

PTX3 Interacts with Inter- α -trypsin Inhibitor IMPLICATIONS FOR HYALURONAN ORGANIZATION AND CUMULUS OOPHORUS EXPANSION^{*†‡}

Received for publication, May 7, 2007, and in revised form, July 26, 2007. Published, JBC Papers in Press, August 2, 2007, DOI 10.1074/jbc.M703738200

Laura Scarchilli[‡], Antonella Camaioni[‡], Barbara Bottazzi[§], Veronica Negri[‡], Andrea Doni[§], Livija Deban[§], Antonio Bastone[¶], Giovanni Salvatori^{||}, Alberto Mantovani[§], Gregorio Siracusa[‡], and Antonietta Salustri^{‡#2}

From the [‡]Department of Public Health and Cell Biology, University of Rome Tor Vergata, 00133 Rome, the [§]Research Laboratory in Immunology and Inflammation, Istituto Clinico Humanitas, 20089 Rozzano, Milan, the [¶]Mario Negri Institute, 20157 Milan, Italy, and the ^{||}SigmaTau SpA, Pomezia, 00040 Rome, Italy

Pentraxin 3 (PTX3) and heavy chains (HCs) of inter- α -trypsin inhibitor (I α I) are essential for hyaluronan (HA) organization within the extracellular matrix of the cumulus oophorus, which is critical for *in vivo* oocyte fertilization and female fertility. In this study, we examined the possibility that these molecules interact and cooperate in this function. We show that HCs and PTX3 colocalize in the cumulus matrix and coimmunoprecipitate from cumulus matrix extracts. Coimmunoprecipitation experiments and solid-phase binding assays performed with purified human I α I and recombinant PTX3 demonstrate that their interaction is direct and not mediated by other matrix components. PTX3 does not bind to I α I subcomponent bikunin and, accordingly, bikunin does not compete for the binding of PTX3 to I α I, indicating that PTX3 interacts with I α I subcomponent HC only. Recombinant PTX3-specific N-terminal region, but not the PTX3-pentraxin C-terminal domain, showed the same ability as full-length protein to bind to HCs and to enable HA organization and matrix formation by *Ptx3*^{-/-} cumulus cell oocyte complexes cultured *in vitro*. Furthermore, a monoclonal antibody raised against PTX3 N terminus, which inhibits PTX3/I α I interaction, also prevents recombinant full-length PTX3 from restoring a normal phenotype to *in vitro*-cultured *Ptx3*^{-/-} cumuli. These results indicate that PTX3 directly interacts with HCs of I α I and that such interaction is essential for organizing HA in the viscoelastic matrix of cumulus oophorus, highlighting a direct functional link between the two molecules.

A few hours before ovulation, up-regulation of hyaluronan (HA)³ synthesis occurs in cumulus cells surrounding the mam-

malian oocyte. This leads to the formation of an expanded and viscoelastic extracellular matrix that plays a fundamental role in fertilization (1). Ultrastructural analysis suggests that HA is organized in twisted or coiled fibrillar elements interconnected to each other to form a mesh-like network (2). Swelling of the fibrils and complete disruption of the gel-like matrix produced by a brief treatment with proteases provided the first evidence for a fundamental role of proteins for organizing HA into a such structure (3). More recently, three proteins have been identified as essential for proper formation and stability of the cumulus cell oocyte complex (COC) matrix: inter- α -trypsin inhibitor (I α I or ITI), tumor necrosis factor-induced protein-6 (TSG6 or TNFIP-6), and pentraxin 3 (PTX3 or TSG14) (4–7). How these proteins influence and integrate their action in assembling the cumulus matrix is, however, not fully understood.

Molecules of the I α I family are serum components consisting of one or two homologous proteins, named heavy chains (HCs), covalently linked to the single chondroitin sulfate chain of bikunin proteoglycan (8). When mouse COCs are induced to expand *in vitro* in the absence of serum or purified I α I, the matrix is not assembled, albeit HA is synthesized at the normal rate, and COCs disaggregate (9–12). *In vivo*, I α I molecules diffuse from blood into the ovarian follicle, and their concentration in the follicular fluid increases during the preovulatory period due to a gonadotropin-dependent increase in vascular permeability (13, 14). In all examined species, HCs are transferred from I α I to the HA synthesized by cumulus cells during COC expansion (14, 15). Mass spectrometric analyses of analogous HC·HA complexes formed in pathological synovial fluids of human arthritis patients indicate that HCs are linked to HA *via* an ester bond, as they are to the chondroitin sulfate chain in I α I molecules, implying a transesterification reaction in the process (16).

TSG6, a protein with the ability to specifically bind to HA and to interact with I α I, is a catalyst in such process (17). This protein is synthesized at inflammatory sites, as well as by cumulus cells in preovulatory follicles (18–22). The crucial role played by HC·HA complexes in cumulus matrix formation is suggested by the evidence that bikunin-null and TSG6-deficient mice,

hormone; COC, cumulus cell-oocyte complex; PBS, phosphate-buffered saline; PBS⁺⁺, PBS with Ca²⁺ and Mg²⁺; PMSG, pregnant mare serum gonadotropin; hCG, chorionic gonadotropin; HRP, horseradish peroxidase; TBS, Tris-buffered saline; BSA, bovine serum albumin; N_{term}, N-terminal; C_{term}, C-terminal.

* This work was supported by grants from Ministero Istruzione, Università e Ricerca (MIUR, Cofin) (to A. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† This article was selected as a Paper of the Week.

‡ The on-line version of this article (available at <http://www.jbc.org>) contains two supplemental figures.

¹ An employee of the pharmaceutical company Sigma Tau, which holds patent rights on PTX3 and fertility.

² To whom correspondence should be addressed. Tel.: 39-06-7259-6168; Fax: 39-06-7259-6172; E-mail: salustri@med.uniroma2.it.

³ The abbreviations used are: HA, hyaluronan; I α I, inter- α -trypsin inhibitor; HCs, heavy chains of I α I; PTX3, pentraxin 3; bPTX3, biotin-labelled PTX3; TSG6, tumor necrosis factor-induced protein-6; FSH, follicle-stimulating

PTX3 Interacts with I α I

both unable to complete the transesterification reaction, exhibit impaired cumulus matrix stability and severe subfertility (4, 5).

PTX3 is a 45-kDa protein predominantly assembled in a multimeric complex of \sim 10 protomers by interchain disulfide bonds (23). It consists of a C-terminal 203-amino-acid pentraxin domain, sharing homology with the classic short pentraxins, C-reactive protein and serum amyloid P component, coupled to an N-terminal portion of 174 amino acids that does not show any significant homology with any other known protein (24, 25). PTX3 synthesis is up-regulated in a variety of cell types, both *in vitro* and *in vivo*, in response to primary inflammatory signals (26). PTX3 synthesis also increases in mouse and human cumulus cells during the time preceding ovulation and localizes in the COC extracellular matrix (6, 7). Although PTX3 does not bind to HA, *Ptx3*-deficient mice, as well as bikunin-null and TSG6-null mice, do show cumulus matrix instability and female infertility. Cumuli from *Ptx3*^{-/-} mice are unable to organize HA into a matrix, albeit HC·HA complexes are normally formed. On this basis, we hypothesized that a direct interaction of PTX3 with TSG6 or HCs or both is required for proper cumulus matrix assembly. This hypothesis found support in the ability of PTX3 to bind to TSG6 (7).

In this study, we have investigated whether PTX3 could interact with HCs. Association between PTX3 and HCs in mouse cumulus matrix is suggested by their colocalization in the ovary, coimmunoprecipitation from cumulus matrix extracts, and binding assays performed with purified molecules. We also show that HCs interact with the N-terminal domain of PTX3 and that this portion of the molecule is required and sufficient for organizing HA and for enabling matrix formation by *Ptx3*^{-/-} COCs induced to expand *in vitro*. Relevance of such interaction is additionally supported by the evidence that a PTX3 antibody blocking PTX3 binding to HCs also prevents the ability of full-length recombinant PTX3 to restore a normal phenotype to *Ptx3*^{-/-} COC stimulated *in vitro*.

EXPERIMENTAL PROCEDURES

Materials—Pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) were from Intervet. Sepharose A, mineral oil, L-glutamine, and sodium pyruvate were obtained from Sigma-Aldrich. *Streptomyces* hyaluronidase was purchased from Calbiochem, and chondroitinase ABC was from Seikagaku (Tokyo, Japan). Affinity-purified rabbit anti-human I α I immunoglobulin was obtained from DAKO Corp. (Carpinteria, CA). Horseradish peroxidase (HRP)-labeled anti-rabbit F(ab')₂ fragment immunoglobulin, HRP-linked streptavidin, and enhanced chemiluminescence Western blotting detection reagent were purchased from Amersham Biosciences. Mouse serum was obtained from Rockland Immunochemicals, Inc. (Gilbertsville, PA). Minimum essential medium, fetal bovine serum, gentamycin, and HEPES buffer solution (1 M) were obtained from Invitrogen. Maxisorp 96-well plates were from Costar Corp. (Cambridge, MA).

Animals—Adult wild type and *Ptx3*-null 129Sv mice, generated as described (27), were injected with 5 IU of PMSG. After 44–48 h, the animals were either sacrificed or injected with 5

IU of hCG. Ovulated COCs were collected from oviductal ampullae 14 h after hCG.

Immunofluorescence Analysis—Ovaries or oviducts were dissected from wild type mice at different times from hCG injection, fixed in 4% paraformaldehyde for 4 h at room temperature, washed in TBS, and embedded in paraffin. Five- μ m sections were blocked in TBS-glycine, 1% BSA for 45 min and then in phosphate-buffered saline (PBS), 3% BSA for 30 min at room temperature. For localization of PTX3 and I α I, sections were incubated with 20 μ g/ml rabbit anti-human PTX3 polyclonal antibody or with rabbit anti-human I α I polyclonal antibody diluted 1:1000 in PBS with 1% BSA for 2 h at room temperature. After washing, sections were incubated with Alexa Fluor 488-labeled anti-rabbit IgG diluted 1:500 (Molecular Probes) for 1 h in PBS with 3% BSA at room temperature. For PTX3 and I α I colocalization in ovulated COCs, oviducts were dissected 14 h after hCG injection, and sections prepared as reported above. Sections were incubated with rabbit anti-human I α I and then with Cy3 goat anti-rabbit IgG diluted 1:400 (Chemicon International) in the same conditions indicated above. Sections were then extensively washed and incubated with biotin-labeled rabbit anti-human PTX3 polyclonal antibody and then with Alexa Fluor 488-labeled streptavidin (Molecular Probes) at the final concentration of 2 μ g/ml for 20 min. Hoechst 33258 was added during the last 5 min for staining nuclei. Finally, the sections were visualized with an Axioplan 2 fluorescence microscope.

Expression and Purification of PTX3 and I α I—Recombinant mouse and human full-length PTX3 and C_{term}-PTX3 and N_{term}-PTX3 fragments were expressed by stable transfected Chinese hamster ovary cells (23). Briefly, a 1311-bp fragment of human PTX3 cDNA, containing the complete coding sequence, a 741-bp fragment coding for C_{term}-PTX3, and a 520-bp fragment coding for N_{term}-PTX3 were subcloned in pSG5 and transfected in Chinese hamster ovary cells. Recombinant full-length PTX3, C_{term}-PTX3, and N_{term}-PTX3 fragments were purified from conditioned medium as described previously (23, 28) Purity of recombinant proteins was assessed by SDS-PAGE followed by silver staining. Cross-linking of C_{term}-PTX3 was performed with bis(sulfosuccinimidyl)suberate as described previously (23).

I α I, purified from human serum (29), and bikunin, prepared by NH₂OH dissociation of purified I α I followed by ion-exchange chromatography (30), were kindly supplied by Dr. Jacques Mizon (Université de Lille II, France). Purity of the isolated proteins was assessed by SDS-PAGE followed by silver staining.

Immunoprecipitation—All coimmunoprecipitations were performed by first binding human I α I antibody to protein A-Sepharose beads. For each coimmunoprecipitation, 10 μ g of rabbit anti-human I α I antibody or rabbit preimmune IgG was added to 30 μ l of protein A-Sepharose suspended in 100 μ l of PBS with Ca²⁺ and Mg²⁺ (PBS⁺⁺). The antibodies were allowed to bind to the beads for 1 h at room temperature while rotating and were then washed three times with 0.1% Tween 20 in PBS⁺⁺. For immunoprecipitation studies of proteins from cumulus matrix extracts, 150–200 ovulated COCs were washed in PBS⁺⁺ and then incubated with 2 IU of *Streptomyces* hyaluronidase in 200 μ l of PBS⁺⁺ in the presence of protease

inhibitor mixture (Roche Applied Science) for 30 min at 37 °C. After digestion, the cells were pelleted by centrifugation at $300 \times g$ for 5 min, and the supernatant, containing matrix proteins, was collected. Matrix extract was precleaned by incubation at 4 °C for 1 h with BSA-prebound protein A-Sepharose beads and divided in equal aliquots (equivalent to 50 COCs). An aliquot was directly denatured in SDS/ β -mercaptoethanol loading buffer, and the others were incubated for 2 h at 4 °C with either immune or preimmune IgG bound to protein A-Sepharose. For immunoprecipitation studies of purified proteins, 1 μ g of mouse recombinant PTX3 was mixed with 2 μ l of mouse serum or 1 μ g of human recombinant PTX3 with 1 μ g of IαI purified from human serum, and the mixtures were incubated with either immune or preimmune IgG bound to protein A-Sepharose, as reported above. After incubation, the beads were washed five times with 0.1% Tween 20 in PBS⁺⁺, and the bound material was eluted with 20 μ l of Laemmli buffer containing 2% SDS and 6% β -mercaptoethanol and stored at -80 °C until use. Samples were boiled at 100 °C for 5 min before gel electrophoresis and Western blot analysis.

Gel Electrophoresis and Western Blot Analysis—Proteins were separated by SDS-PAGE (7.5% polyacrylamide gel) and transferred to Hybond ECL membrane (Amersham Biosciences). The membrane was blocked in 5% milk powder in TBS, 0.05% Tween 20 (TBS/T) for 2 h and then incubated with primary antibodies in TBS/T, 5% BSA at 4 °C overnight. For native and recombinant murine PTX3 immunoblots, biotinylated hamster anti-mouse PTX3 polyclonal antibody was used at a concentration of 1 μ g/ml. For human recombinant full-length PTX3 and N_{term}-PTX3 fragment immunoblots, 1 μ g/ml purified rabbit anti-human PTX3 polyclonal antibody was used. For IαI immunoblots, rabbit anti-human IαI polyclonal antibody was added at a dilution of 1:2000. Membranes were then washed four times for 10 min in TBS/T followed by incubation with 1:1000 HRP-linked streptavidin or 1:10000 HRP-linked anti-rabbit F(ab')₂ fragment immunoglobulin in TBS/T, 5% BSA for 1 h. Following washing, the signal was detected by enhanced chemiluminescence and autoradiography using Hyperfilm ECL (Amersham Biosciences).

Solid-phase Binding Assay—Interaction of PTX3 or PTX3 fragments with IαI was measured as follows. Plates were coated by overnight incubation at 4 °C with 100 μ l of purified IαI (10 μ g/ml) in 20 mM sodium carbonate, pH 9.5. Wells were then washed three times with PBS⁺⁺, 0.05% Tween 20 (PBS⁺⁺/T) and blocked for 2 h at 37 °C with PBS⁺⁺/T containing 1% BSA. Recombinant full-length PTX3 (~45 kDa) or N_{term}-PTX3 fragment (~17 kDa) or C_{term}-PTX3 (~25 kDa) diluted in PBS⁺⁺/T 1% BSA was then added at the concentrations specified under "Results" and incubated for 2 h at 37 °C. Bound PTX3 and PTX3 fragments were revealed by their reaction with biotin-labeled rabbit anti-human PTX3 polyclonal antibody (1 μ g/ml) for 1 h at 37 °C followed by incubation with 100 μ l of HRP-conjugated streptavidin diluted 1:5000 for 1 h at room temperature. The enzymatic reaction was developed using the substrate ditetramethylbenzidine (Sigma) for 10 min.

Interaction of PTX3 with bikunin proteoglycan was assessed by measuring the binding of 500 ng/well PTX3 to plates coated with 100 μ l of 10 μ g/ml bikunin (40 kDa) following the proce-

dures reported above. In competition experiments, biotinylated PTX3 (110 nM) was mixed with unlabeled PTX3 or IαI or bikunin (all at 1100 nM) or with different concentrations of rat monoclonal antibodies MNB4 or rat monoclonal 16B5 before addition to IαI-coated plates.

Dose response binding of IαI to immobilized PTX3 was performed by coating wells with 100 μ l of PTX3 (10 μ g/ml) and adding IαI at the concentrations specified under "Results." The binding was revealed by rabbit polyclonal anti-human IαI antibody (1 μ g/ml) for 1 h at 37 °C followed by incubation with HRP-linked anti-rabbit F(ab')₂ fragment immunoglobulin (1:5000).

Isolation and Culture of COCs—Mice were sacrificed at 44–48 h from PMSG injection, and their ovaries were dissected. COCs were mechanically isolated by puncturing large follicles in minimum essential medium containing 25 mM HEPES, 0.1% BSA, and 50 ng/ml gentamycin. Five to 20 compact COCs were cultured in 20- μ l droplets, under mineral oil, of minimum essential medium supplemented with 3 mM glutamine, 0.3 mM sodium pyruvate, and 50 ng/ml gentamycin, in the presence of 100 ng/ml FSH (highly purified rat-FSH; kindly provided by the NIDDK and the National Hormone and Pituitary Program, National Institutes of Health) and 1% fetal calf serum, or 5% mouse serum where specified, for 16 h at 37 °C, 5% CO₂. In *Ptx3*^{-/-} COC cultures, human recombinant PTX3 or PTX3 fragments were added to the medium at the beginning of culture, at the concentrations indicated in the text.

Quantitation of HA—The amounts of HA synthesized by COC cultures were determined by metabolic labeling. Cultures were carried out in the presence of [³⁵S]sulfate (60 μ Ci/ml) and [³H]glucosamine (100 μ Ci/ml; PerkinElmer Life Sciences). After 16 h of culture, medium and cell matrix were collected separately, and the amount of HA in the two compartments was determined as described elsewhere (12).

RESULTS

PTX3 and IαI Colocalize in the COC Extracellular Matrix—The evidence that both PTX3 and IαI are essential for the organization of HA enriched-matrix prompted us to investigate whether the two proteins colocalize during matrix formation. The temporal pattern of accumulation and distribution of PTX3 and IαI in COCs was analyzed by immunological staining of sections of ovaries isolated from mice treated with PMSG, to stimulate the formation of COCs, and from mice treated with PMSG followed by hCG, to stimulate cumulus matrix synthesis and COC expansion. As shown in Fig. 1A, both proteins were absent in COCs before hCG injection, but they became clearly detectable after hCG treatment. At 6 h from hCG, PTX3 was localized in the matrix surrounding cumulus cells, the most intense staining being found between the innermost layer of corona radiata cells and oocyte. At 9 h from hCG, signal of PTX3 was increased between both corona radiata cells and cumulus cells. A slight positive staining could also be detected around parietal granulosa cells closest to the COC and those lining the antrum, in agreement with the ability of these cells to synthesize HA and to become embedded within the cumulus at ovulation (31, 32). IαI showed a similar localization and temporal pattern of accumulation in the perioovulatory follicle, although it appeared more

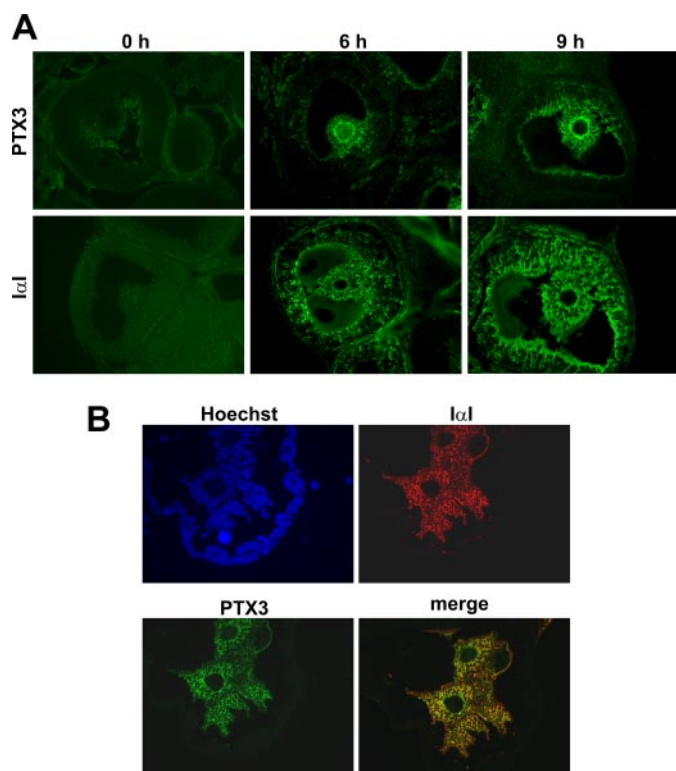


FIGURE 1. Immunolocalization of PTX3 and IαI during *in vivo* mouse cumulus expansion. *A*, ovaries were collected before (0 h) and after 6 and 9 h from an ovulatory dose of hCG. Sections of ovaries were probed with rabbit polyclonal anti-human PTX3 or rabbit polyclonal anti-human IαI and fluorescent goat anti-rabbit IgG secondary antibody. *B*, colocalization of PTX3 and IαI in the extracellular matrix of ovulated COCs was assessed in sections of oviducts collected at 14 h from hCG injection. Sections were first incubated with rabbit anti-human IαI and with the secondary Cy3 goat anti-rabbit IgG (red). After washing, the same sections were probed with biotin-labeled rabbit anti-human PTX3 polyclonal antibody and with Alexa Fluor 488 streptavidin (green). Nuclei were stained with Hoechst 33258 (blue).

uniformly distributed throughout the cumulus. In mice, ovulation occurs 12–13 h from hCG, and COCs, released from the follicles, rapidly move to the oviduct. Double staining of PTX3 and IαI in sections of oviducts collected at 14 h from hCG showed that both proteins are abundant in the extracellular matrix of ovulated COCs and that they colocalize (Fig. 1*B*).

PTX3 Coprecipitates with IαI-HCs—Previous studies have shown that the HCs, but not the bikunin subcomponent of IαI, are integrated in the cumulus matrix through the formation of a covalent linkage with HA (4, 15). To investigate whether PTX3 interacts with HCs in native tissue, we immunoprecipitated HCs from hyaluronidase-digested extracellular matrix of ovulated COCs with anti-human IαI antibody and tested for the presence of associated PTX3 by Western blot analysis (Fig. 2*A*). In agreement with previous reports, immunoblot of matrix extract with IαI antibody revealed a major band of ~85 kDa, corresponding to the molecular mass of free HCs of IαI molecules, and two minor bands of higher molecular mass most likely representing clusters of HCs on a single HA strand resistant to hyaluronidase digestion (15). The same bands were generated by proteins eluted from Sepharose-bound anti-IαI antibody but not from uncoated or control IgG-coated Sepharose beads, indicating that the HCs of mouse IαI were specifically and successfully immunoprecipitated by anti-human IαI anti-

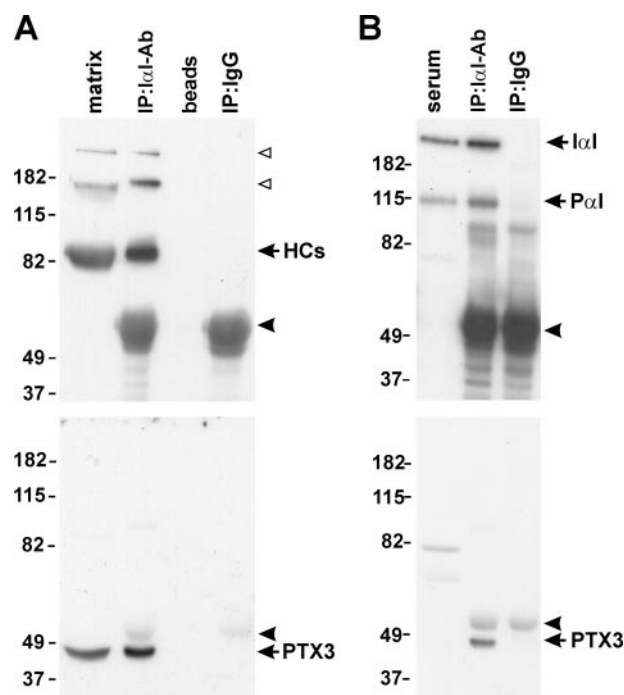


FIGURE 2. PTX3 interacts with HCs of IαI in mouse cumulus matrix. *A*, PTX3 coimmunoprecipitates with HCs of IαI from cumulus matrix extract. *Streptomyces* hyaluronidase-digested matrix of ovulated COCs was immunoprecipitated (IP) with rabbit anti-IαI antibody (IαI-Ab) or with control rabbit IgG bound to protein A-Sepharose and separated by 7.5% SDS-PAGE. The same amount of native matrix extract (matrix) was also loaded to the gel for comparison. The immunoblot was first probed with a rabbit polyclonal anti-human IαI antibody (top panel) and then stripped and reprobed with hamster polyclonal anti-mouse PTX3 antibody (bottom panel). The open arrowheads indicate clusters of HCs on a single HA resistant to dissociation by hyaluronidase digestion (15). The closed arrowheads indicate heavy chains of rabbit immunoglobulins linked to the Sepharose beads and eluted by denaturation of the beads in sample buffer. *B*, 1 μl of mouse serum was mixed with 1 μg of purified recombinant PTX3, and proteins were immunoprecipitated with rabbit anti-IαI antibody or with control rabbit IgG. Samples were analyzed by SDS-PAGE and immunoblotted with IαI antibody (top panel) and PTX3 antibody (bottom panel) as above.

body. The diffuse band at about 50 kDa corresponds to the heavy chains of rabbit immunoglobulins linked to Sepharose beads and eluted in the sample buffer. The same blot, probed with the anti-PTX3 antibody after stripping, showed a positive band of ~45 kDa, corresponding to the molecular mass of PTX3, in both the matrix extract and the material eluted from IαI antibody-Sepharose beads. Reciprocally, HCs were immunoprecipitated from hyaluronidase-digested COC matrix by PTX3 antibody (supplemental Fig. S1).

Although these results suggested an association between PTX3 and HCs of IαI in native tissue, we could not exclude the possibility that the interaction was mediated by other matrix proteins synthesized by cumulus cells. Thus, purified recombinant mouse PTX3 was mixed with mouse serum, which is the physiological source of IαI molecules for cumulus matrix formation, and the mixture was immunoprecipitated with IαI antibody. As shown in Fig. 2*B*, mouse serum contains two forms of IαI family molecules, pre-α-trypsin inhibitor (PαI) (~125 kDa) and IαI (~240 kDa), containing one and two HCs, respectively, and both of them were pulled down by the antibody. Western blot analysis with PTX3 antibody revealed that the recombinant PTX3 was coimmunoprecipitated.

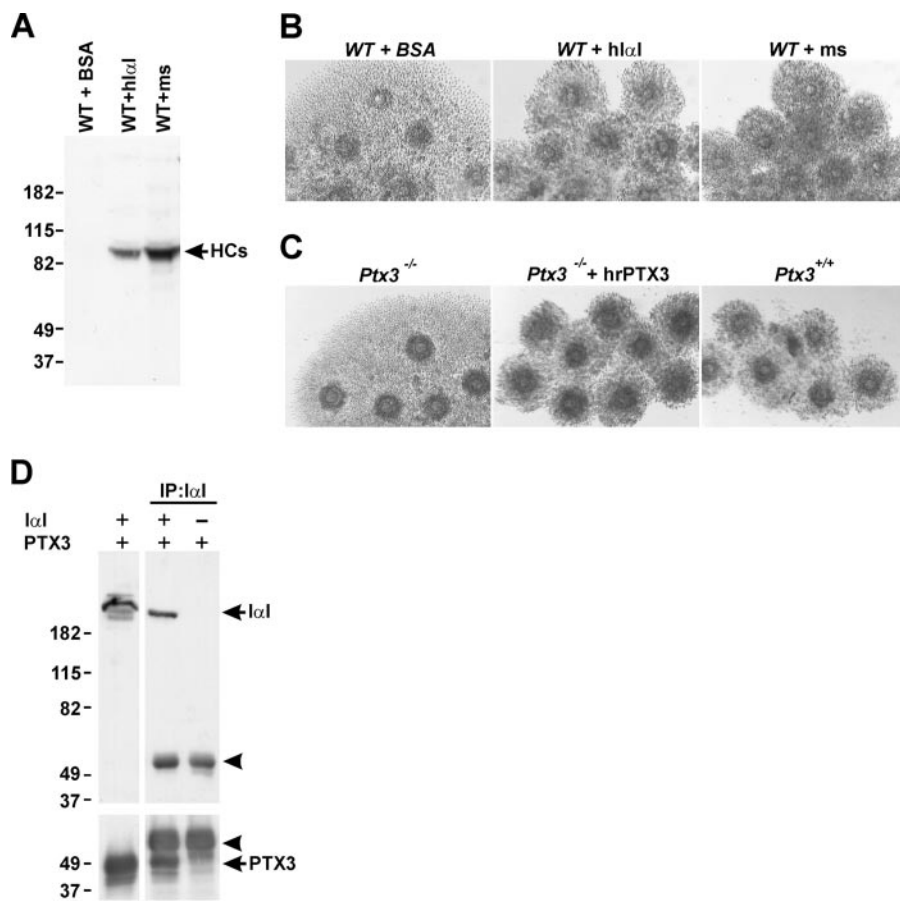


FIGURE 3. Human purified IαI and human recombinant PTX3 promote mouse cumulus expansion and interact in vitro. *A*, HCs of human IαI are integrated in mouse cumulus matrix. IαI Western analysis of *Streptomyces* hyaluronidase-digested matrix from 20 COCs isolated from PMSG-primed wild type mice and cultured in the presence of 100 ng/ml FSH with 1 mg/ml BSA (WT+BSA) or 5 μg/ml human native IαI (WT+hIαI) or 5% mouse serum (WT+ms) for 16 h as above showing that human IαI, as mouse serum, supports matrix organization and retention of cumulus cells around the oocyte. *B*, morphology of COCs treated for 16 h as above showing that human IαI, as mouse serum, supports matrix organization and retention of cumulus cells around the oocyte. *C*, human recombinant PTX3 allows cumulus expansion of *Ptx3*^{-/-} COCs. Morphology of COCs from PMSG-primed *Ptx3*^{-/-} mice cultured for 16 h in the presence of 100 ng/ml FSH and 5% mouse serum without (*Ptx3*^{-/-}) and with 2 μg/ml human recombinant PTX3 (*Ptx3*^{-/-} + hrPTX3) is shown. For comparison, COCs from PMSG-primed *Ptx3*^{+/+} mice were cultured in the presence of FSH and mouse serum (*Ptx3*^{+/+}). Images were captured with an inverted microscope at ×50 magnification. *D*, hrPTX3 and IαI coimmunoprecipitate. One μg of purified hrPTX3 was incubated with rabbit anti-IαI antibody bound to protein A-Sepharose in the presence and in the absence of 1 μg of purified hIαI, and the immunoprecipitated proteins were separated by 7.5% SDS-PAGE. The immunoblot was first probed with a rabbit polyclonal anti-human IαI antibody and then stripped and re-probed with rabbit polyclonal anti-human PTX3 antibody. The closed arrowheads indicate heavy chains of rabbit immunoglobulins eluted by denaturation of the beads in sample buffer.

The primary structure of PTX3 and HCs of IαI is highly conserved between mouse and humans (33, 34), and both proteins have been found in human cumulus matrix (7, 35), suggesting that these molecules play the same role in human as in mouse cumuli. Consistent with this hypothesis, when mouse COCs were stimulated with FSH in the presence of IαI purified from human serum, HCs were integrated in the matrix (Fig. 3A) and were as effective as mouse serum in promoting expansion of COCs (Fig. 3B). Likewise, purified human recombinant PTX3 was able to support *in vitro* the formation and expansion of extracellular matrix by *Ptx3*^{-/-} mouse COCs (Fig. 3C). We then tested whether purified human recombinant PTX3 would bind to IαI purified from human serum by mixing the two molecules and immunoprecipitating with IαI antibody. As shown in Fig. 3D, PTX3 was pulled down by the IαI antibody in the

presence of IαI. Altogether, the results demonstrate a direct binding between PTX3 and IαI.

Characterization of Binding of PTX3 to IαI—To further characterize PTX3/IαI interaction, we performed microtiter plate binding experiments with human purified molecules. As shown in Fig. 4A, at physiological pH in the presence of 1 mM Ca²⁺ and 0.5 mM Mg²⁺ (PBS⁺⁺), human IαI bound to immobilized human PTX3 in a dose-dependent manner. The same result was obtained when soluble PTX3 was tested on immobilized IαI (Fig. 4B). Conversely, under the same experimental conditions, PTX3 did not bind to a well coated with the subcomponent of IαI bikunin (Fig. 4C). Accordingly, the binding of biotinylated PTX3 to IαI was prevented by a 10-fold molar excess of unlabeled PTX3 or IαI but not of bikunin (Fig. 4D). These results, together with the coimmunoprecipitation experiments reported above, suggest that PTX3 specifically and exclusively binds to HCs of IαI.

To determine the effect of ions on the interaction between PTX3 and IαI, enzyme-linked immunosorbent binding assays were performed in the absence of Ca²⁺ and Mg²⁺ and with EDTA (Fig. 5). In this condition, the binding of PTX3 to immobilized IαI diminished to background level, indicating divalent cation dependence. Therefore, the effects of different concentrations of Ca²⁺ and Mg²⁺ on the binding were investigated. In the absence of

added ions but without EDTA, a slight binding of PTX3 to immobilized IαI was observed, likely due to metal ion impurities. The binding did not increase in the presence of Ca²⁺, whereas it was enhanced by Mg²⁺, reaching maximal value at the concentration of 0.5 mM Mg²⁺. Binding of PTX3 to BSA was minimal at each analyzed condition. These data suggest that PTX3/IαI interaction is dependent on the presence of Mg²⁺.

PTX3 Binds to IαI through Its Specific N-terminal Domain—PTX3 is a prototypic long pentraxin consisting of a C-terminal 203-amino-acid domain (C_{term}-PTX3), homologous to classical short pentraxins, and an N-terminal 178-amino acid domain (N_{term}-PTX3), unrelated to other known proteins (24). To determine the role of the two domains in IαI recognition, the human C_{term}-PTX3 region and N_{term}-PTX3 extension were

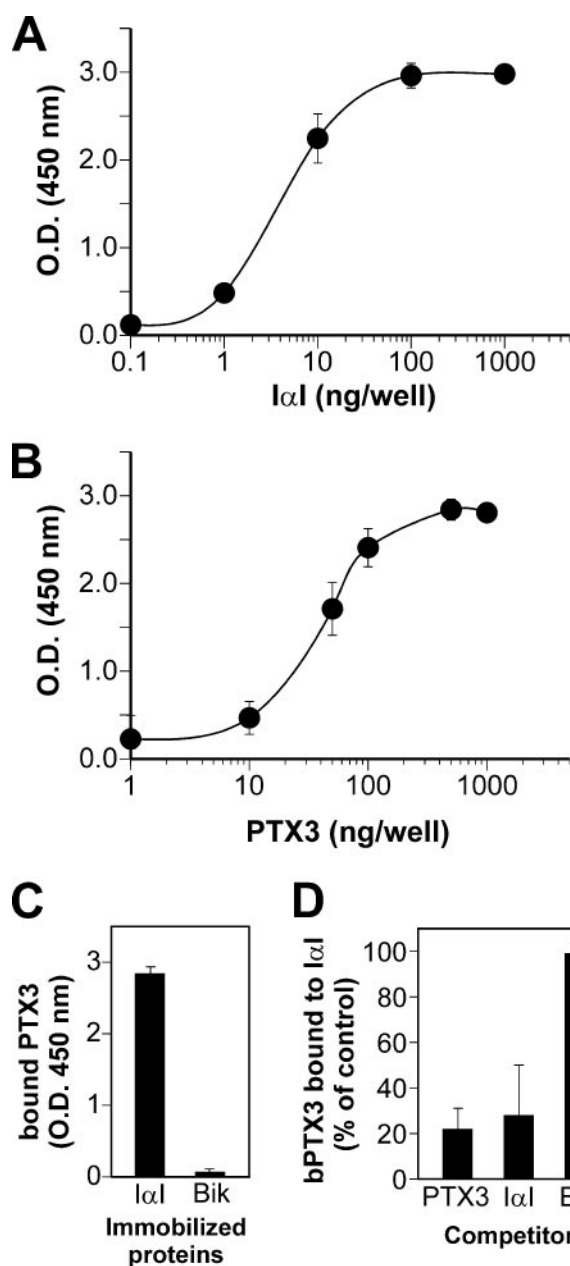


FIGURE 4. PTX3/I α I binding in solid-phase assays. *A* and *B*, dose-response binding of human I α I to human recombinant PTX3 (*A*) and of PTX3 to I α I (*B*) coated onto wells of a microtiter plate. *C*, binding of PTX3 (500 ng/well) to I α I and bikunin (*Bik*)-coated wells. *D*, I α I-coated wells were incubated with biotinylated PTX3 (bPTX3) (110 nM) in the absence or in the presence of a 10-fold molar excess of competitors (PTX3, I α I, bikunin). Data are expressed as the percentage of binding measured in the absence of any competitor. All the assays were performed at pH 7.4 in the presence of PBS with 1 mM Ca²⁺ and 0.5 mM Mg²⁺. The extent of binding was determined by enzyme-linked immunosorbent assay. All data are the mean \pm S.D. of three independent experiments in triplicate.

expressed in Chinese hamster ovary cells and compared with full-length PTX3 for their ability to bind to I α I-coated microplates. C_{term}-PTX3, unlike full-length PTX3 and recombinant N_{term}-PTX3 fragment, does not form multimers under native conditions (supplemental Fig. S2). Previous findings have shown that cross-linking of recombinant C_{term}-PTX3 fragments is required for its binding to immobilized C1q molecule, a component of the complement system that is a well known

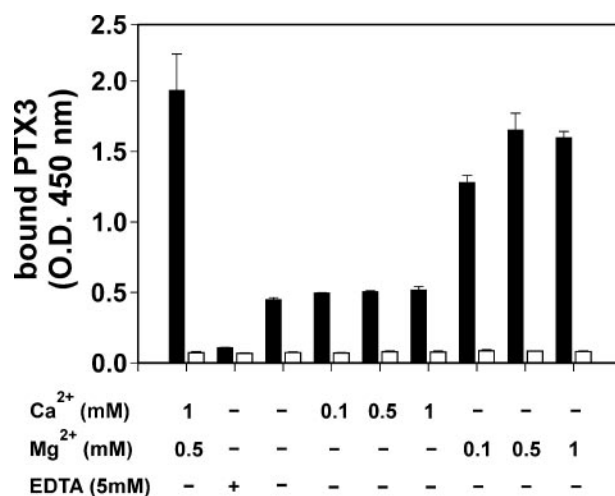


FIGURE 5. Effect of ions on the binding of PTX3 to I α I. Binding of PTX3 (100 ng/well) to immobilized I α I (solid bars) and BSA (open bars) was examined in the presence or absence of EDTA and with or without Ca²⁺ or Mg²⁺. The binding assay was conducted as described under "Experimental Procedures" except that the blocking and washing steps before PTX3 addition were performed in all cases with PBS without Ca²⁺ and Mg²⁺, and dilution of PTX3 was performed using PBS containing the indicated levels of EDTA, Ca²⁺, or Mg²⁺. The extent of binding was determined by enzyme-linked immunosorbent assay. The data are the mean \pm S.D. of three independent experiments in triplicate.

ligand of the long pentraxin PTX3 as well as of short pentraxins (23). Therefore, a fraction of C_{term}-PTX3 was subjected to cross-linking and included in the analysis. As shown in Fig. 6A, N_{term}-PTX3 bound to I α I-coated wells in a dose-dependent manner and with an efficiency comparable with that of full-length PTX3. No interaction was instead observed with cross-linked and native C_{term}-PTX3.

We also found that monoclonal antibody MNB4, which recognizes epitopes 87–99 in the N-terminal extension of human PTX3 (28), prevented full-length PTX3 from binding to I α I (Fig. 6B). No effect was instead observed with monoclonal antibody 16B5, which recognizes epitopes 306–312 in the C terminus of PTX3 (28). Antibody MNB4 did not alter the binding of PTX3 to immobilized C1q, ruling out side effects of this antibody on C-terminal ligand recognition. On the whole, these results demonstrate that the site of interaction of PTX3 with HCs of I α I resides in the N-terminal region of the molecule.

The N Terminus of PTX3 Is Necessary and Sufficient for Stabilizing COC Extracellular Matrix—We then investigated the functional role of N-terminal PTX3 domain in COC matrix organization. We have previously shown that, at variance with COCs from wild type mice, COCs isolated from *Ptx3*^{-/-} mice and stimulated with hormones in the presence of serum (*i.e.* I α I) are unable to retain the neosynthesized HA in the intercellular space and to form a stable viscoelastic matrix (7). As a consequence, cumulus cells dissociate from each other and from the oocyte, settling on the bottom of the plate. The presence of recombinant full-length PTX3 during cumulus expansion stimulation restored a normal phenotype in *Ptx3*^{-/-} cumuli. We show here that recombinant N_{term}-PTX3 can replace full-length PTX3 action in promoting HA organization in the matrix and normal cumulus expansion, whereas recombinant C_{term}-PTX3 is ineffective (Fig. 7, *A* and *B*). In addition, anti-N-terminal PTX3 antibody MNB4, which blocked PTX3

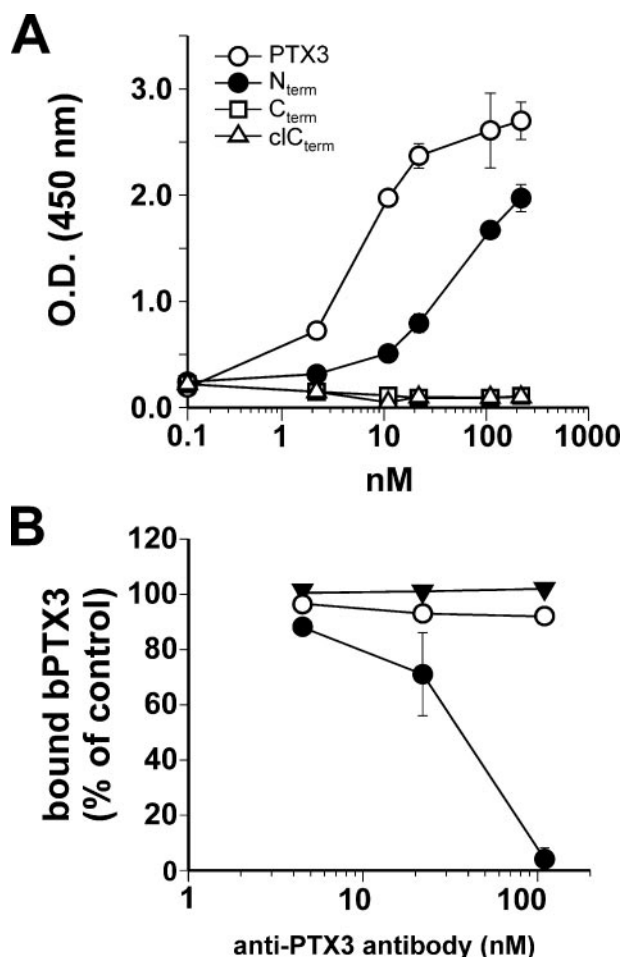


FIGURE 6. PTX3 binds to IαI through its N-terminal domain. *A*, recombinant N_{term}-PTX3 domain binds to immobilized IαI. IαI-coated wells were incubated with different amounts of full-length PTX3 (open circle), N_{term}-PTX3 domain (closed circle), or native (open square) or cross-linked (open triangle) C_{term}-PTX3 domain, all expressed and purified from stable transfected Chinese hamster ovary cells. The relative amount of ligands bound to immobilized IαI was immunodetected by incubation with a rabbit polyclonal anti-PTX3 antibody as described under "Experimental Procedures." The data represent the mean ± S.D. of three independent experiments in triplicate. *B*, monoclonal MNB4 antibody against N-terminal region of PTX3 prevents the binding of full-length PTX3 to IαI. Closed circle and open circle, binding of bPTX3 (110 nM) to IαI-coated wells in the presence of different concentrations of MNB4 (closed circle) or 16B5 (open circle) antibody. Closed triangle, binding of bPTX3 (110 nM) to C1q-coated wells in the presence of different amounts of MNB4 antibody. Data are expressed as the percentage of control (binding measured in the absence of any antibody). Data are the mean ± S.D. of two independent experiments in triplicate.

interaction with IαI, also prevented the ability of full-length PTX3 to allow HA retention and cumulus expansion, whereas the antibody 16B5, recognizing the C-extension, did not (Fig. 7C). These results demonstrate that the essential role played by PTX3 in matrix assembly is specifically related to its N-terminal domain.

DISCUSSION

In the preovulatory follicle, HCs of serum-derived IαI are dissociated from the chondroitin sulfate chain of bikunin and covalently linked to HA strands newly synthesized by cumulus cells (4, 14, 15). We and others have shown that stimuli inducing HA synthesis and the formation of HA·HC complexes by mouse cumulus cells also promote the production by these cells

of the long pentraxin PTX3, which accumulates in the extracellular matrix (6, 7). *In vivo* and *in vitro* observations show that both PTX3 production and HC linkage to HA play a key role for cumulus matrix assembly and stability (4–7). Data reported in the present study indicate that PTX3 interacts with HCs of IαI and that this interaction is essential for organizing HA in the viscoelastic matrix of cumulus oophorus, providing a direct functional link between the two molecules.

The formation of a complex between PTX3 and IαI-HCs in cumulus matrix is suggested by the evidence that PTX3 coprecipitates with IαI-HCs from ovulated cumulus matrix extracts. This interaction is not mediated by other cumulus matrix components since recombinant mouse PTX3, incubated with mouse serum, is pulled down by the IαI antibody. Furthermore, the same result is obtained with purified molecules, recombinant human PTX3, and IαI isolated from human serum. Direct interaction is further corroborated by microtiter plate assays showing that recombinant human PTX3 binds to human serum-purified IαI in a concentration-dependent manner. Noteworthy, PTX3 is unable to interact with the bikunin subcomponent of IαI (consisting of a light chain protein plus a chondroitin sulfate chain), as indicated by the evidence that PTX3 does not bind to immobilized bikunin and that bikunin does not compete for the binding of PTX3 to immobilized IαI. It has been pointed out that bikunin, released during the reaction of HC coupling to HA, does not accumulate in the cumulus matrix, indicating that this subcomponent of IαI is not directly involved in building cumulus matrix (4). All the whole, these findings suggest that PTX3 specifically and exclusively interacts with HCs of IαI, which become integral components of the cumulus matrix.

Our results show that PTX3-IαI interaction is metal ion-dependent, with a Mg²⁺ requirement. It is not known whether Mg²⁺ binding sites are present on PTX3, but HCs likely bind this ion because of the presence in their C-terminal half of a von Willebrand factor type A-domain (8), known to support Mg²⁺ binding in other proteins and to play a key role in ligand recognition. Whether this region of HC is involved in PTX3 binding requires further investigation.

PTX3 consists of a C-terminal 203-amino-acid domain, sharing homology with classic short pentraxins C-reactive protein and serum amyloid P, and an N-terminal 178-amino-acid extension with no significant homology to any known protein, including the other identified long pentraxins (24). Previous findings that classic short pentraxins bind *in vitro* to some extracellular matrix components (36–39) and localize in physiological and pathological extracellular matrices, conferring them stability (40–42), might lead to the hypothesis that PTX3 function in cumulus matrix could be related to its pentraxin region. However, we show here that the pentraxin domain of PTX3 is not involved in HC recognition and in cumulus matrix stability, thus assigning a specific and unique role to PTX3 in matrix organization. Findings reported in the present study show that the binding site for HCs resides in PTX3 N-terminal amino acid sequence, which shares no significant homology with any known protein. The recombinant N_{term}-PTX3 sequence, but not the C_{term}-PTX3, binds to immobilized IαI,

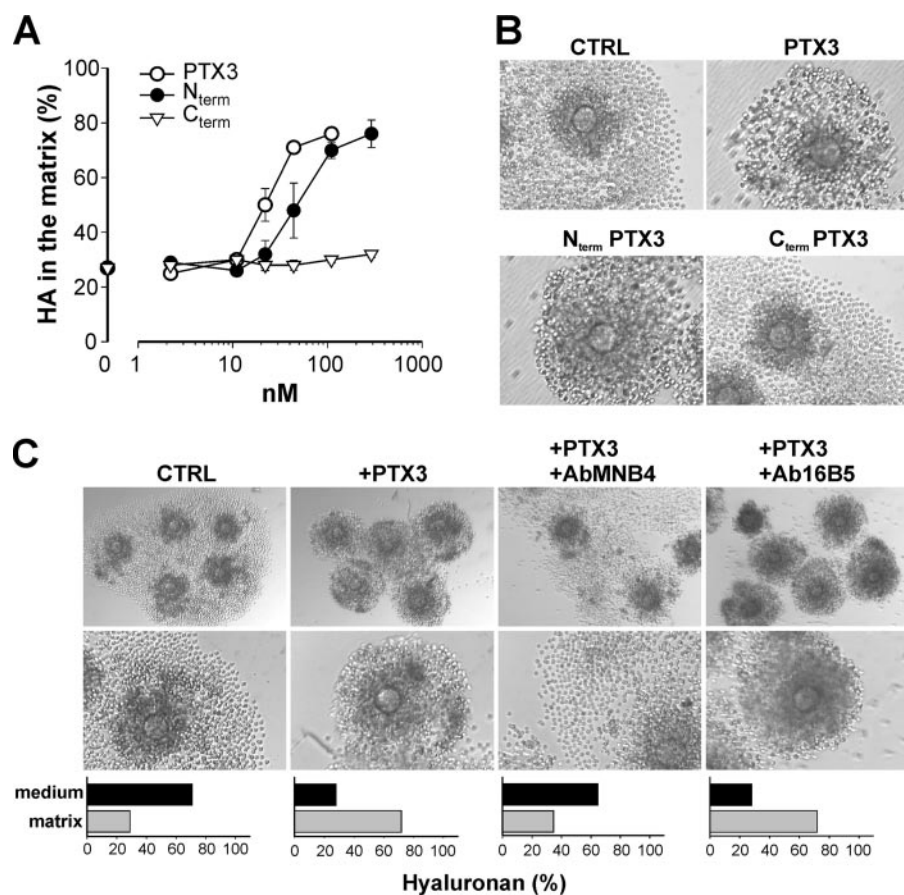


FIGURE 7. N-terminal domain of PTX3 is involved in HA organization and cumulus expansion. Recombinant N_{term} PTX3 domain restores normal phenotype in *Ptx3*^{-/-} COCs. *A*, COCs from PMSG-primed *Ptx3*^{-/-} mice were cultured with 100 ng/ml FSH, 1% fetal calf serum, radiolabeled precursors of glycosaminoglycans, in the absence or presence of increasing concentrations of human recombinant full-length PTX3 (*open circle*), N_{term}-PTX3 (*closed circle*), or C_{term}-PTX3 (*triangle*), and the net amount of newly synthesized HA was retained in the matrix and released into the medium determined as described under "Experimental Procedures." The reported values represent the mean ± S.D. of the percentage of HA retained in the matrix on total synthesized, obtained from three independent experiments. *B*, morphology of COCs cultured as above for 16 h without (CTRL) or with PTX3, N_{term}-PTX3, or C_{term}-PTX3 (all at 110 nM). *C*, MNB4 antibody, recognizing the N-terminal domain, blocks the effect of human recombinant full-length PTX3 on *in vitro* COC expansion. COCs from PMSG-primed *Ptx3*^{-/-} mice were cultured with 100 ng/ml FSH, 1% fetal bovine serum, radiolabeled precursors of glycosaminoglycans and 2 μg/ml full-length PTX3, in the absence or in the presence of MNB4 or 16B5 antibody (both at 10 μg/ml). At 16 h of culture, cumulus expansion and distribution of HA in matrix and medium (histograms) were assessed. Reported data refer to one representative experiment of three performed.

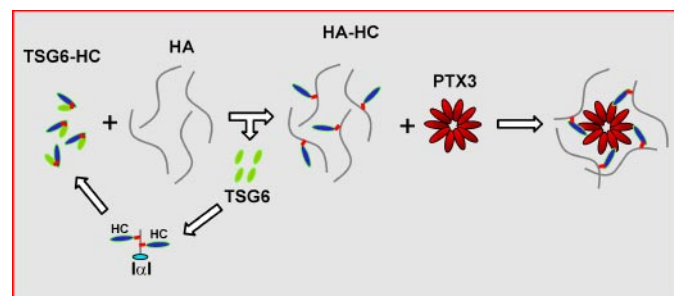


FIGURE 8. Role of PTX3/HC interaction in cumulus matrix assembly. In the proposed model, HCs, transferred from $I\alpha I$ to HA by the catalytic activity of TSG6, bind to distinct protomers of multimeric PTX3, leading to HA cross-linking during the formation of cumulus matrix. The red rectangles represent covalent interactions.

and the binding efficiency is comparable with that of recombinant full-length PTX3. Likewise, the binding of full-length PTX3 to immobilized $I\alpha I$ is selectively inhibited by monoclonal

anti-hPTX3 antibody MNB4, which specifically recognizes the N-terminal region of PTX3.

The biological effects of the N_{term} domain and of the antibody MNB4 provide new insight into the mechanism by which the viscoelastic matrix of the cumulus is assembled. It has been hypothesized that HCs might cross-link separate HA strands through the formation of a covalent bond with one HA molecule and an ionic bond with another one (5, 15, 43). Agarose gel electrophoresis and gel filtration analyses of HC·HA complexes from synovial fluids of patients with rheumatoid arthritis suggest that HC·HA complexes tend to form aggregates (43), a finding that supports a role for HCs in the regulation of HA network formation. However, *Ptx3*-null mice show instability of the cumulus matrix despite the fact that HC·HA complexes are normally formed (7), suggesting that additional molecular interactions involving PTX3 are required for organizing HA in a stable gel-like matrix. Our present findings indeed show that PTX3 binds to HCs and that this interaction has physiological relevance. This is suggested by the evidence that the recombinant N_{term}-PTX3 fragment, which contains the HC binding site, mimics the action of the full-length protein, in that it allows normal *in vitro* expansion of *Ptx3*^{-/-} COCs by holding the newly synthesized HA within the matrix. Furthermore,

anti-PTX3 monoclonal antibody MNB4, which prevents PTX3 from binding to $I\alpha I$, also prevents full-length recombinant PTX3 from restoring normal phenotype in *Ptx3*^{-/-} COCs stimulated *in vitro*.

PTX3 is mostly assembled in a complex consisting of ~10 identical protomers (23). Noteworthy, complete inhibition of PTX3- $I\alpha I$ interaction is achieved by preincubating PTX3 with a concentration of monoclonal antibody MNB4 that is equivalent, in molar terms, to that of PTX3 protomers, suggesting that each protomer might interact with a single HC molecule. Thus, although PTX3 has no direct HA binding capacity (7), it might cross-link several HA strands by binding HCs that are covalently linked to them (Fig. 8). In a previous study, we have shown that PTX3 may also bind multiple molecules of TSG6 (7), a protein synthesized by cumulus cells, with the ability to ionically bind to HA through a link module common to many hyaluronectins (44, 45). Thus, binding of TSG6 to PTX3 might

in principle contribute to additionally cross-link HA chains. However, recent findings cast doubts on the hypothesis of a structural role of TSG6. It has been shown that HA hexasaccharides, which efficiently inhibit the binding of TSG6 to HA, do not inhibit cumulus matrix formation (46). In addition, it has been demonstrated, with purified proteins, that TSG6 acts as a cofactor and catalyst in the transfer of HCs to HA (17), by forming covalent complexes with individual HCs that act as intermediates in the transfer reaction. TSG6 released upon the transfer was shown to quickly combine with α I to form new TSG6·HC complexes and thus be recycled. In agreement, TSG6·HC complexes, but not free TSG6, are found in the expanding cumulus matrix up to a few hours before ovulation (32), suggesting that all the available TSG6 is engaged in transferring HCs to HA (Fig. 8). Thus, it seems unlikely that TSG6 may directly participate in cross-linking HA strands. Rather, binding of TSG6 to PTX3 might favor the interaction of PTX3 with HCs committed to link with HA (those in TSG6·HC complexes), leading to the integration of PTX3 into the matrix at the same time as, and in coordinate fashion to, HCs. Further studies, focused on the identification of sites of interaction among these proteins, are required to validate this hypothesis.

A cooperation between PTX3 and HCs in matrix organization is further supported by the similar temporal pattern of accumulation of the two proteins in the cumulus matrix and by their colocalization. Interestingly, PTX3 is apparently more abundant between cells of the innermost layers. On the basis of the proposed model of interaction, this would imply a greater aggregation for HA strands in this area. Such regional difference in cumulus matrix organization might be responsible for the formation of a postulated gradient of sperm chemoattractants (47) both by determining the tighter arrangement of corona radiata cells and likely by regulating the diffusion of molecules synthesized by the oocyte and cumulus cells.

In conclusion, our findings suggest a direct cooperation between PTX3 and HCs in organizing the HA-rich matrix of ovulated mouse COCs, which is essential for the success of *in vivo* fertilization. The *in vitro* interaction of human PTX3 and α I, and their ability to support expansion of the mouse cumulus additionally, suggest that PTX3-HC association may have physiological relevance for the maturation and function of the human cumulus as well.

Acknowledgments—We are grateful to Dr. Jacques Mizon for providing human purified α I. We thank Graziano Bonelli and Gabriele Rossi for expert technical assistance.

REFERENCES

- Russell, D. L., and Salustri, A. (2006) *Semin. Reprod. Med.* **24**, 217–227
- Yudin, A. I., Cherr, G. N., and Katz, D. F. (1988) *Cell Tissue Res.* **251**, 555–564
- Cherr, G. N., Yudin, A. I., and Katz, D. F. (1990) *Dev. Growth Differ.* **32**, 353–365
- Zhuo, L., Yoneda, M., Zhao, M., Yingsung, W., Yoshida, N., Kitagawa, Y., Kawamura, K., Suzuki, T., and Kimata, K. (2001) *J. Biol. Chem.* **276**, 7693–7696
- Fülöp, C., Szántó, S., Mukhopadhyay, D., Bárdos, T., Kamath, R. V., Rugg, M. S., Day, A. J., Salustri, A., Hascall, V. C., Glant, T. T., and Mikecz, K. (2003) *Development (Camb.)* **130**, 2253–2261
- Varani, S., Elvin, J. A., Yan, C., DeMayo, J., DeMayo, F. J., Horton, H. F., Byrne, M. C., and Matzuk, M. M. (2002) *Mol. Endocrinol.* **16**, 1154–1167
- Salustri, A., Garlanda, C., Hirsch, E., De Acetis, M., Maccagno, A., Bottazzi, B., Doni, A., Bastone, A., Mantovani, G., Beck Peccoz, P., Salvatori, G., Mahoney, D. J., Day, A. J., Siracusa, G., Romani, L., and Mantovani, A. (2004) *Development (Camb.)* **131**, 1577–1586
- Salier, J. P., Rouet, P., Raguenez, G., and Daveau, M. (1996) *Biochem. J.* **315**, 1–9
- Eppig, J. J. (1979) *J. Exp. Zool.* **208**, 111–120
- Salustri, A., Yanagishita, M., and Hascall, V. C. (1989) *J. Biol. Chem.* **264**, 13840–13847
- Chen, L., Mao, S. J., and Larsen, W. J. (1992) *J. Biol. Chem.* **267**, 12380–12386
- Camaioni, A., Hascall, V. C., Yanagishita, M., and Salustri, A. (1993) *J. Biol. Chem.* **268**, 20473–20481
- Powers, R. W., Chen, L., Russell, P. T., and Larsen, W. J. (1995) *Am. J. Physiol.* **269**, E290–E298
- Nagyova, E., Camaioni, A., Prochazka, R., and Salustri, A. (2004) *Biol. Reprod.* **71**, 1838–1843
- Chen, L., Zhang, H., Powers, R. W., Russell, P. T., and Larsen, W. J. (1996) *J. Biol. Chem.* **271**, 19409–19414
- Zhao, M., Yoneda, M., Ohashi, Y., Kuroso, S., Iwata, H., Ohnuki, Y., and Kimata, K. (1995) *J. Biol. Chem.* **270**, 26657–26663
- Rugg, M. S., Willis, A. C., Mukhopadhyay, D., Hascall, V. C., Fries, E., Fülöp, C., Milner, C. M., and Day, A. J. (2005) *J. Biol. Chem.* **280**, 25674–25686
- Day, A. J., and de la Motte, C. A. (2005) *Trends Immunol.* **26**, 637–643
- Wisniewski, H. G., and Vilcek, J. (2004) *Cytokine Growth Factor Rev.* **15**, 129–146
- Fülöp, C., Kamath, R. V., Li, Y., Otto, J. M., Salustri, A., Olsen, B. R., Glant, T. T., and Hascall, V. C. (1997) *Gene (Amst.)* **202**, 95–102
- Yoshioka, S., Ochsner, S., Russell, D. L., Ujioka, T., Fujii, S., Richards, J. S., and Espey, L. L. (2000) *Endocrinology* **141**, 4114–4119
- Mukhopadhyay, D., Hascall, V. C., Day, A. J., Salustri, A., and Fülöp, C. (2001) *Arch. Biochem. Biophys.* **394**, 173–181
- Bottazzi, B., Vouret-Craviari, V., Bastone, A., De Gioia, L., Matteucci, C., Peri, G., Spreafico, F., Pausa, M., D'Etto, C., Gianazza, E., Tagliabue, A., Salmons, M., Tedesco, F., Introna, M., and Mantovani, A. (1997) *J. Biol. Chem.* **272**, 32817–32823
- Breviario, F., d'Aniello, E. M., Golay, J., Peri, G., Bottazzi, B., Bairoch, A., Saccone, S., Marzella, R., Predazzi, V., and Rocchi, M. (1992) *J. Biol. Chem.* **267**, 22190–22197
- Lee, G. W., Lee, T. H., and Vilcek, J. (1993) *J. Immunol.* **150**, 1804–1812
- Garlanda, C., Bottazzi, B., Bastone, A., and Mantovani, A. (2005) *Annu. Rev. Immunol.* **23**, 337–366
- Garlanda, C., Hirsch, E., Bozza, S., Salustri, A., De Acetis, M., Nota, R., Maccagno, A., Riva, F., Bottazzi, B., Peri, G., Doni, A., Vago, L., Botto, M., De Santis, R., Carminati, P., Siracusa, G., Altruda, F., Vecchi, A., Romani, L., and Mantovani, A. (2002) *Nature* **420**, 182–186
- Camozzi, M., Rusnati, M., Bugatti, A., Bottazzi, B., Mantovani, A., Bastone, A., Inforzato, A., Vincenti, S., Bracci, L., Mastroianni, D., and Presta, M. (2006) *J. Biol. Chem.* **281**, 22605–22613
- Michalski, C., Piva, F., Balduyck, M., Mizon, C., Burnouf, T., Huart, J. J., and Mizon, J. (1994) *Vox Sang.* **67**, 329–336
- Capon, C., Mizon, C., Lemoine, J., Rodie-Talbere, P., and Mizon, J. (2003) *Biochimie (Paris)* **85**, 101–107
- Salustri, A., Yanagishita, M., Underhill, C. B., Laurent, T. C., and Hascall, V. C. (1992) *Dev. Biol.* **151**, 541–551
- Ochsner, S. A., Russell, D. L., Day, A., Breyer, R. M., and Richards, J. S. (2003) *Endocrinology* **144**, 1008–1019
- Chan, P., Rislis, J. L., Raguenez, G., and Salier, J. P. (1995) *Biochem. J.* **306**, 505–512
- Introna, M., Alles, V. V., Castellano, M., Picardi, G., De Gioia, L., Bottazzi, B., Peri, G., Breviario, F., Salmons, M., De Gregorio, L., Dragani, T. A., Srinivasan, N., Blundell, T. L., Hamilton, T. A., and Mantovani, A. (1996) *Blood* **87**, 1862–1872
- Jessen, T. E., Odum, L., and Johnsen, A. H. (1994) *Biol. Chem. Hoppe-Seyler* **375**, 521–526
- Hamazaki, H. (1987) *J. Biol. Chem.* **262**, 1456–1460

PTX3 Interacts with $\alpha 1$

37. Swanson, S. J., McPeck, M. M., and Mortensen, R. F. (1989) *J. Cell. Biochem.* **40**, 121–132
38. Zahedi, K. (1996) *J. Biol. Chem.* **271**, 14897–14902
39. Zahedi, K. (1997) *J. Biol. Chem.* **272**, 2143–2148
40. Dyck, R. F., Lockwood, C. M., Kershaw, M., McHugh, N., Duance, V. C., Baltz, M. L., and Pepys, M. B. (1980) *J. Exp. Med.* **152**, 1162–1174
41. Al Mutlaq, H., Wheeler, J., Robertson, H., Watchorn, C., and Morley, A. R. (1993) *Histochem. J.* **25**, 219–227
42. Pepys, M. B. (2001) *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **356**, 203–211
43. Yingsung, W., Zhuo, L., Mörgelin, M., Yoneda, M., Kida, D., Watanabe, H., Ishiguro, N., Iwata, H., and Kimata, K. (2003) *J. Biol. Chem.* **278**, 32710–32718
44. Lee, T. H., Wisniewski, H. G., and Vilcek, J. (1992) *J. Cell Biol.* **116**, 545–557
45. Kohda, D., Morton, C. J., Parkar, A. A., Hatanaka, H., Inagaki, F. M., Campbell, I. D., and Day, A. J. (1996) *Cell* **86**, 767–775
46. Mukhopadhyay, D., Asari, A., Rugg, M. S., Day, A. J., and Fulop, C. (2004) *J. Biol. Chem.* **279**, 11119–11128
47. Eisenbach, M., and Giojalas, L. C. (2006) *Nat. Rev. Mol. Cell. Biol.* **7**, 276–285

**PTX3 Interacts with Inter- α -trypsin Inhibitor: IMPLICATIONS FOR
HYALURONAN ORGANIZATION AND CUMULUS OOPHORUS EXPANSION**

Laura Scarchilli, Antonella Camaioni, Barbara Bottazzi, Veronica Negri, Andrea Doni,
Livija Deban, Antonio Bastone, Giovanni Salvatori, Alberto Mantovani, Gregorio
Siracusa and Antonietta Salustri

J. Biol. Chem. 2007, 282:30161-30170.

doi: 10.1074/jbc.M703738200 originally published online August 2, 2007

Access the most updated version of this article at doi: [10.1074/jbc.M703738200](https://doi.org/10.1074/jbc.M703738200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

Supplemental material:

<http://www.jbc.org/content/suppl/2007/08/07/M703738200.DC1>

This article cites 46 references, 25 of which can be accessed free at

<http://www.jbc.org/content/282/41/30161.full.html#ref-list-1>