHANEADKMPFLHLH-TRPHGPDRSPR--RAAATIPW-F-PSSIDLLMR  254
inh-chicken  180 FV.L....G.P.L.DGD-.....-VAT..AKAA--G-.AR.S--AV..--S.GALS..Q.  230
inh-human    194 LV.LL...L.T.S.RPE-AT...-VAH..TRP.SGGE.AR.STPLMS.PWS..ALR..Q.  251
inh-bovine   188 LA.LL...L.S.STRPE-AT...-VAH..AKP.SGGE.AR.STPPL..PWS.AALR..Q.  245
inh-mouse    195 LV.LL...L.S.SGRPE-TT...-VAH..ARA.SAGE.AR.STPSV..PWS.AALR..Q.  252
                 ..*..***.* *.....  .*** ...**.  .. ..*..*...  .*...* ...**.*

inh-RT       255 PSQQKPEYSDCQREMINISFQELGWDNWIVHPSSFIFYYCHGT------CSA-------L  301
inh-chicken  231 ..EDVAAHTN.R.ASL....E.............V.H....NC--AEPD-GLSHRL-G-  285
inh-human    252 .PEEPAAHAN.H.VAL.........ER...Y.P....H....GCGLHIPP-NLSLPVPGA  310
inh-bovine   246 .PEEPAAHA..H.AAL..........R.....P.........GCGLS-PPQDLPLPVPGV  304
inh-mouse    253 .PEEPAAHAF.H.AAL..........R.....P....H....SCG--MPT.DLPLPVPGV  310
                 *........ *.*...****.****..***.*.**.*.**** ..    .    . .....

inh-RT       302 DRTTA----I----LGIKQCCARVPGTMRSLRFTTTSDGGYSFKYETLPNIIPEECTCI   352
inh-chicken  286 --------VQ--------L...AL........VR.............V...LAQD...V   328
inh-human    311 PP-.PAQPYS--LLP.AQP...AL.....P.HVR.............V..LLTQH.A..   366
inh-bovine   305 PP-.PVQPLS--LVP.AQP...AL.....P.HVR............MV..LLTQH.A..   360
inh-mouse    311 PP-.PVQ--PLFLVP.A.P...AL..S.T...VR............MV..L.TQH.A..   366
                 .. .. .      . ... .***..**.*..*...***********..**.....*.*.
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FIG. 2. Alignment of inhibin α amino acid sequences. The DDBJ/GenBank/EMBL accession numbers are as follows: chicken (I51215), human (A24248), mouse (I48243), and cow (A25732).

Amplification of cDNA was carried out for 1 min at 95°, 1 min at 50°, and 1 min at 72°; this cycle was repeated 30 times. The PCR product was sequenced and used to screen the cDNA library. Several cDNA clones were obtained. One of these clones was sequenced. The cDNA clone was sequenced using ThermoSequenase (Amer-

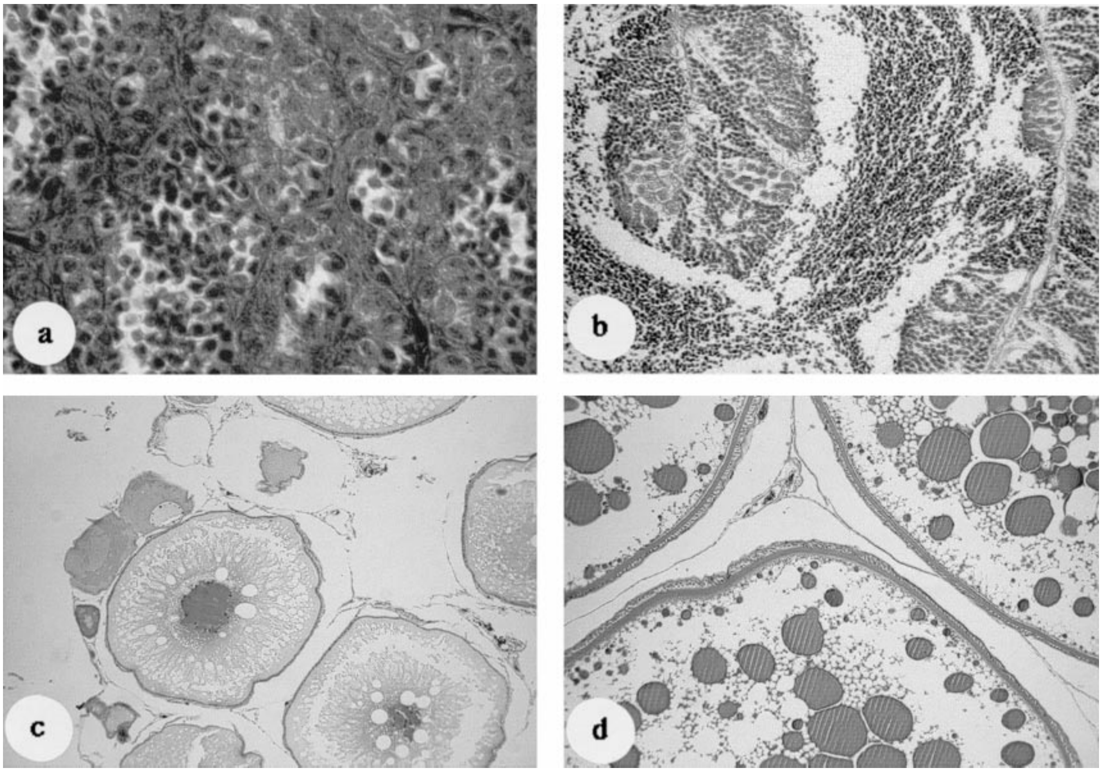

FIG. 3. Histological analysis of rainbow trout testes and ovary from different stages. (a) Seminal lobules in a testis of rainbow trout (*Oncorhynchus mykiss*) sampled in June. On the lobules, Sertoli cells encyst the aggregated spermatocytes, whereas Leydig cells are distributed in the interlobular spaces (×350, H–E stain). (b) Seminal lobules in a testis sampled in October. Large numbers of spermatids occur in the lobular lumens, whereas the lobular walls have many unencysted spermatocytes. The Sertoli cells are no longer detectable among the spermatocytes (×240, H–E stain). (c) An ovary sampled in March. It was occupied by many oocytes having cytoplasmic yolk vesicles and a few oocytes with perinucleoli (×50, H–E stain). (d) An ovary sampled in July. The thick follicles enwrap oocytes that have many yolk globules in their cytoplasm. (×50, H–E stain).

sham–Pharmacia) and an automated DNA sequencer LC4200 (Li-Cor, United States).

RNA Probes

Digoxigenin-labeled sense and antisense RNA probes specific for inhibin α and activin β A and β B (DDBJ/GenBank/EMBL accession Nos. D88462, D88463, and D88464) were generated with a T7 and SP6 Dig RNA labeling kit (Boehringer Mannheim, Germany) with digoxigenin–UTP (Boehringer Mannheim). The inhibin α RNA probe complementary to the 411 nucleotides corresponded to the 3′ noncoding region and coding region (Fig. 1). The activin β A and β B mRNA probes were 310 and 150 nucleotides in length, respectively (Tada *et al.*, 1998). These three regions had no significant identity to each other and to other known genes in GenBank.

In Situ Hybridization

In situ hybridization was carried out using a commercial kit (Nippon Gene, Japan). In brief, a section was de-paraffinized and pretreated with proteinase K (5 μg/ml) for 15 min at 37° for protein removal. The reaction was stopped by washing in glycine–PBS buffer (2 mg/ml) for 10 min. For acetylation, the treated sections were dipped in 100 mM triethylamine for 15 min and then immersed in anhydrous acetic acid for 20 min. The acetylated section was prehybridized in 50% formamide with 4 × SSC at 42° for 30 min. For hybridization, the section was covered with 100 μl of antisense mRNA probe solution (1 μg/ml) and then reacted in a moist chamber at 42° for 16 h. For the negative control, the antisense mRNA probe was replaced by the sense RNA probe. After the hybridization, the section was kept in RNase–NTE buffer (20

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Inhibin-α(411 bp)
5′-TTTCATCTTCTACTACTGTCATGGCACCTGCTCGGCTTTGGACCGCACCACTGCTATTC
TGGGGATCAAACAGTGCTGCGCCCGGGTCCCTGGGACCATGAGGTCACTACGGTTTACCACT
ACGTCTGACGGAGGGTACTCCTTTAAATACGAGACCCTTCCCAACATCATACCAGAGGAGTG
CACCTGTATCTAGGCCTAAAACCTCTGAGATGGGCTCCGTTTTTCCAAGTAAGATTAATAGT
TATGCTGGTGTAATCTAGGCCAGGGCAACTGTCGGAGAAAACCTAGGCTAGACCTATAACTG
ATAAGCCTGTATTTGTCATGCACCAAGATTATAGAGCAAAATAAAGTTTGTTATTGATACAG
CTACACAAGATGCATTTTTAATCAAAATAATTTAAGAAACTG-3′

Activin-βA(310 bp)
5′-CACATGCATACACAGCTTCATTCATTCACACATATCCTACACACTCATTCTCACATATG
CCTGAACAAACACTTGTATATATATTTATGTTTGTTAATAATATTCTCTGGTAGAAACCTTT
TTTTCTTTTGCCAACATATGAACATGTGATTCAATCCCATTTTTTTGTGAACATGTGTTTGC
AAATTCATGTAGGTGTCATTATCAGAGGGATTGGAACCAAATGGTACTTATCGCATTATATC
TTTTCCAAGATTGCCATTTCTATTCTGAAAATGGAGAGTTCTATTTCGATACTCTGACGAAG
GTG-3′

Activin-βB(150 bp)
5′-GAGACTGACTGACCTGCCCGTTTTGTCGGATTACCCCGAGGGGGAAATTTGGGAAGTCT
CTTTTCCGAGATTGGAAATTGGAAGTCTCTTTTCACAGATTAAAAAAAAAGGAGACTACAGA
ATATATTTGTGTGATGGGAGAGCAAGAGG-3′
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FIG. 4. Nucleotide sequences of inhibin α and activin β A and β B cDNA that were used as probes for *in situ* hybridization.

μg/ml) at 37° for 30 min. Detection using the mRNA probe was carried out with a Dig nucleic acid detection kit (Boehringer Mannheim) as described in the technical manual. After rinsing with distilled water, the section was stained with hematoxylin and observed with a light microscope.

RESULTS AND DISCUSSION

The inhibin cDNA consisted of 1305 bp, which encoded 352 amino acid residues (Fig. 1). The DDBJ/EMBL/GenBank accession number of rainbow trout inhibin cDNA is AB044566. The deduced amino acid sequence is aligned with the sequences of other inhibins in Fig. 2. The amino acid sequence identities of the whole region of inhibin among rainbow trout, chicken, and mammals ranged from 35 to 43%. The identities of the mature regions of these proteins were higher than the identities of their whole sequences and were 50 to 60%. The positions of cysteine residues and the N-glycosylation site of the mature protein region were well conserved in rainbow trout inhibin when compared to previously reported inhibin sequences (Fig. 2). These results indicate that the cloned cDNA from the rainbow trout ovary cDNA library encodes inhibin.

In testes sampled from March to April, the seminal lobules appeared to contain only spermatogonia. The lobular walls were divided into many cysts, with each cyst containing some spermatogonia. Sertoli cells were distributed around the cysts. Spermatocytes could be seen in some of the seminal lobular walls from May onward. From July to September, the seminal lobules showed progressive enlargement with increased numbers of spermatocytes on their lobular walls (Fig. 3a). The spermatocytes were present as aggregations, which were encysted by Sertoli cells. Testes sampled in October included large numbers of spermatids in the lobular lumens and spermatocytes on the lobular walls (Fig. 3b). Most of the spermatocytes were reduced in size and showed a scattered distribution on the lobular walls, whereas the Sertoli cells were not present among the scattering of spermatocytes. In November and December, the lumens of all the seminal lobules were filled with a great number of spermatids and/or sperm. The lobular walls at this time had completely lost the spermatocytes. Leydig cells were located in the interlobular interstitial tissues throughout the sampling period.

In ovaries sampled from March to May, most of the oocytes were at the yolk vesicle stage (Fig. 3c), distributed on the ovarian lamellae with small numbers of perinucleoli stage oocytes. Oocytes with cytoplasmic yolk globules progressively increased in size and number from June to August. These were surrounded by thick follicles, consisting of an outer theca cell layer and an inner granulosa cell layer (Fig. 3d). An ovary sampled in June possessed regressive oocytes, whose cytoplasms were invaded by many macrophages and which were enveloped by very thick follicles. In September and October, very large maturation-phase oocytes (migratory nucleus stage to maturation stage) occupied the largest proportion in the ovarian components. Their cytoplasm contained many large yolk globules and oil droplets. Well-developed follicles enwrapped the oocytes. Ovaries sampled in November had a spawning appearance, consisting of the complete loss of maturation-phase oocytes and thickened interstitial connective tissues. In December, small immature-phase oocytes with perinucleoli or cytoplasmic yolk vesicles were again present in the ovaries.

Recently, several types of activin/inhibin families of mammals and *Xenopus laevis* have been cloned and characterized (Oda *et al.*, 1995; Hötten *et al.*, 1995; Lau *et al.*, 1996; Fang *et al.*, 1997). The coding regions of these genes have a significant homology in amino acid and nucleotide sequences. However, there is no significant homology in the 3′ noncoding region of activin/inhibin family genes. The 3′ noncoding region of

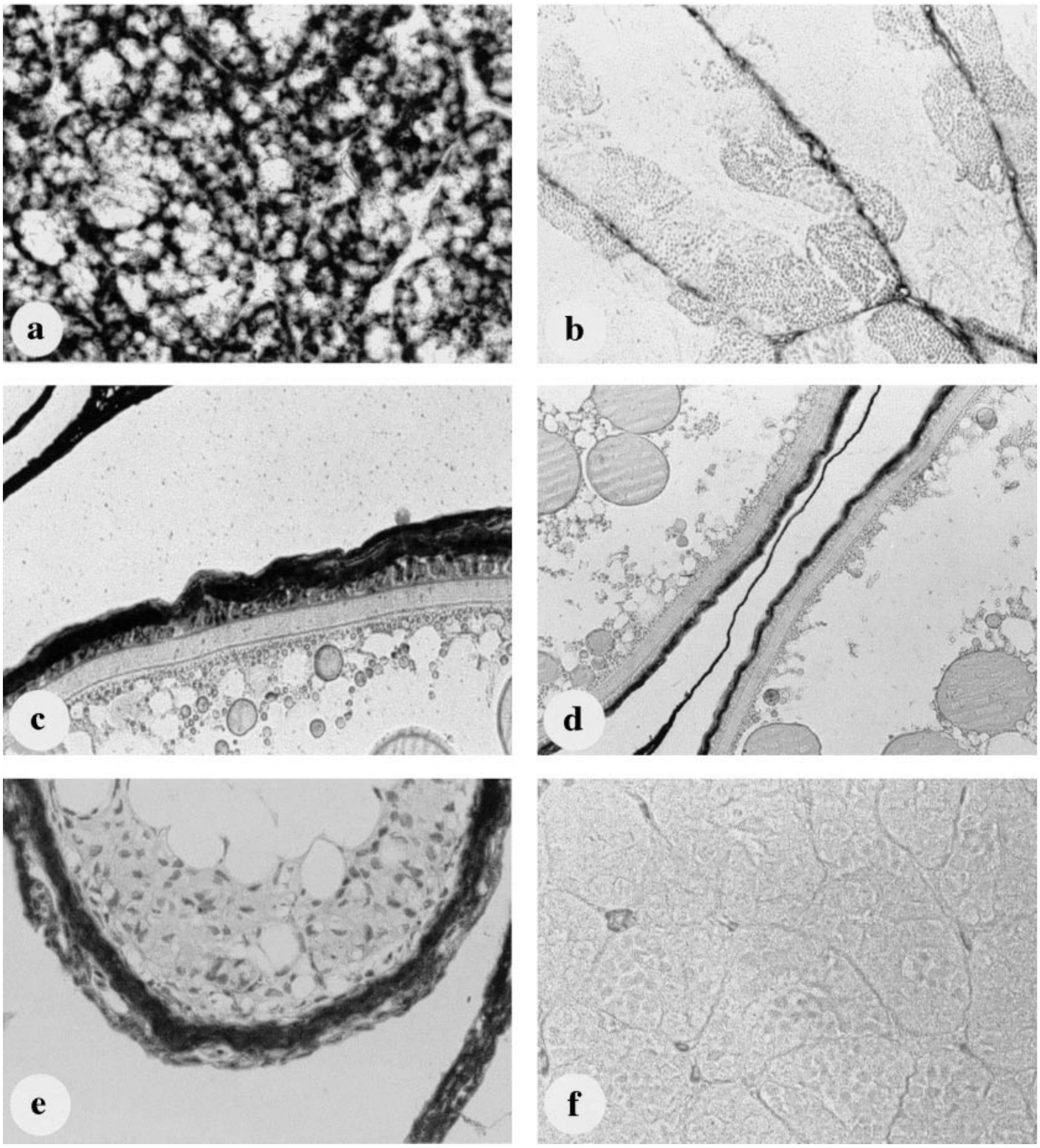

FIG. 5. Distribution of rainbow trout inhibin and activins in testis and ovary. (a) *In situ* hybridization of the α-unit in a testis sampled in June. Positive reactions are visible in the Sertoli cells around the spermatocytic aggregations and in the Leydig cells in the interlobular tissues (×220). (b) *In situ* hybridization of the β B-subunit in a testis sampled in October. Leydig cells of interlobular tissues show a positive reaction (×120). (c) *In situ* hybridization of the β A-subunit in an ovary sampled in September. The theca cell layer shows a positive reaction (×400, counterstained by hematoxylin). (d) *In situ* hybridization of the β B-subunit in an ovary sampled in June. A positive reaction is visible in the theca cell layers. The connective tissue between the oocytes shows a nonspecific reaction (×250, counterstained by hematoxylin). (e) *In situ* hybridization of the α-unit in a regressing oocyte of an ovary sampled in June. A positive reaction is visible in the granulosa cell layer. Many macrophages can be seen invading the cytoplasm (×350, counterstained by hematoxylin). (f) Negative controls of the inhibin α, activin β A, and activin β B in a testis sampled in April, using the sense probes for these mRNAs. The interlobular vessels show a faint nonspecific reaction (×220).

rainbow trout activin β A and β B and inhibin also have no significant identity to each other (Fig. 4). Based on this result, we used the 3′ noncoding region as probes for *in situ* hybridization. Based on the cell localization of positive reactions of the antisense mRNA probes, there were no differences among the

α-unit, β A-subunit, and β B-subunit. In the testes, when the spermatogonia or spermatocytes were forming aggregations on the lobular walls, positive reactions were seen in the Sertoli cells of lobular walls and Leydig cells of the interlobular tissues (Fig. 5a). After the spermatocytes were scattered, only the Leydig cells showed positive reactions (Fig. 5b). The localizations of rainbow trout mRNAs are the same as those reported for rat and newt testes (Kaipia *et al.*, 1992; Yamamoto *et al.*, 1996). In this study, the amount of mRNA expressed at different stages of testis development was not analyzed. In rat, the expressions of inhibin α and activin β A and β B mRNAs have been reported to be stage dependent (Bhasin *et al.*, 1989; Kaipia *et al.*, 1991, 1992), suggesting that production of inhibin and activin in the adult testis may be regulated by germ cell development in a paracrine manner. Further studies are needed to determine the stage-dependent expression of these three genes in rainbow trout.

Theca cell layers of ovarian follicles showed positive reactions in all of the ovaries sampled every month (Figs. 5c and 5d), excluding regressing oocytes. In the regressing oocytes found in the July sample, a positive reaction was observed in the granulosa cell layer of a follicle (Fig. 5e). There was no reaction in the negative controls of testes or ovaries (Fig. 5f), although the interlobular vessels showed a faint nonspecific reaction. The localization of rainbow trout inhibin α and activin β A and β B mRNAs is distinct from that of human. The human inhibin α and activin β A and β B mRNAs are expressed in the normal ovary granulosa cell layer, and inhibin α and activin β A mRNAs are expressed in the human normal theca cell layer (Jaatinen *et al.*, 1994). We previously reported that, between the two rainbow trout activins, the β B gene had a high level of expression and the β A had a relatively low level of expression (Tada *et al.*, 1998). This result was contrary to a report that, in mammals, the expression of β A is higher than the expression of β B (Meunier *et al.*, 1988). The differences in localization of expression of inhibin α and activin β A and β B mRNAs between rainbow trout and human, and the high expression of β B in rainbow trout, may be due to differences in the functions and the mechanisms of action of these proteins. To better understand these differences, further investigations of inhibin α and activin β A and β B in rainbow trout and other fish are needed.

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