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Molecular and cellular changes associated with maturation and early development of sheep eggs

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Introduction

It has long been postulated in non-mammalian species that successful embryogenesis depends directly on an ordered sequence of events in oogenesis (Wilson, 1925). Oogenesis in these species is recognized as the phase of synthesis and storage of intracellular components whilst early embryogenesis is the period of distribution and utilization of stored product. A clear example of the interrelationship between oogenesis and embryogenesis is provided by the eggs of the toad, *Xenopus laevis*. In this species the single-celled egg at fertilization contains all the components and information required for the development of the swimming tadpole which consists of approximately 50 000 cells (Gurdon, 1974). Although this extreme degree of independence from extracellular support is unlikely to apply directly to mammals, it is nevertheless critical to identify the extent to which mammalian embryogenesis is regulated by the products of oogenesis. It is our purpose to answer this question by describing the intracellular events during oogenesis in sheep and relating these to the control of fertilization and early development.

Over what developmental time-scale does oogenesis occur in mammals? The process is initiated when the primordial germ cells invade the genital ridge of the embryo. After colonization of the early gonad the germ cells undergo a period of mitotic activity before entering meiosis and progressing to the dictyate stage of meiotic prophase. At this point the cell cycle is interrupted and the oocyte, containing a large nucleus referred to as a germinal vesicle (GV), remains in meiotic arrest for all but the last few hours of oogenesis. In addition to nuclear arrest the oocyte, surrounded by a single layer of flattened cells, constitutes part of the non-growing or resting pool of primordial follicles for much of its postnatal existence. The limited amount of biochemical evidence available about oocytes in primordial follicles suggests that they are merely synthesizing 'housekeeping' proteins during the resting period. However, a small number of primordial follicles enter the growing pool each day. It is with the developmental events initiated in the oocyte at this time and terminating during embryogenesis when maternal regulation ceases that this paper deals. Although the sheep oocyte and embryo will serve as a model for the paper, information from other mammals is used to compensate for deficiences in our knowledge of oogenesis in this species.

At least three distinct developmental programmes direct the molecular changes which occur during oogenesis and early embryogenesis. A growth programme regulates differentiation in the immature oocyte (Canipari *et al.*, 1984) while a separate maturation programme regulates the reprogramming of the oocyte before ovulation. The entry of the spermatozoon initiates an early embryonic programme which persists until maternal regulation is terminated and development becomes directed by the embryonic genome (Howlett & Bolton, 1985). A later embryonic programme, which will not be discussed in this communication, begins with the expression of the

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embryonic genes and is an important regulator of events culminating in the formation of the blastocyst (Johnson *et al.*, 1984).

If the growth, maturation and fertilization programme provide the framework for early development what molecular and cellular events occur during each of these phases? Furthermore, how is each programme regulated and what effects do early events exert on later development?

Oocyte growth phase

Morphology and biology of oocyte growth

Two features characterize the oocyte at the start of its growth phase. The first is its small size and the second is the total inability of the early oocyte to progress from the G2 to the M phase of the meiotic cycle (Szybek, 1972). Both of these characteristics are radically altered during the growth phase. Firstly, the oocyte, after exit from the resting state, grows rapidly and its volume increases 350-fold within the first 2 weeks (Wassarman *et al.*, 1981). About half the amount of protein required for this rapid growth is synthesized by the oocyte (Schultz *et al.*, 1979). The remainder is almost certainly synthesized by somatic cells and taken up by the oocyte in a non-degraded form (Glass & Cons, 1968).

The highly active metabolic state of the growing oocyte imposes demands on the oolemma which are far in excess of the limited uptake characteristics of this membrane (reviewed by Moor, 1983; Schultz, 1985). Thus, oocytes isolated from direct contact with surrounding follicle cells fail to grow, while those maintaining normal oocyte–follicle cell contacts grown normally *in vitro* (Eppig, 1979). Investigations of the precise nature of these heterologous contacts reveal that they are formed by cellular processes which extend across the zona pellucida and terminate on the oocyte membrane where gap junctions are formed (Anderson & Albertini, 1976). The intercellular passage of molecules through these junctions provides the high substrate uptake required by oocytes during the growth phase (Moor, 1983; Schultz, 1985).

The second feature of the resting oocyte, its inability to progress beyond diplotene, changes during the final part of the growth phase. Studies of mice, pigs and sheep indicate that oocytes acquire the ability to proceed to metaphase I when they reach about 80% of their full size, but are unable to progress beyond this to metaphase II until their growth is complete (Sorensen & Wassarman, 1976; Motlik *et al.*, 1984; R. M. Moor, unpublished observations).

The growth of the oocyte, the dependence on intact oocyte–follicle cell contact and the acquisition of meiotic competence provide valuable markers of cell differentiation and development. What molecular changes occur during this phase of differentiation and what subsequent biological actions do these changes regulate?

Biochemistry of oocyte growth

RNA synthesis. Autoradiographic analyses (Crozet *et al.*, 1981) demonstrate that growing pig oocytes actively synthesize both ribosomal (r)RNA and heterogeneous (hn)RNA. A significant reduction in the synthesis of rRNA is observed as pig oocytes approach their full size. More rigorous quantitative analyses of RNA synthesis have regrettably not yet been undertaken for this or other domestic animals. By contrast the detailed analyses of RNA synthesis and stability undertaken in mouse oocytes provide a model for domestic animals. The major classes of RNA have been studied during mouse oogenesis by pulse-labelling procedures (see Kaplan *et al.*, 1982, for references). Total RNA, rRNA, tRNA, poly(A)$^+$RNA and ribosomes accumulate steadily throughout growth. The bulk of the newly synthesized RNA is remarkably stable during the growth phase but degradation increases, once maturation is initiated. About 20% of maternal RNA is degraded during maturation, a further 40% by the 2-cell stage and 30% on Day 3. Despite

this dramatic post-fertilization loss of RNA, Bachvarova & De Leon (1980) calculated that the amount of mRNA stored in the mouse egg is sufficient to direct protein synthesis up to the 8-cell stage.

In summary, the accumulated information indicates that mammalian oocytes, like those of non-mammalian species (Davidson, 1976), sequester large amounts of RNA during the growth phase. The sequestered ribosomes and RNA are packaged into lattices or ribonuclear particles and remain inactive in the oocyte for extended periods of time. To what extent are proteins like RNA, also synthesized and stored during the growth phase for utilization during early development?

Protein synthesis. Oocyte growth involves not only a substantial enlargement of the cell but also a major reorganization of its metabolism and gene expression. The increase in oocyte volume is accompanied by a proportional increase in the amino acid pool and a linear increase in protein synthesis (Wassarman *et al.*, 1981). Detailed electrophoretic analyses of proteins at different stages of growth reveal that the synthesis of structural and other housekeeping proteins is accompanied by the synthesis of a changing pattern of stage-specific proteins. In a series of investigations on these proteins Wassarman *et al.* (1981) have identified some early proteins whose function becomes critical in later embryonic development. For example, histone-H4, some of the ribosomal proteins and a so-called germinal vesicle associated protein (GVAP) are all sequestered in the nucleus and used during maturation and development. An even more dramatic example of the early synthesis of proteins which will then be sequestered and used specifically for later developmental events relates to the production of three glycoproteins. These products, representing at least 10% of the total synthesis of growing oocytes, are secreted and form the zona pellucida (Wassarman *et al.*, 1981). The smallest of these glycoproteins in the mouse zona will subsequently function as the sperm receptor; modifications to the other zona glycoproteins fulfil the further role of forming the block of polyspermy after fertilization has occurred.

Regulators of oocyte growth. Indirect evidence suggests that the synthesis of at least some of the developmental regulator proteins is controlled by a programme which is unrelated to the growth of the oocyte. For example, growing oocytes freed of follicle cells and cultured thereafter on fibroblast monolayers survive but do not grow (Canipari *et al.*, 1984). Nevertheless, even these non-growing oocytes acquire meiotic competence at precisely the same temporal stage as do their growing counterparts. The results of Canipari *et al.* (1984) suggest that a programme is initiated in the newly activated primordial follicle which then progresses independently through a series of molecular sequences to its conclusion when meiotic competence is attained.

At the completion of the growth phase the oocyte has a store of components required for subsequent development. It has also acquired the ability to progress from the dictyate to the metaphase II stage of the cell cycle. The oocyte is not, however, able to become fertilized or support early embryonic development at this stage. The acquisition of developmental competence depends on a further phase of differentiation, initiated *in vivo* by the release of LH before ovulation. What intracellular changes confer developmental competence on the ovine oocyte?

Oocyte maturation phase

Biology of maturation

After completion of growth the germinal vesicle oocyte is characterized by a relatively impermeable membrane, intimate association with adjacent follicle cells through gap-junctions, a peripheral location of many cellular organelles, a low rate of transcription and a stable pattern of protein synthesis (Fig. 1). The role of the somatic compartment at this time is crucial both for the provision of metabolic support and for the regulation of nuclear and synthetic activity in the oocyte (Moor & Osborn, 1983). The increased amounts of gonadotrophins released at oestrus bind to the follicle cells and alter both the signals (Fig. 2) and their means of transmission within the follicle

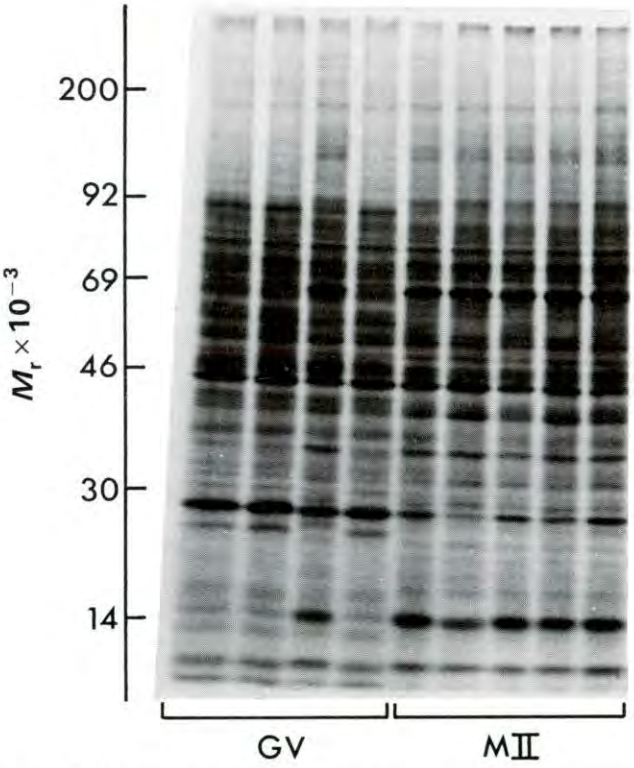

Fig. 1. Fluorographs of [^{35}S]methionine-labelled polypeptides from individual oocytes before the initiation of maturation (GV) and after its completion (MII). Cumulus-enclosed oocytes were removed from the ovaries of sheep 40 h after FSH stimulation (GV stage) or from super-ovulated sheep slaughtered 18 h after the onset of oestrus (MII). Note that the protein pattern in one of the GV oocytes (track 3) has been prematurely activated by the FSH treatment.

(Moor, 1983). The resultant changes in the oocyte and the consequences of these maturational changes on fertilization and early embryogenesis are the subject of the remainder of this paper.

The maturation timetable

The acquisition of developmental competence in the sheep oocyte occurs over a 24-h period before ovulation and involves two phases. In the first 6-h phase the oocyte undergoes few structural or synthetic changes but appears instead to be undergoing programming by the somatic elements (Moor & Warnes, 1977). In the longer (18 h) second phase most components of the oocyte undergo reorganization; it is unlikely that the follicle cells have an important influence during this period.

Membrane-related changes during maturation

Immediately before the LH surge the oocyte is coupled to the surrounding follicle cells through a highly developed system of gap-junctional contacts. These junctions, which occupy approximately 1% of the total membrane area, mediate the entry of nucleosides, sugars, phospholipid precursors, amino acids and signal molecules (Moor, 1983). The intercellular passage of these substrates into the oocyte remains at a consistently high level for the first 12 h of maturation and then declines sharply in the following 3 h. This reduction in junctional transmission is compensated

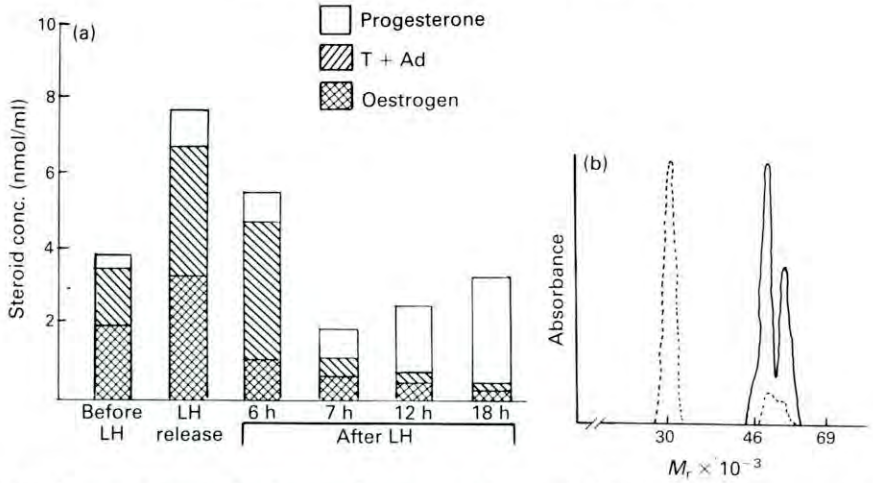

Fig. 2. Changes in intrafollicular signals generated during the 24 h period of maturation in the sheep. (a) The time-dependent fluctuations in both total steroid output and in the proportions of unconjugated oestradiol-17β, androgen (testosterone plus androstenedione) and progesterone in the follicular fluid of preovulatory follicles (Osborn & Moor, 1983b). (b) A densitometric trace of the changes in the profile of polypeptides secreted by the granulosa cells during maturation; a polypeptide complex (M_r 46 000–60 000) secreted before the LH surge (solid trace) disappears and is replaced during maturation by a polypeptide with a relative molecular mass of 30 000 (R. M. Moor & I. M. Crosby, unpublished observations).

for by a significant increase in the carrier-mediated uptake of substrates across the membrane itself (Moor & Smith, 1979).

Degenerative changes in the fine processes and junctions, induced primarily by FSH rather than LH, account for the reduced transmission of substrates into maturing oocytes (Moor & Cran, 1980). Some workers assert that the disruption of junctional contact also prevents the entry of an inhibitor of meiosis into the oocyte and thereby initiates nuclear maturation (Dekel & Beers, 1978). This explanation appears unlikely to apply directly to meiotic regulation in sheep oocytes because nuclear change precedes junctional disruption in this species (Moor *et al.*, 1981). The possibility of an alternative form of junctional regulation has, however, recently been highlighted by Larsen *et al.* (1986). These workers report on an early and selective uncoupling of adjacent cumulus cells in rats injected with hCG, at a stage when the heterologous coupling between the oocyte and the corona is still fully maintained. The corona–oocyte unit may therefore be isolated from the granulosa compartment (and its inhibiting signals) at an early stage in maturation by a band of non-communicating cumulus cells.

It has further been postulated that the disruption of cumulus–oocyte coupling initiates cytoplasmic remodelling in the maturing oocytes of sheep and other species (Szollosi *et al.*, 1978). To what extent and for what purpose does this postulated relocation of organelles take place in the oocyte during maturation?

Structural remodelling during oocyte maturation

Ultrastructural studies by Zamboni (1970) were the first to show that maturation is accompanied by a major reorganization of organelles such as mitochondria, lysosomes and cortical granules within the oocyte. The development of perinuclear microtubule organizing centres (MTOCs) during maturation, and the establishment of a temporal relationship between LH release and intracellular remodelling have extended these initial observations (Szollosi *et al.*, 1972; Kruip

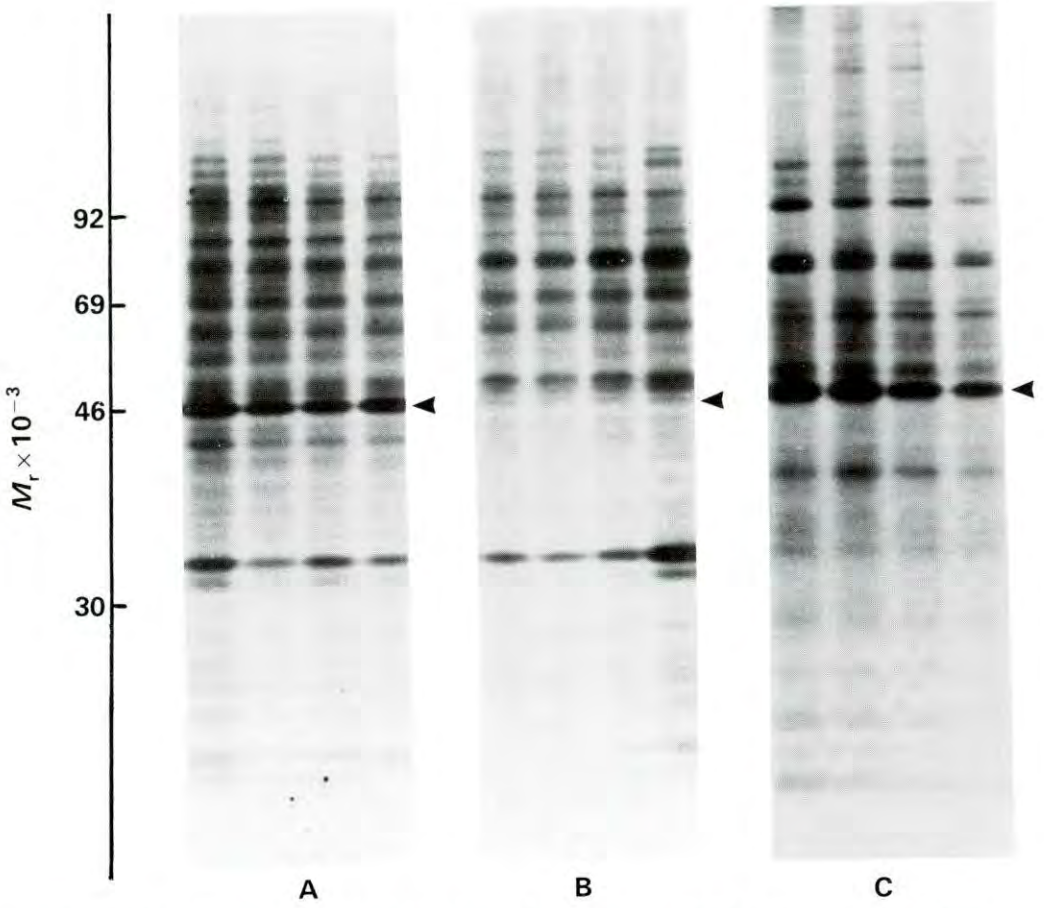

Fig. 3. Effect of somatic cells on the synthesis of β-actin (arrowed) by sheep oocytes incubated in [^{35}S]methionine. Fluorographs on gel A show that individual cumulus-enclosed oocytes synthesize substantial amounts of actin. Actin synthesis is immediately terminated by the removal of the cumulus cells although the synthesis of all the other polypeptides continues (gel B). Actin synthesis is re-initiated in the denuded oocyte when the zona pellucida is removed to enable the gamete to reform junctional contact with a wide range of somatic cells (gel C).

et al., 1983; Van Blerkom & Runner, 1984). The mitochondria are located in a peripheral position whilst rough endoplasmic reticulum (RER) and membrane-bound vesicles including cortical granules occupy a more central position in germinal vesicle oocytes of domestic animals. After the breakdown of the germinal vesicle the RER disappears and clusters of mitochondria and lipid droplets become apparent (Kruip *et al.*, 1983). During the final phase of maturation the clusters of organelles disperse and become centrally located leaving the cortical granules in the relatively organelle-free region immediately adjacent to the plasma membrane.

It is reported by Szollosi *et al.* (1978) that the migration and alignment of cortical granules beneath the plasma membrane depend on the disruption of the junctional contact between the oocyte and somatic cells. Equally, Van Blerkom (1985) argues that the relocation of organelles is mediated by microtubules radiating from perinuclear MTOCs that develop during the GV to metaphase I transition. Although the inductive signals and molecular mechanisms underlying intracellular modelling are still uncertain, no doubt exists about the biological importance of remodelling

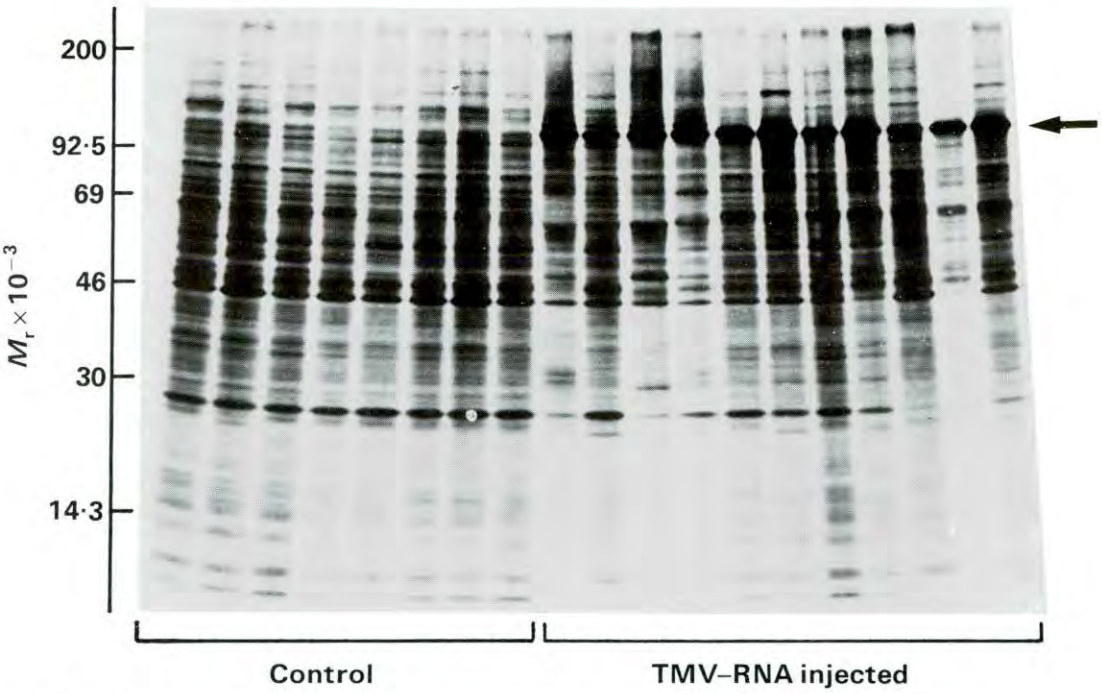

Fig. 4. Fluorograph of ^{35}S-labelled polypeptides from individual oocytes injected with buffer (controls) or with TMV-RNA (10 pg TMV-RNA per oocyte). The dominant early TMV polypeptide (M_r 110 000) is arrowed. Production of TMV-encoded proteins is accompanied by a corresponding decrease in the synthesis of endogenous polypeptides (R. M. Moor, unpublished observations).

during maturation. At fertilization exocytosis of cortical granules occurs, and the membrane and zona pellucida are modified and form a block to the entry of additional spermatozoa. Any failure in the alignment of cortical granules beneath the membrane prevents exocytosis and results invariably in polyspermy.

Reprogramming of protein synthesis

The final 24 h of differentiation in sheep oocytes is accompanied by major changes in protein synthesis (Fig. 1); these changes are obligatory events in the preparation of female gametes for fertilization (Warnes *et al.*, 1977; Thibault, 1977). This reprogramming process involves a slight decline in the overall rate of synthesis and changes in the relative rates of synthesis, degradation and post-translational modification of specific proteins (Wassarman *et al.*, 1981; Crosby *et al.*, 1984; Johnson *et al.*, 1984). Whilst the majority of these changes take place after the breakdown of the nucleus some important changes precede and regulate GVBD in domestic animals (Moor & Crosby, 1986).

Two major questions arise from the reprogramming of protein synthesis during maturation. Firstly, what mechanisms regulate the reprogramming process and secondly, what role do the new proteins play in development?

Regulation of protein reprogramming. Various regulatory mechanisms influence protein synthesis during the maturation process. Indirect evidence from RNA inhibitor studies suggests that new transcription and the resultant limited change in mRNA populations regulates a small number

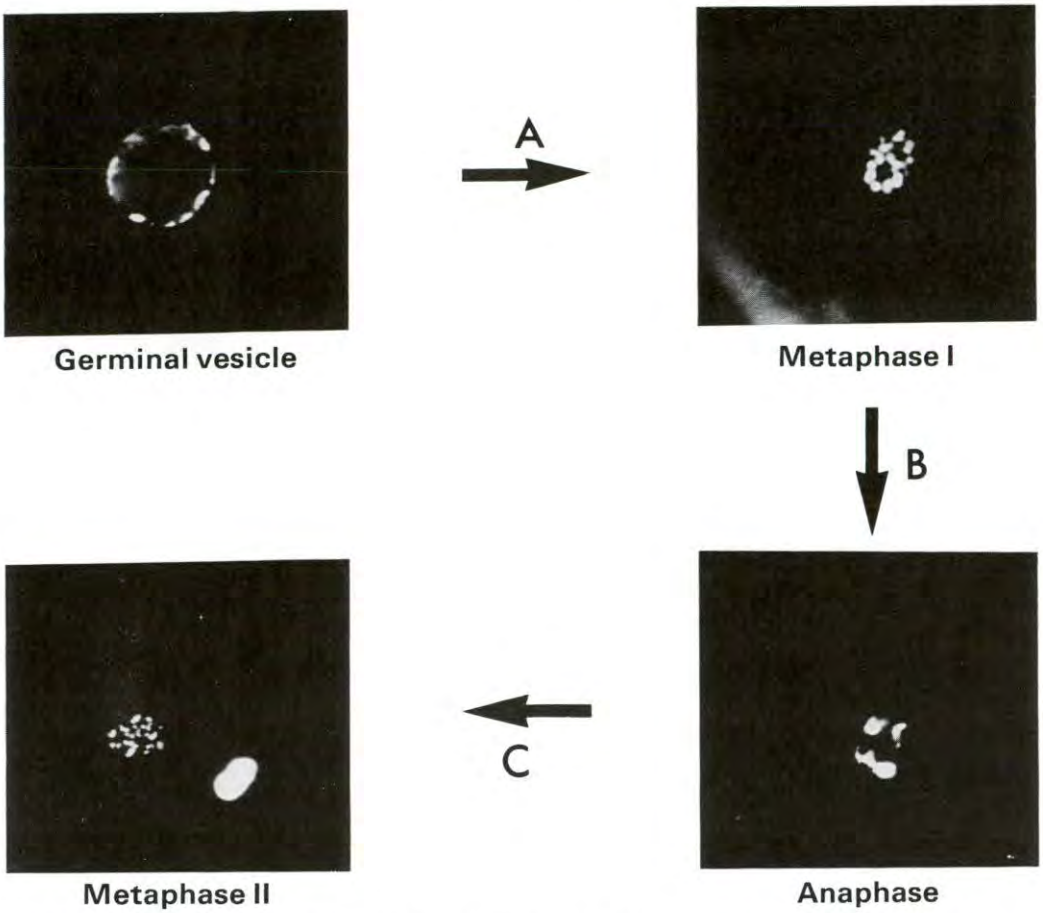

Fig. 5. Changes in the meiotic cycle from the initiation of maturation to its completion. New protein synthesis is required to drive the meiotic cycle into metaphase I (phase A), for the transition from metaphase I to anaphase (phase B) and for the inhibition of the cycle at metaphase II (phase C).

of early protein changes in sheep oocytes (Osborn & Moor, 1983a; Moor & Crosby, 1986). The great majority of changes in the oocytes of sheep are, however, not dependent on new transcription but are induced primarily by alterations to existing polypeptides by phosphorylation or glycosylation (Crosby *et al.*, 1984).

Changes in the rates of utilization of specific subsets of mRNA represent a further major regulator of protein change during maturation. The means by which stored message is selectively activated is unclear but cannot be explained by general changes in the 5′-capped or 3′-tailed status of the mRNA or by variations in the ability to process primary transcripts (see Johnson *et al.*, 1984). On the other hand, translation of at least one species of mRNA, that coding for β-actin, is regulated by the degree of junctional coupling that exists between the oocyte and the adjacent somatic cells (Fig. 3a). Disruption of junctional contact immediately terminates actin mRNA translation in sheep oocytes despite the continual presence of the transcriptions in the cytoplasm (Fig. 3b). Re-establishment of junctional contact with follicle cells or other somatic cells (Fig. 3c) rapidly re-initiates actin mRNA translation (Moor & Osborn, 1983). Experimental evidence from somatic cells suggests that changes in cell cytoarchitecture, induced by alterations to cell

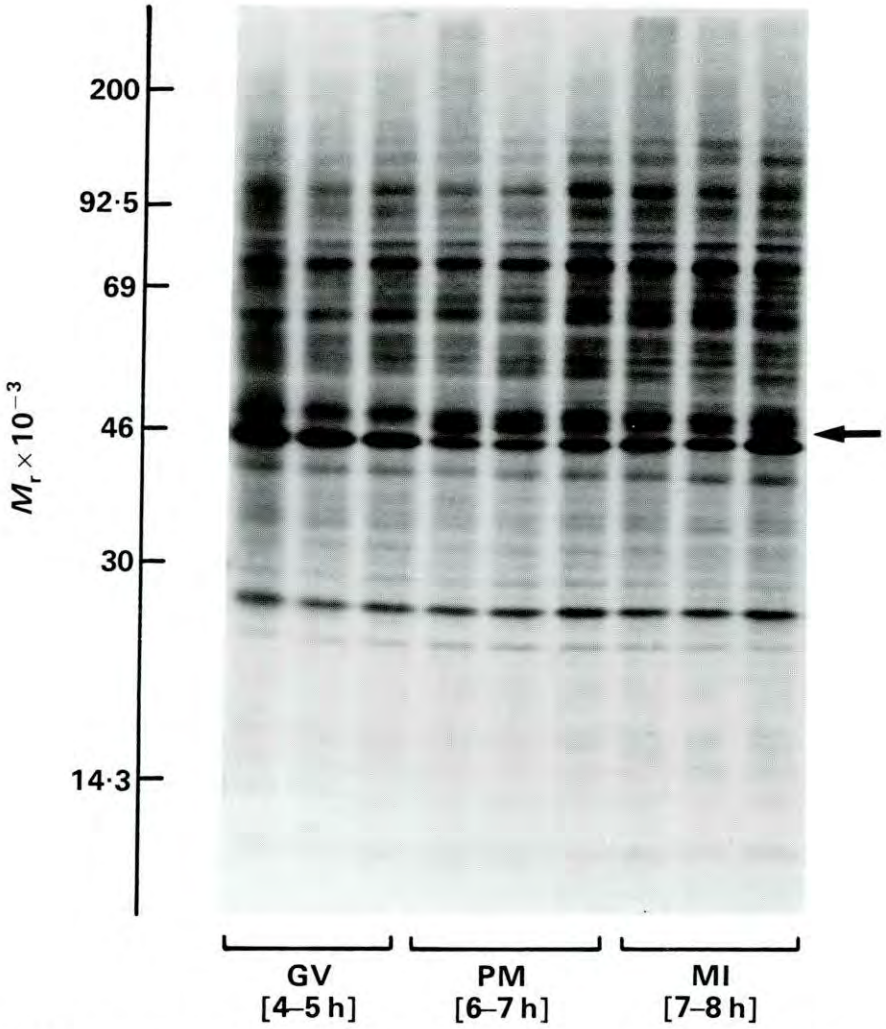

Fig. 6. Fluorographs of polypeptide profiles synthesized by oocytes labelled with [^{35}S]methionine for short periods during the 4 h before germinal vesicle breakdown. The appearance of a polypeptide (arrowed) with a relative molecular mass of 47 000 correlates closely with the breakdown of the germinal vesicle (Moor & Crosby, 1986).

shape and contact, affect actin mRNA translation by changing the association between mRNA, initiation factor and ribosomes (Farmer *et al.*, 1983; Howe & Hershey, 1984). A similar alteration to the cytoarchitecture of the oocyte might occur when junctional contact is disrupted and this could account for the cessation of actin mRNA translation in these cells. An alternative explanation has arisen from a careful examination of the actin mRNA molecule itself (Bachvarova *et al.*, 1987). Their work has indicated that actin mRNA becomes deadenylated in mouse oocytes after junctional disruption; it is postulated that the process of deadenylation may itself regulate translation of actin mRNA. However, junctional coupling, whilst important for the translation of some messages, does not appear to regulate translation of many other mRNA subsets. Although of central importance to maturation, the mechanisms involved in the translation of these other mRNA species are still uncertain. Similar uncertainty surrounds the mechanisms that selectively

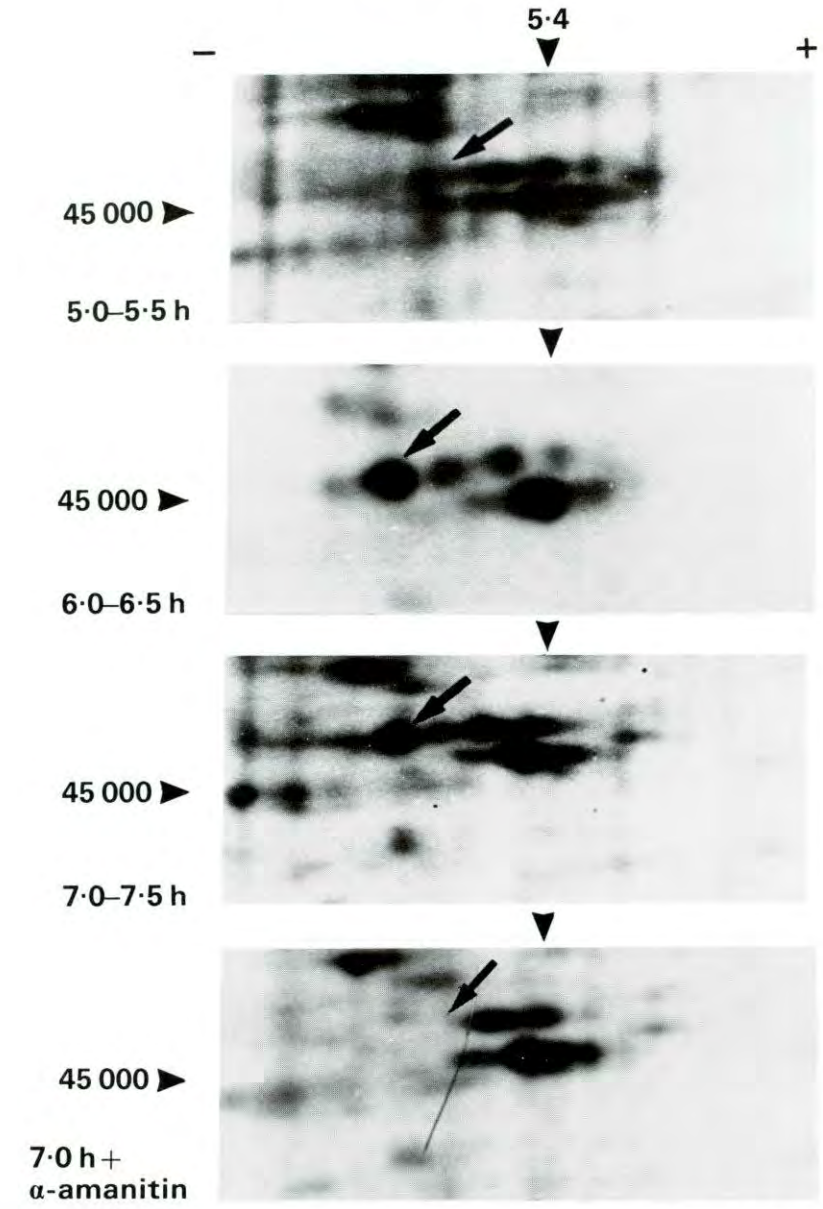

Fig. 7. Two-dimensional separations of the polypeptide m (arrowed) which is closely temporally related to the breakdown of the germinal vesicle. The inhibition of transcription with α-amanitin during the first 2 h after the induction of maturation prevents the synthesis of polypeptide m and blocks the breakdown of the germinal vesicle (Moor & Crosby, 1986).

alter the rate of synthesis and stability of certain polypeptides during maturation. Our approach to the study of these questions has firstly been to determine the efficiency of the translation apparatus in sheep oocytes (R. M. Moor, unpublished observation). Maturing oocytes have been microinjected with Tobacco Mosaic Virus–RNA (TMV–RNA), then labelled with [^{35}S]methionine and the resultant protein profiles quantitated by densitometry. The results clearly indicate that TMV-polypeptides are synthesized by injected oocytes (Fig. 4). In addition, it is clear from the

quantitative analyses that the proportion of endogenous polypeptides synthesized is reduced in direct proportion to the amount of TMV-polypeptide produced (data not shown).

The results demonstrate that exogenous mRNA competes for a strictly limited translational capacity within the oocyte. This is consistent with the hypothesis of Laskey *et al.* (1977) that the translational capacity of the oocyte is fully saturated during maturation. It follows that the translation of new mRNA must be accompanied by a reduction in the translation of existing messages.

Role of proteins synthesized during maturation. The occurrence of abnormalities in oocytes during fertilization is the most obvious consequence of incomplete maturation (Thibault, 1977). Thus, both the block to polyspermy and the cytoplasmic factors required to decondense sperm chromatin are absent in ovine oocytes fertilized during the GV or metaphase I stage of meiosis. Similar effects on fertilization can be induced by altering the steroid signals during the early phase of maturation (Moor *et al.*, 1980). Quantitative analyses of polypeptide patterns indicate that the ability to decondense sperm chromatin is associated with the synthesis of a small group of polypeptides between 12 and 18 h after the induction of maturation (Osborn & Moor, 1983b).

It is, moreover, known that some proteins synthesized during maturation persist throughout early development. While we are unable to ascribe particular functions to these proteins indirect evidence suggests that they may still exert an important effect up to blastulation (Moor & Trounson, 1977). It was observed in those experiments that intrafollicular oocytes matured *in vitro* in the presence of sub-optimal levels of gonadotrophin reached metaphase II, became fertilized and cleaved but failed to blastulate. If some proteins synthesized during maturation are possibly important at the blastocyst stage, others are required immediately after synthesis as regulators of the meiotic cycle. We have used protein blocks and two-dimensional gel electrophoresis to determine the relationship between protein synthesis and progression through the meiotic cycle (Moor & Crosby, 1986).

Meiotic cycle control mechanisms

Relationship between RNA synthesis, proteins and meiosis. Figure 5 shows both the sequence of nuclear events and the stages during meiosis at which new protein synthesis is essential for the continued progression of the cycle. Our results show that an early set of new proteins is required to drive the meiotic cycle from prophase to metaphase I and a second period of synthesis is required to facilitate progression from metaphase I to anaphase (Moor & Crosby, 1986). The third set of meiotic proteins is synthesized in the last 4–6 h of maturation and exerts an inhibitory influence on meiosis. These results are interesting, not only because they identify an essential period of synthesis, but also because they underline important differences between meiosis in rodents and domestic animals. No protein synthesis or transcription is required for the breakdown of the germinal vesicle in mouse oocytes (Stern *et al.*, 1972; Wassarman *et al.*, 1981). By contrast, both transcription and new protein synthesis are essential for the breakdown of the germinal vesicle in sheep oocytes. Experiments using the polymerase II inhibitor, α-amanitin, indicate that a 2 h period of RNA synthesis at the beginning of maturation is a prerequisite for GVBD (Osborn & Moor, 1983a). Moreover, protein synthesis is required 4–6 h later; the germinal vesicle breaks down about 90 min after the synthesis of these proteins (Moor & Crosby, 1986). The results of short-term radiolabelling experiments (Fig. 6) indicate that a protein with a relative molecular mass of ~47 000 and an isoelectric point of 5·8 is probably involved in the transition from prophase to metaphase I. Moreover, this protein is probably coded for by transcripts synthesized at the initiation of maturation since inhibition of transcription selectively inhibits the synthesis of this protein (Fig. 7).

Intrafollicular inhibitors of meiosis. From the above results it is perhaps not surprising that the regulators of meiotic maturation in mice and sheep do not appear to be similar. Exhaustive studies, initiated in amphibian oocytes and extended to the mouse, indicate that a phosphoprotein, a

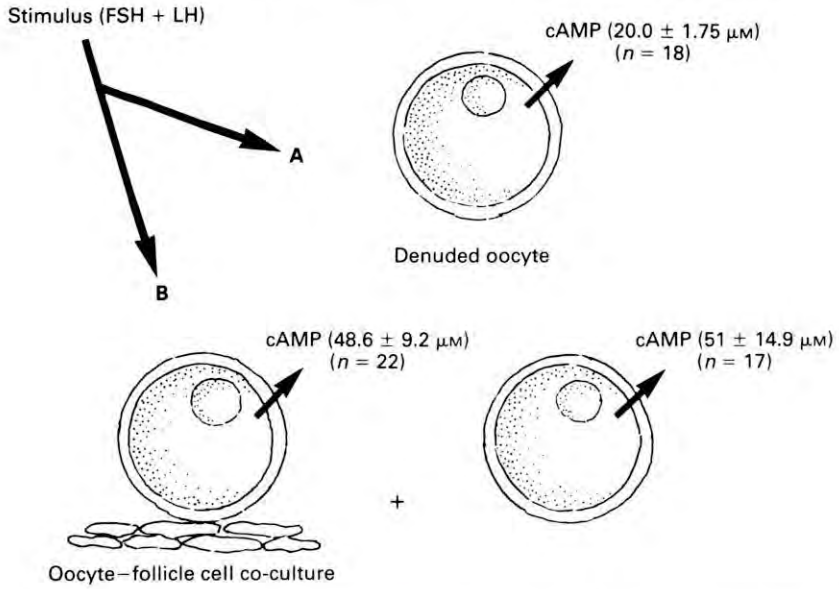

Fig. 8. The effect of gonadotrophins and follicle cell signals on the concentrations of cyclic AMP in ovine oocytes. Oocytes denuded of follicle cells and then exposed to gonadotrophins (panel A) have cyclic AMP concentrations not significantly different from those of untreated oocytes (see Moor & Heslop, 1981). By contrast gonadotrophins added to cumulus-enclosed oocytes (data not shown) or denuded oocytes co-cultured directly on follicle cells or in the same culture dish as follicle cells (panel B) significantly increase intraoocyte concentrations of cyclic AMP (Crosby *et al.*, 1985).

substrate of protein kinase, maintains meiotic arrest in mouse oocytes (Maller & Krebs, 1977; Bornslaeger *et al.*, 1986). A decrease in intraoocyte cyclic AMP and an accompanying decrease in protein kinase results in the net dephosphorylation of the inhibitory phosphoprotein and the consequent resumption of meiosis (Bornslaeger *et al.*, 1986). In sheep oocytes, cyclic AMP concentrations do not decline at the initiation of maturation but instead increase significantly at this time (Moor & Heslop, 1981). It appears further that the increase in intraoocyte cyclic AMP levels during maturation is initiated by gonadotrophins acting on the follicle cells (Crosby *et al.*, 1985). Signals from the follicle cells, acting as distance activators, stimulate the adenylate cyclase system, and consequently increase the cyclic AMP concentration, in the oocyte (Fig. 8). However, even though cyclic AMP does not appear to act as the meiotic inhibitor in sheep oocytes no other inhibitory factor has yet been identified in this species. It should be stressed that the requirement for early transcription and protein synthesis before GVBD in sheep oocytes presents novel possibilities for cell cycle regulation which warrant investigation.

The end of oogenesis is marked by the completion of intracellular reorganization and the formation of the second metaphase plate. However, it is not until the pool of stored mRNA is exhausted in early embryogenesis that the oocyte programmes cease to be a dominant directive force in development. At this time maternal regulation is replaced by the regulation exerted by the embryonic genome. The period from the end of oogenesis to the onset of genomic control by the embryo will be designated as the early embryonic phase. This phase encompasses both fertilization and the earliest materno–embryonic recognition processes. Since both these events are the subject of detailed consideration elsewhere (First & Parrish, 1987; Sasser & Ruder, 1987) attention will be focussed here solely on the utilization of maternal transcripts during early embryogenesis.

Early embryonic phase

The early embryonic phase, representing a period of distribution and utilization of stored components, is under dual molecular control in mouse eggs (Pratt *et al.*, 1983). The maturation programme, which confers on the oocyte the potential to initiate development, continues throughout early development to direct the housekeeping functions of the cell. Superimposed on this is a sequence of specialized changes in synthesis which are initiated or accelerated by sperm penetration. Howlett & Bolton (1985) postulate further that this fertilization programme is not only the direct initiator of embryogenesis but also performs the function of terminating the oocyte programme.

The results of our experiments in sheep are entirely compatible with the above dual control concept. Protein synthesis in unfertilized sheep oocytes continues unchanged for many hours after ovulation (I. M. Crosby and R. M. Moor, unpublished observations). Fertilization imposes on this oocyte-directed pattern of synthesis specific but limited changes in protein profiles. Despite similarities in control mechanisms, the duration of the resultant early embryonic phase differs markedly between species. Thus, the transition from the maternally-directed early embryonic phase to the later embryo-directed phase occurs at the mid-2-cell stage in mice, the 4–8-cell stage in rabbits and the 8–16-cell stage in sheep (Manes, 1973; Young *et al.*, 1978; I. M. Crosby & R. M. Moor, unpublished observations). It is with the molecular events that occur in the egg before the transition that our current work is directed.

Protein and RNA utilization during the early embryonic phase

Protein modulation. Fertilization is the trigger for a number of protein changes in the sheep egg. Firstly, studies on the effects of acrosin suggest that certain macromolecules associated with the zona undergo limited proteolysis during fertilization (Brown, 1986). The glycoproteins most affected by sperm enzymes are probably not native to the egg: these glycoproteins are produced by the oviduct and thereafter bind avidly to the zona pellucida (Brown, 1986). Other modifications induced by fertilization include changes to two intracellular polypeptides (M_r = 47 000 and 67 000), but we are not able at present to ascribe specific functions to these proteins.

Once these fertilization-induced modifications are completed no other consistent changes in the polypeptide profiles are seen until the onset of genomic control by the embryo (I. M. Crosby & R. M. Moor, unpublished observations).

Although the profile of polypeptides remains constant at this time major changes occur in the rates of amino acid incorporation during the early embryonic phase (I. M. Crosby & R. M. Moor, unpublished observations). Between the 4-cell and 8-cell stage the rate of [^{35}S]methionine incorporation, after correction for changes in precursor uptake, declines precipitously and remains low until after the transition to the embryo-directed phase of development. The fall in amino acid incorporation, and thus in the synthesis of new protein, does not imply that sequestered proteins are also degraded during this period. Indeed, indirect evidence presented earlier suggests that proteins synthesized during oogenesis persist until at least the blastocyst stage.

RNA utilization. No direct evidence exists in sheep on the rate of mRNA degradation during the maternally-directed early embryonic phase. By contrast, careful measurements in mouse eggs show that virtually all the maternally derived mRNA is degraded by the late 2-cell stage in this species (Bachvarova & De Leon, 1980). Whilst most of this loss occurs at the 2-cell stage, earlier losses at the 1-cell stage have also been detected (Piko *et al.*, 1984). It is tempting to speculate on the possibility that the drop in protein synthesis in the 8-cell sheep egg reflects a similar but delayed period of degradation of maternal transcripts. However, until measurements of poly(A)$^+$RNA levels are made in cleaving sheep eggs nothing definite on RNA metabolism can be concluded from our experiments.

Concluding remarks

Oogenesis in the sheep represents a period of synthesis and sequestration of products required for the support of early embryogenesis. However, the fragmentary nature of our knowledge about these early processes in sheep is clear: corresponding information on other large mammals is even more sparse. The progressive miniaturization of molecular technology and the increased availability of oocytes and embryos from in-vitro sources provide the embryologist with unique opportunities for critical experimentation. It is imperative that work on the oocytes and embryos of domestic animals should be based on these modern embryological techniques.

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