Human Reproduction Update 1999, Vol. 5, No.4 pp. 293–301

Hyaluronan and proteoglycans in ovarian follicles

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Proteoglycans are macromolecules formed by a protein backbone to which one or more glycosaminoglycan side chains are co-valently attached. They can be secreted by the cells, retained at the cell surface, or stored in intracellular vacuoles. Hyaluronan is an extremely long glycosaminoglycan which, at variance with other glycosaminoglycans, is released into the extracellular matrix as a free polysaccharide not co-valently linked to a core protein. Both proteoglycans and hyaluronan influence many aspects of cell behaviour by multiple interactions with other molecules. They are involved in matrix formation, cell–cell and cell–matrix adhesion, cell proliferation and migration, and show co-receptor activity for growth factors. Both proteoglycan and hyaluranon synthesis change significantly during ovarian follicle development and atresia. This review describes the structure of these molecules and their possible function in ovarian physiology.

Key words: hyaluronan/ovarian follicles/proteoglycan/regulation of hyaluronan and proteoglycan synthesis

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Introduction

Pioneering work during the 1950s suggested that granulosa cells of mammalian follicles synthesize glycosaminoglycans at the time of antrum formation and secrete them into the follicular fluid (Zachariae, 1957, 1958). These glycosaminoglycans were identified as chondroitin sulphate, dermatan sulphate and a heparin-like substance (Jensen and Zachariae, 1958; Gebauer *et al*., 1978). They are co-valently linked to core proteins to form proteoglycan molecules. The biochemical properties of the intact, native proteoglycans in porcine follicular fluid have been investigated to determine their structures and potential functions in ovarian follicles (Yanagishita *et al*., 1979). Granulosa cells cultured *in vitro* synthesize and secrete proteoglycans with chemical and physical properties very similar to those isolated from follicular fluid (Yanagishita and Hascall, 1979). Thus, cell cultures have been used extensively to study parameters involved in the regulation of proteoglycan synthesis and degradation by granulosa cells. Proteoglycan synthesis by granulosa cells is significantly altered in response to gonadotrophins, which suggests that these macromolecules have defined functions during folliculogenesis.

Granulosa cells which closely surround the oocyte in the antral follicle, called cumulus cells, synthesize essentially the same spectrum of proteoglycans (Salustri *et al*., 1989). However, during the preovulatory period, cumulus cells—in contrast with the other granulosa cells—synthesize a large amount of hyaluronan which is organized between the cells to form a muco-elastic matrix (Salustri *et al*., 1992). Mouse cumulus cell–oocyte complexes have been successfully cultured *in vitro* and have been used to study the synthesis of hyaluronan and its organization in the extracellular matrix.

In the past few years, proteoglycans and hyaluronan have been identified as important in a large range of biological processes. This chapter describes some possible roles they may have in ovarian physiology.

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General characteristics of hyaluronan and proteoglycans

Hyaluronan is a glycosaminoglycan that consists of a polymer of (β1,4-glucuronic acid–β1,3-*N*-acetylglucosamine)$_n$ disaccharides, where n can be more than 10 000 (Figure 1). The resulting large, polyanionic macromolecules (≥5000 kDa) exist in solution as relatively stiff, random coils that occupy large domains in solution. Thus, at a concentration of ~1 mg/ml of solvent, the domains of individual molecules begin to overlap. A family of three eukaryotic hyaluronan synthases (Has1, 2, 3) with considerable sequence identity have been recently identified (for review see Weigel *et al*., 1997). These enzymes contain several transmembrane domains and appear to be associated with the plasma membrane. Evidence favours a mechanism for hyaluronan synthesis in which the polymer is elongated at the reducing end, with extrusion of the elongating chain into the extracellular space. Unlike other glycosaminoglycans, hyaluronan is not assembled on a core protein to form a proteoglycan.

Proteoglycans consist of a core protein with co-valently attached glycosaminoglycan chains of variable length and composition (for reviews see Hascall *et al*., 1991; Wight *et al*., 1991). Proteoglycans involved in follicle development and function contain two types of glycosaminoglycans, chondroitin sulphate and heparan sulphate with initial backbone disaccharide repeats of (β1,4-glucuronic acid–β1,3-*N*-acetylgalactosamine)$_n$ and (β1,4-glucuronic acid–α1,4-*N*-acetylglucosamine)$_n$ respectively, where n is usually less than a few hundred (Figure 1). Like other proteins, the core proteins are synthesized in the rough endoplasmic reticulum, and the glycosaminoglycan chains are assembled on appropriate serine residues by multi-enzyme complexes as they traverse through the Golgi cisternae and the trans-Golgi network. Additional modifications in the glycosaminoglycans, i.e. addition of sulphoesters on various hydroxyl groups and 5-epimerization of some glucuronic acids to iduronic acid, occur during or shortly after chain elongation (Figure 1). The formation of iduronic acid is extensive in heparan sulphate. If it occurs in chondroitin sulphate, the glycosaminoglycan is referred to as dermatan sulphate. These modifications can be essential for determining the biological activities of the completed proteoglycans. It has not been determined whether the chondroitin sulphate chains on proteoglycans in the ovarian follicle have iduronic acid and hence would be designated as dermatan sulphate proteoglycans. For simplicity, we refer to this class of proteoglycans as chondroitin sulphate proteoglycans throughout this chapter. The mature proteoglycans can be either: (i) secreted, thereby contributing to formation of the extracellular matrix; (ii) retained at the cell surface via either an intercalated transmembrane domain or a lipid, glycosylphosphatidylinositol (GPI) anchor; or (iii) retained in intracellular vacuoles such as in the storage granules in mast cells.

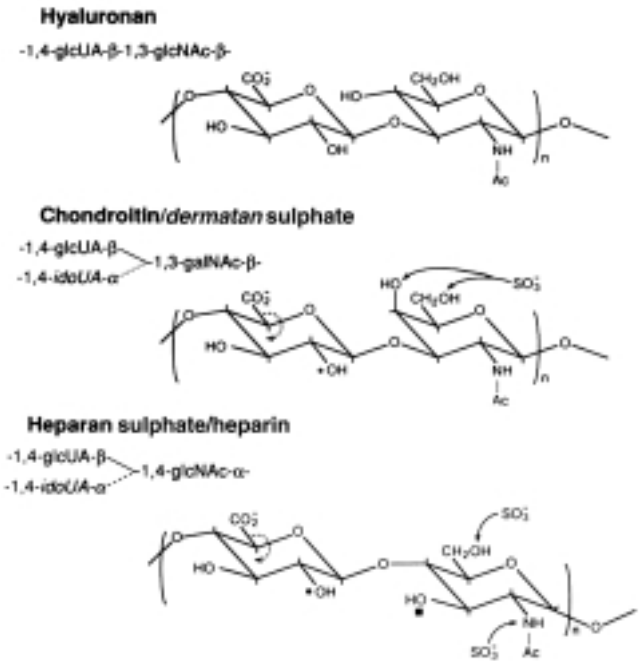

Figure 1. Structure of the repeating disaccharides of glycosaminoglycans. The dashed circular arrows indicate the enzymatic conversion by 5-epimerases of glucuronic acid to iduronic acid for the chondroitin sulphate and heparan sulphate structures. This post-elongation modification converts chondroitin sulphate to dermatan sulphate. The percentage of iduronic acid in heparan sulphate is generally less than 50%, while in heparin it is much higher. Positions of frequent sulphation are indicated by the various arrows. In the chondroitin sulphate structure, * indicates the 2-position of the hexuronic acid which is less frequently sulphated and typical for dermatan sulphate; • indicates the similar substitution for heparan sulphate; and ■ indicates the location on the 3-position of the glucosamine which is infrequently sulphated, a substitution which is, however, required for heparin's anti-coagulant activity. In summary, heparin differs from heparan sulphate as having a higher degree of sulphation (>1.5 versus 0.5–0.8 sulphate per disaccharide), a more frequent 3-*O*-sulphation of glucosamine, and a higher content of iduronic acid (>50% versus <50%).

Proteoglycans are synthesized by all cells. However, their concentrations, the core proteins, and the types and structures of their glycosaminoglycan chains differ in different tissues and often in the same tissue during differentiation, ageing or pathological processes. The mature macromolecules are involved in cell–cell and cell–matrix adhesion, in cell migration and proliferation, in co-receptor activity for various growth factors such as the fibroblast growth factor family, in binding to other molecules such as low-density lipoproteins (LDL) thereby affecting their interaction with uptake receptors,

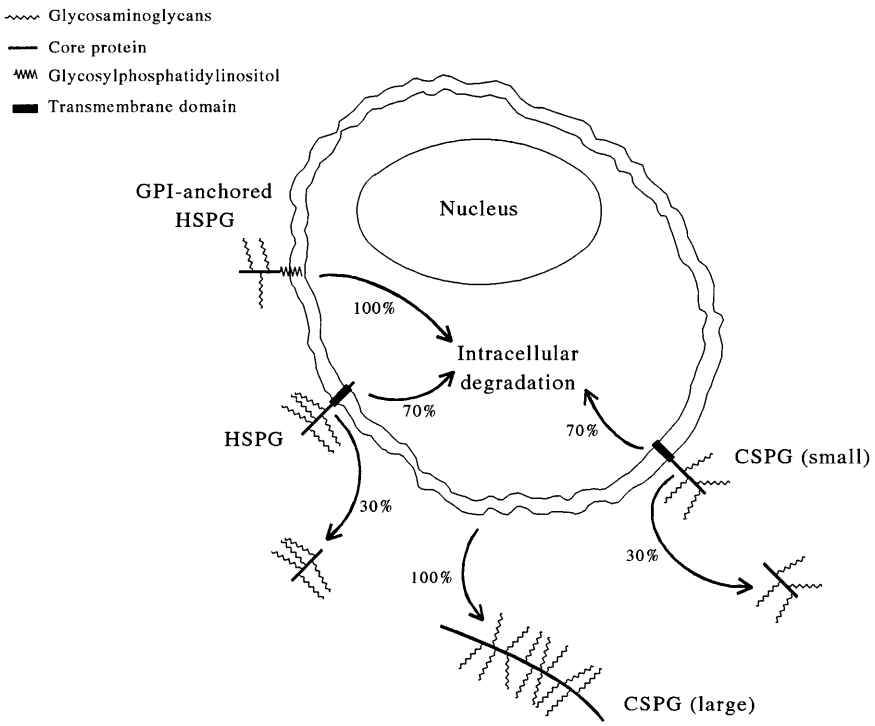

Figure 2. Schematic diagram of proteoglycans synthesized by granulosa cells and their secretion or catabolic fates. CSPG = chondroitin sulphate proteoglycan; GPI-anchored HSPG = glycosylphosphatidylinositol-anchored heparan sulphate proteoglycan; HSPG = heparan sulphate proteoglycan.

in activation or inhibition of proteases, and in matrix formation (for reviews see Wight *et al*., 1991; Salmivirta *et al*., 1996).

Biochemical properties of proteoglycans

During growth of the ovarian follicle, intercellular spaces filled with fluid appear and then coalesce to form a central cavity, the antrum. During this time, [^{35}S]sulphate incorporation increases in granulosa cells and glycosaminoglycans on both chondroitin sulphate and heparan sulphate proteoglycans that accumulate in the follicular fluid (Jensen and Zachariae, 1958; Gebauer *et al*., 1978). The major proteoglycans in porcine follicular fluid are large chondroitin sulphate proteoglycans with estimated average molecular weights of 2000–3000 kDa (Figure 2). Each macromolecule contains 10 to 20 chondroitin sulphate chains of average molecular weight ~55 kDa (Yanagishita *et al*., 1979). The large solvent domains occupied by these proteoglycans exceed the molecular weight cut-off for the blood–follicle barrier (Shalgi *et al*., 1973), and this likely contributes to their accumulation in the follicular fluid where they reach a concentration of 1–2 mg/ml.

Granulosa cells also produce two other distinct proteoglycans that are primarily associated with the cytoplasmic membrane: heparan sulphate proteoglycans with an average molecular weight of 400–500 kDa, and a chondroitin sulphate proteoglycan with an average molecular weight of 300–400 kDa (Yanagishita and Hascall, 1983a,b) (Figure 2). Both contain only a few glycosaminoglycan chains (approximately three to five), with an average molecular weight of ~60 kDa. Most (~85%) of the heparan sulphate proteoglycans are membrane-spanning molecules, quite likely in the syndecan family, while the remainder are bound by a GPI anchor, quite likely in the glypican family (Yanagishita and Hascall, 1984a; Yanagishita and McQuillan, 1989). These proteoglycans remain on the cell surface for only 2–3 h (Yanagishita and Hascall, 1984b). Approximately one-third of the membrane-spanning species are shed into the medium, most likely after a proteolytic cleavage in the ectodomain near the plasma membrane surface. These released heparan sulphate species accumulate in the follicular fluid. The remainder of the membrane-bound (and all of the GPI-anchored) heparan sulphate proteoglycans are internalized and eventually completely degraded after reaching the lysosomal compartment (Yanagishita and Hascall, 1984b). The chondroitin sulphate proteoglycans associated with the cell surface have similar fates.

While these cell surface proteoglycans have not yet been identified precisely, these types of proteoglycans participate in a variety of biological processes, including mediating cell–matrix interactions as in focal adhesions, acting as co-receptors for growth factors such as fibroblast growth factors, or participating in cellular uptake pathways. Their biological functions in ovarian biology remain to be determined.

Gonadotrophin regulation of proteoglycan synthesis

Synthesis of proteoglycans by granulosa cells is regulated by gonadotrophins. Daily injection of purified follicle stimulating hormone (FSH) over 2 days into hypophysectomized, diethylstilboestrol-treated immature female rats stimulated the incorporation of intraperitonally injected [^{35}S]sulphate into ovarian proteoglycans almost 10-fold (Mueller *et al*., 1978). Histochemical analyses of ovarian sections show that proteoglycans secreted by granulosa cells accumulate in the developing antrum. FSH also stimulates proteoglycan synthesis by granulosa cells *in vitro*. Similar effects have been obtained by treating the cells in cultures with dibutyryl cyclic adenosyl monophosphate (cAMP) and phosphodiesterase inhibitors (Schweitzer *et al*., 1981; Yanagishita *et al*., 1981). Since FSH increases cAMP concentrations in granulosa cells, it is likely that the action of this hormone is mediated by this cyclic nucleotide. Granulosa cells isolated from porcine large follicles increase proteoglycan synthesis in response to FSH much less than do granulosa cells isolated from small follicles (Schweitzer *et al*., 1981). In addition, FSH treatment of rat granulosa cells isolated from large follicles stimulates the synthesis of the smaller chondroitin sulphate proteoglycan without altering the rate of synthesis of the larger chondroitin sulphate proteoglycan (Yanagishita *et al*., 1981), which is the predominant proteoglycan in the follicular fluid. Thus, the response of granulosa cells to FSH in proteoglycan synthesis changes with follicular maturation. In addition, granulosa cells in the large follicles have luteinizing hormone (LH) receptors, and LH seems to exert an inhibitory effect on proteoglycan synthesis by ovarian tissues (Gebauer *et al*., 1978). Such hormonal regulation may account for the observed decrease of total glycosaminoglycan concentration in the follicular fluid with the increase of the follicle size (Ax and Ryan, 1979; Grimek and Ax, 1984; Grimek *et al*., 1984).

Prostaglandins, epidermal growth factor (EGF) and testosterone, like FSH, can stimulate proteoglycan synthesis by granulosa cells, and insulin-like growth factor-1 (IGF-1) increases the stimulatory effect of FSH (Yanagishita *et al*., 1981; Adashi *et al*., 1986; Salustri *et al*., 1990a). Thus, it is likely that these local factors participate in the regulation of proteoglycan synthesis *in vivo*.

Proteoglycan functions in the ovarian follicle

The negatively charged sulphate and carboxyl groups and the extended conformation of the glycosaminoglycans chains on the large chondroitin sulphate proteoglycan create a large hydrodynamic domain around the core protein with a high internal charge density, i.e. the macromolecule occupies a large volume of solvent. This creates a swelling pressure by attracting cations, and thus promotes solvent influx into the follicle to form the follicular fluid and to keep the follicle expanded. In addition, the overall structure of this proteoglycan and its large associated solvent domain contribute to the high viscosity of the follicular fluid. An additional rapid increase of follicular fluid occurs just before ovulation. It was found that this increase was accompanied by moderate increase of osmolarity in the ovulatory follicle (Smith and Ketteringham, 1938). These workers proposed that this might depend on depolymerization of macromolecules present in the follicular fluid which, in turn, would promote the secondary increase of fluid volume. Later (Zachariae, 1959), it was suggested that degradation of the 'acid mucopolysaccharides' by the action of 'mucopolysaccharidases' could be involved. However, the presence of enzymes that degrade glycosaminoglycan in the follicle has been never confirmed. Based on the knowledge that glycosaminoglycans are co-valently linked to a core protein to form proteoglycans, it is possible that the preovulatory increase of proteolytic enzymes might cleave the core protein, thereby generating smaller molecules. Indeed, the core protein of the large chondroitin sulphate proteoglycan is highly susceptible to treatment with proteases, including plasmin, that degrade it into chondroitin sulphate–peptide fragments (Yanagishita *et al*., 1979). Whether this occurs *in vivo* in the preovulatory follicle remains to be established.

In follicles classified as atretic by morphological and steroidal criteria, the concentrations of proteoglycans within the membrana granulosa and the follicular fluid are higher than in the healthy follicle (Bellin and Ax, 1984; Bushmeyer *et al*., 1985; Huet *et al*., 1997). Follicle atresia is the result of an apoptotic process that occurs in granulosa cells, mainly for the lack of an appropriate gonadotrophin stimulus to generate the second messenger cAMP (Chun *et al*., 1994, 1996). High concentrations of glycosaminoglycans in the culture medium inhibit gonadotrophin binding to rat granulosa cells and prevent the stimulation of adenylate cyclase (Salomon *et al*., 1978; Nimrod and Lindner, 1980). Thus, upregulation of proteoglycan synthesis might indirectly participate to promote granulosa cell death by preventing gonadotrophin action (Bellin and Ax, 1984). In addition, cAMP may exert its action on cell survival by increasing the synthesis of: (i) growth factors [fibroblast growth factor-2 (FGF-2) and transforming growth factor-α (TGF-α)] which can suppress granulosa cell apoptosis through autocrine action; or (ii) paracrine signals (IGF-1) for theca cells to increase their production of survival growth factors [FGF, hepatocyte growth factor (HGF), EGF, TGF-α] (for review see Hsueh *et al*., 1994). The ability of growth factors, such as FGF-2, FGF-7 and HGF, to bind to heparan sulphate proteoglycans may also inhibit their activity by preventing the interaction with their receptors if proteoglycans are in excess (Mali *et al*., 1993; Bonneh-Barkay *et al*., 1997; Friedl *et al*., 1997; Bono *et al*., 1998; Filla *et al*., 1998; Kato *et al*., 1998; Rahmoune *et al*., 1998; Zhou *et al*., 1998). Evidence has been provided recently that proteoglycans are directly involved in the apoptosis process in several cell types (Dhodapkar *et al*., 1998; Gonzalez *et al*., 1998; Jourdan *et al*., 1998).

Shortly after the LH surge, the follicle becomes more permeable to elements in blood plasma, and the follicular fluid contains thrombin, antithrombin, fibrinogen and other factors involved in clot formation. The heparan sulphate species released into the follicular fluid by granulosa cells are highly sulphated and contain a high content of the regions with the structure, including glucuronic acid adjacent to 3-*O*-*N*-disulphated glucosamine, required for high-affinity binding to antithrombin III (Andrade-Gordon *et al*., 1992; Hosseini *et al*., 1996). This binding accelerates complex formation of antithrombin III with thrombin and prevents thrombin-mediated conversion of fibrinogen to a fibrin clot. Thus, the heparan sulphate species in the follicular fluid are anti-coagulant. This provides a mechanism for maintaining fluidity of the follicular fluid until ovulation. Vascular permeabilization and fibrin deposition does occur in the outer layers of ovulatory follicles, but a fibrin clot only forms in the remnant antral cavity after ovulation. The heparan sulphate species may also bind to serine protease inhibitors present in follicular fluid and enhance their inhibitory activity, providing a mechanism to limit proteolytic activity to the site of follicle wall rupture at ovulation.

Considerable evidence has accumulated to support a role for membrane-associated heparan sulphate proteoglycans to bind circulating lipoproteins and participate in their uptake by several cells (for reviews see Mahley, 1996; Williams and Fuki, 1997). Such protein–lipid complexes provide the major source of cholesterol for different cell types, including luteinized granulosa cells, and synthesis of progesterone by granulosa luteal cells is strictly dependent on lipoprotein availability (for review see Gore-Langton and Armstrong, 1994). Lack of vascularization of the granulosa cell layer in the follicle and the low permeability of the blood–follicle barrier to large lipoprotein molecules appear to limit progesterone production by granulosa cells until after ovulation. Higher LDL concentrations in human follicular fluid were associated with higher progesterone concentrations and decreased oocyte fertilization rate, suggesting that premature follicle luteinization may compromise the developmental competence of the oocyte (Volpe *et al*., 1991). After ovulation, blood vessels in the theca cell layer penetrate into the ruptured follicle, and the cells in the formed corpus luteum start to synthesize high concentrations of progesterone. The influence of heparan sulphate proteoglycans in controlling the steroidogenic activity of granulosa and luteal cells has not yet been investigated. However, evidence that exogenous glycosaminoglycans added to granulosa cell cultures inhibit LDL degradation and progesterone production support this possibility (Bellin *et al*., 1987).

Regulation of hyaluronan synthesis

Cumulus cells in a Graafian follicle before the preovulatory gonadotrophin surge are closely associated with the oocyte and with each other, thereby forming a compact cumulus cell–oocyte complex (COC). The cells maintain intercellular communication with the oocyte and with each other via an extensive network of gap junctions. The hormonal surge initiates a remarkable series of events which culminate with the rupture of the follicle and the extrusion of a highly expanded (or mucified) COC. An extensive extracellular matrix enriched in hyaluronan is synthesized and organized by the cumulus cells before ovulation.

The cellular events required to initiate hyaluronan synthesis and matrix organization have been extensively studied in mouse COC both *in vivo* and *in vitro*. There is very little extracellular matrix around the oocytes or between the cumulus cells in the mouse COC at the time of the ovulatory gonadotrophin surge. Indeed, histological staining with a biotinylated probe specific for hyaluronan reveals little, if any, of this macromolecule within the follicle (Salustri *et al*., 1992). By 5 h, the presence of a hyaluronan matrix around the cumulus cells is apparent, and the COC is partially expanded. Shortly before ovulation the matrix is fully expanded, and the COC is 20- to 30-fold as large as the initial compact COC (Figure 3). Hyaluronan is the predominant macromolecule in this matrix and is present at ~0.5 mg/ml. Accessory proteins are necessary to stabilize the matrix (Chen *et al*., 1992; Camaioni *et al*., 1993, 1996; Fulop *et al*., 1997a).

The cumulus cells in the initial compact COC are not synthesizing hyaluronan, nor do they have mRNA for hyaluronan synthase 2 (Has2), the enzyme required by these cells to synthesize this macromolecule (Fulop *et al*., 1997b). The cellular events required to initiate hyaluronan synthesis and matrix organization have been studied extensively by isolating the compact COC and culturing them *in vitro* under conditions that promote or inhibit matrix formation. Mouse cumulus cells must interact with two distinctly different factors to synthesize the maximum amount of hyaluronan, an unknown soluble factor released by the oocyte and FSH (Salustri *et al*., 1990b). If these are present in saturating concentrations, the amount of hyaluronan per cell produced by a COC during ~20 h of culture is the same as that in a fully expanded COC isolated shortly after ovulation *in vivo* (Salustri *et al*., 1992). The FSH needs to be present only during the first 2 h in culture, a time period during which the second messenger, cAMP, reaches maximal concentrations in response to the hormone. The oocyte factor does not influence cAMP production and its presence is required from 2 h on to promote and sustain maximal hyaluronan synthesis (Buccione *et al*., 1990; Tirone *et al*., 1997). The oocyte factor may modulate cumulus cell response to FSH by influencing the steady-state content of Has2 mRNA, as observed for urokinase plasminogen activator mRNA (Canipari *et al*., 1995) and LH receptor mRNA (Eppig *et al*., 1997). Cumulus cell masses isolated from rat, pig and cow show only partial or no dependence on the oocyte for gonadotrophin stimulation of expansion (Prochazka *et al*., 1991; Vanderhyden, 1993; Ralph

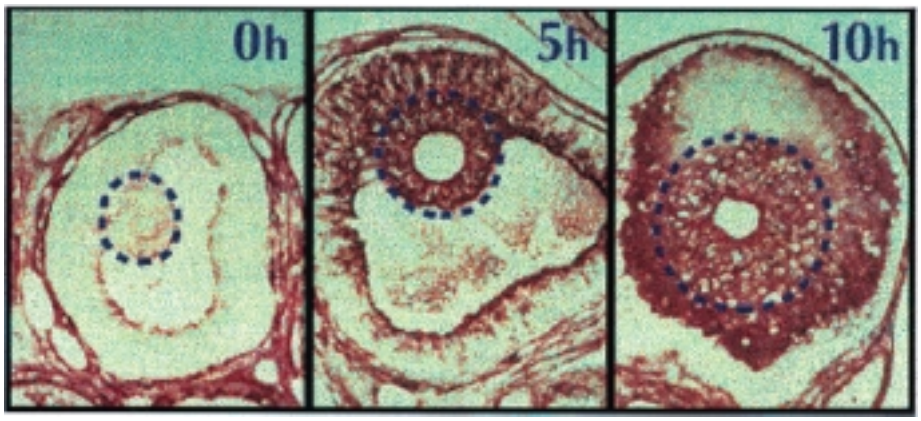

Figure 3. Hyaluronan synthesis during cumulus cell–oocyte complex (COC) expansion *in vivo*. A biotinylated hyaluronan protein was used to stain hyaluronan specifically in mouse follicles at times 0, 5 and 10 h after injection of an ovulatory dose of human chorionic gonadotrophin. The COC is indicated in each section by a dotted blue line.

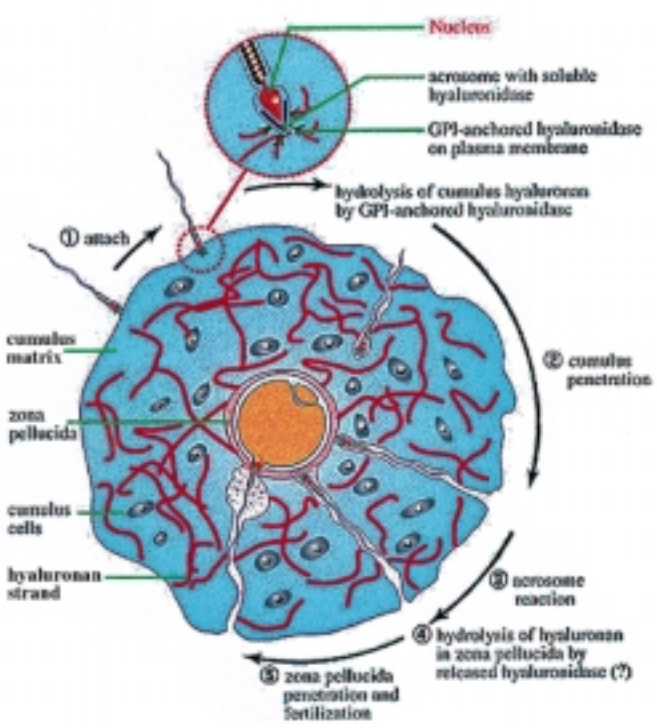

Figure 4. Diagrammatic representation of sperm penetration through the cumulus oophorus matrix. See text for details.

et al., 1995). However, the oocytes of all species analysed are able to substitute for mouse oocytes in inducing expansion in mouse cumulus cells. Thus, the oocytes in all species synthesize the factor which promotes hyaluronan synthesis. Species-specific differences in cumulus cell behaviour may depend on different stability of the factor, or on the extent of the cell response to the factor.

TGFβ_1 can also induce hyaluronan synthesis by FSH-stimulated mouse cumulus cells, but at levels only ~60% of maximum (Salustri *et al*., 1990a; Tirone *et al*., 1997). However, TGFβ_1 is distinct from the oocyte factor since antibodies that neutralize TGFβ_1 action do not affect that of the oocyte factor. This observation, however, does not exclude that the oocyte factor is a member of TGFβ family and triggers similar intracellular signals.

Mouse mural granulosa cells do not synthesize hyaluronan *in vivo*, while they do so if they are cultured *in vitro* with denuded oocytes (Salustri *et al*., 1990a). Since only a small fraction of the oocyte factor escapes into the culture medium when mouse oocytes are cultured enclosed in their cumulus cell mass, the cumulus cells must bind, and likely internalize or inactivate the factor as part of their response (Salustri *et al*., 1992). It is likely, then, that in the follicle there will be a decreasing concentration gradient of the factor away from the oocyte, and mural granulosa cells distant from the oocyte could not receive a sufficient concentration of the oocyte factor to initiate hyaluronan synthesis. This would limit the number of cells that become embedded in the hyaluronan matrix and leave the follicle at the ovulation. It would also ensure that sufficient cells remain to form the corpus luteum.

EGF, at a concentration range present in the follicular fluid, induces full expansion and stimulates maximal hyaluronan synthesis by in-vitro-cultured cumulus cell–oocyte complexes (Downs, 1989; Salustri *et al*., 1990a). The additive effect of FSH and EGF at suboptimal doses, and their similar kinetics of action, suggest that they could work in combination *in vivo* to ensure full expansion (Tirone *et al*., 1997).

Hyaluronan functions in ovulation and fertilization

Soluble factors produced by cumulus cells during the preovulatory period are essential for the oocyte to acquire the ability to be fertilized and to sustain normal embryo development. When the ovulatory gonadotrophin surge occurs, cumulus cells retract their cytoplasmic projections and lose intercellular contact with each other and with the oocyte.

However, they are subsequently embedded in the hyaluronan network and remain closely associated to the oocytes. The oocyte is also firmly held by the matrix. Histochemical and ultrastructural studies suggest that hyaluronan is present in the outer third of the mouse and hamster zona pellucida, and even in the perivitelline space of opossum, pig and human oocytes (for review see Talbot, 1985). Further, when the formation of the zona pellucida is prevented, the oocyte can escape from the viscoelastic matrix of the expanded cumulus mass (Liu *et al*., 1996; Rankin *et al*., 1996).

The accumulation of hyaluronan creates a spongy, elastic—and hence reversibly deformable—matrix that facilitates the extrusion of the oocyte at ovulation. When the follicle wall is ruptured, the expanded COC deforms considerably as it passes through the small hole in the membrana granulosa, thereby bringing the oocyte outside of the follicle within the greatly expanded cumulus mass (Chen *et al*., 1993). This expanded COC probably also facilitates its capture by the fimbria of the oviduct and its transport to the site of fertilization (Mahi-Brown and Yanagimachi, 1983).

The extracellular matrix of the ovulated COC may present a physiological barrier for penetration by functionally or enzymatically deficient spermatozoa. A good correlation exists between the ability of spermatozoa to penetrate a highly viscous solution of sodium hyaluronan and both sperm motility and fertilization efficiency (Neuwinger *et al*., 1991; Aitken *et al*., 1992). Therefore, this procedure is used in clinics for sperm preparation or evaluation of functional sperm competence. As noted above, the extracellular matrix of the cumulus oophorus is more complex than a simple hyaluronan solution, with specific molecules that link hyaluronan strands and limit their extension. In spite of this, spermatozoa take only about 2 min to pass through the cumulus cell layer (Talbot, 1985) and only a few seconds to penetrate the zona pellucida (Cummins and Yanagimachi, 1982). This must depend in large part on the hyaluronidase activity of a GPI-anchored protein, namely PH-20, that is present on the plasma membrane of the sperm head (Lin *et al*., 1994) (Figure 4). Antibodies generated against PH-20 which block its hyaluronidase activity inhibit penetration of the cumulus cell mass by acrosome-intact spermatozoa. The same enzyme is present at the inner acrosomal membrane, and inside the acrosome in a soluble form (Cherr *et al*., 1996; Meyer *et al*., 1997). This may serve to hydrolyse hyaluronan in the zona pellucida following the acrosome reaction, thereby facilitating the penetration of spermatozoa through the zona.

Concluding remarks

It is clear that proteoglycans and hyaluronan participate in many facets of ovarian development, ovulation and fertilization, and some of these possibilities are discussed in this review. Future work should focus on identifying the proteoglycans involved, and on defining their precise structures, metabolism and functions. The recent identification of the gene sequence of some proteoglycan core proteins has opened new prospectives for studying in-vivo and in-vitro expression of specific classes of proteoglycans. Characterization of the glycosaminoglycan chains remains essential for these studies, as the specificity of the biological activity of proteoglycans often depends on their carbohydrate component.

Acknowledgements

This work was supported by a joint grant CRG 950829 from the NATO, by a grant from MURST 40% (to A.S.), and by NIH grant HD34831 and the Cleveland Clinic Foundation (to V.C.H.). We thank Seigakagu Corporation for permitting us to reproduce Figures 3 and 4 from website www.glycoforum.gr.jp/science/hyaluronan/hyaluronanE.html.

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Received on October 14, 1998; accepted on April 16, 1999