Pyriform Cell Differentiation in *Podarcis sicula* Is Accompanied By the Appearance of Surface Glycoproteins Bearing α-GalNAc Terminated Chains

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ABSTRACT

The present histochemical and cytochemical study using a lectin panel (WGA, GSI-A4, GSI-B4, PSA UEA-I, PNA, LCA, Con-A , DBA, MPA, BPA) has demonstrated that, in *Podarcis sicula*, the differentiation of small follicle cells into pyriform cells by means of intermediate cells is accompanied by the appearance of glycoproteins bearing α-GalNAc terminated O-linked side chains on the cell surface. The distribution of DBA- and MPA-binding sites over the follicular epithelium changed during the different stages of oocyte growth. DBA- and MPA-binding sites first appeared at the beginning of folliculogenesis within the zona pellucida (ZP) and on the surface of small cells, i.e., the stem cells of pyriform cells. Afterward, labeling was evident on the cell surfaces of intermediate cells and, later on, also of pyriform cells. On the other hand, no labeling was detected on the small cells located under the basal lamina, which, reportedly, do not differentiate into pyriform cells (Filosa et al. J. Embryol. Exp. Morphol., 1979; 15:297-316). Once pyriform cells were differentiated, the distribution of DBA- and MPA-binding sites over the follicular epithelium remained unchanged until intermediate and pyriform cells underwent apoptosis (Motta et al. J. Exp. Zool., 1996; 276:233-241) and the follicular epithelium transformed into a monolayer composed of small follicle cells only (Filosa Mon. Zool. Ital., 1973; 7:151–165). During this stage of oocyte growth, DBA and MPA labeling gradually decreased to completely disappear in the follicular epithelium of vitellogenic follicles. It is noteworthy that the observed changes in the distribution of DBA- and MPA-binding sites represent the first evidence recognized by lectins of a gradual modification of surface glycoprotein distribution over the follicular epithelium in the ovarian follicles of nonmammalian vertebrates so far studied. Finally, the zona pellucida (ZP), characterized by the presence of GalNAc, GluNAc, Man, and Gal, was demonstrated to be first synthetized by the oocyte and later on by the follicle cells. Anat Rec 263:1–9, 2001.

Key words: lectin; pyriform cells; follicle cells; zona pellucida; reptiles

The follicular epithelium surrounding previtellogenic oocytes of squamate reptiles is characterized by the presence of a peculiar type of follicle cells, the pyriform cells. Such cells are connected to the oocyte by means of intercellular bridges (Ghiara et al., 1968; Neaves, 1971; Bou-Resly, 1974; Filosa, 1976; Filosa and Taddei, 1976; Andreuccetti et al., 1978, 1979; Hubert, 1985; Klosterman, 1987; Ibrahim and Wilson, 1989; Taddei and Andreuccetti, 1990; Motta et al., 1995, 1996), allowing the transfer of RNA, mitochondria, ribosomes, and vesicles to the oocyte (Motta et al., 1995). In this regard, pyriform cells may be considered as a kind of nurse cell similar to those present in polytrophic ovarioles of insects (for review see Mahajan-Miklos and Cooley, 1994). Unlike insect nurse cells, pyriform cells differentiate from somatic cells, i.e., the small follicle cells; these are stem cells which, once their plasma

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TABLE 1. Lectins, buffers, concentrations used, carbohydrate specificity, and competing sugars*

Lectin	Buffer	Concentration (μg/ml)	Specificity	Inhibitory sugars
Arachis hypogaea (PNA)	PBS	30	βGal1,3GalNac>αandβGal	D-Gal
Bauhinia purpurea (BPA)	PBS	30	GalNAc>Gal	D-GalNAc
Canavalia ensiformis (ConA)	TBS-Ca	30	αMan>αGlc>αGlcNAc	Methyl-α-Man
Dolichos biflorus (DBA)	PBS	30–60	αGalNAc1,3GalNAc>GalN	αD-GalNAc
Griffonia simplicifolia I-A4 (GS-I-A_4)	PBS-Ca	30	αGalNAc≫αGal	D-GalNAc
Griffonia simplicifolia I-B4 (GS-I-B_4)	PBS-Ca	30	αGal	D-Gal
Maclura pomifera (MPA)	PBS	30–60	GalNAc>Gal	D-GalNac
Pisum sativum (PSA)	TBS	30	αMan>αGlc=αGlcNAc	Methyl-α-Man
Triticum vulgare (WGA)	PBS	15–30	βGlcNAc1,4βGlcNAc>GlcNAc>Neu5Ac	D-GlcNAc
Ulex europaeus I (UEA-I)	PBS	30–60	αL-Fuc	L-Fuc
Lens culinaris (LCA)	PBS	30	αMan>αGlc=αGlcNAc	Methyl-α-Man

*From Goldstein and Poretz, 1986: *Gal,* galactose; *GalNAc,* N-acetylgalactosamine; *GlcNAc,* N-acetylglucosamine; *Fuc,* fucose; *Man,* mannose; *Methyl-α-Man,* methil-alfa-mannoside; *Neu5Ac,* sialic acid.

membrane has fused with the oocyte, differentiate into pyriform cells by means of intermediate cells (Andreuccetti et al., 1978; Filosa et al., 1979). At the onset of fusion of the membranes (100 μm follicles), the follicular epithelium appears homeomorphic and monolayered for the presence of small cells but progressively (follicles 150–200 μm) becomes multilayered and polymorphic due to the presence of small, intermediate, and, at a later stage, pyriform cells (Filosa, 1973; Andreuccetti et al., 1978). Such an organization is maintained in follicles up to 1500 μm in diameter. At the end of the previtellogenic stage (1,500–2,000 μm follicles), the follicular epithelium gradually undergoes changes as a result of apoptosis of the intermediate and pyriform cells (Motta et al., 1996). Therefore, in vitellogenic follicles (2,000–9,000 μm in diameter), the follicular epithelium appears to be made up again of a single layer of small cells (Filosa, 1973). The changes observed in the organization of the follicular epithelium may be accompanied by changes in the follicle cell surface. It is well known that surface glycoconjugates are involved in cell–cell interaction (O'Rand, 1988). In the reproductive system, carbohydrates appear to modulate sperm–egg interaction (Wassarman, 1999), sperm–oviductal adhesion (DeMott et al., 1995), and implantation of the embryo (Lee et al., 1983). Here, we studied the distribution and nature of glycoconjugates during oocyte growth in *Podarcis sicula* by using lectins that bind specific nonreducing terminal sugars of glycoprotein oligosaccharide chains. Reactivity to lectins was studied by both light and electron microscopy, and the nature of reactive glycoconjugates was investigated by β-elimination, for the removal of O-linked oligosaccharide side chains (Egea et al., 1989; Martinez-Menarguez et al., 1993) and membrane lipid extraction (Suzuki et al., 1993). The present observations demonstrate that, in reptiles, the distribution of lectin-binding sites over the follicular epithelium changes during follicle development and that surface glycoconjugates bearing terminal α-N-acetylgalactosamine residues accompany pyriform cell differentiation. The oligosaccharide composition and probable origin of zona pellucida (ZP) are also discussed.

MATERIALS AND METHODS

Ovaries were collected from five sexually mature females of the lizard *Podarcis sicula* Raf, collected in the neighborhood of Naples. The ovarian follicles were isolated under a dissecting microscope and fixed in 4% paraformaldehyde, 0.1% glutaraldehyde, in 0.1 M sodium cacodylate pH 7.4, for 2.5 hr at room temperature, washed 3×10 min in buffer, treated with 0.2 M NH_4Cl for 1 hr to aminidate free aldehyde groups, and washed again 3×10 min in buffer. The samples were then dehydrated in ethanol and singly flat-embedded in the epoxy resin Embed 812 (Polysciences, Milan, Italy). Semithin sections (1 μm thick) were cut with a diamond knife, mounted on cleaned glass slides, and dried at 45°C overnight. Sections were deplasticized on slides by treatment with potassium methoxide (modified from Maxwell, 1978), washed in running water for 15 min, and in 0.01 M phosphate buffered saline pH 7.4 (PBS), for 3×5 min. They were then incubated for 20 min in the dark with fluorescein-labeled (FITC) lectins (Table 1) at 15–60 μg/ml in the appropriate buffer (see below), washed twice in PBS, once in PBS plus 0.05% para-phenylendiamine and mounted with 90% glycerol in PBS plus 0.05% para-phenylendiamine. The buffers used were: PBS (MPA, WGA, UEA-I, DBA, BPA, PNA, LCA, Sigma, Milan, Italy); PBS supplemented with 0.5 mM $CaCl_2$ (GS-I-A_4 and B_4, EY, Florence, Italy), tris-buffered saline 0.05M, pH 7.4 (TBS) supplemented with 1 mM $MnCl_2$ and $CaCl_2$ (ConA); TBS (PSA). Table 1 summarizes the lectins and the working conditions used in the present study. Sections were observed under a fluorescence microscope and photographed by using Kodak Ektachrome 160 or 320 T with 40× and 63×-immersion objectives. As control for sugar specificity, lectins were preincubated with the appropriate hapten sugar (0.2 M) (purchased

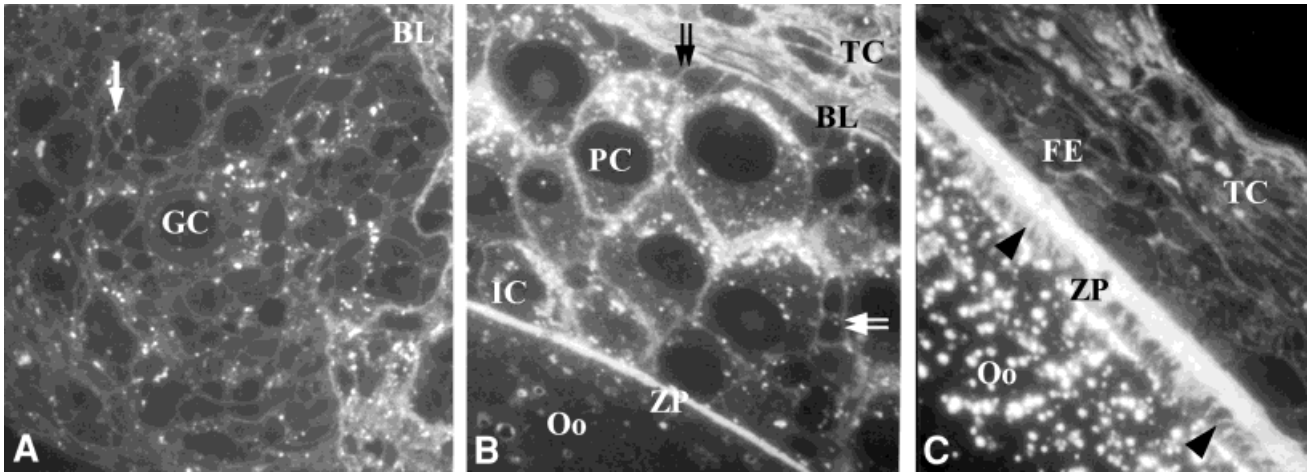

Fig. 1. Deplasticized 1-μm-thick sections of samples labeled with wheat germ agglutinin (WGA). Light microscope. **A:** Germinal bed. Positive labeling is evident along the surface and inside the cytoplasm of several germ (GC) and stroma cells (arrow). BL, basal lamina. Original magnification, ×415. **B:** Follicle 1,200 μm in diameter. Labeling is observed on the surface of small (double arrows), intermediate (IC) and pyriform cells (PC), as well as on the zona pellucida (ZP), the basal lamina (BL), the theca cells (TC) and the cytoplasmic vacuoles present inside the oocyte (Oo) and follicle cells. Original magnification, ×440. **C:** Follicle approximately 2,000 μm in diameter with regressing follicular epithelium. Follicular epithelium (FE), ZP, TC are still lectin labeled. A positive reaction is observed in the crypts (arrowheads) of oocyte cortex where vitellogenin is taken up and inside the cytoplasmic vacuoles present in the Oo cortex. Original magnification, ×620.

from Sigma Chemical Co.) for 30 min before section incubation.

To test whether selected lectins bound glycolipids or glycoproteins, deplasticized sections were treated with chloroform/methanol (2:1 vol/vol) for 10 min at room temperature, and extensively rinsed in methanol and water before lectin labeling (Suzuki et al., 1993). Different procedures of β-elimination for the removal of O-glycosidically linked oligosaccharide side chains were tested before labeling with three selected lectins, DBA, MPA, and Con A, which bind terminated sugars, respectively, in O-linked and N-linked glycoconjugates: deplasticized sections were incubated in 0.1 N NaOH for 48 hr at 37°C (Egea et al., 1989), in 0.1 N NaOH for 2 hr at 37°C (modified from Egea et al., 1989), or in 0.5 N NaOH in 70% ethanol for 12 days at 4°C (Martinez-Menarguez et al., 1993) and extensively rinsed in PBS. Parallel control sections were treated in the same way but without NaOH before lectin labeling. The data reported in this study were obtained using the first method, which proved to be the most appropriate one. Sections were microscopically examined, and the labeling was defined as positive or negative by the same observer.

Semithin sections of the same samples processed with biotinylated lectins and streptavidin gold (see below) were also tested by silver-enhancement according to Gualtieri and Andreuccetti (1995). In this regard, 1-μm sections were deplasticized as above, washed in distilled water and in PBS, incubated with biotin-labeled lectins at 150 μg/ml in PBS-BSA 0.5% (bovin serum albumin) for 2 hr at room temperature, washed twice in PBS-BSA, incubated with streptavidin gold/PBS-BSA 1:10 (vol/vol) for 1 hr, washed in PBS and in distilled water, and, finally, silver enhanced (Gualtieri and Andreuccetti, 1995) and observed under a light microscope. Lectin labeling at ultrastructural level was carried out by a two-step postembedding technique by using biotin-conjugated lectins and streptavidin gold 10 nm (EY, Florence, Italy). Shortly, samples were fixed and embedded in the epoxy resin Embed 812; subsequently, thin sections (60–80 nm thick) were cut with a diamond knife, mounted on nickel grids, and treated by means of an antigen unmasking technique (Maxwell, 1978). For this purpose, sections on grids were treated in 0.01 M citrate buffer pH 6.0, at 95°C for 10 min, washed in distilled water and PBS (modified from Stirling and Graff, 1995), and incubated with biotinylated lectins and streptavidin gold as described above. Sections were finally stained with uranyl acetate and lead citrate (Reynolds, 1963), and observed under a Philips 301 electron microscope.

RESULTS

Eleven lectins (PNA, BPA, ConA, DBA, MPA, GSI-A4, GSI-B4, PSA,WGA, UEA-I, LCA) were tested for their ability to bind different constituents of the ovarian follicle. WGA, LCA, ConA exhibited the same binding pattern during the different stages of the oocyte growth (Fig. 1). In fact, binding sites for WGA, LCA, and ConA were always detected in early meiotic oocytes and stroma cells of the germinal bed, i.e., a lenticular area of the ovary containing oogonia and pre- and early diplotene oocytes (Andreuccetti et al., 1990) (Fig. 1), as well as follicle and theca cells, ZP, oocyte cytoplasm (Fig. 1B,C). UEA-I, PNA, BPA, GSI-A4, and GSI-B4 never showed any ability to bind oocytes and follicles. PSA lectin exhibited a positive binding capacity for all different constituents of previtellogenic and vitellogenic follicles but not for any constituent of the germinal bed (data not shown).

Unlike the others lectins, DBA and MPA displayed marked changes in their binding pattern during oocyte growth (Figs. 2, 3). DBA labeling first appeared in early diplotene oocytes surrounded by follicle stem cells. In such oocytes, a positive reaction was detectable both in the perivitelline space, where ZP was depositing, and inside the oocyte (Fig. 2A) but not in the leptotene-pachytene

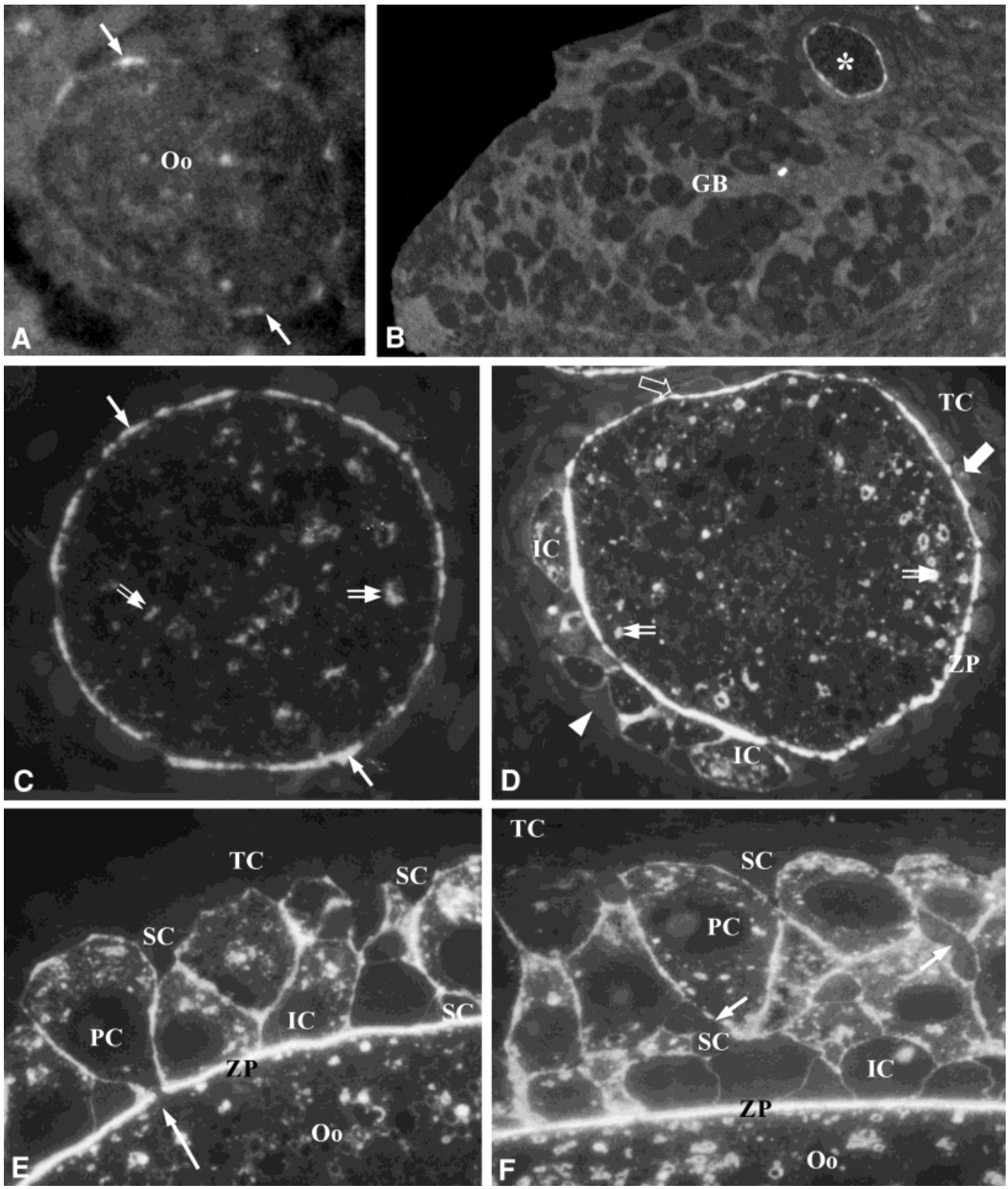

Fig. 2.Deplasticized 1-μm-thick sections of samples labeled with *Dolichos biflorus* lectin (DBA) at the light microscope. **A:** Early dyplotene oocyte. Lectin labeling is evident in a few regions (arrows) where zona pellucida (ZP) is deposited, as well as inside the oocyte (Oo). Original magnification, ×1,000. **B:** Germinal bed (GB). No positive labeling is evident on the germ cell clusters and on the stroma cells. Note the presence of a recently formed primary follicle (asterisk): positive staining is evident on the zona pellucida; follicle and theca cells are not labeled. Original magnification, ×225. **C:** Primary follicle 70 μm in diameter. Staining is evident on the zona pellucida (arrows) and cytoplasmic vacuoles (double arrows). A weaker reaction is observed on the nuclei of small cells, the surfaces of which are not stained; theca cells are not labeled. Original magnification, ×1,250. **D:** Follicle 150 μm in diameter. The surface of a small cell (white arrow) in contact with the oocyte and the intermediate cells (IC) are labeled. Note a small cell (open arrow) with a completely lectin-stained surface. Zona pellucida (ZP) and cytoplasmic vacuoles (double arrows) are also labeled. No labeling is evident on the small cells (arrowhead) located next to the basal lamina and on the theca cells (TC). Original magnification, ×433. **E:** Follicles 700 μm in diameter. Labeling is evident along the surface of small cells (SC) in contact with the Oo, IC, and pyriform cells (PC), ZP. Small cells under the basal lamina and TC are not labeled. Long arrow, intercellular bridge. Original magnification, ×540. **F:** Follicle 1,500 μm in diameter. *Dolichos biflorus* lectin (DBA) -binding sites are distributed as in Fig. 2E. Note also that the surface of contact between two small cells (arrow) located among pyriform cells is not labeled by lectin. ×540.

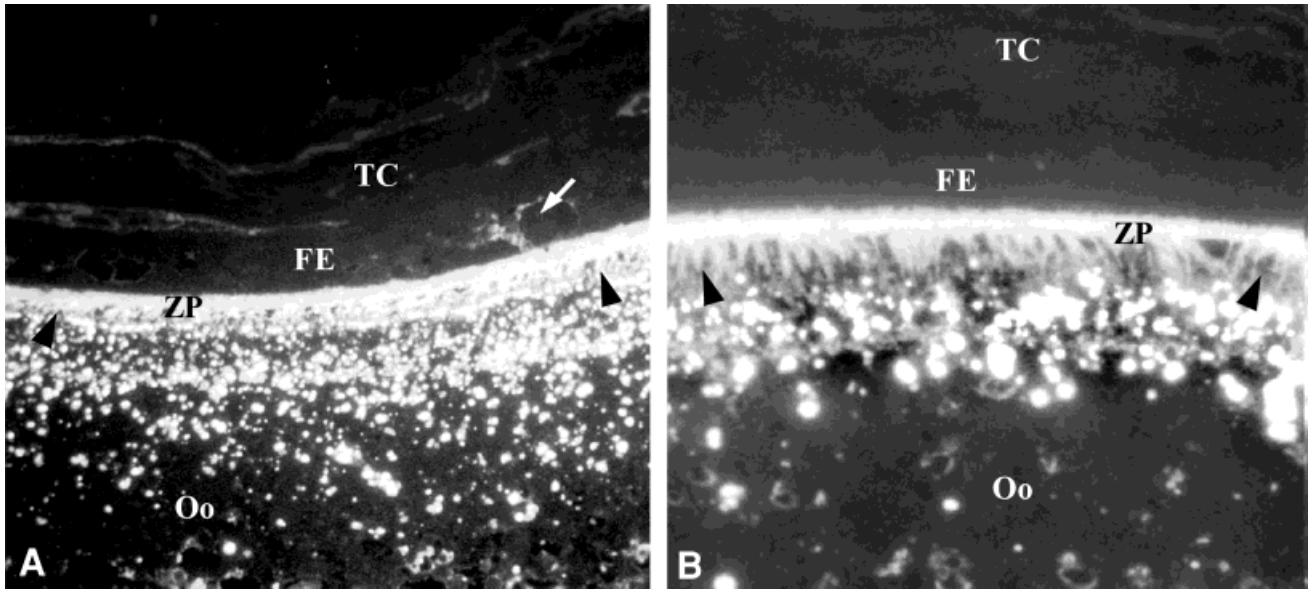

Fig. 3. Deplasticized 1-μm-thick sections of samples labeled with *Dolichos biflorus* lectin (DBA) at the light microscope. **A:** Follicle 2,000 μm in diameter. The follicular epithelium (FE) is positively labeled only on regressing pyriform cells (arrow), whereas the zona pellucida (ZP), the crypts (arrowheads) of the oocyte surface, and the oocyte (Oo) cortex are positively labeled. TC, theca cells. Original magnification, ×516. **B:** Vitellogenic follicle 3,000 μm in diameter. Labeling is evident on the ZP, the crypts (arrowheads) of the oocyte surface, and inside the Oo cortex. FE and TC are not labeled. Original magnification, ×930.

oocytes and stroma cells (Fig. 2B). Once the primary follicle was formed (70–100 μm follicles), DBA labeling became evident on discrete oocyte cytoplasmic regions and on ZP material that was actively deposited on the oocyte surface (Fig. 2B,C).

It is noteworthy that labeling on ooplasm and ZP remained unchanged during the subsequent stages of oocyte growth (Figs. 2C–F, 3A,B). No binding was evident in small follicles and theca cells (Fig. 2A–C).

In 150- to 200-μm follicles, in which small cells were seen to differentiate into intermediate cells (Andreuccetti et al., 1978; Filosa et al., 1979), DBA labeling was observed on the surface and within the cytoplasm of small follicle cells (Fig. 2D). Furthermore, besides the small cells facing the oocyte, the surface and the cytoplasmic granules of intermediate cells were also DBA-reactive (Fig. 2D). In larger follicles (400–500 μm in diameter), the surface and the cytoplasm of pyriform cells were also stained by DBA (Fig. 2E). The same labeling was found in follicles 600–1,500 μm in diameter (Fig. 2F). During the stages when intermediate and pyriform cells are known to undergo apoptosis (Motta et al., 1996) (1,500–2,000 μm follicles), DBA binding on the follicular epithelium gradually decreased (Fig. 3A) to become undetectable at the end of the previtellogenic stage (approximately 2,000 μm follicles). In vitellogenic follicles (>2,000 μm in diameter), DBA bound the crypts where vitellogenin was taken up, as well as the primary yolk globules, whereas the theca and the follicular epithelium were negative (Fig. 3B).

It is noteworthy that, during the previtellogenic phase of oocyte growth (100–2,000 μm in diameter), the small cells that were not in direct contact with the oocyte and the theca cells were not labeled by DBA (Fig. 2D–F); conversely, the small cells located along the perivitelline space showed positive labeling (Fig. 2F). Results with MPA, which has the same sugar specificity as DBA, were similar to those obtained with DBA, except for a weak labeling on the theca cells and on the nuclei of all small follicle cells (data not shown).

Lectin binding was abolished by preincubation with the corresponding inhibitory sugars (Fig. 4A). No changes in labeling were detected when lipid extraction with chloroform/methanol was performed on sections before labeling with DBA. To verify whether the residues detected by DBA lectin belonged to N- or O-linked chains, some sections were processed for β-elimination, according to the second procedure described in the Materials and Methods section. Beta-elimination abolished labeling on the follicular epithelium, ZP, and oocyte (Fig. 4B) but failed to modify labeling with ConA, showing that N-linked sugars (Cummings et al., 1989) were not affected under these conditions (Fig. 4C).

Silver enhancement (Fig. 5A) and EM observations (Figs. 5B–F) confirmed and extended the results obtained by fluorescence microscopy. In particular, ultrastructural observations of previtellogenic follicles showed that DBA labeled the oocyte (Fig. 5B), the follicle cells surface (Fig. 5C), various cytoplasmic vacuoles (Fig. 5B,C), and the ZP (Fig. 5B). Labeling was absent from control sections (Fig. 5D). No reaction was evident in the small cells that were not in contact with the oocyte surface and the ZP either located among the pyriform cells (Fig. 5E) or under the basal lamina (Fig. 5F).

DISCUSSION

In our experiments, the distribution of binding sites for the lectins WGA, PSA, LCA, and ConA remained unchanged throughout oocyte growth of *Podarcis sicula*, whereas labeling with DBA and MPA markedly changed

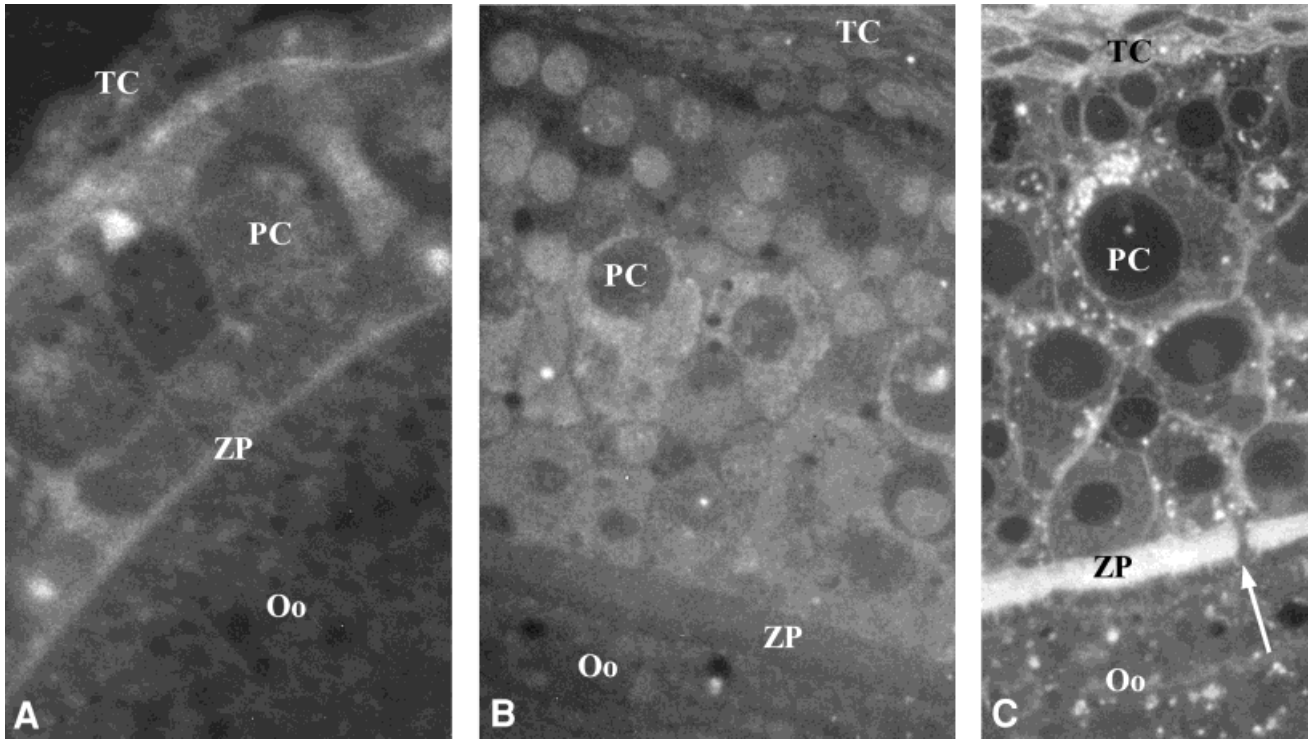

Fig. 4. **A:**Follicle 500 μm in diameter: control section. No labeling is observed. Oo = Oocyte; PC = Pyriform cells; TC = Theca cells; ZP = Zona pellucida. × 740. **B:** Follicle 1200 μm in diameter. No DBA labeling is evident after β-elimination. Oo = Oocyte; PC = Pyriform cells; TC = Theca cells; ZP = Zona pellucida. × 620. **C:** Follicle 1200 μm in diameter. ConA labeling remains unchanged after chloroform treatment for lipid extraction. Oo = Oocyte; PC = Pyriform cells; TC = Theca cells; ZP = Zona pellucida; Long arrow = Intercellular bridge. × 620.

in follicle cells. On the contrary, no binding sites for GSI-A4, GSI-B4, BPA, UEA-I, PNA were found during the different stages of oocyte growth. DBA and MPA labeling were absent from the germinal bed, being first evident at early stages of the ovarian follicle assembly, when stroma cells are organized around the oocyte. Once the primary follicle has been formed, DBA and MPA labeling was detectable on the surface of small follicle cells facing the oocyte as well as on the oocyte proper. Later on, the whole surface of the small cells next to the ZP, as well as that of intermediate and pyriform cells, was labeled by MPA and DBA, whereas small cells located under the basal lamina were negative. In the vitellogenic stages, follicle cells were no longer stained by MPA and DBA. The temporal and spatial distribution of MPA- and DBA-binding sites over the different follicle cells during the previtellogenic and vitellogenic stages strongly suggests that the appearance of GalNAc residues may be linked to the differentiation of small cells into pyriform cells by means of intermediate cells. Experiments of β-elimination and lipid extraction demonstrated that the sugar residues identified by DBA and MPA belonged to terminal O-linked side chains of glycoproteins located on the surface and in the cytoplasm of oocytes and follicle cells. In particular, it can be hypothesized that α-GalNAc residues, which were exposed on the surface of follicle cells upon contact with the oocyte surface, might play an important role in determining contact or fusion between the oocyte and the follicle cell membrane, as well as in maintaining the differentiated state of the pyriform cells. Interestingly, the small cells that were located both under the basal lamina and among the pyriform cells but that were not in contact with the oocyte surface were never labeled with DBA and MPA lectins. In this regard, the apparent reactivity upon small cell surfaces located among pyriform cells may be due to neighboring pyriform cells closely attached to small cells. It can be postulated that oocytes, as already suggested for mammals (Vanderhyden et al., 1990), might induce both the specific lectin receptor expression within the surface of follicle cells and the subsequent differentiation of small cells into pyriform cells. In this regard, irrespective the role of surface α-GalNAc terminated proteins during oocyte growth in *P. sicula*, the present data demonstrated that, unlike the situation in mammals, where cumulus cells were generally poorly reactive to most lectins (Shalgi et al., 1991), follicle cells of *Podarcis* exposed a large amount of α-GalNAc sugar residue with a distribution undergoing remarkable changes throughout oocyte growth. Such changes represent for the first time in a nonmammalian species evidence of a gradual modification of lectin binding sites over the follicular epithelium in the follicles of nonmammalian vertebrates so far studied. Heterogeneity for lectin binding within the follicular epithelium has already been reported in mammals and has been related to the differentiation program, which induces the granulosa cells to respond differently to the gonadotropin hormones (Erickson et al., 1985; Aviles et al., 1994).

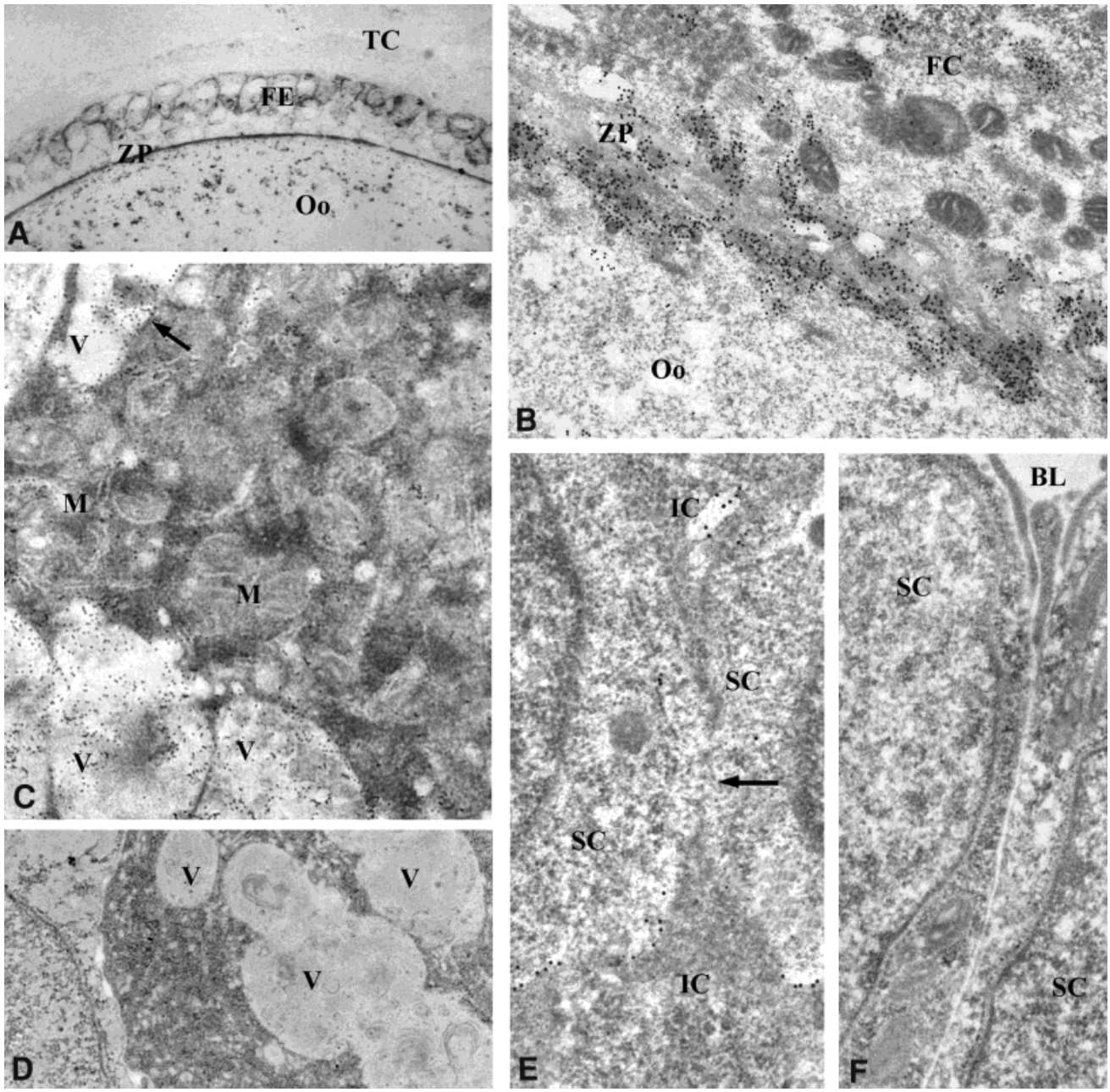

Fig. 5. **A:** Follicle 1,200 μm in diameter. Section labeled with *Dolichos biflorus* lectin (DBA) biotin and streptavidin gold, and silver enhanced. The labeling pattern is the same as that of Fig. 2E. FE, follicular epithelium; Oo, oocyte; TC, theca cells; ZP, zona pellucida. Original magnification, ×650. **B:** Electron micrograph of a postembedded follicle 1,200 μm in diameter labeled with DBA biotin and streptavidin gold. Labeling is evident on the oocyte surface and on the ZP components. FC, follicle cells (Oo = oocyte). Original magnification, ×20,000. **C:** Follicle 1,200 μm in diameter. Labeling is evident inside cytoplasmic vacuoles (V) and along the cell surface of a pyriform cell (arrow). No labeling is evident on mitochondria (M). Original magnification, ×27,000. **D:** Follicle 1,200 μm in diameter. Control section preincubated with α-D-GalNAc before labeling. No labeling is evident on the surface and cytoplasm of a pyriform cell. V, vacuoles. Original magnification, ×16,000. **E:** Follicle 1,200 μm in diameter. No labeling is evident on the contact surface of two small cells (SC) among two intermediate cells (IC). Original magnification, ×28,000. **F:** Follicle 1,200 μm in diameter labeled with DBA biotin and streptavidin gold. No labeling is evident on the SC located under basal lamina (BL). Original magnification, ×20,000.

Unlike the follicular epithelium, the ZP did not show any changes in carbohydrate composition and spatial distribution during oocyte development. Indeed, the *Podarcis* ZP, which was found to be rich in GalNAc, GluNAc, Man, and Gal residues, exhibited the same lectin-binding pattern throughout oocyte growth. In mammals, differences in the carbohydrate composition and/or spatial distribution within the ZP of preantral, antral, and ovulated oo-

cytes have been extensively documented in several species (Kaufman et al., 1989; Roux and Kan, 1991; Shalgi et al., 1991; Araki et al., 1992; Aviles et al., 1994; Parillo et al., 1998; Parillo and Verini Supplizi, 1999). Such differences have generally been related to the unequal distribution and composition of sperm receptors that are localized in the mammalian ZP (Bleil and Wassarman, 1980; Yamagata, 1985; Wassarman, 1995). Unfortunately, in reptiles, there are no data either on ZP saccharide composition or on the recognition and binding between spermatozoa and the egg coats.

The present data provide information on the probable origin of the ZP. In particular, light microscope observations strongly suggest that ZP components might be first synthesized by the oocyte. In fact, light and electron microscopic observations revealed for the first time the presence of DBA- and MPA-binding sites within the oocyte and the perivitelline space. Afterward, DBA- and MPA-binding sites were evident within follicle cells and in the intercellular spaces in continuity with the perivitelline space where the ZP is actively deposited, thus suggesting that follicle cells may contribute to ZP synthesis. The participation of both the oocyte and follicle cells in the ZP synthesis has been documented, among the reptiles, in *Tarentola* (Andreuccetti and Carrera, 1987) and in different mammals (Gwatkin et al., 1979; Wolgemuth et al., 1982; Tesoriero, 1984; Parillo and Verini Supplizi, 1999).

In conclusion, our results demonstrate that, in *Podarcis*, during oocyte growth, only follicle cells show marked differences in the pattern of lectin-binding sites, which may be related to the differentiation of small follicle cells into pyriform cells and that follicle cells and the oocyte cooperate to synthesize ZP.

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