Development of the follicle in egg-laying species such as the chicken is regulated by systemic factors as well as by the highly orchestrated interplay of differentially expressed genes within this organ. Differential mRNA display analysis of defined phases of follicle development resulted in the characterization of coagulation factor XIII A. It is expressed and produced by cells of the theca externa in a highly regulated manner during distinct growth phases of the follicle. Transcripts for factor XIII A are already detectable at the beginning of follicle development and peak at the end of phase 2. Protein levels, however, still increase during phase 3, peak shortly after ovulation, and persist until the postovulatory tissue is completely resorbed. Factor XIII A is secreted as a monomer into the extracellular matrix of the theca externa and is not associated with factor XIII B as is the case in plasma. Our data suggest that, due to its transglutaminase activity, factor XIIIA stabilizes the follicular wall by cross-linking matrix components. Thus, coagulation factor XIII A might play a key role in coping with the massive mechanical stress exerted by the large amount of yolk accumulating during the rapid growth phase of the oocyte.

Reproduction in the mature hen depends on the coordinate differentiation and growth of oocytes. In the case of the domesticated chicken (Gallus gallus domesticus), fully developed oocytes are laid as eggs every 25 h. The oocytes develop in follicles, highly specialized structures of the ovary consisting of the oocyte proper in the center of the follicle, surrounded by concentric layers of cells, acellular material, and structures, including the perivitelline membrane (the equivalent of the zona pellucida in mammals), granulosa cells, a basement membrane, and the basement membrane. We found, one of the zona pellucida proteins, ZPC, is synthesized by granulosa cells and deposited in polarized fashion into the cell layers during development of the follicle and its remodeling in preparation for and following ovulation.

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The abbreviations used are: POF, postovulatory follicle; DD, differential display; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; RACE, rapid amplification of cDNA ends; HRP, horseradish peroxidase.
ially expressed genes within different cells, dependent on growth phases of the follicle. Thus, as part of our second approach we have started to screen for such genes using the differential mRNA display methodology. Here we report results obtained in such a screen for genes expressed at various levels during different phases of follicle development. We show that the avian homologue of human coagulation factor XIIIa is expressed and secreted by theca cells and propose that it stabilizes the follicular wall by cross-linking matrix components during the last growth spurt of the oocyte, in preparation for ovulation of the follicle, for its subsequent transition into the postovulatory follicle and resorption.

### EXPERIMENTAL PROCEDURES

**Differential Display PCR**—Total RNA from chicken follicles was prepared with TRI reagent (Molecular Research Center, Inc.). Poly(A)^+ RNA was prepared with the Oligotex mRNA kit (Qiagen). 1 μg of poly(A)^+ RNA was mixed with 50 pmol of oligo-dT primer, incubated at 70 °C for 10 min, and chilled on ice. First strand cDNA was synthesized in 20 μl of buffer containing 2.5 μM of each dNTP, 1 μM of diluted cDNA, 10 mM Tris-HCl (pH 8.3), 40 mM KCl, 6 mM MgCl₂, 1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, and 200 units of Superscript II reverse transcriptase (Life Technologies, Inc.) at 42 °C for 50 min. The reaction mixture (20 μl) for differential display (DD-PCR) contained 1 μl of diluted cDNA (1:15), 1 μM of arbitrary primer (5'-TACAACGAGG-3'), 1 μM of anchored primer (5'-TAC-AC-3'), 2.5 μM of each dNTP, 5 μCi of [α-32P]dATP, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.25 mM MgCl₂, 2.5 units of Taq polymerase (Life Technologies, Inc.). After an incubation of 4 min at 94 °C, the mixture was subjected to 40 low stringency cycles (94 °C for 30 s, 40 °C for 30 s, 60 °C for 60 s) as described in the indicated references. The antisera were designated Ac18 (against the activation peptide) and A3 (against the carboxyl terminus).

**Immunoprecipitation and Western Blotting**—For immunoprecipitation, 1 ml of conditioned medium (from 293 cells expressing FXIIIA and mock-transfected cells) was incubated for 24 h at 4 °C with 5 μl of antisera (Ac16) or preimmune serum and with 4 μg of Protein A-Sepharose (Amersham Pharmacia Biotech). Isolation of the glutathione S-transferase fusion proteins in DH5α cells (8) and production of the respective polyclonal antibodies (9) were carried out as described in the indicated references. The antisera were designated Ac18 (against the activation peptide) and A3 (against the carboxyl terminus).

**Expression of Chicken FXIIIA**—The full-length cDNA of chicken FXIIIA was cloned into the Xbal and Smal sites of pCIneo (Promega) and expressed in the human embryonic kidney cell line 293. Transfection of the cells was performed using Lipofectin reagent (Life Technologies, Inc.). Stable transformants were selected by the addition of 500 μg/ml G418 to the medium (Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.), 10% fetal calf serum, 584 μg/ml glutamine). Cells expressing factor XIIIa were washed three times with 2 ml each of phosphate-buffered saline (PBS), scraped from the dishes with a rubber policeman, and centrifuged at 2000 × g for 5 min, and the cell pellet was solubilized by addition of 150 μl of buffer/dish (200 mM Tris maleate, pH 6.5, 2 mM CaCl₂, 0.5 mM phe-noethylsulfonfluoride, 2.5 μM leupeptin, and 1.4% Triton X-100). The cell extracts were centrifuged at 300,000 × g for 40 min at 4 °C and the pellet discarded.

**Purification of Glutathione S-Transferase Fusion Proteins and Antibody Production**—cDNA fragments encoding amino acid residues 1–45 (corresponding to the activation peptide) and amino acid residues 674–735 (corresponding to the carboxyl terminus) of chicken factor XIIIa were cloned into pGEX-5X-1 (Amersham Pharmacia Biotech). Expression of the glutathione S-transferase fusion proteins in DH5α cells (8) and production of the respective polyclonal antibodies (9) were carried out as described in the indicated references. The antisera were designated Ac18 (against the activation peptide) and A3 (against the carboxyl terminus).

**Coagulation Factor XIIIa Stabilizes the Follicular Wall**

![Figure 1](image-url)
RESULTS

In a first step to identify genes involved in the progression of follicle development, mRNAs isolated from three distinct pools of differently staged follicles (small white, <2 mm; large white, 2–4 mm; small yellow, 4–8 mm), covering phases 1 and 2 of follicle development, were subjected to differential mRNA display analysis (12). We have isolated several fragments derived from genes that appeared up- or down-regulated as judged from the intensities on the gel, e.g., fragments 12 and 13 displayed in Fig. 1A. However, only six of these fragments (derived from mRNAs displayed using the given primer combination) exhibited differential expression, as evaluated by Northern blotting (Fig. 1, B and C). To obtain sequence information from the coding regions corresponding to the selected fragments, these fragments were used to screen a chicken follicle cDNA library (7). BLAST searches against available data bases demonstrated that only one of the clones (number 12) was identical with a known chicken gene; it codes for a chicken chondrocyte transglutaminase (13, 14). Thus, we focused our attention on clone 12, and the sequence of the corresponding full-length transcript with a length of 4.5 kilobase pairs was obtained by 5′-RACE. The sequence defined an open reading frame of 2208 base pairs (GenBank accession number AJ278103). Sequence alignments (Fig. 2) clearly identified the obtained cDNA as the chicken homologue of human factor XIIIA (15). The ATG serving as the translational start site was assigned based on the following considerations: First, it is located in a sequence context fulfilling the rules for a translation initiation site (16), and second, the corresponding chicken protein starts with the same sequence motif as human factor XIIIA (Fig. 2). The overall identity at the amino acid level between chicken and human factor XIIIA is 65%, displaying 100% identity around the active site cysteine, which catalyzes the acyl transfer reaction common to all transglutaminases (for review see Ref. 17).

Mammalian plasma factor XIIIA is part of a large tetrameric complex consisting of two molecules of XIIIA and two molecules of XIIIB. The cellular form of factor XIIIA, however, is a homodimer consisting of two A subunits only. An interesting functional feature of human factor XIIIA is a thrombin cleavage site between Arg-37 and Glu-38 (see Fig. 2). Cleavage of FXIIIA by thrombin at this site leads to liberation of the amino-terminal so-called activation peptide and subsequently to the dissociation of the A and B subunits. Thus, in the absence of the inhibitory B subunits, the thrombin-modified A subunits as-
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Fig. 3. Western blot analysis of chicken factor XIIIA in plasma and follicles. Plasma (P) and serum (S) samples (0.5 μl) (panel A) and chicken follicle extracts (50 μg of protein) (panel B) were electrophoretically separated under reducing (r) or non-reducing (n) conditions and analyzed by Western blotting using the indicated antibodies as described under “Experimental Procedures.” The primary antibody was visualized with HRP-goat-anti-rabbit IgG (1:10,000) and a chemiluminescence system. Exposure time was 1 min for A and 20 s for B. The positions of the heterotetramer, homodimer, and monomer of factor XIIIA are indicated.

To characterize the corresponding chicken protein further, we produced two antibodies directed against the putative activation peptide at the amino terminus (Ac16), and against the extreme carboxyl terminus of chicken XIIIA (A3), respectively. Using these antibodies in combination with a commercially available antibody against human XIIIA, we first characterized the plasma form of chicken XIIIA (Fig. 3A). Under non-reducing conditions, Ac16 recognizes all three forms of chicken factor XIIIA in plasma, i.e. the heterotetramer (2A2B), the homodimer (2A), and the monomer (A), respectively (lane 1). The occurrence of both the homodimer (2A) and the uncleaved monomer (A) in plasma is due to the inherent instability of the tetrameric complex during electrophoresis. Under the same conditions, A3 and the commercial anti-human-XIIIA antibody recognize the heterotetramer in plasma (lanes 3 and 5). Independently of the enzyme’s activation status, A3 reacts with the dimer as demonstrated by comparing plasma versus serum samples electrophoretically separated under non-reducing conditions (lanes 3 and 4). A3 only weakly recognizes the monomer of chicken XIIIA under the conditions tested (the monomer becomes visible only after prolonged exposure, lane 3). Removal of the activation peptide and dissociation of the A and B subunits was demonstrated by the absence in serum of any detectable heterotetramer using A3 and anti-human XIIIA (lanes 4 and 6). In addition, under reducing conditions, all of the XIIIA present in plasma (i.e. not activated) migrates as monomer and is strongly recognized by Ac16 (lane 7). Upon activation, in serum, hardly any reactive band is visible under reducing conditions, indicating that the activation peptide is removed (lane 8). Upon prolonged exposure of the blot, however, a faint band with a size similar to that of the monomer becomes apparent; most likely it represents a small residual amount of non-activated XIIIA (also visible as dimer under non-reducing conditions in lane 2). Thus, activation of chicken factor XIII, as it occurs during blood clotting, obviously follows a similar process as described for mammals (17).

Having characterized the circulating forms of chicken factor XIIIA, we studied the protein produced by chicken follicles. Under both reducing and non-reducing conditions, the only band detected by Ac16 is the monomer, indicating that XIIIA is not associated with the B subunit and that follicular XIIIA does not form a dimer as it does in the circulation (Fig. 3B, lane 1). This observation is substantiated by using A3, which reacts strongly with the dimer (2A) and the heterodimer of XIIIA (2A2B), but only weakly with the monomer (A), as demonstrated on plasma and serum samples. In the follicle this antibody visualizes only the monomer under the conditions applied (Fig. 3B, lanes 3 and 4).

Human factor XIIIA does not contain a hydrophobic leader
sequence and thus belongs to a group of proteins which are secreted from cells by an "unusual secretory pathway" (for review see Ref. 18). Chicken factor XIIIA also does not contain a hydrophobic leader sequence (Fig. 2). To test whether chicken factor XIIIA can be secreted from cells, we expressed the full-length cDNA in 293 cells and tested for the expressed protein in cell extracts and the medium. As seen in Fig. 4A, transfected 293 cells express large amounts of monomeric chicken factor XIIIA (lane 2). Again, antibody A3 does not detect a band corresponding to the homodimer seen under the same conditions in the plasma (Fig. 4, lanes 4 and 6). Furthermore, significant amounts of the non-activated monomeric protein are indeed secreted into the medium, as demonstrated by immunoprecipitation experiments using Ac16 (Fig. 4B, lanes 1–5).

To evaluate the expression pattern of XIIIA in chicken tissues, we examined all major organs (follicle, eye, intestine, liver, kidney, muscle, brain, heart, lung, skin, adrenal, and spleen) and relevant cell lines by Northern blot analysis (data not shown). The only site in female chicken expressing detectable transcripts for factor XIIIA is the follicle and, of the cell lines tested, HD11 cells, a chicken macrophage cell line (19).

From these experiments we conclude that in the chicken circulating factor XIIIA is highly homologous to human factor XIIIA and that it is associated into a tetrameric complex constituting plasma factor XIII in a similar way as described for the mammalian system. Expression analysis did not reveal where the circulating protein is produced. This situation is reminiscent of that in humans where the question about the site of synthesis of plasma-borne XIIIA is still not resolved (17). However, the follicle produces the monomeric form of factor XIIIA, and its regulated expression during follicle development suggests that it plays a role in this complex process.

To study this unexpected finding, we examined in close detail the expression pattern of follicular XIIIA during the entire sequence of follicle development, starting at phase 1 to the complete involution of the postovulatory follicle. Such an experiment is hampered by the fact that follicles taken from the last growth phase (F8–F1) contain up to 15 g of yolk. RNA preparation from such follicles is very inefficient, and data obtained cannot be quantified appropriately. Thus, we have included follicles from phase 1 up to the smallest follicles at the beginning of phase 3 (F8–F6), and representative postovulatory follicles covering the time period from after ovulation until almost complete resorption. Detailed evaluation on follicles during the last rapid growth phase will be presented in a separate experiment below. As shown in Fig. 5, transcripts for factor XIIIA are already present at the very beginning of follicle development, and the amount significantly increases until follicles reach the end of phase 2 (5–8 mm). This result confirms the initial finding from the differential mRNA display approach that led to the characterization of factor XIIIA in follicles. Apparently, mRNA levels decrease at the beginning of the rapid growth phase (approximately 8 mm) and further decrease after ovulation (Fig. 5, top). However, protein levels seem to increase during the last phase of follicle development, reach the highest levels shortly after ovulation, and stay high until the postovulatory tissue is completely resorbed (Fig. 5, bottom). This observation suggests that factor XIIIA is not catabolized during follicle development, but rather becomes deposited in the extracellular matrix of the follicle wall. To further evaluate this question and to pinpoint the site of synthesis of factor XIIIA in the follicle, we turned to the largest follicles (F4–F1) of the final growth phase. Despite problems to isolate RNA from follicles of this size, they can be dissected to separate and prepare the theca and granulosa cell layers free of most of the yolk (20). RNA and protein extracts were prepared from these tissues and subjected to Northern and Western blot analysis. The most striking result from this experiment was that expression of factor XIIIA in the follicle is restricted to the theca layer and virtually absent from the granulosa cell layer (Fig. 6A). Immunohistochemical analysis of the follicles showed that factor XIIIA is present in the theca externa and in the basement membrane but not in theca interna (Fig. 6B, Panels 1 and 2). Laminated staining of the theca externa, which is similar to that seen with anti-collagen-1 antibodies (Fig. 6B, Panel 3) and anti-fibronectin antibodies (Fig. 6B, Panel 4), together with the fact that granulosa cells do not express the protein, suggest that factor XIIIA is secreted from theca cells and deposited in the acellular matrix of the theca externa and possibly in the basement membrane.

To evaluate whether factor XIIIA is enzymatically active in the matrix of the follicle wall, an activity assay based on fluorescein-labeled cadaverine as substrate was performed on thin sections of the follicle. Background labeling of the sections was controlled by using EDTA (Fig. 7, A* and B*), which completely blocks the transglutaminase reaction. As seen in Fig. 7A,
distribution of the transglutaminase activity visualized by incorporation of the labeled substrate is identical with the distribution of the immunoreactive enzyme seen in Fig. 6B (Panels 1 and 2). Furthermore, the activity pattern is maintained in the early postovulatory follicle (Fig. 7B), when the highest amount of factor XIIIA is present in the follicle (Fig. 5). Note that at this stage the perivitelline membrane is lost (together with the ovulated oocyte) and the granulosa cell layer starts to disintegrate, whereas the theca externa reaches its largest extension.

**DISCUSSION**

Differential mRNA display screening for genes expressed during specific stages of chicken follicle development resulted in the identification of chicken factor XIIIA. The transglutaminase is expressed by cells of the theca externa and deposited in certain areas of the follicular wall. Usually, factor XIIIA is part of a large tetrameric complex consisting of two molecules of XIIIA and two molecules of XIIB constituting plasma coagulation factor XIII. Upon activation by thrombin, factor XIII catalyzes the last step of the blood coagulation pathway by cross-linking soluble fibrin monomers to insoluble fibrin, which forms and stabilizes the final clot. Besides its abundant presence in plasma, factor XIIIA can also be found in certain cells like platelets (21), monocytes/macrophages (22), and some mononuclear cells in the skin (23). The cellular form of factor XIII, however, is a homodimer consisting of two A subunits.
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Fig. 7. In situ activity assay for factor XIIIa in chicken follicles. In situ activity assays on cryostat sections of chicken follicles (6 A, A*) and post-ovulatory follicle (POF1) (B, B*) were performed in the presence of CaCl$_2$ (A, B) or EDTA (A*, B*) as described under "Experimental Procedures." Cross-linked fluorescein-cadaverine appears in green. Anatomically distinct parts of the follicles are indicated (TH, theca externa; TH in, theca interna; GC, granulosa cells; *, basement membrane; †, perivitelline membrane).

The present immunohistochemical studies on the distribution of factor XIIIa in the chicken follicle (Fig. 6B) demonstrate that this protein is present in the theca externa and possibly in the basement membrane, although this could be demonstrated only with one of the antibodies used (compare Fig. 6A with 6B). Factor XIIIa is completely absent from the theca interna, the granulosa cell layer, and the perivitelline membrane. These results are supported by data obtained from expression studies showing that cells in the theca are the source of the protein. The possibility that factor XIIIa is at least partially recruited from the plasma cannot be excluded at this point. However, it seems rather unlikely, because factor XIIIa in plasma always exists as a heterotetramer complexed to factor XIIIb. Because we could not detect any such complexes in the follicle (Fig. 3B), we suggest that factor XIIIa found in the follicle wall is produced by cells within the follicle, as is the case in the calcifying cartilage discussed above. As shown in Fig. 6B, the localization of XIIIa coincides with that of collagen-1 and fibronectin, both major extracellular matrix components of the theca externa. Because many extracellular matrix proteins, including collagen and fibronectin (32) are substrates for transglutaminases, we assume that factor XIIIa plays a central role in constructing and stabilizing the extracellular matrix of the theca externa. This may provide the mechanical strength necessary for progression through the rapid phase of oocyte growth.

This assumption also applies to the integrity of the postovulatory follicle, which, after loss of its balloon-like tension by expelling the oocyte, does not disintegrate right away but stays metabolically active for several more days (2). There is another aspect about factor XIIIa function in the theca externa: It was recently shown that transglutaminases and especially factor XIIIa serve as substrates for cell adhesion (33). Upon inspec-
tion of the ultrastructure of the theca externa (Fig. 6B), it becomes obvious that most of this tissue is a matrix, with only a few cells scattered in this layer. On the other hand, a massive presence of factor XIIIA in this tissue suggests that it serves other functions than catalysis of cross-linking of matrix proteins. For instance, factor XIIIA may be a true structural component of the matrix-like fibronectin, serving as an anchor for the adhesion of the fibroblast-like cells present in this stratum. Finally, follicular factor XIIIA may also serve the same function as in the blood. Ovulation of the follicle is started by rupture of the theca along the stigma, a special region of the follicle that is almost devoid of blood vessels. However, small capillaries are expected to rupture upon ovulation and factor XIIIA in the follicular wall might prevent microbleeding during this event.

REFERENCES
1. Perry, M. M., Gilbert, A. B., and Evans, A. J. (1978) J. Anat. 125, 481–497
2. Johnson, A. L. (1990) Crit. Rev. Poult. Biol. 2, 319–346
3. Nimpf, J., and Schneider, W. J. (1998) Atherosclerosis 141, 191–202
4. Schneider, W. J., Osanger, A., Waclawek, M., and Nimpf, J. (1998) Biol. Chem. 379, 965–971
5. Waclawek, M., Foisner, R., Nimpf, J., and Schneider, W. J. (1998) Biol. Reprod. 59, 1230–9
6. Bausek, N., Waclawek, M., Schneider, W. J., and Wohlrab, F. (2000) J. Biol. Chem. 275, 28866–28872
7. Bujo, H., Hermann, M., Kaderli, M. O., Jacobsen, L., Sugawara, S., Nimpf, J., Yamamoto, T., and Schneider, W. J. (1998) EMBO J. 17, 5165–5175
8. Herz, J., Goldstein, J. L., Strickland, D. K., Ho, Y. K., and Brown, M. S. (1991) J. Biol. Chem. 266, 21232–21238
9. Nimpf, J., George, R., and Schneider, W. J. (1988) J. Lipid Res. 29, 657–667
10. Vieira, P. M., Vieira, A. V., Sanders, E. J., Steyerer, E., Nimpf, J., and Schneider, W. J. (1995) J. Lipid Res. 36, 601–610
11. Raghunath, M., Hopfner, B., Aeschlimann, D., Luthi, U., Meuli, M., Altermann, S., Gobet, R., Bruckner-Tuderman, L., and Steinmann, B. (1996) J. Clin. Invest. 98, 1174–1184
12. Liang, P., and Pardee, A. B. (1992) Science 257, 967–971
13. Nurminskaya, M., and Linser, T. F. (1996) Develop. Dyn. 206, 260–271
14. Kurta, C., Magee, C., Nurminsky, D., and Linser, T. F. (1998) J. Cell Biol. 142, 1135–1144
15. Grundmann, U., Amann, E., Zettlmeissl, G., and Kupper, H. A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8024–8028
16. Raghunath, M., Hopfner, B., Aeschlimann, D., Luthi, U., Meuli, M., Altermann, S., Gobet, R., Bruckner-Tuderman, L., and Steinmann, B. (1996) J. Biol. Chem. 271, 28866–28872
17. Bujo, H., Hermann, M., Kaderli, M. O., Jacobsen, L., Sugawara, S., Nimpf, J., Yamamoto, T., and Schneider, W. J. (1998) EMBO J. 17, 5165–5175
18. Herz, J., Goldstein, J. L., Strickland, D. K., Ho, Y. K., and Brown, M. S. (1991) J. Biol. Chem. 266, 21232–21238
19. Nimpf, J., George, R., and Schneider, W. J. (1988) J. Lipid Res. 29, 657–667
20. Vieira, P. M., Vieira, A. V., Sanders, E. J., Steyerer, E., Nimpf, J., and Schneider, W. J. (1995) J. Lipid Res. 36, 601–610
21. Raghunath, M., Hopfner, B., Aeschlimann, D., Luthi, U., Meuli, M., Altermann, S., Gobet, R., Bruckner-Tuderman, L., and Steinmann, B. (1996) J. Biol. Chem. 271, 28866–28872
22. Nimpf, J., George, R., and Schneider, W. J. (1988) J. Lipid Res. 29, 657–667
23. Vieira, P. M., Vieira, A. V., Sanders, E. J., Steyerer, E., Nimpf, J., and Schneider, W. J. (1995) J. Lipid Res. 36, 601–610
24. Aeschlimann, D., Wetterwald, A., Fleisch, H., and Paulsson, M. (1993) J. Cell Biol. 120, 1461–1470
25. Aeschlimann, D., Kummer, O., and Paulsson, M. (1995) J. Cell Biol. 120, 1461–1470
26. Yoshimura, Y., Okamoto, T., and Tamiura, T. (1985) Jpn. Poult. Sci. 22, 274–278
27. Dahl, E. (1970) Z. Zellforsch. Mikrosk. Anat. 109, 195–211
28. Porter, T. E., Hargis, B. M., Silsby, J. L., and Halawani, M. E. E. (1989) Endocrinology 125, 109–116
29. Chapeau, C., Engelhardt, H., King, G. J., and Etches, R. J. (1996) Poult. Sci. 75, 1536–1545
30. Asem, E. K., and Novero, R. P. (1994) J. Reprod. Fertil. 101, 375–384
31. Moshier, D. F., Fogerty, F. J., Chernousov, M. A., and Barry, E. L. (1991) Ann. N. Y. Acad. Sci. 614, 167–180
32. Ueki, S., Takagi, J., and Saijo, Y. (1996) J. Cell Sci. 109, 2727–2735