Regression of the Epithelium in Late Previtellogenic Follicles of *Podarcis sicula:* A Case of Apoptosis

CHIARA M. MOTTA, SILVANA FILOSA, AND PIERO ANDREUCCETTI
*Department of Evolutive and Comparative Biology, University of Naples
Federico II, Naples, Italy*

ABSTRACT   We investigated the reorganization of the follicular epithelium at the end of previtellogenesis in the lizard *Podarcis sicula.* In particular, we determined the mechanism of intermediate and pyriform cell regression and the fate of their different subcellular constituents. Morphological and biochemical analyses revealed the presence of hallmarks of apoptosis such as nuclear changes, chromatin condensation, protein scaffolds, RNA synthesis, and DNA fragmentation. It was therefore concluded that the remodelling of the follicular epithelium is associated with programmed cell death. Evidence was also obtained that during regression, the cytoplasmic constituents of intermediate and pyriform cells are transferred into the oocyte while the remnants of the nuclei are recycled by the small cells. The functional significance of these events is discussed.
© 1996 Wiley-Liss, Inc.

In *Podarcis sicula,* as in most Squamata (Hubert, '77; Klosterman, '87; Andreuccetti, '92), the follicular epithelium of previtellogenic oocytes is polymorphic, being characterized by the presence of three distinct cell types: small, intermediate and pyriform cells. The small stem cells are gathered immediately below the connectival theca; the intermediate cells are located close to the zona pellucida, while the large, flask-shaped pyriform cells are located in the inner portion of the epithelium (Filosa, '73; Andreuccetti et al., '79).

The small cells differentiate during primary follicle formation, constituting a homomorphic and unilayered epithelium. When the follicle reaches about 150 µm in diameter, the membrane of the small cell fuses with the oocyte membrane to form an intercellular bridge. This event triggers the development of the cell into an intermediate cell and, following a further differentiation, into a pyriform cell (Andreuccetti et al., '78). The progression of such processes of proliferation and differentiation of the small cells will determine an increase in the total number of follicle cells that parallels the increase in surface occurring in the growing oocyte (Filosa et al., '79).

The intermediate and pyriform cells play a fundamental role in oogenesis since they synthesize various materials then transferred to the oocyte via intercellular bridges. RNAs (Motta et al., '95) and probably also a large variety of different subcellular components (Taddei, '72; Andreuccetti et al., '79) reach the oocyte, thus significantly contributing to its growth. The intermediate and py-riform cells, however, are destined to degenerate as vitellogenesis approaches. In fact, in follicles ranging 1,600–2,000 µm, the epithelium gradually regresses to a homomorphic condition that persists throughout vitellogenesis (Filosa, '73).

In the present work, we investigated the mechanism of degeneration of intermediate and pyriform cells to clarify the fate of these cells at the end of previtellogenesis. In particular, since tissue remodelling has been associated with programmed cell death (Ellis et al., '91), we examined at the morphological and biochemical level the possibility that these follicle cells regress by apoptosis.

The presence of the hallmarks of apoptosis was investigated in fully differentiated and regressing epithelia (follicles ranging 300–1,600 µm and 1,600–2,000 µm, respectively). The appearance of the characteristic morphological alterations, such as chromatin condensation and membrane blebbing (Wyllie, '81) was examined at the light and electron microscope. In addition, we determined the transcriptional activity in the epithelial cells by autoradiography, since apoptosis is accompanied by the activation of several specific genes (Fesus et al., '91; Schwartzman and Cidklowski, '93). In particular, the tissue transglutaminases would be responsible for the reorganization of the cytoplasm proteins and the formation of scaffolds highly re-

sistant to detergents (Piacentini et al., '91). The nucleases instead would be responsible for the cleavage of the DNA in fragments, most often of internucleosomal size (Arends et al., '90; Earnshaw, '95). The presence of scaffolds in the epithelial cells was determined by the technique previously described by Piacentini et al. ('91) while the occurrence of DNA fragmentation was analyzed by nick translation according to Gold et al. ('93). Finally, since the nucleases are expected to cut but not to digest the DNA (Arends et al., '90), we measured the nuclear DNA content of the follicle cells by cytophotometry.

MATERIALS AND METHODS
Animals

Adult female specimens were collected near Naples and maintained in the laboratory under natural conditions of light and temperature. They were supplied with water and meal worms ad libitum.

Light microscopy

The ovaries, dissected out from anesthetized animals, were fixed in ethanol and acetic acid (3:1, v/v), processed for embedding in wax, serially sectioned and stained with haemathoxylin-eosin, trichromic stains or pyronin as described by Mazzi ('77).

Electron microscopy

Ovaries were fixed in a phosphate-buffered solution of formaldehyde and glutaraldehyde (pH 7.4; Karnowsky, '65), postfixed in 2% osmium tetroxide, dehydrated and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and observed under a Philips model 301 electron microscope (Eindoven, The Netherlands).

Tritiated uridine incorporation

Each female received an i.p. injection of 200 μCi of tritiated uridine (specific activity 5 Ci/mmol; Amersham International, UK). Ovaries were dissected out 24 hours following injection of radiolabel. For autoradiography, slides were washed in ice-cold 5% trichloroacetic acid, coated with NTB2 emulsion (Kodak, Rochester, NY) and incubated for 1 month at 4°C. They were developed in Unifix and D19 (Kodak) and stained with haematoxylin. Labelling specificity was verified in slides previously treated for 1 h at 37°C with a 1 mg/ml solution of pancreatic RNAse A (Maniatis et al., '82). Grain counts were corrected for background and statistically analysed by ANOVA.

Nuclear DNA content

Manually isolated follicular epithelia were fixed in ethanol:acetic acid (3:1 v/v), rinsed in 45% acetic acid and squashed. Nuclei were stained with Feulgen and the nuclear DNA content was evaluated by cytophotometry using a two-wavelength method (Patau, '52; Motta et al., '91).

In situ nick translation (ISNT)

ISNT was performed as described in Gold et al. ('93). Ovaries were fixed in 2% paraformaldehyde in 0.1 phosphate buffer and embedded in wax. Sections were incubated in 10× nick translation buffer (Maniatis et al., '82) containing a mixture of dATP, dDTP, dCTP and digoxigeninated dUTP and 5 U of DNA polymerase I. The reaction was stopped in TE buffer. Sections were then treated with PA-digoxigenin antibody at a dilution of 1:250. Reaction was visualised by NTB/BCIP. All reagents were purchased from Boehringer (Mannheim, Germany).

Scaffold isolation

Epithelia were treated according to Fesus et al. ('89). Briefly, the follicular cells were lisated in a buffer containing 0.2 mM PMSF, 0.4 mM iodoacetamide and 0.5% Triton X-100. Following centrifugation, the pelleted nuclei were dissolved using 6 M guanidine-HCl. Protein scaffolds were collected by centrifugation and boiled for 5 minutes in 2% SDS solution containing 0.05 mM β-

---

Fig. 1. Changes in the organization of the follicular epithelium during previtellogenesis. **a:** Follicle 1,000 μm in diameter. Polymorphic and multilayered epithelium with small (arrowheads), intermediate (small arrows) and pyriform cells (PC). T, connectival theca. **b:** Follicle 1,200 μm in diameter. The intermediate cells (IC) are bell-shaped, show a large nucleus and nucleolus (arrowheads) and a cytoplasm containing ribosomes and mitochondria. The pyriform cell (PC) shows a conspicuous nucleus (N) and nucleolus and a cytoplasm engulfed with mitochondria. The intercellular bridge (IB) crosses the zona pellucida (ZP). Oo, oocyte. **c:** Follicle 1,700 μm in diameter. The small cells (arrowheads) form a bilayer below the connectival theca (T). **d:** Follicle 1,600 μm in diameter. The regressing cells (arrows) show shrunken cytoplasm and large intercellular spaces. The nuclei, apparently intact, show large nucleoli. **e:** Follicle 1,600 μm in diameter stained with pyronin. The nucleolus of the regressing cell (arrow) shows a reduced affinity for staining as compared with those of the surrounding healthy cells (arrowheads). **f:** Follicle 1,800 μm in diameter. The epithelium is typically bilayered and composed mostly by small cells (SC). **g:** Follicle of about 2,000 μm in diameter. The epithelium is mainly composed of small cells. A few large cells can still be recognized (arrows) together with cells with clearly pycnotic nuclei (arrowheads). a, c, d, g: trichromic stain; e, pyronin stain. Magnification = a, c–e, g: ×600; b: ×2,600; f: ×1,500).
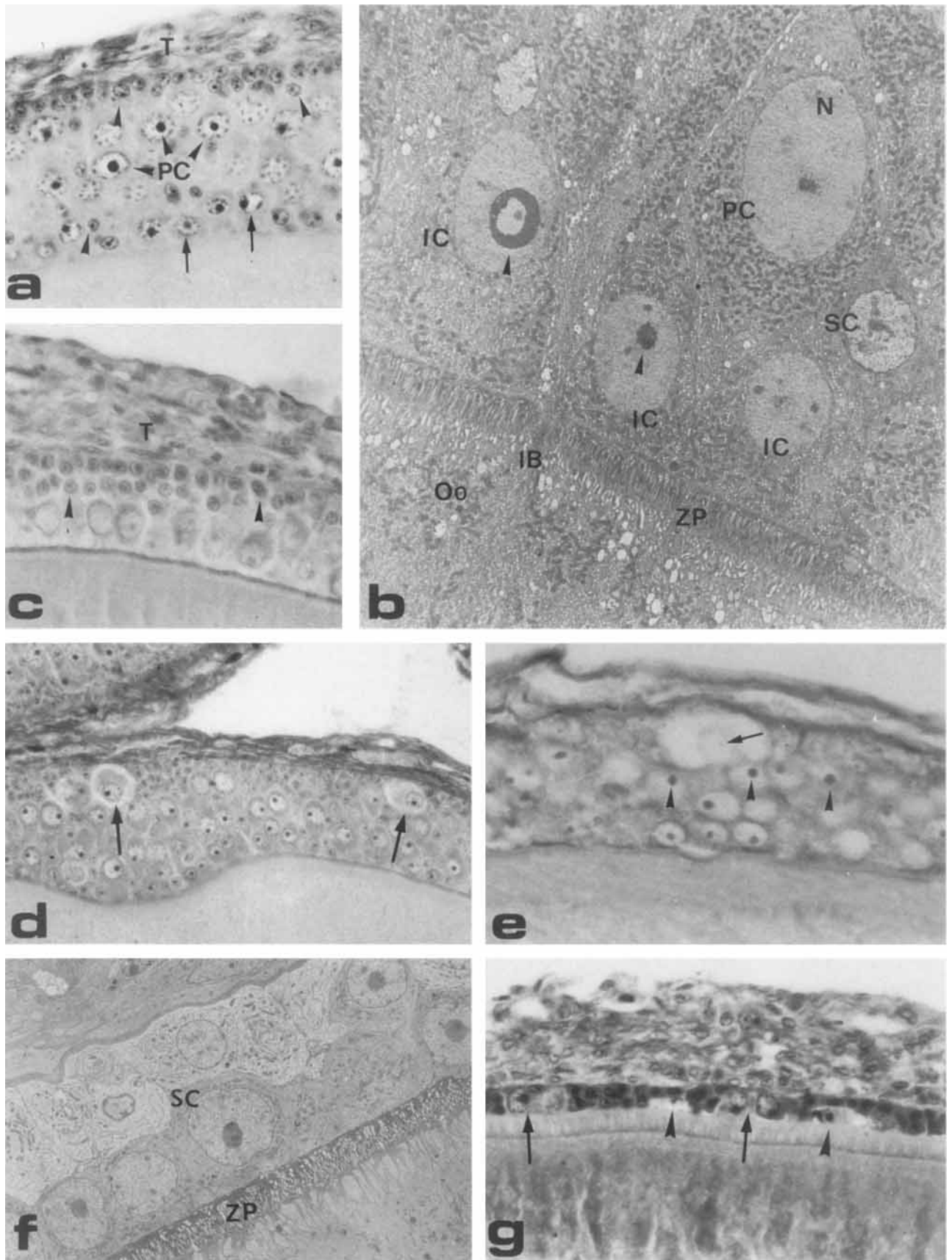
Figure 1.

mercaptoethanol. The suspension was observed either directly under a phase-contrast microscope (Leitz, Wetzlar, FRG) or following air-drying and staining on a slide.

RESULTS

The follicular epithelium of oocytes ranging 300–1,600 μm in diameter is typically polymorphic and multilayered with clearly distinguishable small, intermediate and pyriform cells (Fig. 1a,b). The small cells, either concentrated in the outer region or located close to the zona pellucida, show a small, round nucleus surrounded by a scarce cytoplasm. The intermediate cells show a typical bell shape, a large nucleus and a conspicuous nucleolus. Their cytoplasm displays an increased number of subcellular components. The pyriform cells have a large body containing a vesicular nucleus and a prominent nucleolus. Their cytoplasm is engulfed with mitochondria, Golgi bodies, ribosomes and different vesicles. All the cells lay close to each other and no evidence of cell degeneration is found.

In follicles ranging 1,600–1,800 μm in diameter, the epithelium undergoes a reorganization. The intermediate and pyriform cells become distinctly separated by an increasing number of small cells that also form a thick layer below the basal lamina (Fig. 1c). A few cells scattered throughout the epithelium show clear evidence of alteration: the cytoplasm has shrunken and is surrounded by a large, empty space (Fig. 1d). In these cells, the morphology of the nucleus is apparently unaltered but the nucleolus may show a reduced size and affinity for pyronin staining (Fig. 1e).

In follicles ranging about 1,800 μm in diameter, the epithelium is thinner and bilayered with a few largest cells laying against the zona pellucida (Fig. 1f). In follicles about 2,000 μm in diameter, the epithelium is composed of a single layer of small cells with a few scattered large cells. Several cells with pycnotic nuclei are also present (Fig. 1g).

In follicles larger than 1,600 μm, at the electron microscope, several intermediate and pyriform cells show clear evidence of alterations. The nucleus appears irregularly outlined (Fig. 2a) and/ or may show a progressive condensation and margination of chromatin (Fig. 2b,c). The nuclear membrane may be swollen (Fig. 2b) and show a reduced number of pore complexes, especially in correspondence with the dense aggregates of chromatin. The cytoplasm contains a large variety of organelles but these appear less abundant than in the previous stages (Fig. 2a,b). By contrast,

vacuoles of varying size and contents are present in large number (Fig. 2b,d). In both intermediate and pyriform cells, the intercellular bridges are still open and filled with different subcellular components that apparently are being transferred toward the oocyte (Fig. 2a–d; see also Motta et al., '95). The cytoplasm in many follicle cells is so reduced that the cell membrane lies close to the nuclear membrane (Fig. 2c).

In follicles ranging 1,800–2,000 μm in diameter, cells in late stages of degeneration become more frequent. Electron-dense vesicles containing remnants of chromatin and nuclear membrane are found together with vesicles containing mitochondria, glycogen, rough endoplasmic reticulum, or lipid droplets. These vesicles are often embedded in the cytoplasm of the small cells (Fig. 2e,f).

In follicles ranging 300–1,600 μm (Fig. 3a), intermediate and pyriform cells actively incorporate tritiated uridine and show significantly labelled nucleoli, nucleoplasm and cytoplasm. Significant labelling is also present on the oocyte cytoplasm. In follicles larger than 1,600 μm (Fig. 3b,c), a significant labelling is present only in the nucleoplasm and the cytoplasm of follicle cells and in the oocyte cytoplasm. A low but significant labelling is also present in the nucleoplasm and cytoplasm of the regressing follicle cells (Fig. 3c).

The cytophotometrical analysis (Fig. 3d) reveals that the nuclear DNA content does not differ in follicle cells obtained from small (1,200 μm) and large (1,800 μm) follicles. In both cases, in fact, the DNA contents range between 105 and 165 ar-

Fig. 2. Morphological alterations in epithelial cells from follicles 1,600–2,000 μm in diameter. a: Pyriform cell showing an irregularly outlined nucleus (N), a large nucleolus (Nu) and a few dense clumps of chromatin (small arrows) bordering the nuclear envelope. The cytoplasm contains a few mitochondria and a large multivesicular body (large arrow). An open intercellular bridge (IB) is still evident across the zona pellucida (ZP). Note the presence of empty intercellular spaces (asterisks). b: Pyriform cell with chromatin condensed in marginally located patches (small arrows). The nuclear envelope shows several blebbings (arrowheads). Small vesicles (V) and mitochondria (M) are evident in the cytoplasm. c: An intermediate cell with reduced volume of cytoplasm. The cell membrane lays in close contact with the nuclear envelope (arrowheads). Clumps of chromatin are also evident (arrows). d: A regressing pyriform cell (PC) showing an irregularly shaped nucleus (N) and large vacuoles (arrowheads) containing different materials. e, f: Small cells (SC) containing large vacuoles, probably remnants of regressing cells. Fragments of cisternae and large clumps of electron-dense materials are present as well as multivesicular bodies (arrows). Magnification = a–c: × 4.500; d: × 3,000; e, f: × 7,000.

Figure 2.

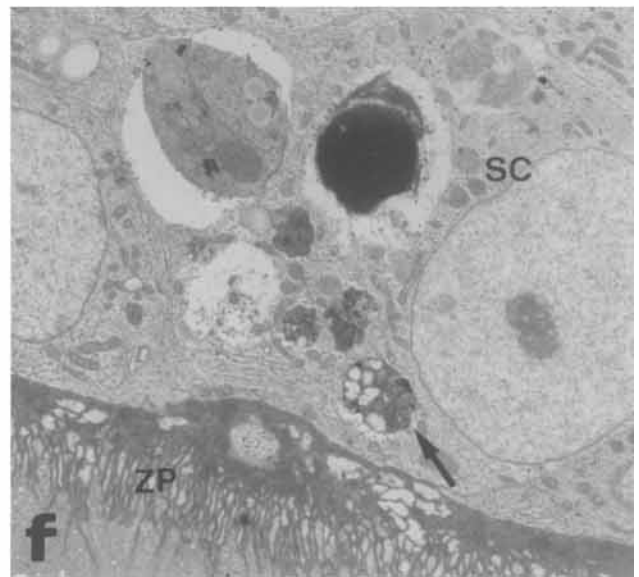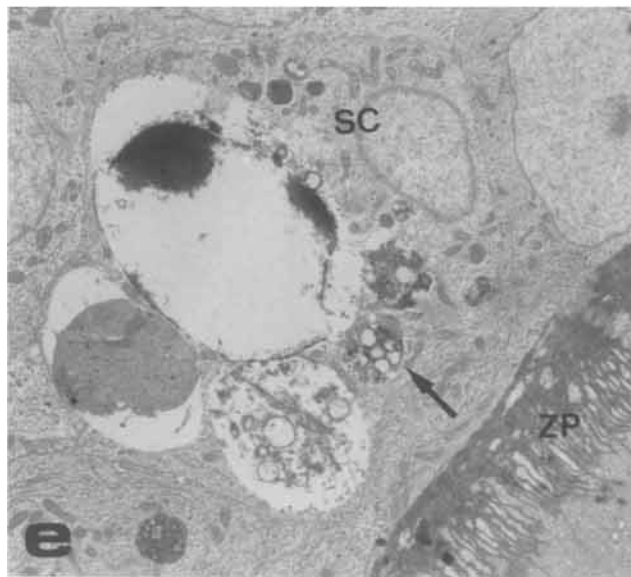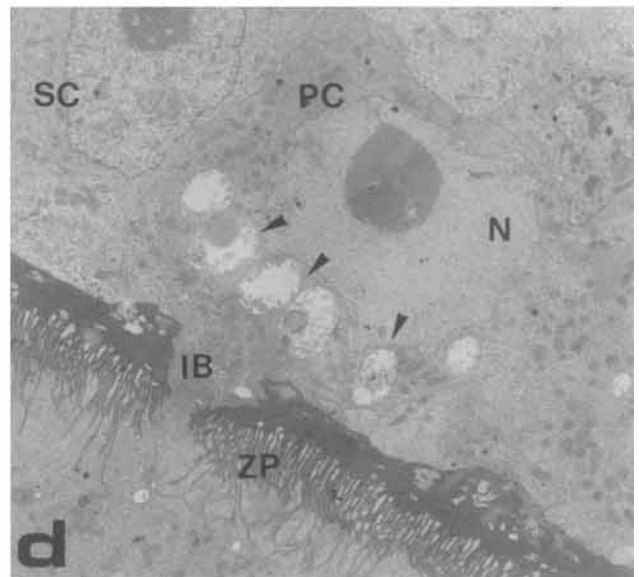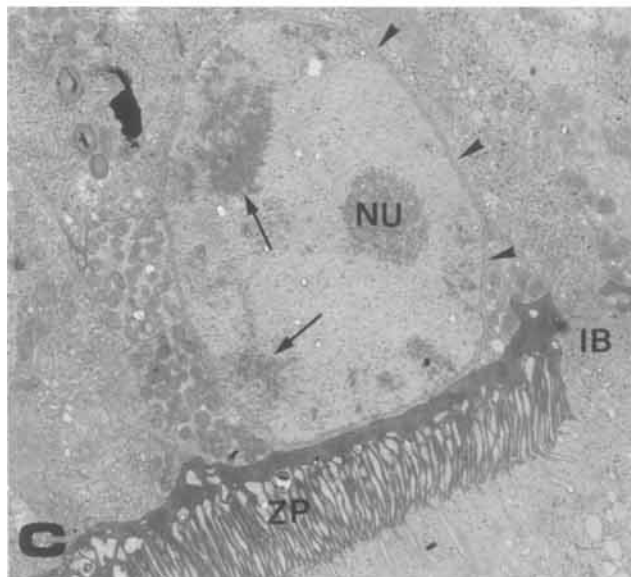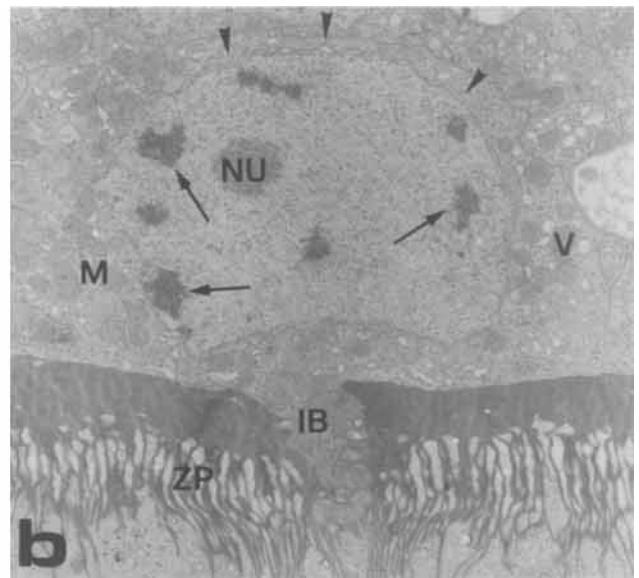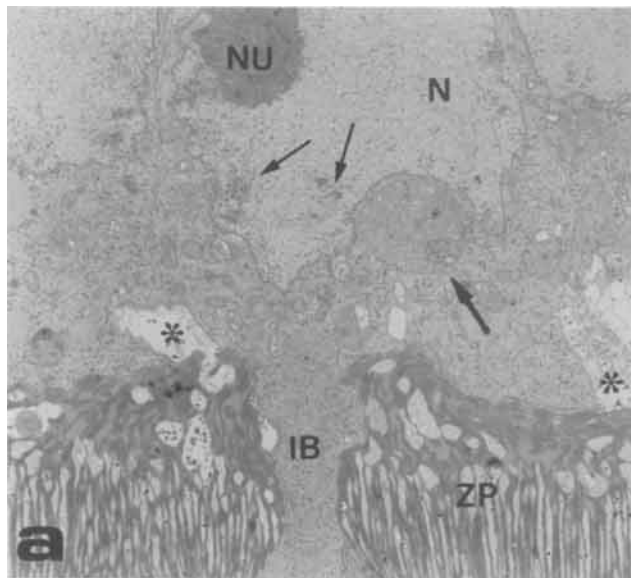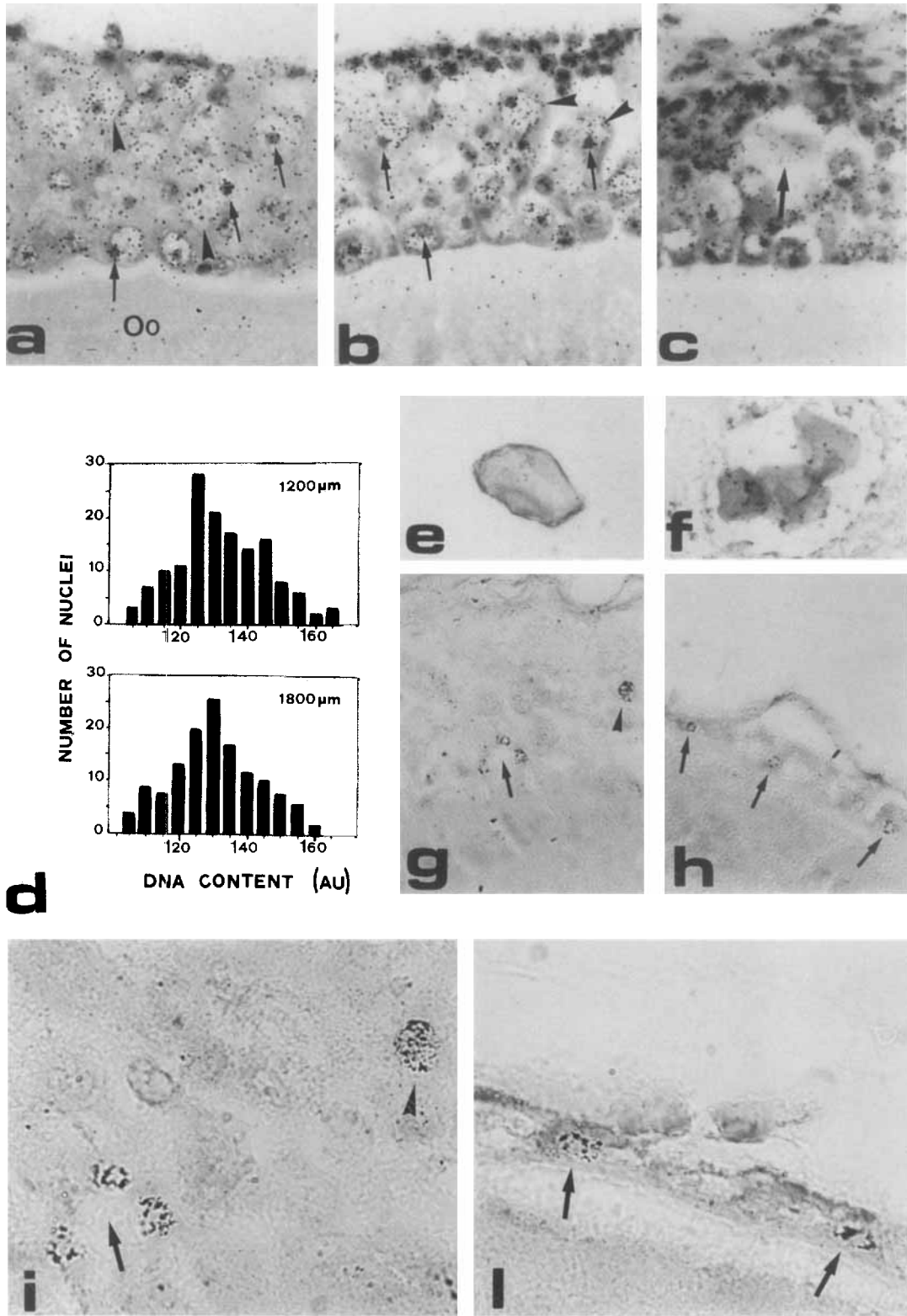Fig. 3. Biochemical events occurring in degenerating follicle cells. a–c: Variation in gene expression as revealed by in vivo incorporation of tritiated uridine. **a:** Follicle 1,400 μm in diameter. A significant labelling is present on the nucleolus (arrows), the nucleoplasm (arrowheads) and the cytoplasm of the follicle cells and also on the oocyte (Oo) cytoplasm. **b:** Follicle 1,700 μm in diameter. Labelling is present on the nucleoplasm (arrowheads) and cytoplasm of follicle cells and on the cytoplasm of oocyte. The nucleoli of the intermediate and pyriform cells are unlabelled (arrows). **c:** Follicle 1,700 μm in diameter. Significant labelling is present on the cytoplasm of a follicle cell undergoing

bitrary units (AU), with averages of 134 ± 18 AU in small follicles and 136 ± 14 AU in large follicles. These mean values correspond to the 2C nuclear DNA content measured in typically diploid erythrocytes (135 ± 13 AU) or fibrocytes (130 ± 8; data not shown).

Protein scaffolds are obtained only in samples prepared from epithelia dissected out from previtellogenic follicles larger than 1,600 μm with a regressing epithelium. At the phase-contrast microscope, the scaffolds appear as small, empty sacks showing an irregular shape (Fig. 3e). When the suspension containing the scaffolds is dried on a slide and stained, the scaffolds show high affinity for eosin (Fig. 3f).

Following in situ nick translation, several labelled epithelial cells are commonly found in follicles ranging 1,500–1,800 μm in diameter. About 200 nuclei of both intermediate and pyriform cells may be positive in each follicle (Fig. 3g,i). In follicles larger than 1,800 μm, a significant labelling is observed over almost all the nuclei of the large cells (Fig. 3h,l). No labelling is present on follicle cells from follicles smaller than 1,500 μm.

DISCUSSION

From these results, it is evident that the remodelling of the epithelium in large previtellogenic follicles involves the degeneration of intermediate and pyriform cells and that the process occurs via programmed cell death. The degenerating cells, in fact, show the typical features of apoptosis: the shrinkage of the nucleus and of the cytoplasm, the condensation and margination of chromatin, a blebbing of the nuclear membrane and an extensive vacuolization of the cytoplasm. During the final stages, the nuclear material becomes clearly pycnotic and dispersed in a series of dense vesicles. Vesicles containing organelles can also be seen.

degeneration (arrow). **d:** Nuclear DNA content (in arbitrary units, AU) in cells from small (1,200 μm) follicles as compared with cells from large (1,800 μm) follicles. Significant differences cannot be noticed. **e, f:** Activation of a tissue transglutaminase as revealed by the formation of protein scaffolds. Scaffold at the phase-contrast microscope (e) and following air drying on a slide and staining with eosin (f). **g–l:** DNA fragmentation in apoptotic cells as revealed by in situ nick translation. Significant labelling can be observed in follicles ranging 1,500–1,800 μm (g, i) on nuclei of both intermediate (arrows) and pyriform (arrowheads) cells. In follicles larger than 1,800 μm (h, l) labelling is present on most large cells (arrows). Magnification = a, b: × 500, c: × 350; e: × 450; f–h: × 250; i: × 1,300; l: × 750).

Both intermediate and pyriform cells maintain a significant level of mRNA, but not of rRNA synthesis during regression (see also Motta et al., '95). This reveals that significant changes of the metabolism occur as the cells approach degeneration.

The recovery of scaffolds only in the epithelial cells of large follicles demonstrates that the process of remodelling also involves a significant reorganization of the cytoplasmic proteins. These structures, resistant to detergent and chaotropic factors, would play an essential role in avoiding the leakage of materials outside the regressing cells, thus preventing the onset of inflammatory processes (Piacentini et al., '91). In P. sicula, the cells in degeneration are scattered among healthy cells and no leucocytes are present either in the epithelium or in the theca.

As expected, the early phases of apoptotic regression of intermediate and pyriform cells do not involve any significant decrease in the nuclear DNA content. The nucleases, however, are activated and consequent DNA fragmentation occurs as it is revealed by the in situ nick translation technique. Both "domain" and "internucleosomal" fragmentation may occur in apoptotic cells, usually in temporal sequence (Gold et al., '93), although the absence of internucleosomal fragmentation has been often reported (Cohen et al., '92; Falcieri et al., '93; Cinti et al., '95). In our species, the reduced number of regressing cells present in the epithelium (see Fig. 3g–l) does not allow an investigation by gel electrophoresis of the presence of megabase or oligonucleosomal fragments (unpublished results).

The occurrence of apoptosis in systems undergoing remodelling is certainly not surprising: much evidence has already been obtained from a large number of different tissues, including granulosa cells in mammals (Hughes and Gorospe, '91). The degeneration of intermediate and pyriform cells via apoptosis, however, is interesting if it is considered that these cells are nurse cells connected with the oocyte via an intercellular bridge. Why, when their function is completed, do these follicle cells degenerate via apoptosis and are not simply reabsorbed by the oocyte?

This event is apparently occurring for the cytoplasm. The ultrastructural observations indicate that most of the cytoplasm and of the organelles are released into the oocyte. During regression, the cell volume appears significantly reduced while the intercellular bridges remain open and are filled with organelles. A passage of vesicles, mitochondria and other organelles toward the

oocyte is supported by previous observations (Andreuccetti et al., '79; Andreuccetti, '92; Motta et al., '95) while the transferring of RNA was recently demonstrated in long-term experiments of in vivo administration of tritiated uridine (Motta et al., '95).

The nuclear materials apparently have a different fate. In fact, nuclear remnants cannot be found in the intercellular bridges or in the cortical region of the oocyte. By contrast, electron-dense vesicles containing pycnotic chromatin and fragments of nuclear membrane are present in several small cells. The different fate of the nuclear materials may be explained, taking into account the considerable impact that this material may exert on the differentiating oocyte. DNA fragments released into the oocyte cytoplasm, in fact, may interfere with the regular completion of meiosis following the germinal vesicle breakdown. Nuclear materials, therefore, are first degraded and fragmented in apoptotic bodies that are then engulfed by the small cells.

This DNA would then probably play a fundamental role in sustaining the active proliferating phase of these small cells during the last stages of previtellogenesis. It is not clear, however, how the apoptotic bodies are engulfed into the small cells since signs of endocytosis were never found.

Which factor(s) is involved in the control of differentiation and regression of intermediate and pyriform cells is not yet clear. The process is certainly under genetic control as demonstrated by the occurrence of apoptosis, a genetically directed process, and by the persistence of RNA synthesis. In other systems, it was demonstrated that terminal differentiation and aging are also related to the expression of the tissue transglutaminase (Tacher and Rice, '85; Thomazy and Fesus, '89). In *P. sicula*, this enzyme is apparently expressed only during late previtellogenesis as the finding of scaffolds suggests. We can therefore postulate that degeneration of intermediate and pyriform cells is in some way also related to the expression of this enzyme.

In conclusion, in *Podarcis sicula* the intermediate and pyriform cells initially support the oocyte growth by assuming the role of nurse cells. Later, at the end of previtellogenesis, they complete their life cycle and undergo apoptosis, thus indicating that the remodelling of the epithelium is associated with programmed cell death. The cytoplasmic materials from the regressing cells are recovered by the oocyte while the nuclear remnants are recycled by the small cells that become

the only constituents of the follicular epithelium during vitellogenesis. The different fate of the cellular constituents could be a fundamental event that sustain the differentiation of both epithelium and oocyte.

ACKNOWLEDGMENTS

LITERATURE CITED

Andreuccetti, P. (1992) An ultrastructural study of differentiation of pyriform cells and their contribution to oocyte growth in representative squamata. J. Morphol., *212*:1–11.

Andreuccetti, P., E. Limatola, and G. Ghiara (1979) Secretory activity of pyriform cells during the oocyte growth in *Lacerta sicula*. J. Submicr. Cytol., *11*:369–377.

Andreuccetti, P., C. Taddei, and S. Filosa (1978) Intercellular bridges between follicle cells and oocyte during the differentiation of follicular epithelium in *Lacerta sicula*. J. Cell Sci., *33*:341–350.

Arends, M.J., R.G. Morris, and A.H. Wyllie (1990) Apoptosis. The role of the endonuclease. Am. J. Pathol., *136*:593–608.

Cinti, C., S. Santi, A.R. Mariani, M. Columbaro, M. Vitale, and E. Falcieri (1995) Different patterns of DNA cleavage in Molt-4 apoptotic cells. Eur. J. Histochem. *39*/suppl. *1*:48.

Cohen, G.M., X. Sun, R.T. Snowden, D. Dinsdale, and D.N. Skilleter (1992) Key morphological features of apoptosis may occur in the absence of intenucleosomal DNA fragmentation. Biochem. J., *286*:331–334.

Earnshaw, W.C. (1995) Nuclear changes in apoptosis. Curr. Op. Cell Biol., *7*:337–343.

Ellis, R.E., J. Yuang, and H.R. Horvitz (1991) Mechanisms and functions of cell death. Annu. Rev. Cell Biol., *7*:663–698.

Falcieri, E., A.M. Martelli, R. Bareggi, A. Cataldi, and L. Cocco (1993) The protein kinase inhibitor staurosporine induces morphological changes typical of apoptosis in Molt-4 cells without concomitant DNA fragmentation. Biochem. Biophys. Res. Commun., *193*:19–25.

Fesus, L., P.J.A. Davis, and M. Piacentini (1991) Apoptosis: Molecular mechanisms in programmed cell death. Eur. J. Cell Biol., *56*:170–177.

Fesus, L., V. Thomazy, F. Autuori, M.P. Ceru, E. Tarcsa, and M. Piacentini (1989) Apoptotic hepatocytes become insoluble in detergents and chaotropic agents as a result of transglutaminase action. FEBS Lett., *245*:150–154.

Filosa, S. (1973) Biological and cytological aspects of the ovarian cycle in *Lacerta sicula sicula*. Mon. Zool. Ital., *7*:151–165.

Filosa, S., C. Taddei, and P. Andreuccetti (1979) The differentiation and proliferation of follicle cells during oocyte growth in *Lacerta sicula*. J. Embryol. Exp. Morphol., *54*:5–15.

Gold, R., M. Schmied, G. Rothe, H. Zischler, H. Breitschopf, H. Wekerle, and H. Lassmann (1993) Detection of DNA fragmentation in apoptosis: Application of in situ nick transla-

tion to cell culture and tissue sections. J. Histochem. Cytochem., *41*:1023–1030.

Hubert, J. (1977) Facteurs et modalités de la differentiation des 3 types cellulaires de la granulosa du follicle ovarien chez des lacertiliens. Bull. Soc. Zool. Fr., *102*:151–158.

Hughes, F.M., and W.C. Gorospe (1991) Biochemical identification of apoptosis (programmed cell death) in granulosa cells: Evidence for potential mechanism underlying follicular atresia. Endocrinology, *129*:2415–2422.

Karnowsky, M.J. (1965) A formaldehyde-glutaraldehyde fixation of high osmolarity for use in electron microscopy. J. Cell Biol., *27*:137a.

Klosterman, L. (1987) Ultrastructural and quantitative dynamics of the granulosa of ovarian follicles of the lizard *Gerrhonotus coeruleus* (Fam. Anguidae). J. Morphol., *192*:125–144.

Maniatis, T., E.F. Fritsch, and J. Sambrook (1982) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Mazzi, V. (1977) Manuale di Tecniche Istologiche e Istochimiche. Piccin Editore, Padova.

Motta, C.M., P. Andreuccetti, and S. Filosa (1991) Ribosomal gene amplification in oocytes of the lizard *Podarcis sicula*. Mol. Rep. Dev., *29*:95–102.

Motta, C.M., S. Filosa, and P. Andreuccetti (1995) Role of py-riform cells during the growth of oocytes in the lizard *Podarcis sicula*. J. Exp. Zool., *273*:267–256.

Patau, K. (1952) Absorbtion microphotometry of irregularly-shaped objects. Chromosoma, *5*:341–362.

Piacentini, M., V. Thomazy, E. Tarcsa, M.G. Farrace, F. Autuori, and L. Fesus (1991) Role of tissue transglutaminase in the formation of apoptotic bodies. In: Chemical Carcinogenesis 2. A. Columbano, ed. Plenum Press, New York, pp 461–471.

Schwartzman, R.A., and J.A. Cidlowski (1993) Apoptosis: The biochemistry and molecular biology of programmed cell death. Endocrine Rev., *14*:133–151.

Tacher, S.M., and R.H. Rice (1985) Keratinocyte-specific transglutaminase of cultured human epidermal cells: Relation to cross-linked envelope formation and terminal differentiation. Cell, *40*:685–688.

Taddei, C. (1972) Significance of pyriform cells in ovarian follicle of *Lacerta sicula*. Exp. Cell Res., *72*:562–566.

Thomazy, V., and L. Fesus (1989) Differential expression of tissue transglutaminase in human cells. Cell Tissue Res., *255*:215–219.

Wyllie, A.H. (1981) Cell death: A new classification separating apoptosis from necrosis. In: Cell Death in Biology and Pathology. I.D. Bowen and R.A. Lockshin (eds.) Chapman and Hall, London and New York, pp 9–34.