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Expression of an Intracisternal A-Particle-like Element in Rat Ovary

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We have isolated a rat intracisternal-A particle element (IAP)-like element (IAP-LE) from ovarian granulosa cells that appears to be identical to the rat EST clone AA964260. The compiled cDNA sequences contain several putative in-frame translation initiation codons with the largest capable of encoding a 365 amino acid protein with a reverse transcriptase domain in the N-terminus as well as a bipartite nuclear localization signal sequence in the middle. Northern blotting shows a major ~7 Kb transcript and a minor ~5 Kb transcript that are abundantly expressed in the ovary. In situ hybridization histochemistry using ovaries from gonadotropin-treated immature rats and regularly cycling adult rats show that this transcript is predominantly localized to granulosa cells of all healthy follicles, including primary follicles, and to newly-formed and healthy corpora lutea. This cell-specific expression pattern of the IAP-LE gene is distinct from those of the several known retroviral elements, suggesting the potentially novel functional importance of the IAP-LE gene. Taken together, our results demonstrate abundant and cell-specific expression of a novel IAP-LE in rat granulosa cells.

Endogenously expressed, highly-repetitive retroviral-like sequences have gained substantial interests during the last decade due to their limited expression in specific cell types as well as their potential to affect the hormone-induced expression of nearby genes, cellular transformation, and oncogenesis (1–4). Included among these retroviral sequences are intracisternal A-particle (IAP) elements that are present at a high number of copies per cell in mammalian species (5) including the human (6), rat (7), mouse (8), and hamster (9). However, unlike other viruses, IAPs are defective in horizontal transmission from one cell to another due to impairment and/or absence of the envelope protein. In contrast, their intact reverse transcriptase/integrase activity allows proviral integration and thus they are implicated as retroposons (10). IAP retrotranspositions in proto-oncogenes such as cytokines, c-mos, growth factors and receptors have been suspected to play a significant role in altering cellular function from gene expression to transformation (11–16). In addition, IAP is expressed during the early G1 phase of the cell cycle (17) and in turn, high expression of IAPs has been positively correlated to the preneoplastic stage of tumors.

Ovarian follicles continue to grow, differentiate, and transform throughout adult life. Granulosa cells are recruited from their G0 dormant status in primordial follicles, undergo mitosis in a hormone-responsive manner, and transform to luteal cells upon ovulation (18–20). The ovary has been reported to express two endogenously expressed retroviral-like genes in a cell-specific manner (21–22). Although the functional significance of these endogenously expressed retroviral-like genes in granulosa cells is unknown, they are speculated to function as retroposons to alter specific genes and to be potentially involved in ovarian tumorigenesis. We report here the cloning of a cDNA that is highly homologous to the rat IAP and is highly expressed in a cell-specific manner during follicular growth, differentiation, and transformation.

MATERIALS AND METHODS

Materials

Unless specifically stated, all molecular biological enzymes were purchased from New England Biolabs (Beverly, MA). All radioisotopes and olignonucleotides were obtained from New England Nuclear (Boston, MA) and Integrated DNA Technology Inc. (Coralville, IA), respectively.

Animals

All rats were housed in a photoperiod of 14 h light/10 h darkness with light on at 0500 h and handled according to the NIH guidelines for care and use of animals and University of Kentucky Institutional Animal Care and Use Committee.

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Superovulation immature rat model. Twenty-one-day-old Sprague-Dawley female pups with nursing mothers were purchased from Harlan Breeding Company (Indianapolis, IN). At 22 or 23 days of age, rats were injected s.c. with pregnant mare's serum gonadotropin (PMSG, 10 IU, Sigma). Forty-eight hours later, rats were injected s.c. with human chorionic gonadotropin (hCG, 10 IU, Sigma).

Adult cycling rat model. Adult virgin female Sprague-Dawley rat (150–180 g body weight) were purchased from Charles River Breeding Company (Wilmington, MA). Estrous cyclic stages were determined by daily vaginal smears, and only rats demonstrating minimally two consecutive 4-day cycles were used for the experiments. Rats were killed by decapitation at 1000 h of estrus, metestrus, diestrus, and proestrus and at 1400 h, 1600 h, 1800 h, 2000 h, and 2200 h of proestrus. Serum LH concentrations in trunk blood as determined by RIA indicate the peak of the LH surge at 1800 h.

Cloning and Sequencing of the IAP-LE cDNAs

A partial PCR clone OKPS#108 (referred as IAP-LE hereafter) with a 588-bp insert was originally obtained during the course of subtraction cloning procedure that was previously reported (23). DNA sequences of the clone IAP-LE were determined with a Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham, OH) using M13 forward and backward primers. Further 5′- and 3′-sequences of the IAP-LE mRNA were obtained using commercially available kits for 5′-RACE (BRL) and 3′-Genewalk (Clonetech) according to the manufacturer's procedures. The fully combined sequences reported here have been confirmed using RT-PCR cloning of the IAP-LE transcript.

Genomic Southern Blotting (24–25)

Rat testis genomic DNA was digested with *Bam*HI, *Eco*RI, or *Hin*dIII, separated on 0.8% agarose gel, transferred onto a nytran membrane (Schleicher & Schleicher), and baked at 80°C for 2 h. The blots were hybridized with [α-^{32}P]dCTP-labeled probes generated from the clone IAP-LE insert at 42°C in a solution consisting of 50% fomamide, 5× SSPE, 2× Denhardt's reagent, 10% dextran sulfate, 0.1% SDS, and 100 μg/ml salmon sperm DNA. After washing to the stringency of 0.1× SSC plus 0.1% SDS at 68°C, blots were exposed to a phosphorscreen for overnight.

Northern RNA Blotting (24–25)

RNA was prepared from the indicated tissues by homogenization in guanidine isothiocynate and centrifugation through cesium chloride. Approximately 40 μg of each was separated by electrophoresis on denaturing 1% agarose/formaldehyde gels. RNA was transferred to a nytran membrane, vacuum-baked, and detected by hybridization to the insert from the IAP-LE that had been labeled with [^{32}P]dCTP using random hexamer primers and the Klenow fragment of *Escherichia coli* DNA polymerase. Hybridization was performed at 42°C in 50% formamide, 5× SSPE, 2× Denhardt's reagent, 10% dextran sulfate, 0.1% SDS, and 100 μg/ml salmon sperm DNA. The membranes were subsequently washed in 0.1× SSC plus 0.1% SDS at 68°C and exposed to a phosphorscreen. After removal of probe in 20% SDS at 65°C, the membranes were re-hybridized to the cDNA clone CHO-B (26), which detects the LLRep3 gene family, to assess the amount of RNA present in each lane.

In Situ Hybridization

Twenty-micrometer cryosections of frozen ovaries collected from animals stated above were mounted onto superfrost/plus microscope slides (Fisher, PA). Sections were fixed, pretreated, and hybridized with antisense and sense RNA probes. [^{35}S]UTP-labeled RNA probes were synthesized off the PCR-generated DNA templates to encode the IAP-LE clone (this study), LH-receptor (27), inhibin-α (28), or VL-30 (29). PCR clones were sequenced to confirm their identities. RNA probes (1×10^7 cpm/ml in hybridization buffer: 50% formamide, 5× SSPE, 2× Denhardt's reagent, 10% dextran sulfate, 0.1% SDS, 500 μg/ml yeast RNA, and 500 μg/ml polyA) were applied to sections and the sections were incubated in a humidity chamber at 47°C for 16–18 h. After hybridization, sections were treated with RNAse A (20 μg/ml) at 37°C for 45 min, washed in increasingly lower concentrations of SSC down to 0.1× SSC at 65°C, and dehydrated through an ethanol series. Slides were then exposed to Kodak XAR-5 film for 2 days and subsequently to a phosphorimager screen, and processed for liquid emulsion autoradiography using NTB-2 emulsion (Kodak, Rochester, NY) for 2 weeks. Developed sections were stained using hematoxylin and photographed.

RESULTS

During the course of a PCR-based subtraction cloning approach that we used to identify poly A-tailed RNA species enriched in rat granulosa cells in response to forskolin plus progesterone (23), we isolated a clone OKPS#108 with a 588 bp cDNA insert. A BLAST search showed that the 522-bp portion of OKPS#108 cDNA matched nearly perfectly (2-bp mismatch) with the corresponding region of the rat EST clone AA964260, indicating that these two cDNAs are derived from the same gene (Fig. 1). This cDNA displayed high homology to the rat IAP nucleotide sequences (U23776). Further upstream and downstream sequences were obtained using rapid amplification of cDNA ends and genewalk approaches based upon the known gene structure of the IAP family. The 1351-bp sequences compiled from these approaches were aligned with the corresponding region of the rat IAP sequences (U23776) (Fig. 2). Because of the diverse homology throughout the sequences, alignment was made in three different parts of the OKPS#108 (21% homology from nucleotide 1665 to nucleotide 1880, 75% homology from nucleotide 1881 to nucleotide 2066, and 87% homology from nucleotide 2067 to nucleotide 2991). The 5′-end of the IAP-LE sequence that is not homologous to the rat IAP indeed showed the sequence homology to the mouse IAP element reported in mouse chromosome sequences (AC006508). Thus, we conclude that the IAP-LE cDNA is highly likely a variant of the rat IAP family and designated the clone OKPS#108 as the IAP-like element (IAP-LE). The 1351-bp IAP-LE sequence contains several putative open-reading frames with the largest capable of encoding a 365 amino acid protein (Fig. 3). The second largest open-reading frame is directed by a Kozak consensus translation initiation codon (the critical nucleotides are indicated as black diamonds), suggesting a possibility that this may be in use. The putative protein contains a reverse transcriptase domain in the N-terminus (the bolded amino acids) as well as a bipartite nuclear localization signal sequence in the middle (the boxed amino acids). When the putative protein sequences were analyzed against the nr data base using the

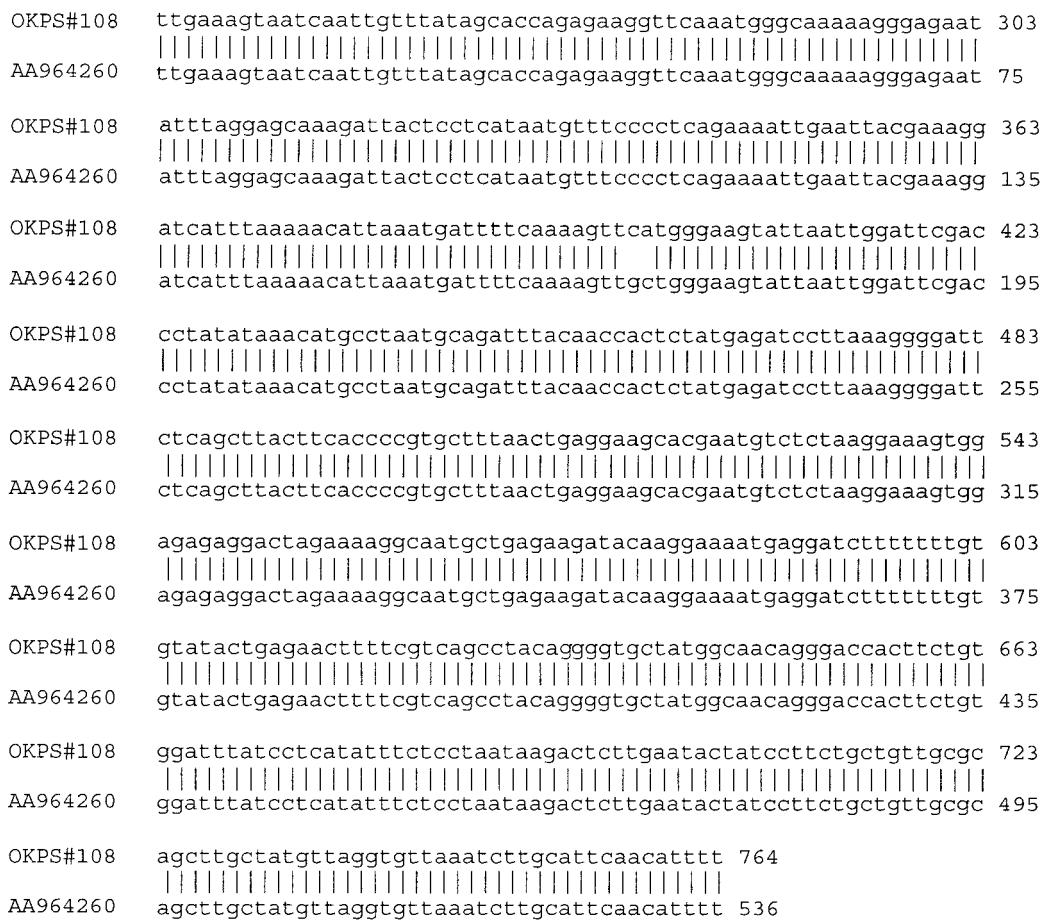
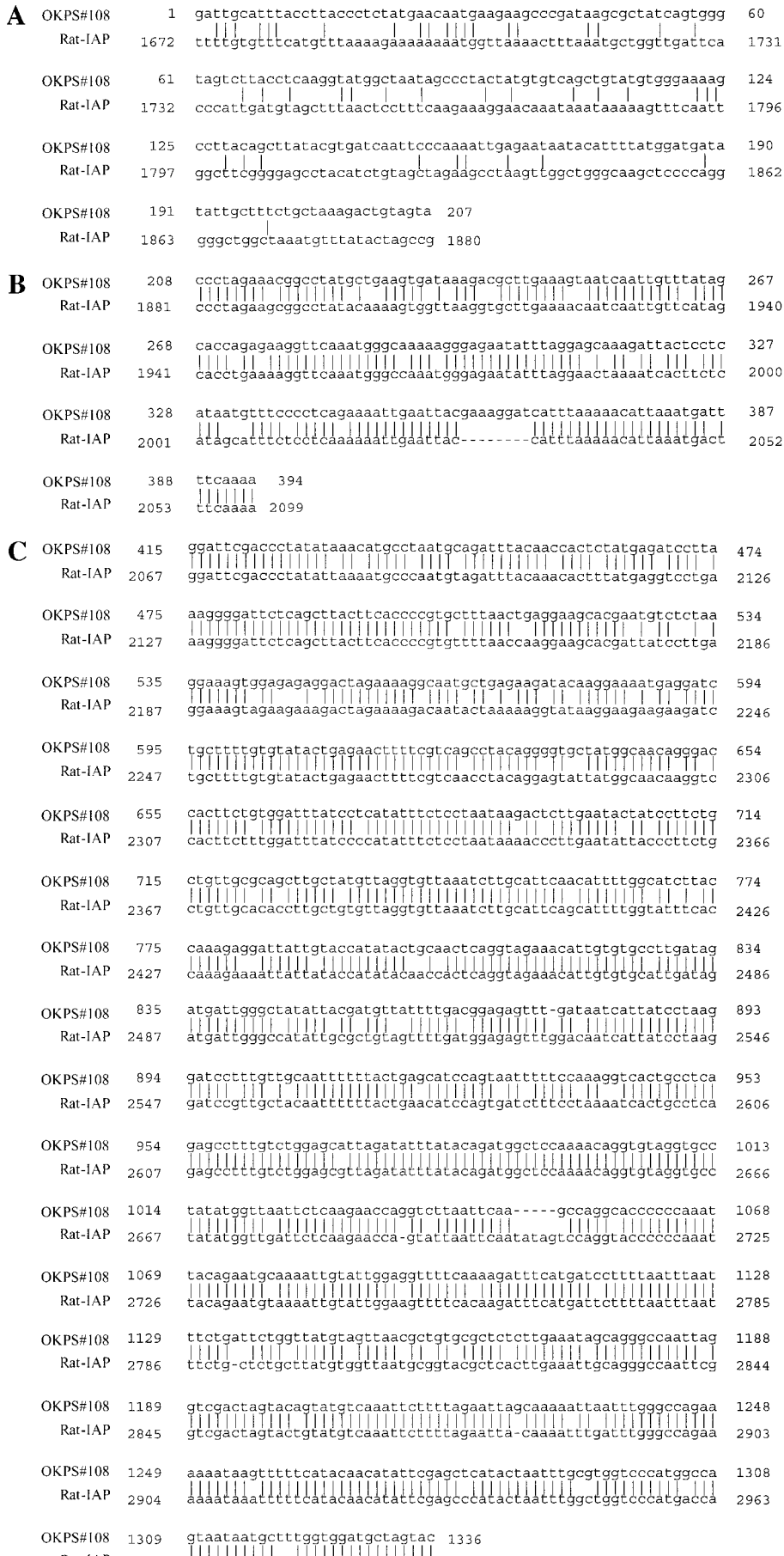


FIG. 1. The clone OKPS#108 is identical to the rat EST clone AA964260. BLAST search using the 588 bp of the clone OKPS#108 cDNA against the est data base identified the rat clone AA964260 that was generated from ovarian RNA. The alignment between these sequences was generated using the NCI BLAST program. The nucleotide number designated for the clone OKPS#108 is derived from the compiled sequences shown in Fig. 3.

BLAST-P program, the probable pol polyproteins derived from the rat, mouse, and hamster IAP sequences were identified with the highest identities ranging from 44 to 48%. Additional proteins that showed high identity (over 40%) include Simian SRV-2 retrovirus and ovine pulmonary adenocarinoma virus. Thus, the IAP-LE may encode an endogenously expressed nuclear retroviral polymerase-like protein.

To gain insight into the pattern of endogenous expression of the IAP-LE gene, we performed genomic Southern blotting using the probe against the IAP-LE cDNA (Fig. 4A) and Northern blotting (Fig. 4B). Results show that the IAP-LE gene is repetitive in good agreement with the characteristics of the IAP family (5) and is highly expressed as a major ~7 kb transcript and a minor ~5 Kb transcript in gonadal tissues. We next examined its mRNA expression at the cellular level in ovaries of immature female rats treated with exogenous gonadotropins (Fig. 5A) and adult cycling rats (Fig. 5B). The specificity of the IAP-LE signal was determined by comparison between the antisense and sense probes. The IAP-LE antisense probe intensely hybridized to granulose cells of follicular structures including corpora lutea whereas the IAP-LE sense strand did not hybridize. In addition, the IAP-LE antisense signal was cell-specific. Because inhibin-α mRNA expression profiles have been well established (28), we used the inhibin-α probe as a control. As ex-

FIG. 2. The clone OKPS#108 is highly homologus to the rat intracisternal A-particle (IAP) element (U23776). The alignment between these sequences were generated using the NCI BLAST program. The nucleotide number designated for the clone OKPS#108 is derived from the compiled sequences shown in Fig. 3. (A) The clone OKPS#108 (1–207) shows 21% identity to the rat IAP (1665–1880). (B) The clone OKPS#108 (208–394) shows 75% identity to the rat IAP (1881–2066). (C) The clone OKPS#108 (415–1336) shows 87% identity to the rat IAP (2067–2991).


A
OKPS#108 1 gattgcatttaccttaccctctatgaacaatgaagaagcccgataagcgctatcagtggg 60
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OKPS#108 268 caccagagaaggttcaaatgggcaaaaagggagaatatttaggagcaaagattactcctc 327
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OKPS#108 328 ataatgtttcccctcagaaaattgaattacgaaaggatcatttaaaaacattaaatgatt 387
Rat-IAP 2001 atagcatttctcctcaaaaaattgaattac---------catttaaaaacattaaatgact 2052
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Rat-IAP 2053 ttcaaaa 2099
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OKPS#108 1309 gtaataatgctttggtggatgctagtac 1336
Rat-IAP 2964 gtaataatgccctggtggatgctagtac 2291

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   1 - GATTGCATTTACCTTACCCTCTATGAACAATGAAGAAGCCCGATAAGCGCTATCAGTGG - 59
                          ♦     ♦        M  K  K  P  D  K  R  Y  Q  W
  60 - GTAGTCTTACCTCAAGGTATGGCTAATAGCCCTACTATGTGTCAGCTGTATGTGGGAAAA - 119
       V  V  L  P  Q  G  M  A  N  S  P  T  M  C  Q  L  Y  V  G  K
 120 - GCCTTACAGCTTATACGTGATCAATTCCCAAAATTGAGAATAATACATTTTATGGATGAT - 179
       A  L  Q  L  I  R  D  Q  F  P  K  L  R  I  I  H  F  M  D  D
 180 - ATATTGCTTTCTGCTAAAGACTGTAGTACCCTAGAAACGGCCTATGCTGAAGTGATAAAG - 239
       I  L  L  S  A  K  D  C  S  T  L  E  T  A  Y  A  E  V  I  K
 240 - ACGCTTGAAAGTAATCAATTGTTTATAGCACCAGAGAAGGTTCAAATGGGCAAAAAGGGA - 299
       T  L  E  S  N  Q  L  F  I  A  P  E  K  V  Q  M  G  K  K  G
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       E  Y  L  G  A  K  I  T  P  H  N  V  S  P  Q  K  I  E  L  R
 360 - AAGGATCATTTAAAAACATTAAATGATTTTCAAAAGTTCATGGGAAGTATTAATTGGATT - 419
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       V  E  R  G  L  E  K  A  M  L  R  R  Y  K  E  N  E  D  L  F
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       L  C  I  L  R  T  F  R  Q  P  T  G  V  L  W  Q  Q  G  P  L
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       R  I  I  V  P  Y  T  A  T  Q  V  E  T  L  C  A  L  I  D  D
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 960 - TTGTCTGGAGCATTAGATATTTATACAGATGGCTCCAAAACAGGTGTAGGTGCCTATATG - 1019
       L  S  G  A  L  D  I  Y  T  D  G  S  K  T  G  V  G  A  Y  M
1020 - GTTAATTCTCAAGAACCAGGTCTTAATTCAAGCCAGGCACCCCCCAAATTACAGAATGCA - 1079
       V  N  S  Q  E  P  G  L  N  S  S  Q  A  P  P  K  L  Q  N  A
1080 - AAATTGTATTGGAGGTTTTCAAAAGATTTCATGATCCTTTTAATTTAATTTCTGATTCTG - 1139
       K  L  Y  W  R  F  S  K  D  F  M  I  L  L  I  *
1140 - GTTATGTAGTTAACGCTGTGCGCTCTCTTGAAATAGCAGGGCCAATTAGGTCGACTAGTA - 1199

1200 - CAGTATGTCAAATTCTTTTAGAATTAGCAAAAATTAATTTGGGCCAGAAAAAATAAGTTT - 1259

1260 - TTCATACAACATATTCGAGCTCATACTAATTTGCGTGGTCCCATGGCCAGTAATAATGCT - 1319

1320 - TTGGTGGATGCTAGTACCAGCCCGGGCCATCG - 1351
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FIG. 3. Nucleotide seqeuences of the rat IAP-like element (IAP-LE). The overlapping sequences spanning 1351 bp contains several putative open-reading frames with the largest capable of encoding a 365 amino acid protein. The second largest open-reading frame is directed by a Kozak consensus translation initiation codon, suggesting a possibility that this may be in use. The putative protein contains a reverse transcriptase domain (bold amino acids) in the N-terminus as well as a bipartite nuclear localization signal sequence (boxed amino acids) in the middle. Thus, the clone OKPS#108 may encode an endogenously expressed nuclear retroviral polymerase-like protein.

pected, granulosa cells of antral follicles express inhibin-α mRNA. The granulose cell layers of the same antral follicles also express IAP-LE mRNA. The intensity of both inhibin-α and IAP-LE signals dramatically decrease in atretic and degerating follicles as opposed to healthy follicles. Interestingly, little or undetectable expression of both the inhibin-α and IAP-LE genes is seen in theca-interstitial cells, cumulus cells, and oocytes. In contrast to inhibin-α mRNA expression profiles in the rat ovary (28), granulosa cells of preantral

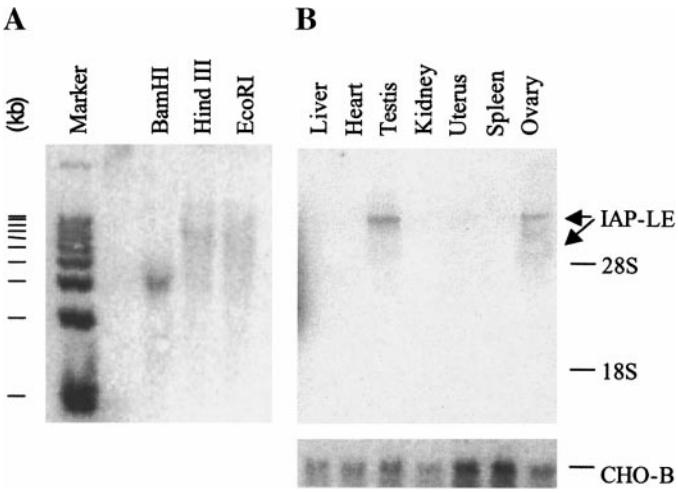

FIG. 4. The IAP-LE gene is expressed in ovary. The characteristics of the IAP-LE gene were examined by genomic DNA Southern blotting (A) and tissue RNA northern blotting (B) using the probe against the portion of the IAP-LE (nucleotides 243–764 of Fig. 3). The enzymes used in digesting rat testis DNA are indicated along with 1 kb size DNA size marker in A. Multiple hybridized bands are prominent in samples digested with *Hin*dIII or *Eco*RI. Analyses using total RNA samples isolated from various tissues of lactating rats are shown in B. Sample sources are indicated along with the migration of 28S and 18S ribosomal RNA. The same blot was stripped and reprobed for CHO-B mRNA as an internal control.

follicles, including primary follicles, highly express IAP-LE mRNA. Interestingly, Following ovulation in gonadotropin-stimulated immature rats as well as in regularly cycling rats, the IAP-LE mRNA signal in granulosa cells is maintained and substantially increased in newly formed corpora lutea. In order to determine the specificity of IAP-LE mRNA expression in preantral follicles, the pattern of IAP-LE mRNA expression was compared to inhibin-α, LH-receptor, and VL-30 transcripts using adjacent sections of immature rat ovaries (Fig. 6A). IAP-LE mRNA is solely expressed in granulose cells of primary and secondary follicles, which do not express inhibin-α. These follicles express LH-receptor mRNA and low VL-30 mRNA in theca-interstitial cells. In contrast, granulose cells of antral follicles highly express both IAP-LE mRNA and inhbin-α mRNA. We also compared IAP-LE mRNA expression in newly-formed corpora lutea with VL-30 mRNA expression using a superovulation model (immature rats primed with PMSG for 48 h followed by hCG for 48 h) (Fig. 6B). Newly-formed corpora lutea highly expressed both IAP-LE and VL-30 mRNAs. Within the same section, small healthy antral follicles clearly show intense expression of the IAP-LE, but not VL-30, gene. In addition, degenerating follicles show weak expression of the IAP-LE gene.

In summary, our results demonstrate that the IAP-LE gene is expressed in the rat ovary in a cell-specific manner, that the IAP-LE gene is highly expressed in granulosa cells of primary, secondary, tertiary, and preovulatory follicles as well as newly formed corpora lutea, that IAP-LE mRNA expression is distinct from another retroviral-like VL-30 gene.

DISCUSSION

In this study, we have shown that the IAP-LE gene is expressed in gonadal tissues. Our Northern blotting results show that the IAP-LE gene is expressed as a ~7-kb major transcript and a ~5-kb minor transcript, indicating the existence of the upstream and/or downstream sequences of our compiled ~1.4-kb sequences. Although similar rat IAP transcripts have been detected in placenta using the mouse IAP sequences 3′ to the pol region (29), the same probe failed to detect any transcripts in ovary or testis (29). Our probe against the IAP-LE cDNA detected positive signals from all steroidogenic tissues examined, including ovary, testis, adrenal gland and placenta (data not shown). This discrepancy may be accounted for the differences in nucleotide sequences of the previously reported rat IAP family members (5, 7, 29) and the IAP-LE gene. It is possible but doubtful that the IAP-LE probe failed to detect IAP sequences since their sequences are highly homologous. It is then probable that the IAP-LE gene is a variant of the rat IAP family with dissimilar sequences in the env region of the IAP sequences that was used in detecting IAP-related sequences in placenta but not in ovary or testis (29). There exist multiple splice variants of the IAP sequences, several of which have been fused, via retrosplicing events to other sequences and thereby influencing the transcription of neighboring genes (30–31). Thus, it is tempting, although premature, to propose that similarly generated chimeric transcripts are expressed in rat granulosa cells. Alternatively, the IAP-LE capable of encoding the reverse transcriptase/integrase may be transposed itself via the reverse transcription of an RNA intermediate (5, 10, 31–32).

Using the partial sequences that correspond to a polymerase portion of the IAP family, we were able to perform in situ hybridization to assess IAP-LE mRNA expression at a cellular level. Under the stringent conditions that specifically detect IAP-LE mRNA, we have shown that IAP-LE mRNA is highly expressed in granulosa cells of all healthy follicles and newly formed healthy corpora lutea. The cell-specificity and hormonal regulation of IAP-LE mRNA expression in the ovary contrasts to the other two already-known retroviral-like elements, VL30 (21) and the type OST-1 (22). VL-30 mRNA has been shown to be weakly expressed in theca cells of antral follicles and luteal cells and is induced in granulosa and interstitial cells by LH (21) presumably at the level of transcription (33). In contrast, IAP-LE mRNA is predominantly expressed by granulosa cells of all healthy follicles and only a subset of luteal cells. No dramatic induction was seen

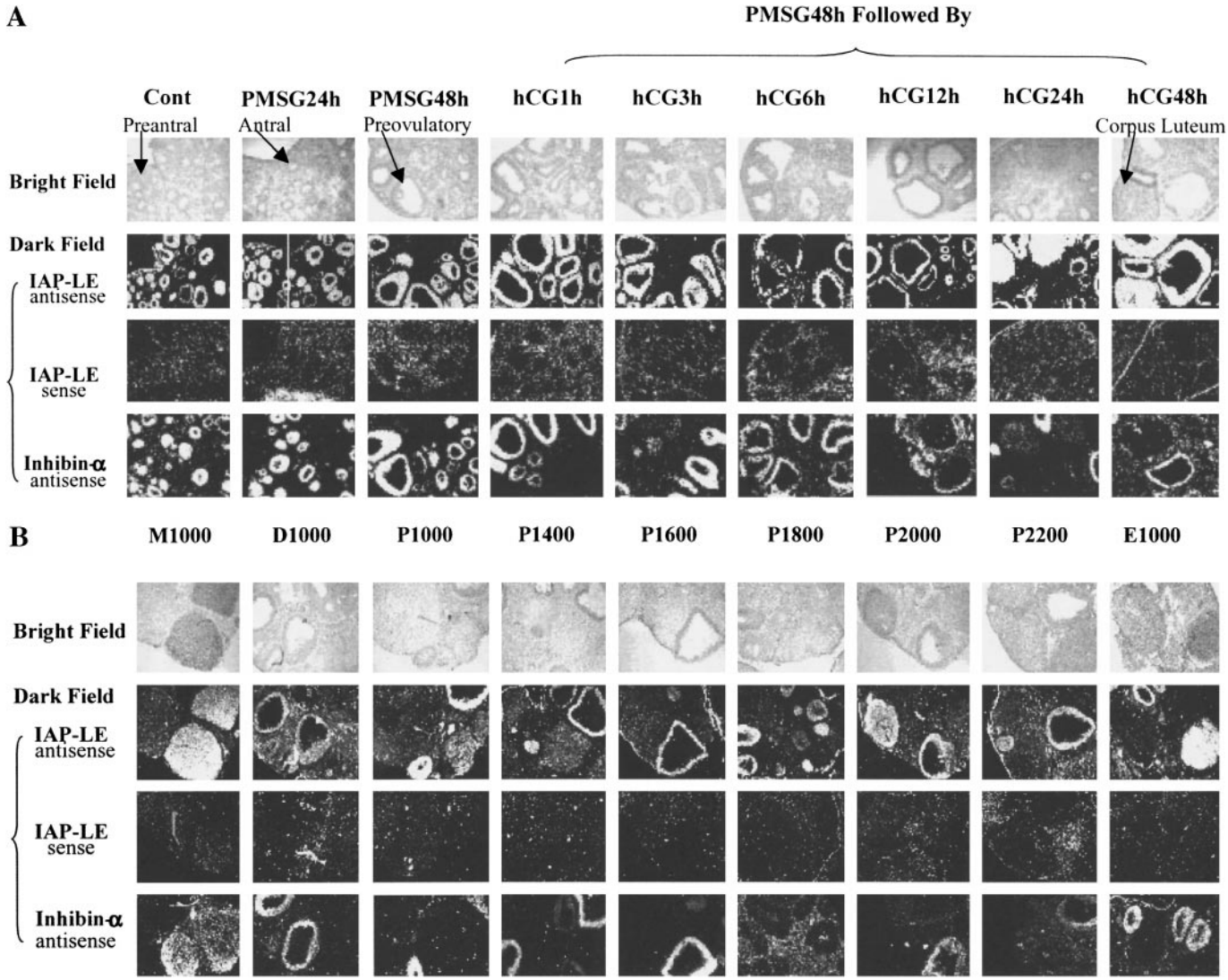

FIG. 5. The IAP-LE gene is expressed in a cell-specific manner. Ovarian sections of gonadotropin-treated immature rats (A) and adult cycling rats (B) were hybridized with ^{35}SUTP-labeled riboprobes and subjected to subsequent liquid emulsion autoradiography. Each panel shows a dark-field of antisense and sense of IAP-LE probes along with a positive control antisense of inhibin-α probe and a bright field. Ovarian sections of gonadotropin-treated immature rats (A) are from non-treated (control), PMSG for 24 h (PMSG24h), PMSG for 48 h (PMSG48h), PMSG for 48 h followed by hCG for 3 h (hCG3), 6 h (hCG6), 12 h (hCG12), 24 h (hCG24), and 48 h (hCG48). Ovarian sections of adult cycling rats (B) are taken throughout the rat estrous cycle. Letters indicate the cyclic stages: metestrus (M), diestrus (D), proestrus (P), and estrus (E). Numbers indicate the colony time. Photographs are taken at 50× magnification. Notice strong IAP-LE mRNA expression in granulosa cells of all types of follicular structures including preantral, antral, and preovulatory follicles, and in a subset of corporal lutea.

by LH, indicating that these two retroviral-like element genes are controlled by different mechanisms. On the other hand, OST-1 mRNA was found in granulosa cells of developing follicles at the stage of preantral and early antral follicles but in theca cells of preovulatory follicles. In contrast, IAP-LE mRNA is found consistently and predominantly in granulosa cells of all healthy follicles, preantral, antral, and preovulatory follicles. Another difference is luteal expression of these two genes. OST-1 mRNA is scarcely found in luteal cells whereas IAP-LE mRNA is strongly expressed in a subset of corpora lutea. Curiously, both OST-1 and IAP-LE mRNAs are detected in ovarian surface epithelial cells. Taken together, our results along with the previous published data indicate that the ovary expresses several different retroviral like element genes in a cell-specific manner and that these genes are differentially expressed in different endocrine status. The precise role(s) that these retroviral sequences play in the ovary, including their potential involvement in proliferation, transformation, and tumorigenesis, remains to be determined.

Our results demonstrating IAP-LE mRNA expression in all healthy follicles including early preantral

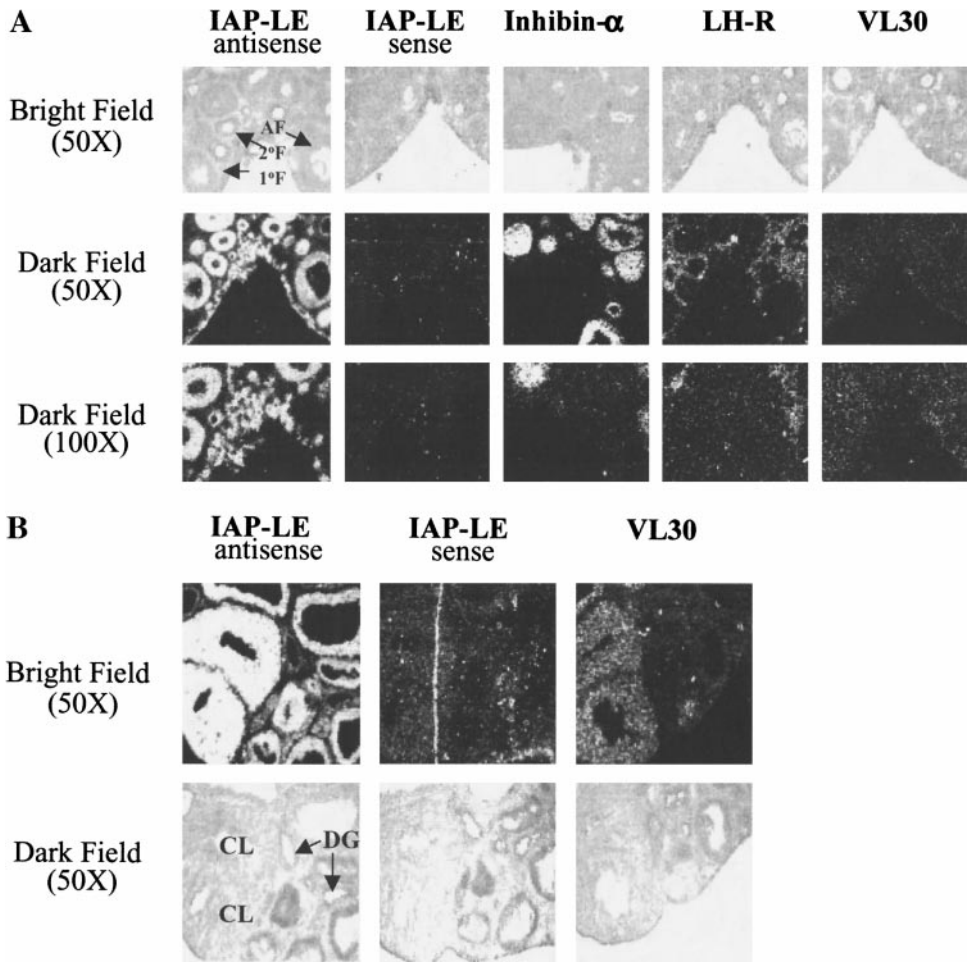

FIG. 6. Comparision of cellular localization of the IAP-LE, inhibin-α, LH-receptor (LH-R), and VL-30 transcripts in rat ovarian sections. The same follicular structures from immature rats without any hormone injection (A) or with a PMSG (48 h) injection followed by a hCG (48 h) injection (B) were oriented for comparison. In A, notice the high expression of the IAP-LE gene in primary follicles whereas none of the inhibin-α, LH-receptor, and VL-30 transcripts are localized to this structure. In contrast, granulosa cells of secondary and antral follicles express inhibin-α mRNA. LH-receptor mRNA is localized to theca-interstitial cells whereas low levels of VL-30 mRNA are seen in this ovary. None of the transcripts were localized to cumulus cells or oocytes. Magnification shown is 50× and 100×. In B, notice high IAP-LE mRNA expression in newly-formed corpora lutea (CL) and little IAP-LE mRNA expression in degenerating follicles (DG). Magnfication shown is 50×.

follicles suggest a possibility that IAP-LE mRNA may be a marker for granulosa cells upon recruitment to G1 since IAP expression has been linked to early G1 phase of the cell cycle (17). In the ovary, IAP-LE gene expression does not appear to be an indicative of proliferating cells because differentiated granulosa cells of antral and preantral follicles express high levels of IAP-LE mRNA. It is also unlikely that the IAP-LE gene serves as a marker of health condition of cells because neither theca cells or cumulus cells or oocytes express detectable levels of IAP-LE mRNA and because IAP-LE mRNA is detected, although at a decreased level, in atretic follicles unless they are degenerating.

The IAP-LE product may possibly contribute to recruitment of dormant primordial follicles to primary follicles, since primary follicles but not primordial follicles express IAP-LE mRNA. IAP gene family has been reported to be involved in cell proliferation, differentiation, and transformation by modulating the expression of the nearby gene(s) upon insertion as a retrotransposon (11–16). It is possible that IAP-LE may contribute to the expression of a particular gene(s) that is necessary for follicular growth. A few genes including the FSH receptor have been identified to be expressed in granulosa cells of preantral follicles and suspected to play a role in early folliculogenesis (19, 34–35). Several growth factors including FSH, c-kit, and GDF-9 all appear to promote the transition of primary and/or secondary follicles to antral follicles (36–37), although it is not clear what initiates recruitment of primordial follicles. The IAP-LE gene itself appears not to respond to any of these growth factors because IAP-LE mRNA levels at the level of individual granulosa cells appear more or less similar among pre-

antral and antral follicles. This is in a remark contrast to the inhibin-α gene that is known to be a direct target of FSH action (28, 38). It is tempting, although premature, to suggest that the IAP-LE gene product may be involved in interplays among various hormone to modulate follicular recruitment and development. It is noteworthy that anti-Mullerian hormone has been suggested to inhibit follicular recruitment (39).

IAP-LE gene expression remains high, exhibiting a slight decrease, throughout the preovulatory LH surge, indicating that the IAP-LE gene promoter does not respond to cAMP in a remarkable contrast to many gonadotropin-responsive genes such as the inhibin-α gene (40) and LH receptor gene (27, 41). Instead, the pattern of IAP-LE gene expression throughout folliculogenesis, ovulation, luteal formation, and luteal regression closely resembles FSH receptor gene expression (27, 34–35).

Taken together, our results demonstrating cell-specific expression of the IAP-LE sequences in ovarian granulosa cells may shed light on potential role(s) that retroviral elements could play in folliculogenesis and transformation.

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