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The Comparative Cell Biology of Accessory Somatic (Follicle or Granulosa) Cells in the Animal Ovary

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A comparative account is given of recent advances in the cell biology of ovarian follicle or granulosa cells in mammals, non-mammalian vertebrates and invertebrates by comparing and contrasting their morphological organization, localization, cytological and chemical characteristics, and functions in relation to oocyte growth, which show great diversity. Their structural and chemical characteristics are discussed in relation to the nucleus, cytoplasmic organelles and inclusions, enzyme systems, cytoskeleton and plasma membrane specializations including junctional complexes which show variations in their development and distribution. The regulation of multiplication of FCs is also discussed. A very limited follicle cell-oocyte association or its complete absence is observed in some groups of invertebrates and amphioxus. Wherever FCs are present their comparative functions are discussed in relation to: (i) provision of nutrient substances and informational molecules to the growing oocyte, (ii) yolk synthesis, (iii) phagocytosis, (iv) steroid hormone synthesis, (v) synthesis and secretion of regulatory proteins and glycoproteins, (vi) zona pellucida or chorion formation, (vii) regulation or meiosis or maturation of oocyte, (viii) determination of animal-vegetal and bilateral symmetries of the egg, and (ix) ovulation, transportation and fertilization of egg. Comparative cellular and molecular aspects of follicle cell oocyte interceptio' s and the regulatory mechanisms involved as well as gaps in our knowledge about the molecular aspects of various functions of follicle and of interactions between follicle cells and oocyte are emphasized for a better understanding of diversity in the patterns and regulation of oocyte growth, differentiation and maturation in animals.

Key Words: Chorion, Cytoskeleton, Egg symmetries, Enzymes, Follicle cells, Granulosa cells, Growth factors, Informational molecules, Junctional complexes, Nucleus, Oocyte maturation, Organelles, Ovulation, Phagocytosis, Regulatory factors, Steroidogenesis, Transport mechanisms, Yolk synthesis

I. Introduction

It is well known that the female germ cells or oocyte in the ovaries of animals grow, differentiate and mature in close morphological association with accessory somatic cells, which because of this nurse cell function have generally been designated as nurse, follicle or ganulosa cells or trophocytes (Raven 1961, Guraya 1978, 1985, 1986, 1989, 1996, 1997, 1998, Adiyodi & Adiyodi 1983, Browder 1985). In this review, accessory somatic cells, which show a great diversity in their origin, proliferation, development, abundance and-structural organization will be referred to as follicle or granulosa cells (FCs or GCs). Our knowledge of these cells at the cellular and molecular levels especially in relation to their roles in the regulation of oocyte growth and differentiation is still meagre from a comparative point of view (Guraya 1978, 1985, 1986, 1989, 1996, 1997, Adiyodi & Adiyodi 1983, Browder 1985, Saidapur 1989a). Although in these reviews, the various authors discuss the structural and functional aspects of FCs in different groups of animals, very little or no attempt has been made to give a consolidated account of their cell biology or functions by comparing and contrasting their cellular and histochemical, biochemical (or molecular) aspects throughout the animal kingdom.

The FCs of developing ovarian follicles of different groups of animals especially of vertebrates including mammals have been extensively subjected to various new techniques of morphology including ultrastructure,

histochemistry, biochemistry, autoradiography, molecular biology, in vitro experiments etc. These studies have revealed a considerable diversity in the morphological (or ultrastructural), histochemical and biochemical including molecular characteristics of FCs in different groups of animals (both invertebrates and vertebrates). The purpose of this most timely review is to summarize and integrate these results from a comparative point of view for a deeper insight into the cell and molecular biology of FCs in relation to oocyte growth throughout the animal kingdom. The gaps in our knowledge about their comparative cell biology will also. be emphasized for further investigations. The cellular and molecular aspects of mammalian GCs have been most extensively investigated during the recent years especially by *in vitro* experiments. In the light of various advances made in this regard, the emphasis will be on the comparative cell biology of FCs in other animal groups especially in invertebrates, which have received relatively little attention for their cell and molecular biology as well as for their endocrine and paracrine functions (Adiyodi et al. 1997). Such comparisons at the cellular and molecular levels will certainly help us better to understand the diversity in the regulation and coordination of proliferative, developmental, maturational and degenerative processes of FCs and oocytes as well as evolutionary steps in the development of different patterns of contacts (or environmental, nutritional and regulatory interactions, between oocytes and FCs during the evolution of different groups of animals. Actually, complex interactions between the oocyte and the FCs occur throughout the follicular development or growth, which are required to be understood at the cellular and molecular levels.

II. Characteristics of Follicle or Granulosa Cells

A. Morphological Organization and Localization

Follicle cells and developing oocytes provide one of the most complex examples of cell - cell interactions because there is great diversity in the presence, morphological organization and contacts or interrelationships between FCs and oocytes as a result of variable patterns of oogenesis in different groups of invertebrates and vertebrates. Even the FCs associated with same oocyte show polymorphism at some stages of its growth, possibly to meet the nutritional or metabolic and regulatory requirements of developmental processes in the growing oocytes which store not only the organelles and nutritive substances (or yolk) but also the informational molecules such as various RNA'S, DNA, proteins, etc. to support the developmental processes of the embryo (Biggers & Schuetz 1972, Browder 1985, Davidson 1986, Guraya 1985, 1986, 1989, 1997, Henning 1992, Russo et al. 1992, Kress 1996). The variations in the development and organization of FCs around the growing oocyte in different groups of animals are indicative of variations in the contributions of organelles or substances or various regulatory macromolecules by the FCs to the growing oocyte, which will be described at the cellular and molecular levels in this review.

1. Invertebrates

In comparison to the enormous number of invertebrate species found in different habitats, relatively few species of invertebrates have been investigated for the ultrastructure of their gonads or ovaries, with special reference to FCs (Bigger & Schuetz 1972, Adiyodi & Adiyodi 1983, Browder 1985).

Ovarian structures of Porifera form masses of oocytes which derive from choanocytes or pinacocytes (Fell 1983). In most species the oocytes are individually scattered throughout a large part of endosome. The oogonia regularly aggregate into temporary groups. Two patterns of FCs and oocyte interrelationship are reported in sponges (Fell 1983).. They occur either as nurse cells or FCs. A variety of cell types can function as nurse cells in different species of sponges, and more than one type of cell may serve this function of supplying macromolecules and/or organelles to developing oocytes (and/or embryos). The accumulation of nurse cells around growing oocyte is believed to be a chemotactic response to a substance (s) produced by the oocytes. In many sponges the eggs are enclosed within a follicular epithelium consisting of a single layer of

flattened pinacocytes or collenzcytes or 2-3 layers of follicular cells during the period of major growth of oocyte, suggesting their nutritive (nurse) function. The follicular epithelium becomes progressively attenuated as the oocyte increases in size. In some cases, follicular cells associate with oocytes of relatively small size. The nurse cells of many sponges come to lie among the typical follicular cells to reach the surface of the oocytes. The nurse cells of some calcareous sponges apparently fuse with the oocytes or are engulfed by them as evidenced by the presence of various stages of their degeneration in the cytoplasm of vitellogenic oocytes (Fell 1983). In some other sponges, the engulfed nurse cells continue to remain metabolically active as evidenced by their presence even in the blastomeres, suggesting their continued nutritive role. The factors regulating the variations in the behaviour of nurse or FCs in sponges are required to be determined at the molecular level in vitro experiments with special focus on interactions between somatic cells and female germ cells.

In the ovary of Cnidaria, which together with Porifera and Ctenophora, represent the first multicellular animals to be evolved, forming definite tissues and producing diverse sex organs. The interstitial cells differentiate not only into oocytes but also into somatic cells (Beams & Kessel 1983). With the growth, some interstitial cells enlarge to form a few oocytes whereas others are engulfed by the oocytes and serve as food for them. No FCs forming an epithelium is reported in Cnidaria. However, in Trachylina, the ovary of the hydrozoan jelly fish is placed within the ectodermal layer and is covered by a thin epidermal layer which separates the oocytes from their external environment and in some places it apparently extends inward beneath the oocytes, suggesting some oocyte-somatic cell association.

Generally, there are neither nurse cells (or FCs) associated with the growing oocytes of invertebrates belonging to various phyla such as Platyhelminthes (Gremigni 1983, Davis & Roberts 1983); Mesozoa (McConnaughey 1983); Nemertina (Bierne 1983); Gnathostomulida (Mainitz 1983); Rotifera (Gilbert 1983); Gastrotricha (Hummon & Hummon 1983); Nematoda (Foor 1983); Acanthocephala (Guraya 1969, Parshad & Guraya 1977, 1978, Crompton 1983); Priapulida (Norrevang & vander Land 1983); Sipuncula (Rice 1983); Annelida (Olive 1983); Pogonophora (Bakke 1983), Pentastomida (Norrevang 1983); Phoronida (Emig 1983); Bryozoa Ectoprocta (Hayward 1983), Bryozoa Entoprocta (Franzen 1983) and Chaetognatha (Alvarino 1983); in some nemertines the secondary cells are believed to be follicular cells which contribute to the formation of oocyte envelope (Bierne 1983). However, in some groups of these invertebrates the somatic cells derived from the ovarian epithelial covering or peritoneum (in coelomic oogenesis as in Sipuncula and Bryozoa Ectoprocta or vasoperitoneal oogenesis as in phoronida); infoldings of the ovary may sometimes get dispersed or extend to a variable degree among the growing oocytes. But it remains to be determined whether they function as nurse or FCs in the synthesis and transfer of nutrients to the growing oocytes. In some turbellarians, the female gonads consist of a collection of follicles, each composed of one oocyte surrounded by several nutritive cells (Gremigni 1983); sometimes, one of these nutritive cells completely envelopes the oocyte and is termed as accessory cell. In some rotifers the follicular layer is syncytium with 8-10 nuclei and attached to the oocytes and vitellarium by desmosome-like structures (Gilbert 1983). Its functional significance remains to be determined. However, Bentfeld (1971a,b) suggested three functions for this syncytial follicular layer (i) a supporting structure for young and developing oocytes; (ii) a source of material from which the final membrane covering the egg is formed; and (iii) nourishment of the oocytes through the transfer of autolysed cytoplasmic material. The supporting syncytial tissue of ovarian balls in Acanthocephala is believed to be involved in the uptake of nutrients and exchange of materials with the environment (Crompton 1983). In the phoronida, the oocytes grow inside the vasoperitoneal tissue which first becomes enlarged and then degenerates. In pentastomid ovary atleast one (sometimes two or three) of the wall cells around the place where oocyte bulges is believed to act as a sort of follicular cell or cells (Norrevang 1983). Their cell processes cover some surface of the oocyte towards the haemocoel, even in oocytes that are almost mature.

In some sipunculates, polychaetes and tardigrades the so-called nurse cells are found to be abortive germ cells or oocytes which contribute to the growth of the definite oocyte as evidenced by the close association between them and growing oocytes (Rice 1983, Olive 1983, Bertolani 1983). Weglarska (1979), using electron microscopy, has shown that in the ovary of the tardigrade (*Macrobiotus richtersi*, the cytoblasts divide and form a cluster of cystocytes which are connected with each other by cytoplasmic bridges only one of each cluster develops into the oocyte. The nurse cells are joined to the oocyte by fusosomes and during early vitellogenesis, they are similar in appearance to the oocyte.

In the Bryozoa Entoprocta (e.g. *Luxosoma vivipara*), a single layer of follicular cells distinctly demarcated from the adjoining tissue surrounds the growing oocyte (Nielsen 1966). In Barentsia discreta, some mesenchymal cells with condensed nuclei are often seen on the surface of ovary (Mukai & Makioka 1980). In the branchiopods there is present a squamous FC having an irregular outline because of its long cytoplasmic processes (Chuang 1983).

In Crustacea, Myriapoda, Insecta and Arachnida, the FCs are well-developed to constitute a single-layered follicular epithelium around the growing oocytes at various stages of development (Raven 1961, Mathews 1962, King 1972, Adiyodi & Subramoniam 1983, Sareen & Adiyodi 1983, Browder 1985, Bownes 1994). With the growth of their oocyte, the FCs increase in number by mitosis and meanwhile their size and shape vary. Generally, they become stretched and flattened in vitellogenic oocytes. In insects, besides FCs the nurse cells are also present (King 1972).

Among molluscs, FCs are present in association 'with the growing oocytes (deJong Brink et al. 1983, Hadfield & Switzer-Dunlop 1984, Kress 1986). But their number and arrangement vary in single oocytes in different groups of molluscs. Three types are distinguished; in the first type the growing oocytes become completely enveloped by an increasing number of FCs, which often form a syncytium; in the second type, the oocyte is enveloped by a distinct (small) number of FCs, but it keeps contact with the basal lamina of the acinar wall; and in the third type, the oocyte in its early stage of development gets completely surrounded by a limited number of FCs. In later stages, FCs are detached from the apex of the oocyte which now bulges freely into the lumen of the acinar. However, the oocyte keeps contact with the acinar wall by a stalk. In the cephalopod Alloteuthis subulata Lam. the oocyte at the beginning of stage II becomes totally surrounded by a flat and smooth follicular epithelium of cube-shaped FCs and with oocyte growth the FCs increase in number and height (Bottke 1974). Soon a network of infoldings of the follicular epithelium penetrates the oocyte giving it a rather complex structure.

Among echinoderms, the FCs show variations in their localization and morphology (Kanatani & Nagahama 1983, Browder 1985). In the sea-urchin ovary each oocyte is well-embedded in a nest of surrounding somatic cells which show similar morphological features as they occupy all the spaces between the oocytes and extend long cytoplasmic processes. In contrast, in ovaries which are filled with growing eggs, only a few somatic cells are found, mainly in the superficial layers. Primary oocytes of star fishes are surrounded by atleast one layer of FCs; each follicle cell possesses a single flagellum. These flagellated cells surround all the primary oocytes of various stages and show long cytoplasmic processes which penetrate the vitelline layer to the egg surface to form desmosome junctions (Schroeder et al. 1979). These authors have also reported the presence of an additional cell type in the ovaries of Asterias rubens. These cells are distributed separately or in small groups near the invaginations of the haemal system and show the ultrastructural features characteristic of steroid-producing cells in vertebrates (Guraya 1976a,b).

As the oocytes of Urochordata-Ascidiacea grow, they progressively protrude from the ovarian wall and are surrounded with a layer of flattened follicular epithelial cells (Kessel 1983). The follicle becomes bilayered as a layer of cuboidal cells constituting the inner-follicular cells lies adjacent to the oocyte and an outer layer of squamous-type cells forms the outer follicular cells. Another type of follicular cell, the so-

called test cell lies in indentations of the oocyte or egg surface (Guraya 1968b, 1972 b). The test cells are believed to originate by division of the inner follicular cells.' The follicular wall in growing and mature oocytes of the Cephalochordate (e.g. Branchiostoma) ovaries do not show much development and differentiation (Guraya 1967, 1968 a, 1972 a, 1983). A follicular epithelium comparable to that of tunicates, cyclostomes and higher chordates (Guraya 1978, 1986, 1989, 1996) is not developed in Cephalochordates (Guraya 1967, 1968 a, 1972 a, 1983). However, electron microscope studies have revealed the presence of some niches, in growing oocytes of amphioxus adjacent to their plasma membrane (Reverberi 1966, 1971). This author interpreted these formations as homologues of the test cells of ascidians but according to Guraya (1978) these nichés appear to represent only islands of zona material possibly secreted by the oocyte.

2. Vertebrates

In their morphological organization and development, FCs in the ovaries of vertebrates including mammals show a great diversity. As the young oocyte of the sea lamprey Petromyzon marinus enlarges the FCs form a follicular layer (Lewis & McMillan 1965). The nuclei of its squamous cells are closely applied to the oocyte. In the adult lamprey FCs are less tightly applied to the surface of the oocyte, are low cubical to cubical in shape, and may overlap and become three to four layers deep. Meanwhile some FCs at various stages of their growth and differentiation are incorporated into the growing previtellogenic oocyte and increase its mass of RNA-rich cytoplasm. During further growth, the oocyte gets surrounded by a single layer of squamous FCs. With the rapid increase in the size of the egg, the single follicular layer becomes thinner and is closely applied to the zona pellucida although on some eggs it may evaginate at one point to form a hollow, cortical stalk of columnar cells. The FCs in the adult river lamprey (Lampetra flaviatilis) and brook lamprey (L. planeri) do not constitute a regular layer all around the oocyte but are grouped at the vegetative pole (Larsen 1970, Busson-Mabillot 1967a, b).

The growing oocytes of cartilaginous fishes show a well-developed follicular epithelium (Guraya, 1978, 1986, 1996). In the early stages of previtellogenic oocyte growth in the dogfish (Scoliodon sorrakowah) it consists of a single layer of FCs which undergo changes in form and size corresponding to further growth of the oocyte. In some species, FCs remain of uniform size, but in others two cell types are developed, a large cell with a reticular nucleus and abundant cytoplasm, and a small columnar cell with a densely staining nucleus (Chieffi 1961). In the bony fishes, a few FCs are seen close to the very young oocytes. With the growth of oocyte, FCs increase in number by mitosis to constitute a continuous follicular epithelium which retains its single-layered structure throughout oocyte growth (Guraya 1978, 1986, 1996). However, in some fish species, the follicular epithelium may acquire a pseudostratified structure for some stages of oocyte growth because of the placement of its nuclei in three to four layers. The follicular epithelium again becomes a monolayer in the advanced stages of oocyte growth, suggesting that the pseudostratified condition may reflect cellular reserve to be used during the rapid growth of the oocyte. In their shape and size, FCs and their nuclei undergo conspicuous changes in teleosts (Guraya 1986, 1996). In the later stages of vitellogenic oocytes, the follicular epithelium is usually stretched, forming some intercellular spaces.

In the amphibian ovary the young oocytes are associated with the fibroblast-like cells which differentiate in time to form a single-layer follicular epithelium as in toleosts (Guraya 1978, Browder 1985, Saidapur 1989b). It remains a monolayer throughout oocyte growth in Anura, Apoda and Urodela. With oocyte growth, FCs and their nuclei undergo changes in their morphology. To start with they are spindle-shaped and flattened parallel to the oocyte surface, but in the subsequent stages of oocyte growth they become first cubical and then columnar in shape. In large yolky eggs, the follicular epithelium becomes thin and size of FCs is reduced as in teleosts (Guraya 1996). The morphological organizations of follicular epithelium is basically similar in teleosts and amphibians.

In different groups of reptiles the development and structure of the follicular epithelium vary greatly (Guraya 1978, 1989 for reviews). This layer shows greater development and complexity in lizards and snakes than in tortoises, turtles and crocodiles. The young oocyte in reptilian ovary is surrounded by a single-layered follicular epithelium of flattened FCs having similar morphology. After the oocyte has left the germinal bed, it becomes first bilayered and then multilayered in the ovaries of lizards and snakes, but in the tortoises, turtles and crocodiles, it remains single-layered as in birds. Three types of cells (small and intermediate cells and large pyriform cells) are differentiated in the follicular epithelium of lizards and snakes; the small cells can be further distinguished into basal and apical cells, depending on their location in the follicular epithelium. The various cell types are distinguishable by their size, by the nucleolar size; and by their chromatin distribution (Guraya 1978, 1989). Small FCs are generally distributed in the inner and outer regions of the polymorphic follicular epithelium and are designated apical and basal cells, respectively. Small basal cells proliferate and give rise to the intermediate and pyriform cells. Each flask-shaped pyriform cell develops a narrow protoplasmic prolongation which traverses the zona pellucida. Thus the cytoplasm of the growing previtellogenic oocyte and the pyriform cells becomes confluent through these cytoplasmic prolongations (Guraya, 1978, 1989).. At the point of communication, a notch develops in the zona pellucida that corresponds to an ultrastructural intercellular bridge (Guraya 1978, 1989).

In the chelonian and crocodilian ovary, the single-layered follicular epithelium remains monomorphic throughout oocyte growth (Guraya 1978, 1989); no intercellular bridges are formed. However, Rahil and Narbaitz (1973) have distinguished two types of FCs in the turtle Pseudemys elegans on the basis of the density of their cytoplasmic matrices, which appear to correspond to light and dark cells of the follicular epithelium in birds. The dark cells are the degenerating FCs which are resorbed *in situ*.

In the ovaries of some lizards and snakes, several nuclei are distributed irregularly in the peripheral ooplasm of growing previtellogenic follicles as soon as they start projecting from the germinal bed (Guraya 1978, 1989). These nuclei are of those FCs that have migrated from the follicular epithelium into the ooplasm of growing oocytes. The number of migrated nuclei is increased with follicular growth. But number varies in different follicles of the same size. These migrated or engulfed nuclei finally disintegrate and their nucleoproteins are-resorbed in the ooplasm before yolk deposition begins. During disintegration and resorption, they are associated with clear spaces which appear to play an important role in their dissolution.

The pyriform cells in vitellogenic follicles of lizards and snakes are greatly reduced in size and the follicular epithelium becomes single-layered and monomorphic again (Guraya 1978, 1989). All that remains are small FCs. Pyriform cells are believed to disappear by degenerative process and their degeneration is one of the reasons for the reduction of the follicular epithelium. Guraya and Varma (1976) have observed the formation of some bag-like structures which project from the follicular epithelium into the oocytes of the house lizard (Hemidactylus flaviviridis) and consist of FCs and zona pellucida; the significance of their formation remains to be determined. In the fully mature vitellogenic eggs, the cells of the follicular epithelium are stretched, decreased in size, and separated from each other. The formation of large intercellular spaces between FCs in large follicles of the turtle (P. elegans) ovary suggests an extracellular route for the transport of nutrients to the surface of oocyte (Rahil & Narbaitz 1973).

The primordial oocytes in the bird ovary are associated with some flat FCs which increase in number by mitosis to form a single layer of cubical cells in growing previtellogenic follicles (Guraya 1978, 1989, Callebaut et al.1991). The general patterns of the follicular epithelial changes are similar in different species of birds investigated so far. The follicular epithelium of the oocytes of intermediate size gradually develops pseudostratified structure; sometimes it produces a wrong impression of being multilayered. FCs get enlarged by developing more cytoplasm and organelles. The follicular epithelium attains its maximum thickness in the intermediate stages of

oocyte growth but its width is again decreased in the large vitellogenic follicles as the pseudostratified epithelium is finally changed into a single-layered epithelium consisting of cubical and flat cells. Its pseudostratified structure is believed to be an adaptation to meet the requirements of the rapid oocyte growth. Both lightly and darkly stained FCs are observed in the follicular epithelium of various bird species (Guraya 1978, 1989, Callebaut 1991). The darkly stained FCs are the degenerating cells as they are gradually resorbed to provide the products of their degeneration such as DNA, RNA, protein and lipoproteins to the growing avian oocyte as will be discussed in Section IV.

In the mammalian ovary, FCs are usually called GCs, which are believed to derive from mesonephric cells during embryogenesis (Guraya 1997, 1998). They increase in number during follicle growth by mitosis, possibly in response to oestrogen, follicle-stimulating hormone (FSH) or some other intraovarian factors (Section III), thus forming unilaminar, bilaminar, trilaminar and multi-laminar follicles (Guraya 1985, 1997, 1998, Driancourt et al. 1993, Adashi & Leung 1993, Eppig 1994). The gonadotrophins (FSH and LH) appear necessary for the normal development of both preantral and antral follicles. The GCs are either polygonal or cuboidal in shape with a granular cytoplasm. The processes of granulosa cell multiplication and antrum development are basically similar in mammals. With the development of antrum or antral cavity in the multi-layered follicular epithelium or granulosa, the follicle is usually called Graafian follicle. Such a cavity containing follicular fluid (liquor folliculi) forms the characteristic feature of the follicles in the ovaries of eutherian mammals.

With the increase in diameter of follicle in both the preantral and antral follicles, the mean number of GCs per follicle is increased (Guraya 1985, 1997, Driancourt et al. 1993, Adashi & Leung 1993). Corresponding to the increase in size of follicles, the mitotic index of GCs is also increased in follicles; the mitotic index is generally higher in normal follicles than in early atretic follicles. Mitotic activity within the granulosa is such that a constant thickness is maintained during much of the follicular growth.

With accumulation of follicular fluid (FF) in the antrum, GCs in the mammalian follicles are separated into a membrana or mural granulosa lying adjacent to the basement lamina and several to many concentric layers forming the cumulus oophorus around the oocyte (Guraya 1985, 1997, Lipner 1988, Driancourt et al.1993). The fluid filled cavity, the antrum, is generally juxtaposed between the cumulus and mural GCs except at a point of attachment of the oocyte-cumulus cell complex to the mural GCs. The GCs lying inner to the basement lamina are larger than the other ones, with regularly arranged nuclei. The several layered membrana granulosa lying inner to the basement or basal lamina of antral follicles in some mammalian species such as rabbit, human, buffaloes, etc. shows Cell-Exner bodies which consist of a central fluid-filled cavity surrounded by a ring of one or two layers of GCs (Guraya 1985, 1997). It is still not known why such bodies are formed in the membrana granulosa of some species of mammals while in others they are absent. Morphogenetic studies of GCs at the molecular level especially their cell surface matrix may help us to explain such differences in their formation. The viscosity of the primary follicular fluid formed in between GCs may be responsible for the arrangement of GCs specific to Cell-Exner bodies (Guraya, 1985, 1997). A cluster of GCs forms the cumulus oophorus of variable size around the oocytes in mammals. The functional significance of variations in the degree of development of cumulus oophorus in mammals remains to be determined in relation to oocyte growth, maturation and fertilization (Eppig 1994). However, the layer of GCs lying immediately outer to the zona pellucida is usually called corona radiata (Guraya 1985, 1997). GCs are larger in preovulatory follicles after the LH surge than in other nonatretic antral follicles (Guraya 1985, 1997). Their size is again further increased close to the time of ovulation after the LH surge.

B. Nuclear and Cytoplasmic Components

In their morphology (including ultrastructure) histochemistry and biochemistry, as well as their distribution and quantity, the nuclear and cytoplasmic components of follicle (granulosa) cells show great variations not only with the stages of development and ,differentiation of follicular epithelium during oocyte growth and maturation but also with the species.

1. Nucleus

In its morphology and location the follicle cell nucleus shows variations that depend on the species and stages of development and differentiation of follicular epithelium in invertebrates (Adiyodi & Adiyodi 1983, Kress 1986), nonmammalian vertebrates (Guraya 1978, 1986, 1989, 1996) and mammals (Guraya 1985, 1997, 1998). It is interesting to mention here that nucleus of nurse or follicle cell becomes polyploid in some invertebrates (Adiyodi & Adiyodi 1983) suggesting an increase in the amount of their DNA. Callebaut et al. (1990) using 3H-thymidine have demonstrated a peak of activity of premitotic DNA synthesis in the follicle cell nuclei in the avian ovary. DNA synthesis has been correlated to structural changes in the follicular epithelium during the growth of the avian oocyte.

The chromatin masses reacting positively for DNA and proteins are generally distributed adjacent to the nuclear envelope of FCs of invertebrates and vertebrates (Adiyodi & Adiyodi 1983, Guraya, 1978, 1985, 1986, 1989, 1996, 1997, 1998). Their amount and distribution varies with the growth and differentiation of FCs. Electron microscopic studies have demonstrated nuclear pores in the nuclear envelope and electron dense heterochromatin masses of variable size, which are generally distributed adjacent to the nuclear envelope. The number of nuclear pores varies, depending upon the amount of ribosomes present in the cytoplasm of FCs. A single or more than one nucleolus of variable size is present. The electron dense heterochromatin masses may also be seen elsewhere within the nucleoplasm. All these nuclear components, which show great variations with the species as well as with the stages of development of follicular epithelium, are related to the production and accumulation of RNA and proteins in the cytoplasm of FCs.

The presence of primarily homogeneously distributed chromatin having a fine fibrillar granular texture in the nuclei of pyriform FCs in lizards and snakes and of large FCs in elasmobranchs (Guraya 1978, 1986, 1989, 1996) appears to be consistent with the suggestion that those FCs express a large portion of their genome in accordance with their highly versatile functions, especially in relation to the production of RNA'S, abundant ribosomes or ribonucleoproteins which are transported to the growing oocyte (Section IV).

The nucleolus or nucleoli of FCs consist of fibrillar and granular components. The molecular immunohistochemistry has demonstrated DNA and RNA in the fibrillar components of the nucleolus. The nucleolus is now known to constitute a very original nuclear territory that reflects the compartmentation of nuclear functions which implies specific RNA polymerases, specific RNAs and specific proteins (Hernandez-Verdun 1991). It forms the site of an intense exchange between the nucleus and cytoplasm that involves specific shuttled proteins and specific relationships with the nuclear envelope. Nucleoli are also involved in the transport of certain messenger RNA'S. All the cytological, histochemical and autoradiographic features of follicle cell nuclei are indicative of transcription of ribosomal genes (Guraya 1978, 1985, 1986, 1989, 1996, 1997).

2 .Cytoplasmic Organelles and Other Components

FCs wherever present have various organelles including mitochondria with simple cristae, Golgi Complex (consisting of a series of compact, smooth membrane lamellae and vesicles of different sizes), elements of granular endoplasmic reticulum (consisting of vesicles, lamellae, cisternae and associated ribosomes), some elements of smooth reticulum (varying among different groups of animals at different stages of oocyte growth in their relative proportion and distribution within the cytoplasm), numerous free ribosomes, multivesicular bodies. lysosomes, centrioles etc. (Adiyodi & Adiyodi 1983, Browder 1985, Guraya 1978, 1985, 1986, 1989, 1994, 1996, 1997, 1998). Besides these usual organelles, the annulate lamellae may also be present Lipid bodies of variable size, which consist mainly of phospholipids, glycogen granules and inclusions of diverse ultrastructure and nature consisting of lipids and carbohydrates, etc. are present, varying in their amount at different stages of follicle growth. These organelles and other cell components and inclusions vary with the species and with the stage of

development of follicular epithelium during oocyte growth. During hibernation of the lizard *Lucerta sicula*, a fraction of ribosome population of FCs aggregates to form ribosomal bodies consisting of stacks of ribosomal tetrameres embedded in a matrix and flanked by cisternae, which represent a storage form of inactive ribosomes. Relatively less attention has been paid to the study of organelles in the FCs of invertebrates at the ultrastructural level, although their growing oocytes have been extensively studied for the ultrastructure of organelles and their roles in vitellogenesis (Biggers & Schuetz 1972, Adiyodi & Adiyodi 1983, Browder 1985). Ultrastructural studies of squamous follicular cells in brachiopod (Chuang 1983) have shown the presence of nucleus with condensed nucleoprotein material forming an incomplete layer next to the nuclear membrane, subspherical mitochondria, some elongated profiles of ergastoplasm, sparse discrete ribosomes, lipid droplets, cortical granules and oval microsomes lying along the major axis of follicular cell whose function remains to be determined.

Histochemical studies have demonstrated RNA in the cytoplasm of FCs in arthropods (Sareen & Adiyodi 1983); the cytoplasmic RNA may be due to ribosomes lying free or in association with elements of granular endoplasmic reticulum. In an ultrastructural study on the follicular cells of Orchestia gammarella, Rateau and Zerbib (1978) showed that in the vitellogenic phase, protein synthetic activity is evident in FCs in the ergastoplasmic (granular endoplasmic reticulum) and Golgi complex regions suggesting that they may take part in the secretion of some substances. A complex but permeable network of intercelllular spaces between Fcs facilitates the entry of extracellular protein in the oocyte. The follicular cells among mollluscs have been shown to possess ultrastructural features or organelles (e.g. granular endoplasmic. reticulum, free ribosomes, mitochondria and large Golgi Complex, which are indicative of protein synthesis (Bottke 1974, deJong Brink et al. 1983, Kress 1986). The accessory cell of Arbacia punctulata, Lytechinus variegatus and L. picta show four types of inclusions showing variable size and ultrastructure (Verhey & Mayer 1967). The accessory cells also show lipid droplets of uniform size and extensive accumulation of glycogen. The follicular cells of starfishes show a number of electron-dense bodies of varying sizes, well developed Golgi complexes, rough endoplasmic reticulum and mitochondria (Kanatani & Nagahama 1983).

Ultrastructural, histochemical and autoradiographic studies on FCs in the ovaries of non-mammalian vertebrates such as lampreys, fishes, amphibians, reptiles and birds have shown that they become metabolically very active with the maturation of follicular epithelium especially in the previtellogenic and early vitellogenic follicles (Guraya 1978, 1986, 1989, 1996, Browder 1983). Histochemical studies have demonstrated the presence of proteins, carbohydrates, RNA, lipids and various enzymes in the cytoplasm of their FCs, which show variations in their amounts during different stages of follicle growth RNA metabolism has been studied in the FCs of *Bufo arenarum* oocytes (Sanchez-Riera et al. 1988a, b).

There is seen an extensive development of various cytoplasmic organelles such as granular endoplasmic reticulum, free ribosomes, large Golgi Complex, mitochondria etc. in FCs of developing oocytes in the ovaries of non-mammalian vertebrates such as fishes, amphibians, reptiles and birds (Guraya 1978, 1986, 1989, 1996, Browder 1983). The presence of these organelles provides an evidence of their increased metabolism as they are indicative of protein synthesis. In their amount and distribution, these organelles vary greatly not only in the FCs of different groups of non-mammalian vertebrates but also in the follicular epithelium of the same species (e.g. lizards, snakes and cartilaginous fishes) which show polymorphic nature of their FCs during the growth of previtellogenic oocytes because of their variable size and morphology (Section IIA).These differences in the amounts of various organelles related to protein synthesis suggested that the amounts of proteins synthesized by the follicular epithelium are variable not only with the stages of follicle growth in the same species but also among different groups of animals. The correlative immunocytochemical and in vitro biochemical studies are required to determine more precisely the qualitative and quantitative aspects of synthesis of various RNA's and their roles in protein

synthesis in FCs at different stages of follicle growth in the same species as well as in different groups of non-mammalian vertebrates. The follicular epithelial cells of bony fishes and amphibians resemble each other for their morphology (including ultrastructure and histochemistry), suggesting great similarities in their metabolic activities in relation to RNA's and proteins synthesis (Guraya 1978, 1996). It will be interesting to mention here that in non-mammalian vertebrates there appears to be correlation between the degree of development and differentiation of nuclear and cytoplasmic components in FCs and nucleoli in the oocyte nucleus or germinal vesicle. The follicular epithelium in teleosts and amphibians is relatively less developed than in reptiles and birds and correspondingly nucleoli of their germinal vesicle are extensively developed because of amplification of ribosomal genes in teleost oocytes (Guraya 1986, Davidson 1986), suggesting relatively less production of ribosomes by their follicle cell nucleus as compared to elasmobranchs, reptiles and birds.

Besides the various organelles related to protein synthesis, the follicular epithelium also shows lipid droplets of variable size, which consist of mainly phospholipids (Guraya 1978, 1980, 1989, 1996). In their amounts and distribution, the lipid droplets vary at different stages of follicle growth in the same species. For example, the large FCs of elasmobranchs and pyriform cells of lizards and snakes in pre-vitellogenic follicles show more lipid bodies of variable size. During the later stages of oocyte growth in the dogfish (*Scoliodon sorrakowah)* ovary, when an extensive deposition of yolk occurs, the highly sudanophilic lipid droplets form large aggregations in the FCs and simultaneously develop triglycerides in addition to phospholipids (Guraya 1978). Lance and Callard (1969) also observed an increase in lipid droplets during maturation of the follicular epithelium in the ovary of Squalus acanthias. These lipid accumulations are utilized either for some developmental processes of oocyte or for lipid yolk deposition as evidenced from their disappearance in vitellogenic follicles.

The results of various studies have shown that the FCs surrounding the growing oocytes of nonmammalian vertebrates synthesize proteins, carbohydrates, lipids and ribonucleoproteins (Guraya 1978, 1986, 1989, 1996, Browder 1985,). Their amounts and nature vary greatly from species to species and also from one group of vertebrates to another apparently depending on the nutrient, metabolic and developmental requirements of the developing oocyte. Most and possibly all of the proteins, ribonucleoproteins, lipids, carbohydrates, possibly some organelles etc. are ultimately delivered to the growing oocytes where they are either used for growth processes or stored for use during early embryogenesis. The phospholipids of lipid bodies also appear to be utilized for the construction of cellular lipoprotein membranes of cell organelles, and for various other metabolic activities concerned with the maturation of the follicular epithelium and oocyte (Guraya 1978, 1980, 1989, 1996). The carbohydrates including glycogen may be the source of energy production, which is required for various synthetic or metabolic activities in the FCs and growing oocytes of non-mammalian vertebrates as they attain a large size by developing more ooplasmic components.

Histochemical studies of healthy follicles of all sizes in the ovaries of mammals have demonstrated a feebly sudanophilic Golgi zone, granular mitochondria consisting of lipoproteins, abundant cytoplasmic RNA and some deeply sudanophilic lipid bodies of variable size in the cytoplasm of GCs (Guraya 1985, 1997). The lipid bodies consisting of phospholipids are sparsely distributed in the membrana granulosa as well as in the cumulus oophorus. it will be interesting to mention here that highly sudanophilic lipid droplets of large size described in buffalo primordial follicles continue to be seen in the cuboidal follicular epithelium of primary follicles (Guraya 1997). These lipid masses consisting of phospholipids disappear during further growth of the follicle, suggesting that their accumulation and disappearance are apparently related to some developmental processes of the follicle growth in buffaloes. These lipid droplets of exceptionally large size form a unique feature of buffalo primordial and primary follicles as no such lipid masses are observed in the follicular epithelium of corresponding follicles of other mammalian species (Guraya 1985, 1997).

Extensive electron microscopic studies on GCs of different mammalian species demonstrate the presence of various organelles such as a Golgi complex, granular ER in the form of tubules, small vesicles and cisternae, many free ribosomes, numerous clusters of polyribosomes, rod-shaped or spherical mitochondria with simple lamellar cristae, lysosomes, lipid droplets and some glycogen granules (Guraya 1985, 1997). Ultrastructural changes occur in the mitochondria and endoplasmic reticulum during the preovulatory period.-:, which are indicative of the luteinization process in GCs of preovulatory follicles (Guraya 1985,1997, Gore-Langton & Armstrong 1988). In recent years, the use of quantitative cytochemistry and electron microscopy has revealed regional specializations of granulosa as distinct differences occur ,between the peripheral portion of the membrana granulosa, the periantral granulosa cells and the cumulus oophorus in regard to organelles (mitochondria and ER),lipid droplets, enzyme systems, lysosomal permeability, carbohydrates, hormone receptors for FSH, hCG and prolactin, and steroidogenesis (Guraya1985, 1997, Gore-Langton & Armstrong 1988, Greenwald & Terranova 1988, Lipner 1988, Greenwald & Roy 1994). Clear differences between mural and cumulus cells in both their structure, functions and fate are reported. Regulatory factors for these regional specializations of granulosa cells as well as their biological significance remain to be determined at the subcellular and molecular levels. However, just before ovulation gonadotrophins stimulate cumulus cells to produce and secrete hyaluronic acid that disperses the cumulus cells and embeds them in a mucus-like matrix (Guraya 1985, 1997); the oocytes also appear to produce a paracrine factor essential for cumulus expansion (Eppig 1994). The process called cumulus expansion or mucification in mammals results in the ovulation of an expanded cumulus oophorus. But mural granulosa cells generally do not undergo expansion, but instead become luteal cells. Cumulus and mural cells also differ in regard to the distribution of their LH receptors, and the mRNA coding for LH receptors, steroidogenic capabilities and mRNA coding for cholesterol side-chain cleavage, cytochrome P 450, IGF-I mRNA, mullerian inhibiting substance, expression of Cdc 25 genes and various other uncharacterized chemical substances (see references in Eppig 1994, Wu & Walgemutch 1995, Guraya 1997). Singh et al. (1993) after studying the effects of various combinations of EGF, TGF-b, FSH, LH, androsterone and oestradiol on oocyte cumulus cell complexes in vitro, have suggested that these hormones may have differing roles in cumulus expansion and meiotic maturation and that their interactions may be part of a complex system regulating the growth and maturation of oocytes during follicular development *in vivo*.

Various ultrastructural and histochemical characteristics of granulosa cells in mammals suggest that these are actively involved in the synthesis of lipids, carbohydrates and proteins as already stated. Regulation of their synthesis is relatively less understood. But during the recent years, detailed in vivo and in vitro biochemical analyses have been made of the metabolism of granulosa cells and of the properties of follicular fluid (Guraya 1985, 1997, Gore-Langton & Armstrong 1988) and such studies continue unchecked. This increase in the number of such studies is due to the fact that it is easy to obtain granulosa cells in relatively pure form and in large numbers. These can also be easily collected at known stages of maturation after the stimulation of follicle growth and ovulation with various hormones. Therefore, these days granulosa cells of mammalian ovary form a strong candidate for the most extensively studied endocrinological cell in the body and very useful information has been obtained in regard to synthesis of carbohydrate - proteins and steroid hormones by these cells, as well as mechanism of hormone action on them in relation to synthesis of various substances (Guraya 1985, 1998a, Gore-Langton & Armstrong 1988, Yoshimura 1995). Actually data on the factors regulating the metabolic activity of granulosa cells in mammals continue to spill out in the scientific literature as will be discussed here. In the last decade, extensive in vitro biochemical studies have revealed the nature of steroidogenesis by granulosa cells and their ability to convert pregenolone to progesterone and androgens to oestrogens (Gore-Langton & Armstrong 1988, Guraya 1997). In the ovary, the androgens are believed to be supplied by the theca of maturing follicle, which are then converted

into oestrogens by the granulosa. However. very little or no attempt has been made to make in vitro biochemical studies on FCs of invertebrates and nonmammalian vertebrates (Adiyodi & Adiyodi 1983, Guraya 1978, 1986, 1996). Such comparative in vitro biochemical studies form promising area for future investigations. The results of such studies will be very useful to understand the evolutionary aspects of synthesis of different molecules in FCs of different groups of animals and the molecular regulatory mechanisms involved in their synthesis.

Histochemical and biochemical studies have demonstrated the presence of various enzyme systems especially those related to steroid biosynthesis in the follicle (granulosa) cells of some invertebrates (Adiyodi & Adiyodi 1983) nonmammalian vertebrates (Guraya 1978, 1986, 1989, 1996, Browder 1985) and mammals (Guraya 1985, 1997). Relatively invertebrates have received less attention for the study of enzyme systems in FCs. However, Teshima and Kanazawa (1971) have observed that the ovaries of the crab Portumus trituberculatus posses the enzymes, steroid 17α-hydroxylase, steroid 21-hydroxylase and steroid C17-C20 lyase involved in the conversion of progesterone to 17α-hydroxyprogesterone, testosterone and deoxycorticosterone. The echinoderm ovaries also contain enzymes essential for steroid biosynthesis (Schaemakers et al. 1976, Schaemakers & Voogt 1980). The cellular source of these enzymes in the crab and echinoderm ovaries remains to be determined with histochemical techniques.

The presence of various hydroxy steroid dehydrogenases such as Δ5-3β hydroxy steroid dehydrogenases (3β-HSDH), 3a, 17α and 17β-HSDH has been demonstrated histochemically in the follicular epithelium of some species of cyclostomes, and cartilaginous and bony fishes, amphibians, reptiles and birds (Guraya 1978, 1986, 1989, 1996). In these studies, the localization of 3β-HSDH has been investigated relatively in more species of non-mammalian vertebrates, in comparison to other steroidogenic enzymes. Alkaline phosphatase and glucose-6-phosphate dehydrogenase (G6»PDH) have been studied for some non-mammalian vertebrate species. Further correlative histochemical and biochemical studies are required to be made of various enzyme systems in the follicular epithelium of different groups of non-mammalian vertebrates. The results of such studies will be very useful for the better understanding of metabolic differences between their FCs.

The more details of distribution and functional significance of various enzyme systems including phosphatases, G6DH, hydroxy steroid dehydrogenases etc. are available for the mammalian granulosa cells (Guraya 1985, 1997, 1998). Alterations in some enzyme activities occur in the granulosa of developing follicles. But their precise significance in relation to the functions (e.g. mitosis, formation of hormone receptors and maturation of their biosynthetic potential and steroidogenesis) of granulosa cells remain to be determined more precisely (Gore-Langton & Armstrong 1988). The changes in enzyme systems in relation to steroid biosynthesis in the granulosa cells of developing and maturing follicles of mammalian ovary form the subject of some reviews (Gore-Langton & Armstrong 1988, Hinshelwood et al. 1994, Guraya 1997). Regulatory mechanism involved in the expression of genes encoding steroidogenic enzymes in the granulosa cells during follicular development, maturation and ovulation in mammals are also being investigated (Hinshelwood et al. 1994, Erickson et al. 1994. Such studies need to be undertaken for FCs of invertebrates and non-mammalian vertebrates. Analysis of the transcription factors involved in regulating the expression of these genes will lead to discovery of the factors involved in that most mysterous of events, the dramatic process of follicle cell differentiation and development that occurs with incredible regularity during follicular growth and maturation in every female animal of reproductive age. Certain steroidogenic enzymes are present in preantral follicles of mammals but they are not maximally expressed. 3β-HSDH activity is strongly expressed in rat granulosa cells of preovulatory follicles but is only weakly expressed in earlier antral stages as demonstrated with histochemical techniques (Hoyer & Anderson 1977). This is in agreement with the biochemical evidence that induction of 3b-HSDH activity needs FSH stimulation of the granulosa cells (Dorrington & Armstrong 1979,

Hinshelwood et al. 1994). Michael et al. (1996) have investigated the follicular variation in ovarian 11b-HSDH activity which varies between follicles of the human ovary. The co-culture of granulosa-lutein cells with low enzyme activity can suppress the ovarian 11b-HSDH in cells from different follicles, suggesting the potential for paracrine inhibition of ovarian 11b-HSDH activity in human granulosa-lutein cells.

3. Cytoskeletal Elements

The cytoplasmic filaments and microtubules that form cytoskeleton vary greatly in amount and distribution in granulosa cells of different portions of the mammalian follicules (Guraya 1985, 1997). Microfilaments consisting of actin and myosin, occur at cytoplasmic locations where a contraction might move portions of the granulosa cells or partly change their shape during follicle growth, suggesting their role in the morphogenetic processes of the granulosa (Guraya 1997). However, Carnegie et al. (1988) suggested that microfilaments and microtubules are important in granulosa cell steroidogenesis, which are believed to function to facilitate the movements of cholesterol from lipid droplets to mitochondria possibly through an effect on subcellular organelles distribution by altering the morphology of the granulosa cells; the latter show morphological and chemical heterogeneity in different regions of granulosa as already discussed here (Guraya 1985, 1997). Vogl et al. (1993) have also proposed the involvement of mechanoenzymes (kinesin and cytoplasmic dynein) in the movement of endoplasmic reticulum in the mammalian Sertoli cells but this suggestion is required to be extended and confirmed with experimental studies not only for Sertoli cells but also for granulosa cells.

Very little attention has been paid to the study of cytoskeletal elements in FCs of non-mammalian vertebrates (Guraya 1978, 1986, 1989, 1996) and invertebrates (Adiyodi & Adiyodi 1983). However, Anderson (1967) has reported the presence of fine filaments and microtubules in FCs of teleost. Amphibian FCs show microfilaments and microtubules especially in the follicle cell processes extending through the zona pellucida (Guraya 1978). Bundles of microfilaments are reported in the cytoplasm of pyriform cells of follicular epithelium of lizards (Guraya 1978). Conspicuous bundles of microtubules are present in the intercellular bridges which are formed by the pyriform FCs and the oocyte of lizards and snakes. They appear to consitute an intercellular transport system. Fine filaments and microtubules are also reported in FCs of some avian species (Guraya 1978). Microtubules are present in FCs of the cephalopod A. subulata (Bottke 1974) and Opisthobranch mollusc, Runcina (Kress 1986). Observations on the starfish Asterina pectinifera follicles with phase contrast and differential interference microscopes and the electron microscope have shown that each follicle cell possesses a single flagellum (Schroeder et al. 1979). These flagellated FCs surround all the primary oocytes of various stages. The functional significance of their flagellum remains to be determined.

4. Plasma Membrana Specializations and Junctional Complexes

The cellular and molecular aspects of development and differentiation of plasma membrane specializations, junctional complexes and cytoskeletal elements of granulosa in the maturing follicles have been investigated for various mammalian species (Espey 1978, Guraya 1985, 1997, Greenwald & Terranova 1988, Lipner 1988, Greenwald & Roy 1994). These studies indicate that granulosa cells during follicle growth undergo a series of complex cellular and molecular changes in plasma membrane structure and function as judged by the formation of surface projections (microvilli, blebs with bundles of microfilaments, granulosa cell processes, cilia, and a variety of junctional complexes (devices of cellular association). In the invertgebrates (Adiyodi & Adiyodi 1983, Khan & Salaunddin 1983, Kress 1986), FCs form processes which extend through the vitelline membrane or chorion or zona pellucida and have been extensively investigated with electron microscope. But junctional complexes between their FCs have received relatively less attention although desmosome attachments are formed between the plasma membranes of follicle cell processes' and oocyte(Kishimoto 1986). In the cephalopod, the

laterally neighbouring FCs are closely connected with each other by desmosomes of the zonula adherens types (Bottke 1974). Yoshizaki and Patino (1995) have suggested that the vertebrate ovarian follicle contains homocellular (follicle cell - follicle cell) and heterocellular (follicle cell - oocyte) gap junctions which consist of connexin proteins. For the first time these authors have reported the presence of at least two hormone responsive connexin gene transcripts (mRNAs) in *Xenopus*. The poor development of junctional complexes in the follicular epithelium of non-mammalian vertebrates and invertebrates appears to be due to the formation of spaces between FCs as these intercellular spaces are required to facilitate the transport of exogenous yolk material though the follicular epithelium for its incorporation into the ooplasm. Ultrastructural studies have demonstrated that certain osmiophilic, membranous structures of specialized nature project from FCs into the avian oocyte, which have been termed premitochondria or unique organelles, transosomes, lining bodies, macrobodies, vesicular bodies and terminal membranes. Their basic structure appears to be similar (Guraya 1978).

Gap junctions in the mammalian granulosa involve in cell-to-cell attachment and communication and in the transport of hormones and nutrients within and through the avascularized follicular epithelium, as well as in the hormone receptor relationships (Guraya 1997); internalization of certain gap junctions by the granulosa cells represents a mechanism for clearing the surface of used recepotrs provided there is a turnover and reformation of gap junctions. In addition, the junctions formed between adjacent granulosa cells provide cell-to-cell cohesion and contribute to the structural integrity of the granulosa layer during formation of antral cavity. The lack of a vascular system within the granulosa of ovarian follicles appears to be replaced by the membrane specialisations of the granulosa cell itself thus becoming a communicating cell system, or a functional syncytium (Eppig 1994). Actually, granulosa cells are electrically and most likely metabolically coupled by large gap junctions (Lipner 1988, Eppig 1994, Guraya 1997). The micropapillae appearing as hemispherical, lens-shaped thickenings of oocyte membrane located mainly on the microvilli are believed to enhance the adhesion of the oocyte microvilli to the follicle cell processes which traverse the zona pellucida. The distal bulbous extremities of FCs terminate at the oocyte surface by tight junction. Many of the junctions between the oocyte and granulosa cells are identified as gap junctions which are extremely small in size as compared to those gap junctions formed between granulosa cells.

Gap junctions and paracrine factors constitute the the modes of intercellular communication between oocytes and FCs. Gap junction mediated cell-to-cell communication links FCs both to other FCs and to the oocyte. These membrane specializations provide the pathway for metabolic cooperation and, possibly, signaling by low molecular weight compounds such as cyclic adenosine monophosphate (cAMP). Gap junctions are known to be highly permeable to small molecules and facilitate electrical coupling between cells (Edelman & Crossin 1991, Green 1992, Guraya 1995). The gap junctions between the oocyte and surrounding FCs may provide the physical means for physiological integration between these two cell types during follicular growth, meiotic arrest, and its resumption. Oocyte growth is known to require gap junctional communication with the granulosa cells as the mammalian oocyte fails to grow in culture when this communication is disrupted, but growth continues at almost normal rates when gap junctional communication is maintained (Eppig 1994 for review). The gap junctions facilitate the transfer of nutrients and metabolites to the oocyte and possibly also introduce low molecular weight signals required for normal oocyte growth between their FCs and oocytes. Such studies are required to be made for invertebrates and non-mammalian vertebrates as our knowledge is very meagre about the localization and structural-functional aspects of gap junctions. In addition to providing a pathway for metabolic cooperation between the oocyte and its surrounding somatic cells during follicular development, the cumulus-oocyte gap junctions transmit an as yet unidentified signal that triggers meiotic maturation of mouse oocyte (Fagbohun & Downs 1991). Gap junctions are known to be

aggregations of intercellular membrane channels that provide a pathway for the passage of ions and small molecules netweem c;pse;u a[[psed ce;s (Bennett et al. 1991). Therefore, coexpression of gap junction proteins in the memmalian cummulus oocyte complex is of great interest to determine the molecular mechanisms involved in the transport processes (Valdimarsson et al. 1993).

Follicle cells and oocytes especially in mammals are also known to produce signaling molecules that are too large for transmission by gap junctions (Eppig 1994, Guraya 1997). The initial step in the transmission of these paracrine signals, therefore, is secretion into the extra cellular space followed by binding to target cells. Although both gap junctions and paracrine factors are potentially bi-directional, gap junctions mediate most of the follicle cell-to-oocyte communication while paracrine factors are believed to mediate oocyte-to-follicle cell communication which is required to be determined from comparative point of view for different groups of animals.

Various experiments demonstrate the absence of a blood-follicle barrier. Besides the intercellular junctional devices, there is also present an intercellular matrix consisting of complex carbohydrates with 1, 2-glycolytic acidic groups, D-mannacyl and αD-glucoacyl residues as revealed with cytochemical techniques (Guraya 1985, 1997, Lipner 1988). Carbohydrates contain hyaluronic acid, sulfate glycosaminoglycans and isomeric chondroitin sulfates. The carbohydrates may be involved in the adhesion of granulosa cells as well as in the reception and transmission of signals for the regulation of granulosa cell multiplication and functions. Such cytochemical studies remain to be extended to FCs of invertebrates and nonmammalian vertebrates as no or very little work has been carried out previously (Adiyodi & Adiyodi 1983, Guraya 1978, 1986, 1989, 1996). However, in recent years our knowledge has greatly increased about the biosynthesis, transport and functions of glycoconjugates due to the use of modern molecular probes (Wieland & Reutter 1994). Such studies need to be carried out on FCs of different groups of animals. Potassium channels and currents are of great interest for *Xenopus* oocytes and FCs (Greenfield et al. 1990a,b, Wibrand et al. 1992, Chiang et al. 1997).

III. Regulation of Follicle (Granulosa) Cells Multiplication

Very little or no attempt has been made previously to study the regulation of multiplication of FCs in the ovaries of different phyla of invertebrates and non-mammalian classes. But in mammals, the most potent stimulators of granulosa cell replication and growth evaluated recently in vitro experiments include a variety of mitogenic and growth factors such as low molecular weight peptides, epidermal growth factor (EGF), fibroblast growth factor (PFG), platelet-derived growth factor (PDGF), insulin or insulin-like peptides or growth factors (IGF), transferrins, steroids, thyroxine, and follicular fluids (FF) (Guraya 1985, 1997, Tsafriri 1988, Tonett & di-Zerega 1989, Ling et al. 1993, Tsafriri & Adashi 1994, Erickson et al. 1994, Yoshimura 1995) the FF contains somatomedins and somatomedin binding proteins. The concept of classical endocrine control of granulosa cell multiplication and functions has now been extended to a more complex regulatory systm including paracrine and autocrine modulating mechanisms which involve the role of locally produced polypeptides or growth factors. Among these factors, IGF and its binding proteins (IGFBPs) have been shown to play an important role in the control of folliculogenesis. Growth hormone (GH) is known to amplify the gonadotrophin actions in the process of follicular development, atleast in part, stimulating IGF-I production; IGF-I as well as IGFBPs are produced in the granulosa cells. IGF-I acts synergistically with gonadotrophins in the stimulation of a variety of granulosa cell functions; the granulosa cells possess specific IGF type I receptors; the expression of genes encoding these receptors is investigated. The oocytes are also believed to produce some factors that affect the proliferation of granulosa cells in mammals. Such factors need to be characterized.

Follicular theca components secrete substances that also enhances granulosa cell multiplication in vitro (Tsafriri 1988). Such potent stimulators (e.g. growth factors) of follicle cell replication and growth

are required to be investigated for various groups of invertebrates and nonmammalian vertebrates which show proliferation of FCs to constitute the follicular epithelium which shows a great diversity in the morphological organization as already discussed in Section IIA. The results of such comparative studies will be very rewarding for the better understanding of evolution, chemistry and functions of various graowth factors or stimulators identified for the multiplication and growth of mammalian granulosa cells.

The EGF is a polypeptide hormone or parahormone containing 53 amino acids. It is now known to be involved in the regulation of various types of gonadal cells, inhibiting the production of steroids by granulosa cells (Guraya 1986, 1997, Gore-Langton & Armstrong 1988, Tonetta & Dierega 1989, Driancourt et al. 1993, Adashi & Leung 1993, Yoshimura 1995). Its effect on cell multiplication, protein synthesis and the production of progesterone and inhibin by mammalian granulosa cells has now been studied (Tsafriri 1988, Yoshimura 1995, Guraya 1997). Thecal cells lying outer to the granulosa of mammalian ovary are also believed to produce factors which can promote the proliferation of granulosa cells, another aspect of the concept of the intrafollicular regulataors helping the dominant follicle to grow while the other undergo atresia (Greenwald & Terranova 1988, Greenwald & Roy 1994, Erickson et al. 1994, Guraya 1997). Soluble factors from thecal cells can exert a local regulation of the multiplication of granulosa cells, and this has been determined using the synthesis of DNA in granulosa cells as a marker (Bendell et al. 1988). The thecal cells invoke responses in granulosa cells greater than that obtained by EGF and cell numbers increase considerably in the presence of thecal cell secretion. This study suggests the possibility of involvement of another factor in the paracrine regulation of granulosa cell growth, thus providing the dominant follicle(s) with autonomy over other follicles in the cohort in the mammalian ovary (Guraya 1997). Such forms of paracrine regulation must exist in the ovary, and also act in the opposite direction, because it is well known that the thecal layers diffeentiate strongly when growing follicles attain a specific size and complexity. It will be surprising if the thecal and granulosa components of the follicle do not interact together in such ways for causing its growth and maturation. Some somatic cells constituting theca are also reported for the growing follicles in different groups of nonmammalian vertebrates (Guraya 1978, 1986, 1989, 1996). But interactions between their FCs and thecal cells remain to be investigated at the cellular and molecular level to determine the regulatory mechanisms involved in their proliferation, growth and diffeentiation. The effects of IGF-I, TGFα and LH have been shown on the probiferation of chicken granulosa cells and production of progesterone in chicken granulosa cells (Onagbesan & Peddie 1995). Some somatic cells may also surround the follicular epithelium in some invertebrates (Adiyodi & Adiyodi 1983, Kress 1986). Their paracrine functions in relation to the multiplication of FCs also remain to be investigated by *in vitro* experiments.

There are some controversial reports about the nature of the paracrine system in the mammalian follicles (Adashi & Leung 1993, Ling et al. 1993, Erickson et al. 1994, Yoshimura 1995). IGF-I and EGF are believed by one group of workers to show no effect on DNA synthesis in rat granulosa cells obtained from follicles of oestrogen-treated immature rats (Dorrington et al. 1980), whereas another group observed that EGF alone consistently stimulates the synthesis of DNA in porcine granulosa cells (May et al. 1988). Similarly IGFb has a significant effect on cell division, which can be augmented by FSH, whereas other investigations suggest that neither the somatomedians nor IGF,B can increase DNA synthesis unless it is already stimulated by EGF, and a combination of all three appear to be most potent (May et al. 1988). The interactions between FSH, EGF, IGGF-I, FGF and TGFb appear to be important regulatory factors in the growth and multiplication of granulosa cells and their responses to TGFb may depend on the ability to affect the production of the extracellular matrix, including the formation of fibronectin and collagen (Dorrington et al. 1988, Tonetta & diZerega 1989, Ling et al. 1993 Erickson et al. 1994, Yoshimura 1995, Guraya 1997). The well-known homology between TGFb, inhibin,

Mullerian duct-inhibitory factor and activin (Guraya 1997, 1998) suggests that this whole family of compounds plays major role in the regulation of reproductive processes. The nature of factors regulating follicle cell multiplication still remains to be determined at the molecular levels not only in the ovaries of more species of mammals but also of diverse groups of invertebrates and nonmammalian vertebrates. Yoshimura (1995) has given an account of the current status of regulatory system and physiological significance of some local factors in the ovary during its development and maturation as also discussed by Guraya (1997). The results of such studies continue to be very rewarding for the elucidation of molecular aspects of regulation of follicle growth and maturation in in vitro experiments on the ovaries. Such studies may help us to increase the follicular responsiveness to various chemical agents including hormones during the non-breeding seasons in animals. FSH but not LH alone stimulates the differentiation of granulosa cells and promotes antrum formation in mammals (Guraya 1985, 1997). The study of biology and expression of receptors for gonadotrophins, steroids and the regulatory mechanisms involved will be very rewarding to determine their precise roles in granulosa cell multiplication (Hsueh & Schomberg 1993, Park-Sarge & Mayo 1994). Antrum formation in the mammalian granulosa appears to reflect changes in FSH dependent secretory function of the granulosa cells as well as influx of interestiatial fluid. Increased follicular vascularisation and steroid effects on follicular vascular premeability may also be involved in antral fluid accumulation (McNatty 1978, Tsafriri & Adashi 1994, Adashi & Leung 1993). Follicular fluid is present in many eutherian mammalian species; some exceptions are moon rats (Echinosorex gymnurus) and Madagascar hedgehogs (Setifer semtosus) (Mossman & Duke 1973).

The details of origin, types and physicochemical characteristics of follicular fluid in mammals, which are described in various previous reviews (Guraya 1985, Lipner 1988, Driancourt et al. 1993, Adashi & Leung 1993) will be omitted here. Follicular fluid contains several proteins, amino acids, sugars, enzymes, mucopolysaccharides, gonadotrophins, steroids, prostaglandins etc., which are identified for different species of mammals and their possible functions are also discussed (McNatty 1978). The electrophoretic pattern of follicular fluid is essentially similar to that of plasma. The various studies indicate a structural homology between the follicular fluid and blood plasma proteins (Schomberg 1993, Park-Sarge & Mayo 1994).

IV. Functions

The precise functions of the follicle or granulosa cells in relation to regulation of oogenesis still remain to be determined more precisely at the cellular and molecular levels. This is due to the fact that in various phyla of lower invertebrates, FCs are either absent or poorly developed but oocyte growth and maturation are still completed. With the evolution of higher invertebrates and vertebrates, the ovary attained a very complex morphological organization by developing FCs and various other somatic cells; meanwhile blood vascularity and innervation also developed in the ovary. Various recent investigations have suggested that wherever FCs occur in association with the growing and maturing oocyte, they perform several functions. These functions include (i) provision of nutrients and informational molecules to the, growing oocytes, (ii) synthesis of yolk, (iii) phagocytosis, (iv) synthesis of steroid hormones, (v), synthesis and secretion of regulatory proteins and glycoproteins, (vi) formation of zona pellucida or chorion, (vii) regulation of meiosis or maturation of oocyte, (viii) the determination of animal-vegelal and bilateral symmetries of the egg, and (ix) ovulation.

A. Provision of Nutrient Substances and Informational Molecules

Ultrastructural, histochemical and autoradiographic studies have revealed that FCs surrounding the growing oocytes of invertebrates and vertebrates including mammals become metabolically very active in the production of proteins, ribonucleoproteins, carbohydrates and lipids (see Section *IIB*). The amounts and nature of such substances synthesized by the follicular epithelial cells is variable among different groups of animals and even with the species,

apparently depending on the requirements of nutrients and informational molecules by growing oocyte. Most of the proteins, ribonucleoproteins, lipids, carbohydrates, ions, etc., are ultimately delivered to the growing oocytes where they are either used for growth processes or stored for use during early embryogenesis (Guraya 1978, 1985, 1986, 1989, 1997, 1998, Adiyodi & Adiyodi 1983, Browder 1988, Callebaut 1991). Substances may reach the oocyte surface either through the intercellular spaces or via the FCs. Whatever route is followed, the exact mechanisms of their transference through the follicular epithelium as well as from the follicular epithelium into the oocyte are still not clear. The processes of membrane diffusion, active transport, pinocytosis, phagocytosis etc. have been suggested to play important roles in their transport into the oocytes, as a direct continuity does not occur between the cytoplasm of oocyte and FCs in most cases. Pinocytotic activity at the oocyte surface appears to be of common occurrence. Callebaut (1991) has produced ultrastructural evidence for the delivery of lipids to the ooplasm by the granulosa or FC extensions through the meshwork of avian zona pellucida as was also suggested for the transport of some organelles and lipids by the granulosa cell processes in mammals (Guraya 1985). However, the oocyte growth in mammals is found to require gap junctional communication with the granulosa cells (Guraya 1985, 1997, 1998, Browder 1985, Eppig 1994; Eppig et al.1996). This requirement is supported by the fact that oocytes do not grow in culture when this communication is disrupted, but oocyte growth continues at almost normal rates when gap junctional communication is maintained. The gap junctions function to transfer nutrients and metabolites to the oocyte and possibly also to introduce low molecular weight signals needed for normal oocyte development. Such studies remain to be carried out on other animal species to determine the development and function of qap junctions in the transfer of nutrients and low molecular weight signals. It will be interesting to mention here that oocyte growth and development in amphibians can occur autonomously in vitro, in the absence of FCs even though Xenopus oocytes and companion FCs are conjoined by functional gap junctions (Eppig et al. 1996).

The direct communication between the oocyte and FCs (especially large pyriform FCs) through intercellular bridges has been reported in the previtellogenic and early vitellogenic oocytes of lizards and snakes (Guraya 1978, 1989). These intercellular bridges disappear in the large, growing vitellogenic follicles, and meanwhile the pyriform FCs degenerate and finally disappear. The pyriform cells of follicular epithelium in lizards and snakes are comparable to nurse cells in the ovaries of insects (Norrevang 1968) and various other invertebrates. (Adiyodi & Adiyodi 1983, Section IIA), which are either engulfed by the oocyte or they contribute their organelles to the growing oocytes in addition to ribonucleoproteins, lipids, carbohydrates etc.

In the growing previtellogenic follicles of bird ovaries, highly specialized organelles called 'lining bodies' are contributed by the follicular epithelium to the ooplasm, where they are believed to play some important role in early yolk formation (Guraya 1978, 1989). They are associated with some RNA component derived from the FCs. The precise functional significance of these lining bodies, which are not found in the follicles of other vertebrates (turtles being an exception) and invertebrates remains to be determined at the molecular level with modern biological probes.

FCs as such are sometimes taken into the ooplasm of growing oocytes where they degenerate and disappear, e.g. in some cyclostomes, birds, reptiles (Guraya 1978, 1989). In some groups of invertebrates the nurse cells either fuse with oocyte or taken into it (Adiyodi & Adiyodi 1983; Section IIA). In lizards and snakes, many follicle cell nuclei are taken into the ooplasm of previtellogenic oocytes where they disintegrate and disappear. FCs as such or their nuclei appear to be taken into the ooplasm by phagocytosis. Depending upon the species, FCs or nurse cells or interstitial cells or abortive germ cells (oocytes) etc. are also engulfed by the growing oocytes of sponges, coelenterates, sipunculates, lamprey etc. (see Section IIIA). The regulatory mechanisms involved in their phagocytosis remain to

be determined. In other words, what are the specific roles of FCs themselves and growing oocyte in this process of phagocytosis of cells or nuclei as such. 'Finally these phagocytosed FCs or their nuclei disintegrate probably by the activity of some lysosomal or hydrolytic enzymes which remain to be investigated. But the engulfed somatic or FCs or abortive germ cells apparently contribute to the storage of reserve informational ooplasmic macromolecules such as proteins, RNAs and DNA and organelles which will be required for oocyte growth and early embryogenesis. But further studies using molecular probes are required to follow the specific roles of these maternal informational molecules contributed by FCs/nuclei during oogenesis. However, their migration into the ooplasm closely corresponds to the rapid growth of previtellogenic oocytes.

In the growing previtellogenic and early vitellogenic follicles of bird ovaries several FCs degenerate in situ, and their products including DNA, RNA, proteins, etc. are apparently transported to the oocyte for growth processes of oocyte and early embryogenesis. Similarly, some FCs also degenerate *in situ* in the follicular epithelium of turtle ovaries (Guraya, 1978, 1989). Various RNAs, DNA and proteins derived from these degenerated FCs must also be transported to the growing oocyte. But this suggestion also needs to be extended and confirmed with autoradiographic and immunocytochemical techniques.

In tunicates, many FCs as such migrate into the ooplasm of early oocytes (Guraya 1968b, 1972b, 1978) where they do not degenerate but give rise to test cells which form a unique feature of their oocytes. The extensive development of cytoplasmic organelles such as large Golgi complex, granular endoplasmic reticulum, mitochondria etc. as well as of secretary granules in the cytoplasm of test cells in tunicates suggests that they possibly elaborate carbohydrate-protein complexes and pigments which may be required for oocyte growth, yolk deposition, and possibly some other roles during fertilization when they are finally released into the perivitelline space (Norrevang 1968, Guraya 1978). But the precise functions of test cells remain to be determined.

B. Yolk Synthesis

During vitellogenesis, the oocytes of some invertebrates e.g. arthropods, molluscs, echinoderms (King 1972, Adiyodi & Adiyodi 1983, Browder 1995) and nonmammalian vertebrates e.g. cyclostomes, fishes, amphibians, reptiles and birds (Guraya 1978, 1986, 1989, Browder 1985) increase markedly in volume. However, the exact role of follicular epithelial cells in synthesis of yolk materials is still not clear as very divergent views are expressed for invertebrates (King 1972, Adiyodi & Adiyodi 1983, Clifford et al. 1994, Bownes 1994, Zimowska et al. 1995) and vertebrates (Wallace 1978, Guraya 1986, 1989). But it is generally believed now that FCs either don't play a direct role or play a minor role in the synthesis of vitellogenin for the oocytes. Some yolk peoteins are synthesized by FCs in Drosophila and transported directly to the oocyte surface as revealed by molecular probes (Bownes 1994). The substances required for formation of yolk bodies are mainly produced outside the oocyte in other distant organs e.g. intestine in nematodes, fat body in arthropods, liver in cephalopods and vertebrates and are transported through the circulatory system to the ovary, where they are incorporated into the growing oocytes as storage materials (Biggers & Schuetz 1972, Browder 1985, Soltani-Mazouni & Soltani 1995, Morales et al. 1996). The oocytes receptor for low density lipoprotein and vitellogenin has been characterized for quail (Elkin et al. 1995).

Oestrogen in nonmammalian, vertebrates induces the hepatic synthesis of yolk precursor (Guraya 1978, 1986, 1989, 1996, Browder 1985). But the precise nature of factors responsible for directing the transport of vitellogenin towards the oocyte remain to be determined. However, the plasma membranes of the oocyte itself undergoes morphological changes by developing numerous large, almost filamentous microvilli, that greatly increase the surface area of the oocyte, especially during yolk deposition. Deep crypts or invaginations between microvilli and a large number of endocytotic (micropinocytotic), pits, add additional area for incorporation of vitellogenin into the oocyte (Wallace 1978, Guraya 1986, 1989, 1996, Adiyodi & Adiyodi 1983, Browder 1985, Bownes 1994). The role of microvilli and endocytotic pits in the uptake of

yolk materials is further supported by the fact that they become very few in postvitellogenic oocytes. The morphological and molecular characteristics of the cell surface are known to change during oocyte growth and differentiation. But precise nature of such molecular changes are required to be determined in future investigations as the secreted yolk proteins are known to enter the oocyte by receptor-mediated endocytosis. During yolk deposition, the yolk proteins synthesized outside the oocyte are transported through the intercellular spaces of FCs, which are simultaneously widened to permit the rapid passage of substances through them. But the precise regulatory mechanisms or force involved in directing the transfer of yolk materials through the intercellular spaces of the follicular epithelium to the oocyte surface remain to be determined. In this regard in vitro experiments may be helpful.

C. Steroid Hormone Synthesis

With growth of oocytes, the cells of follicular epithelium in the ovary of nonmammalian vertebrates develop enzyme systems especially steroid dehydrogenases (Section IIB) which are believed to be indicative of steroid hormone synthesis (Guraya 1978, 1979, 1986, 1989, 1996, Saidapur 1989a,b). But organelles such as mitochondria with tubular cristae, agranular endoplasmic reticulum etc., which are specific to steroid-secreting cells (Guraya 1976a,b, 1979) do not show any appreciable development in the follicle or granulosa cells of maturing follicles. The significance of this discrepancy remains to be determined. But these results are in agreement with the morphological (including ultrastructural) and histochemical observations on the follicle or granulosa cells of maturing follicles in the mammalian ovary, which have also indicated that the granulosa cells are indeed metabolically active in protein rather than steroid hormone synthesis in vivo (Guraya 1985, 1997). FCs of maturing follicles in vertebrates are unlikely to synthesize steroids in vivo because they lack the NADPH-producing enzymes typical of steroid-secreting cells (Guraya 1976a, 1978, 1985, 1986, 1996, 1997, 1998). At this stage of our knowledge, no definite statement can be made in regard to the secretion of steroid hormones by FCs of maturing follicles in vivo. The presence of enzymes related to steroid biosynthesis (Section II B) may simply indicate that the maturing FCs develop the potential of steroid hormone synthesis which possibly occurs in them during the pre- and postovulatory periods (Guraya 1976a, 1985, 1986, 1989, 1993a, 1996, 1997). The absence of organelles specific to steroidogenic tissues during the maturation of follicle wall also suggests the strong possibility that perhaps different pathways of steroid hormone synthesis may be functioning in the follicular epithelium, which are required to be investigated in future comparative in vivo and *in vitro* biochemical studies on FCs of vertebrates including mammals. Recently, several studies have been made of the effects of gonadotrophins, growth factors steroid hormones and other chemicals on steroid-hormone synthesis receptors and other metabolic and synthetic activities of chicken GCs *in vitro* (Asem & Conkright 1995, Conkright & Asem 1995, Yoshimura et al. 1995, Maestro et al. 1995, Soboloff et al. 1995, Dean et al. 1995a, b, Caicedo et al. 1997, Swada et al. 1997, Chiang et al. 1997).

Divergent views are expressed about the site(s) of synthesis of 17β–oestradiol in the Graafian follicles of mammals (Guraya 1985, 1997). Some workers believe that it is produced by the theca interna whereas others claim that theca interna produces the androgens which are transported to the granulosa cells where they are aromatized into 17β-oestradiol (Guraya 1985, 1997); the latter view is supported by various in vitro biochemical experiments which have been carried out on the mammalian granulosa cells during the recent years (Guraya 1997). Lederer et al. (1995) have demonstrated the variable proliferative and steroidogenic capabilities of rat granulosa cells of different sizes. It is proposed that changes in the population of granulosa cells could account for both the slower growth rate of large antral follicles compared with small antral follicles and the inverse relationship between follicular oestradiol secretion and DNA synthesis of granulosa cells. The principal steroid hormones formed by the mammalian granulosa cells *in vitro* are progesterone and 17α-hydroxy-progesterone. The lack of adequate blood-borne nutrients and oxygen supply to FCs in vivo is believed

to be limiting factor for steroid biosynthesis, but nutrients and oxygen become available after ovulation when FCs are luteinized and start secreting progesterone (Guraya 1985, 1997) as also described for the postovulatory follicles of nonmammalian vertebrates (Guraya 1976a, 1993a, Saidapur 1982).

As compared to nonmammalian vertebrates and mammals, not much attention has been paid to the study of the role of FCs in the synthesis of steroid hormones in the ovaries of invertebrates (Adiyodi & Adiyodi 1983, Adiyodi et al. 1997). But it is believed that if FCs are involved in hormone production then it is unlikely that hormone is steroid because the cells do not possess the ultrastructural characteristics specific to steroid-producing cells (extensive smooth endoplasmic reticulum and mitochondria with tubular cristae, as also stated for FCs of vertebrates. On the other hand they have the features of protein-synthesizing cells (see Section IIB) and therefore, might produce a proteinaceous or glycoproteinaceous hormone. However, this also seems unlikely as FCs do not release their contents into the blood in invertebrates e.g., arthropods, molluscs and echinoderms. Although these considerations make it improbable that their FCs are endocrine cells as such a conclusion cannot be drawn on a morphological basis. Further correlative in vivo and in vitro experiments are required to determine the role of FCs in steroid hormone synthesis in invertebrates.

D. Secretion of Regulatory Proteins and Glycoproteins

Biochemical studies have demonstrated the synthesis of nonsteroidal substances (constituted by proteins and glycoproteins) in the granulosa cells of successive stages of follicle growth in mammals, which function as local autocrine and paracrine regulators of follicle/ovarian functions (Tsafriri 1988, Yoshimura 1995, Guraya 1997). Very little or no attempt has been made to carry out comparable biochemical studies on the synthesis of such regulatory chemical components by FCs of invertebrates (Adiyodi & Adiyodi 1983, Adiyodi et al. 1997) and nonmammalian vertebrates (Guraya 1978, 1986, 1989, 1996). Thus this section will discuss the results of various biochemical studies carried out on the synthesis of nonsteroid substances as well as the hormonal regulation of their synthesis in the mammalian granulosa cells which have been extensively investigated using in vitro experiments in the recent years (Guraya 1997).

FSH has been shown to modify the biosynthesis of specific types of proteins in a concentration and time-dependent manner in rat granulosa cells cultured in vitro (Knecht et al. 1986); the granulosa cells are well known to have receptors for FSH. The synthesis of some proteins is increased by adding large amounts of FSH, whereas the amount of others is decreased. Similar observations are made for protein secretion, as the granulosa cells secrete several proteins in, increased and decreased amounts as FSH is raised. Synthesis of proteins and glycoproteins by granulosa cells has been related to the formation of follicular fluid, in the supply of nutrients to the ovum and the formation of various intrafollicular inhibitors and stimulators which accumulate in the follicular fluid (Guraya 1985, 1997, Ireland 1987, Lipner 1998, Tsafriri 1988, Tonetta & diZerega 1989, Driancourt et al. 1993, Adashi & Leung 1993, Tsafriri & Adashi 1994, Yoshimura 1995). Actually, besides the steroids (progestins, oestrogens, androgens) and gonadotrophins (LH, FSH and prolactin), follicular fluid has been shown biochemically to accumulate diverse chemical substances in variable amounts (Guraya 1985, 1997, Ireland 1987, Tsafriri 1988, Adashi & Leung 1993, Tsafriri & Adashi 1993). These substances, which form potential intragonadal or local regulators of follicular growth and function, include oocyte maturation inhibitor, a polypeptide of about 1,500 daltons; luteinization stimulator; luteinization inhibitor, a complex protein; follicle stimulating hormone inhibitor; steroid-binding proteins; enzymes (plasminogen, proteases); fibronectin; inhibitory proteins, a protein about 14,000 daltons; insulin; protein; relaxin, a polypeptide about 9000 daltons oxytocin; inhibin activity (FSH-suppressing activity), a protein of high molecular weight; activin or follicle stimulating hormone releasing protein; mucopolysaccharides (hyaluronic acid, chondrcitin sulfate); proteoglycans; prostaglandins'- gonadotrophin releasing hormone (GnRH); GnRH-like proteins and

various other polypeptides or growth factors. Tsafriri (1988) and Guraya (1997) have reviewed the previous literature in regard to the problems involved in the site of synthesis, isolation, purification, chemical characterization and biochemical effects of these local nonsteroidal regulators cf ovarian function (Tonetta & diZerega, 1989) and thus the details in this regard will be -omitted here. Their functions as local autocrine and paracrine regulators of follicle growth and atresia, and ovum maturation are discussed earlier by Guraya (1997).

All these non-steroidal substances, which function as local regulators of follicle functions including multiplication of GCs (Section III), steroidogenesis (Section IV C), oocyte growth and maturation (Section IV G) etc. remain to be investigated for invertebrates and nonmammalian vertebrates. However, some studies in this regard including their synthesis and mechanism of action have been made on the GCs of chicken follicles *in vitro* (Vanmontfort et al. 1995, Soboloff et al. 1995, Kawashima et al. 1995, Onagbesan et al. 1996, Chen & Johnson 1996, Li et al. 1997). The precise functions of most of the various intragonadal hormonal and nonhormonal and putative regulatory factors in relation to follicle growth and selection, ovum maturation and ovulation are still controversial (Guraya 1985, 1997, 1998, Ireland 1987, Hartshorne 1988, Tsafriri 1988, Driancourt et al. 1993, Greenwald & Roy 1994). There exists the intriguing possibility that many of the various intragonadal factors including growth factors function in the endocrine, paracrine and autocrine way to control development and maturation of dominant follicles during the oestrus cycles of mammals. Such functions remain to be defined more precisely at the molecular level. However, Yoshimura (1995) has suggested that the biological effects of IGF-1 are modulated by a family of binding proteins (IGFBPS) in a complex manner. IGFBPs appear to neutralize the actions of gonadotrophin and IGF-I, possibly via their ability to sequester IGF-I in the process of follicular growth.

Recent studies have demonstrated the secretion of fibronectin by granulosa cells from small follicles of the rat ovary, a property that is inhibited as these cells begin to respond to gonadotrophins (Skinner et al.1985). FSH is thus associated with an increase in aromatase activity and the production of oestrogens (Section IVC). Fibronectin can be used as a marker of granulosa cell maturation, and it may be functioning in the promotion of cell adhesion and multiplication, as in many endothelial and mesenchymal cells and leukocytes (Lipsky et al. 1993). It may also exert its effects on the oocyte as it is known to affect differentiated state of several other systems.

Many polypeptides, especially growth factors are believed to play roles, in the growth and multiplication of granulosa cells and have a direct effect on their differentiation as already discussed in Section III. Their production as well as their paracrine or autocrine functions within the ovary have been investigated (Schomberg 1988, Tsafriri 1968, Tonetta & diZerega 1989, Tsafriri & Adashi 1994, Guraya 1997, 1998), but very little or no attention is paid to the study of synthesis and functions of growth factors in the follicular epithelium of oocytes in invertebrates (Adiyodi & Adiyodi 1983) and nonmammalian vertebrates (Jones 1978, Guraya 1986,1989, 1996). Various authors have given an excellent review of ovarian cellular responses to growth factors in mammals, which still remain to be defined more precisely at the molecular level. The peptides namely epidermal growth factors (EGF), insulin, insulin-like growth factors (IGF) I & II, fibroblast growth factor (FGF), transforming growth factor-Beta (TGF-b), platelet derived growth factor (PDGF) and various others are observed to influence the granulosa cell multiplication, metabolism, steroid production and receptor expression. Schomberg (1988) has proposed three working hypotheses by which growth factors and reproductive hormones can interact to modulate follicle growth and selection, viz. (i) reproductive hormones may be involved in the modulation of production of and or cellular responsiveness to growth factors, (ii) growth factors may modulate the production of and/or cellular responsiveness to reproductive hormones, and (iii) the target cells for certain growth factors or reproductive hormones may show a direct response only to their specific regulators, but interact with each other via gap-junction mediated second messenger systems (as also reviewed by Tsafriri 1988,

Adashi & Leung 1993, Driancourt et al. 1993, Tsafriri & Adashi 1994, Guraya 1997, 1998). The growth factors appear to act at several sites including the granulosa cell membrane which has been shown to possess specific binding sites for certain of the peptides (Davoren et al. 1986, Tsafriri 1988, Schomberg 1988, Tonetta & diZerega 1989, Adashi & Leung 1993, Driancourt et al. 1993, Tsafriri & Adashi 1994). These binding sites (or receptors) mediate the effects of peptides on the function of granulosa cells. This model is complicated by the fact that there exist some compounds which are sufficiently alike as they show affinity for receptors other than those normally their own. For example, insulin/IGFI/IGFII system, where each member possess demonstrable, high affinity, specific binding sites, yet is capable of eliciting a response by interacting at lower affinity with "sibling" receptors (Davoren et al. 1986). The physiological significance of structural homologies between inhibin and TGFP relaxin and its 3-dimensional similarity to insulin and the binding of transforming growth factor to epidermal growth factor receptors still remains to be determined (Hartshorne 1988, Schomberg 1988, Tsafriri 1988, Guraya 1997, 1998). The concept of autocrine control is quite relevant to the differentiating granulosa cells. This is well illustrated by the effects of oestradiol-17β, which is formed by the granulosa cells as discussed in Section IVC. Various other factors produced by the granulosa cells also express receptors for them as well exemplified by insulin like growth factor I (somatomedin C). For the molecular mechanisms of action of various neurotransmitters, peptides and growth factors, which are observed to have a role in folliculogenesis, are recently discussed elsewhere for mammals (Guraya 1997, 1998) and thus will be omitted here. But such studies remain to be carried out for the folliculogenesis in invertebrates and nonmammalian vertebrates. However, Mita and Nagahama (1991) have shown the involvement of G-proteins and adenylate cyclase in the action of gonad stimulating substance on starfish ovarian FCs. Most egg calmodulin is a FC contribution to the cytoplasm of the *Blattella germanica* oocyte (Ziang & Kunkel 1994).

E. Phagocytosis

Follicular cells in the ovaries of various groups of animals become phagocytic by developing the capacity to ingest particulate materials of degenerating oocyte in atretic follicles as is supported by the presence of lysosomes (rich in hydrolytic enzymes), deposits of lipids, pigments and glycogen in their cytoplasm in invertebrates (Adiyodi & Adiyodi 1983), vertebrates including mammals (Byskov 1978, Guraya1976a,1985, 1986, 1989, 1993b,1997, Saidapur 1989a,b). Actually FCs of atretic follicles are transformed into phagocytes in invertebrates and vertebrates. Phagocytosis is especially clear in cells of old follicles, i.e. after the release of the oocyte and in follicular cells surrounding the degenerating previtellogenic and vitellogenic oocytes; the remnants of the oocyte including oocytic organelles, and yolk bodies of vitellogenic follicles are broken down and digested by lysosomal enzymes intracellularly by the follicular cells. Phagocytic activity forming an important feature of FCs is generally greatly accelerated following the massive degeneration of oocytes in different groups of animals under variable physiological and pathological conditions (Guraya 1976a, 1985, 1986, 1989, 1993b, 1997). This further supports the phagocytic function of FCs. FCs of seasonally breeding animals show conspicuous variations in phagocytic activity which is closely related to seasonal oogenetic activity. The precise regulation of transformation of FCs into phagocytes or their phagocytic activity remains to be determined although it forms their specific feature in diverse groups of animals; the causative factors may, therefore, be variable and thus remain to be determined through in vitro experiments. Since phagocytic activity forms a specific feature of FCs in atretic follicles of various groups of animals, the nature and functions of products formed from this activity may be variable depending upon the species and stage of atretic follicle.

F .Formation of Zona pellucida or Chorion

Various terms such as "chorion", vitelline membrane" or "zona radiata" or zona pellucida etc. have been used to describe this noncellular layer which is formed between the surface of oocyte and follicular epithelium in invertebrates (Adiyodi & Adiyodi 1983, Browder

1985, Bownes 1994), non-mammalian vertebrates (Guraya 1978, 1986, 1989, 1996, Cabada et al. 1996) and mammals (Guraya 1985, 1997). In some animals, e.g. insects, the vitelline membrane and chorion form 'two distinct layers (Bownes 1994). The cellular source of zona material, which consists mainly of carbohydrates (Bar-Shira Maymon et al. 1994) and proteins (glycoproteins), is still not accurately determined. Hence very divergent views continue to be expressed in this regard. Depending on the animal species, the zona or chorion material is believed to be secreted by either the oocyte or FCs or both. Bottke (1974) using electron microscopy, has attributed the synthesis of chorion material to FCs in the cephalopod oocyte. However, Cabada et al. (1996) using electron microscopy have suggested that both the oocyte and FCs are directly involved in the synthesis and secretion of the components of the vitelline envelope in Bufo arenarum . The extent to which such a role might differ among animal species as well as between follicles at different stages of growth still remains to be determined in detail. Our knowledge about the origin of the zona pellucida in different groups of animals is mostly the result of various studies carried out with ultrastructural histochemical methods. Very little attempt has been made to apply immunocytochemical methods and autoradiography to determine the precise origin of zona materials. The application of such techniques in fishes (Guraya 1996) and mammals (Guraya 1997) has indicated the role of FCs in the synthesis of some materials. The application of specific techniques for zona components at the mRNA level would be very helpful to determine the precise role oF FCs and oocyte in the synthesis and secretion of zona material (Epifano et al. 1995, Lin et al. 1996). Actually, studies on the synthesis of zona proteins at the transcriptional and translational levels are required for demonstration of how the macromolecular composition of the unique glycoproteins has diverged, whereas the basic physiological functions are highly conserved during evolution of different patterns of fertilization in various groups of animals. From the recent data as discussed in different reviews (Jones 1978, Guraya 1978, 1985, 1986, 1989, 1996, 1997, Adiyodi & Adiyodi 1983), it appears that little progress has been made in problems of cellular origin and naming of egg envelopes in different groups of animals. Therefore, further correlative electron microscopic, autoradiographic, immunocytochemical and biochemical investigations related to synthesis and secretion, and morphological information on the subcellular organization of both FCs and egg are required to resolve the extent difficulties. Monoclonal antibody methods would also be very useful in determining the role of different cell types in the formation of zona proteins. The identification of genes involved in the encoding of proteins required for the formation of vitelline membrane and chorion are of great current interest in Drosophila (Bownes 1994, Romano et al. 1991). The nature of the chemical signals that initiate the formation of chorion and zona pellucida remains to be determined. These may be hormones, genetic, or the result of cell contact and establishment of intercellular communication between the oocyte and FCs (Eppig 1994, Bownes 1994). Whatever the signals may be, regulation of zona development could occur at the level of transcription and/or genome organization and translation, post-translational modification. The molecular mechanism of regulation remains to be determined in future investigations using recombinant DNA and monoclonal antibody techniques. Since biochemical, histochemical and morphological differences occur in acellular layers or the zona pellucida of different animal species, the site of synthesis and post-translational modification possibly also vary among different animal groups depending on their reproductive adaptations to diverse ecological conditions (Guraya 1996). The cellular origin of zona pellucida materials also needs to be investigated, using in vitro biosynthesis of proteins and carbohydrates. In other words, the complementary roles of FCs in their synthesis remain to be defined more precisely in different groups of animals.

G. Oocyte Growth and Meiotic Maturation

The oocytes, in response to an appropriate maturational stimulus, undergo complex structural metabolic, physiological and biochemical changes which are being investigated to solve the problems of growth,

maturation and development in different groups of animals, especially in fishes, amphibians, reptiles, birds and mammals (Jones 1978, Browder 1985, Guraya 1985, 1986, 1989, 1997, Eppig 1994, Jinno et al. 1995, Motta et al. 1995, Crozet et al. 1995, Nagahama et al. 1995, Harrouk & Clarke 1995, Cheenel & Eppig 1995a, b, Skoblina et al. 1995b, Aktas et al. 1995a,b, Eppig et al. 1996), paying little or no attention to such changes in diverse groups of invertebrates and most of which do not show the presence of FCs around the developing oocyte (Section IIA). After the initiation of meiosis during the foetal or embryonic development of female mammals and birds, meiosis becomes arrested at prophase of the first meiotic division (Guraya 1985, 1989, 1997, 1998). But in all other animal groups meiosis is initiated in the sexually mature phases after the proliferation of oogonia (Adiyodi & Adiyodi 1983, Guraya, 1984, 1986, 1989). The factors involved in the regulation of these differences remain to be determined at the molecular level. However, before the initiation of meiosis, the germ cells are called oogonia and thereafter they are referred to as oocytes. Many of the changes during oocyte growth and maturation appear to be mediated or influenced by ions, hormones (especially gonadotrophins and steroids) and endogenous cytoplasmic factors such as maturational promoting factor (MPF) and growth factors (Singh et al.. 1993, Eppig 1994, Skoblina et al. 1995a, b, Hinrichs et al. 1995, Guraya 1997).

Extensive observations made so far have clearly suggested the functional involvement of FCs not only in the regulation of progressive growth and differentiation of the oocyte (Eppig 1994, Bownes 1994, Eppig et al. 1996) but also in mediating meiotic maturation and establishing required conditions for normal fertilization and embryogenesis. In this regard, molecular aspects of communication or interactions between follicle or granulosa cells and oocyte are being investigated to determine the nature of factors involved in these processes as well as the transcription of genes specific to these factors involved in the growth processes of oocytes (Bownes 1994, Clifford et al. 1994, Eppig 1994, Eppig et al.1996).One current example of such a paracrine factor from granulosa cells that affects oocyte growth in rodents is the product of Steel (St) locus and is known as either kitligand (KL) or Steel-factor (SLF). The close relationship of the KL-producing granulosa cells and the oocyte bearing the appropriate receptor for the same suggests an important functional relationship. Striking evidences are produced to show that this functional and symbiotic relationship between germ cells and somatic cells is important for oocyte development and maturation within the follicle as it gets disturbed in the absence or following the loss of FCs during degeneration or mechanical separation leading to termination of communications between FCs and oocyte (Eppig 1994, Guraya 1997). Actually the termination of this communication has been attributed to the disruption of junctional contact between the oocyte and FCs thus preventing the entry of meiosis inhibiting substances into the oocyte and thereby initiating nuclear maturation. Meiotic arrest in mammalian oocytes during the first part of their growth phase is believed to result from either the absence of essential cell cycle regulatory proteins, the presence of meiosis-arresting substances or both. At present atleast three important meiosis inhibitors such as cAMP, peptide designated OMI (oocyte meiosis inhibitor)and the purine metabolite, hypoxanthine, are believed to be involved in the granulosa cell-mediated maintenance of meiotic arrest in mammals (Aktas et al. 1995a, b, Guraya 1997, 1998). The interruption of follicle cell oocyte communication in response to hormones (especially gonadotrophins) is believed to result in a decrease of cAMP as a physiological regulator of meiosis in mammalian oocytes (Aktas et al. 1995a, b, Guraya, 1995, 1997, 1998) the decrease in cAMP is also attributed to an increased activity of phosphodiosterase enzyme or a decrease in adenylate cyclase activity in the oocyte. The interaction of cAMP and follicular OMI is believed to be of great significance in the control of oocyte maturation further supporting the concept of multifactorial regulation of resumption of meiosis. This suggestion is further supported by a model that lays emphasis on synergism between adenosine and hypoxanthine that ultimately modulates oocyte cAMP. Some evidence is also produced for

the synthesis of a meiosis inducing substance by the mammalian granulosa cells,. which stimulates resumption of a meiosis and that effect is possibly mediated via the phosphatidylinositol pathway (Guraya 1997). Aktas et al. (1995a) have observed that increased cAMP reversibly maintains bovine oocytes in meiotic arrest for an extended period without the occurrence of the post-translational protein modifications observed during meiotic ,.resumption or transient arrest. Aktas et al. (1993b) have further suggested that maintenance of meiotic arrest by invasive adenylate cyclase is accompanied through modulation of cellular machinery and regulation of oocyte maturation by cAMP may be a physiological event. Skoblina et al. (1995) have suggested that cAMP, rather than oestradiol-17β, is involved in the actinomycin D-induced inhibition of amphibian oocyte maturation stimulated by gonadotrophins.

Gap junctional communication within the ovarian follicle is believed to be involved in the resumption process of meiosis, but divergent views have been expressed in this regard (Guraya 1985, 1997, Eppig 1994). Some workers believe that the reduction in gap junctional communication between the oocyte-cumulus cell complex and the membrana granulosa decreases the transport of meiosis-inhibiting substances to the oocyte and thus results in the resumption of meiosis. others are of the view that gonadotrophins, induce the generation of a positive maturation-inducing signal within the granulosa cells; the transfer of this signal to the oocyte would need patent gap junctional communication (Fagbohun & Downs 1991). Further investigations are required to resolve this controversy.

Comparative studies are required to define more precisely the cellular source, nature and molecular mechanism of action of such factors in different groups of animals as oocytes in most invertebrate., phyla are either not associated with FCs or show a poor development of follicular epithelium. A deeper understanding of such factors from comparative point of view will be very helpful in the better understanding of role of FCs in the regulation of meiosis. Sakairi and Shirai (1991) have shown the possible MIS production by FCs in spontaneous oocyte maturation of the ascidian. The molecular studies on the oogenesis of the nematode (*Caenorhalliditi: elegans)* have suggested the possibility that germline-soma interactions or interactions between germ cells regulate oocyte maturation or function; and several loci involved in signal transduction appear to play a role in oogenesis (Clifforet et al. 1994). In those groups of invertebrates and amphioxus where FCs are absent, (Section IIA) some well-programmed intrinsic factors may be involved in the regulation of oocyte meiosis which is well conserved during evolution of different groups of animals. With the evolution of follicular epithelium and hormones (gonadotrophins, steroids etc.), this intrinsic regulation might have become extrinsic by the involvement of regulatory factors produced by FCs. Molecular probes need be used to define the precise nature and mechanism of action of intrinsic and extrinsic factors involved in the regulation of oocyte maturation (or meiosis) in different groups of animals.

H. Determination of Animal-Vegetal and Bilateral Symmetries of the Egg

In some animal groups, the animal-vegetal polarity is believed to be determined by an interaction of oocyte and FCs (Adiyodi & Adiyodi 1983, Bownes 1994). The cortex of the basal part of oocyte in some molluscan species appears to be locally influenced by the follicle cell to become the vegetal pole (de Jong-Brink et al. 1983). The interaction of FCs and basal part of the oocyte is believed to determine the localization of morphogenetic factors in this basal part. The vegetal part of the egg not only determines the animal-vegetal axis, but also the bilateral symmetry. In the fruitfly (Drosophila), FCs play an essential role in establishing the polarity of the egg and the embryo that develops from it (Bownes 1994, Clifford et al. 1994, Hong & Haskimoto 1995, Lane & Kalderon 1995, Johnson et al. 1995, , Mahone et al. 1995). The mature Drosophila oocyte shows clear morphological and biochemical asymmetries: its nucleus is positioned anterodorsally, it exhibits a polarized cytoskeleton, and morphogenetic determinants are placed at its anterior and posterior ends. The eggshell, which is produced by FCs, likewise shows obvious anterior-posterior and dorsal-ventral asymmetries. The correct establishment of the anterior-posterior and

dorsoventral pattern of the egg membranes in Drosophila requires signals from the oocyte to FCs which secrete them. For the embryo to develop a normal anterior-posterior and dorso-ventral pattern of structures, signals from FCs to the oocyte during oogenesis are essential. But the molecular mechanisms involved in such an interaction between FCs and oocyte are being investigated, especially for Drosophila melanogaster (Bownes 1994, Gajewski & Schultz 1995, Gonzalez et al. 1995, Hong & Hashimoto 1995, Johnson et al. 1995, Lane & Kalderon et al. 1995, Mehone et al. 1995, Rittenhouse & Berg 1995, Lee et al. 1996, Lee et al. 1996, Grunert & Johnston 1996). The genes expressed in the follicle cell epithelium of Drosophila are known to regulate the localized activation of maternal factors involved in the specification of the dorsoventral axis and terminalia. Genetic studies have indicated that reciprocal signalling occurs between the oocyte and FCs during oogenesis. The ability to detect asymmetry in the oocytes of animals belonging to different phyla appears to be limited by the current paucity of molecular markers which form promising area for future investigations for providing molecular basis of animal-vegetal and bilateral symmetries of eggs which are reported for some groups of animals. Various studies on mutations in the Drosophila genes are being made to determine the precise nature and roles of regulating factors involved in the development of symmetries of the egg (Johnson et al., 1995, Lane & Kalderon 1995, Mahone et al. 1995, Rittenhouse & Berg 1995). Mutations in various genes cause a range of various morphogenetic changes in different components of the Drosophila ovary, which affect the symmetries of the egg.

I. Ovulation

On the basis of morphological and histochemical observations, the follicle or granulosa cells have been involved in the process of ovulation in invertebrates (Adiyodi & Adiyodi, 1983) and vertebrates including mammals (Espey 1978, Guraya 1985, 1986, 1989, 1997, Browder, 1985). During ovulation vesicles derived from the extended rough endoplasmic reticulum of follicle cell fuse with the lateral plasma membrane, thus releasing their contents into the intercellular space between FCs and the oocyte forming the follicular cavity which is considered as an important phase in the ovulation process of vertebrates (Espey 1978, Guraya 1985, 1986, 1989, 1997). The interiorization of the nexus junctions seem to reduce the cell-to-cell contacts and thereby loosen the granulosa cells and the oocyte during ovulation. However, it remains to be determined whether these surface structures are important in the process of ovulation (Espey 1978). The follicle or granulosa cells release acid mucopolysaccharides of high molecular weight into the follicular cavity. These are depolymerized by hyaluronidase, which is also produced by FCs. This causes the swelling of the follicle.

Recent studies have also attributed the formation of hydrolytic enzymes to the follicle or granulosa cells. Such enzymes play an important role in the rupture of follicle by causing hydrolysis of surrounding tissues for the release of egg through the stigmata (Guraya 1997, Mita (1991, 1992, 1993) has investigated the production and characterization of I-methyladenine in the starfish ovarian FCs and its role in spawning.

J. Transportation and Fertilization of Egg

In those animal species where some FCs continue to be present in association with the ovulated eggs e.g. cumulus oophorus of mammalian eggs, they play some important roles in the transportation and fertilization of eggs. An expanded cumulus is believed to be important for ovulation, ovum uptake by oviductal fimboria, sequestering sperm or the fertilizability of the egg (Dunbar & O'Rand 1991, Eppig 1994, Guraya 1997).

K. General Conclusions and Future Perspectives

The ovarian accessory somatic (follicle or granulosa) cells associated with growing oocytes show various patterns of development, distribution, size, abundance and structure in invertebrates, nonmammalian vertebrates and mammals, which appear to be related to the variable mode of oogenesis. In some groups of invertebrates. FCs are either absent or show very poor development. The regulation and functional meaning of all these variations remained to be

determined at the cellular and molecular levels. Wherever FCs are present, they play important roles in (i) providing nutrient substances and informational molecules (e.g. RNA'S, DNA proteins, etc.) to the growing oocyte, (ii) synthesis of yolk, (iii) steroid hormone synthesis and metabolism, (iv) synthesis and secretion of regulatory autocrine and paracrine nonsteroidal substances (proteins or polypeptides, growth factors, hormones etc.), (v) synthesis of chorion or zona pellucida substances, (vi) regulation of meiosis or maturation of oocyte, (vii) determination of animal-vegetal and bilateral symmetries of the egg, (viii) ovulation and transportation and fertilization of egg, and (ix) phagocytic activity.

The diversity in the specializations of plasma membrane including the morphology and numbers of junctions between FCs is closely related to the complexity of the structural organization of the follicular epithelium in different groups of animals. The ways in which FCs influence growth, differentiation, maturation and metabolic activities of oocyte are possibly diverse and remain to be understood at the molecular level. A major goal in future investigations of interactions between the oocyte and FCS and among FCs themselves in different groups of animals should be a convergent application of biochemical, immunological, molecular, biological and physiological techniques to reveal the functional meaning of such interactions.

The precise nature and mechanisms of transport of regulating molecules (including proteins, enzymes as well as receptor-transporter complexes) and nutritive substances (lipids, carbohydrates, proteins, amino acids, vitamins, metals, etc.) from FCs to growing oocytes remain to be determined, but binding proteins and endocytosis appear to play a predominant role in this regard. The growing oocytes of mammals are now shown to provide signals to the follicle or granulosa cells to coordinate their various stage-specific functions, suggesting mutual functional interrelationships, which remain to be understood at the molecular level in different groups of animals showing a great diversity in the morphological organization of follicular epithelium at different stages of oocyte growth in the same or different species of animals. The study of cell adhesion molecules, substrate adhesion molecules, and cell junctional molecules, as well as enzymes will be very rewarding in revealing the nature of the molecules involved in the adhesion, communication and transport processes between FCs and oocytes, and between FCs themselves. Actually, there is a chemical communication between the cells of any tissue or organ of the animal body as well as between the various nuclear and cytoplasmic components of the same cell. Glycoconjugates are now known to play important roles in this regard (Wieland & Reutter, 1993). Such roles are required to be investigated for FCs and oocyte. Advances are made in the identification and characterization of cell adhesion molecules and intracellular glycoconjugates (Greenwald 1992, Lipsky et al. 1993 Wieland & Reutter 1993). Cadherins and integrins are being studied in relation to adhesion of FCs and oocyte (Eppig 1994).

The complexity of follicular epithelium and multiple interactions occurring among FCs and oocytes at the cellular levels make the experimental design of investigations and interpretation of data difficult for the better understanding of regulation of oocyte growth by FCs. Therefore, the use of a combination of traditional techniques and new molecular biology probes in vitro and in vivo in comparative investigations of the synthesis, chemical characterization, and biological activities of proteins, including growth factors, and steroid hormones in the ovarian follicles of animals, which show variable morphological organization (or structural complexity in FCs-oocyte contacts), form promising area for future studies because our knowledge in these areas is very poor, especially for invertebrates and lower vertebrates.

For answering questions about the local regulation of functions of FCs and oocytes, it is important to investigate the differential regulation of gene expression throughout oogenesis and then its correlation with the development of specific functions, or characteristics of FCs. Although, immunocytochemistry, mRNA expression and in situ hybridization reveal synthesis of a peptide, final demonstration of the specific effects of factors isolated from FCs during different stages of oogenesis and subsequent characterization are

essential. Comparative investigations on the identification of the secretary products including proteins, glycoproteins and steroid hormones and their biological roles in the "nurse cell" function of FCs in different groups of animals should be very rewarding to improve our understanding of precise, regulatory mechanisms involved in oogenesis.

However, a very limited FC-oocyte associations or its complete absence in the ovaries of some animals (Section II) clearly suggests that the information required for oocyte growth and differentiation is present in the genome of the oocyte itself. An alternative type of cell-cell interaction may be a more passive process which does not require regulatory (i.e. signal transduction type interactions. The follicle cell may be simply involved in providing a proper environment, metabolites, and nutrients to permit oocyte growth to proceed, suggesting that the primary FC-oocyte interactions are environmental and nutritional rather than regulatory. It also remains to be determined whether follicle cell function in the ovary of the mature (or adult) animal is simply maintained or regulated (i.e., changing). It is also possible that with the evolution of complexity of follicle-oocyte interrelationships, endocrine and local regulatory mechanisms also evolved to ensure the growth, development, differentiation and maturation of various components of the oocyte; about which our knowledge is still meagre.

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