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21. Ovum Maturation

CONTROL MECHANISMS IN OOCYTE GROWTH AND MATURATION

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SUMMARY

Development of fertilizable female gametes involves characteristically nuclear and cytoplasmic transformations during the processes of oocyte growth and meiotic maturation. Present evidence concerning the nature and intra- and extra-oocytic origin of molecules regulating selected aspects of oocyte differentiation are discussed. Particular attention is given to the manner in which specific signals or triggers of oocyte maturation are transduced to change the properties of the oocyte. Data concerning the synthesis and local action of steroid hormones with the amphibian ovary and oocyte are discussed. The effects of secondary cytoplasmic factors, produced in response to steroids, on oocyte growth and maturation as studied in intact and enucleated oocytes are described. The results suggest that the developmental program underlying growth, meiotic arrest, reinitiation of meiosis and subsequent cell division is closely linked to morphological and molecular events occurring in the oocyte membrane and cortex.

INTRODUCTION

Embryogenesis is typically initiated at the time of the union of the male and female gametes. However, the consequences of such a union are determined to a major extent by the maturational stage of the oocyte cytoplasm and nucleus at the time of fertilization [1, 2, 3]. Recent evidence indicates that nuclear and cytoplasmic maturation are not necessarily synchronized and that such differences may be linked to marked alterations in the developmental potential of the gametes [4, 5]. In many species, including most vertebrates, fusion of the sperm of immature, germinal vesicle stage oocytes, arrested at prophase I of meiosis, can be achieved without triggering any subsequent oocyte or embryo differentiation [6, 7]. However, following the interruption of arrested meiosis, oocytes typically develop the capacity to undergo development after fertilization. Comprehending the mechanisms by which oocyte maturation is controlled and mediated is thus crucial to the understanding of a continuum of processes extending from the intra-ovarian through to the extra-ovarian periods of oocyte differentiation. In the present article, I shall review our knowledge concerning the manner by which specific signals or triggers of oocyte maturation are transduced to change the properties of the oocyte and control the expression of the underlying developmental programme inherent within the germ cells. Particular attention will be given to the role of the oocyte cell membrane in the process of amphibian oocyte and follicle differentiation. Amphibians provide an extremely useful model for elucidating hormone-cell interactions, since it has been shown that certain steroid hormones can act as effective initiators and control many aspects of nuclear and cytoplasmic maturation [8, 2]. Steroid hormones, however, do not serve as a universal trigger for interrupting meiotic arrest in oocytes of all species. A variety of hormonal and nonhormonal mechanisms appear to have evolved to control this event and/or other closely associated aspects of oocyte differentiation and early development [5]. In species such as *Spisula solidissima*, it appears that the fertilizing sperm triggers meiosis reinitiation and development by means of some calcium dependent mechanism [9]. Furthermore, in the amphibians, extensive evidence indicates that oocyte nuclear breakdown and cytoplasmic maturation are not directly induced and controlled by the steroid hormones, but occur as a result of the formation or activation of secondary cytoplasmic factors which originate in the cytoplasm or nucleus or as a result of nuclear-cytoplasmic interaction. The term, maturation promoting factor (MPF), has been coined to designate that cytoplasmic factor(s) which acts within the oocyte to cause disintegration of the nucleus or germinal vesicle [10, 11]. Whether such cytoplasmic factors have single or multiple activities within the oocytes remains conjectural.

THE MATURATION TRIGGER AND STEROIDOGENESIS

Present evidence indicates that the primary stimulator of nuclear and cytoplasmic maturation in the amphibian oocyte is progesterone and/or a closely related hormone produced intrafollicularly by the somatic cells. Thus, control of meiosis can be considered in terms of the various factors and cellular mechanisms which regulate the synthesis and secretion of these hormones. Early studies established that a range of common progestational, androgenic and adrenocortical molecules effectively induced *in vitro* maturation of follicular enclosed or isolated oocytes; thus, a high degree of structural specificity

is not associated with biological activity [8, 12, 13]. Oestrogenic steroids are characteristically inactive in initiating maturation, but play a crucial role in the process of vitellogenesis and oocyte growth [2]. Additional studies of molecular structure-function relationships, with regard to ovulation and oocyte maturation, show that many other steroids are biologically inactive and that the physical arrangement of groups on the surface of the hormones plays an important role in determining biological function [14]. Delta-4 as well as Δ^5 steroids are biologically active as are many 5α and 5β reduced compounds of progesterone [15, 16, 17]. Progesterone and other steroids are incorporated extensively into follicles and oocytes and undergo rapid metabolic conversion within these various cells [15, 12, 18]. In many cases it has not been determined whether metabolism of steroids reflects separate or combined enzymatic activities of germinal and somatic cells. Significant oocyte uptake of steroids (oestrogens) inactive in inducing maturation occurs; this suggests that incorporation does not ensure or even reflect biological activity. Interestingly, addition of a sulphate group to pregnenolene renders this steroid biologically inactive and markedly retards its inclusion in the oocyte [1, 12]. The steroids which induce maturation and the role of metabolism in the process of maturation are areas still unresolved. It has been suggested that the stepwise reduction of incorporated progesterone serves to terminate the specific effects of the hormone [17]. *In vitro* stimulation of the synthesis and secretion of a variety of steroids by ovarian follicles, including progesterone and oestrogens, has been reported [19, 20, 21, 18]. Amounts and types of steroids synthesized are related to the state of ovarian follicular growth and differentiation. The exact metabolic pathways by which these follicular steroids are synthesized also remains largely unknown. Bioassay, biochemical and histochemical evidence, however, all indicate that the 3β-hydroxysteroid dehydrogenase-isomerase enzyme complex is intimately involved in mediating maturation [22, 23, 24, 2]. Cyanoketone, a specific inhibitor of this enzyme, blocks maturation induced by pituitary extracts and threshold doses of pregnenolene (enzyme-substrate), whereas it has no inhibitory effects on the maturation inducing activity of progesterone (enzyme product) [25]. Oestrogenic steroids produce similar effects. Pregnenolene conversion to progesterone, as monitored isotopically, is also suppressed by either cyanoketone or oestrogen treatment. A possible role of oestrogens in a direct feedback control of these enzymes has been postulated [26, 27].

MEDIATION OF MATURATION AND MPF PRODUCTION

A short period of oocyte exposure to steroid is sufficient to initiate disintegration of the germinal vesicle. This nuclear breakdown occurs following a delay of many hours [28]. Stimulation of protein synthesis and formation of cytoplasmic MPF are invariably associated with this transformation [10, 28, 29]. When added in the early post-steroid exposure period, inhibitors of protein synthesis, such as cycloheximide, readily prevent maturation and MPF formation. Insensitivity to cycloheximide, however, develops some hours prior to actual disintegration of the germinal vesicle. We have shown that cycloheximide inhibition of oocyte maturation is readily reversed. These results suggest that certain steroid induced events are initiated even in the presence of cycloheximide [30]. Oocytes treated with steroid and cycloheximide for 24 h and subsequently washed underwent maturation without additional steroid treatment. Presumably, the initial steroid exposure imprints the steroid maturational message into oocyte, and cycloheximide is rapidly lost from its site of action. Furthermore, the time course of oocyte maturation following washing was not markedly different or was only slightly delayed compared with the time course of maturation observed in freshly treated oocytes. The lack of accelerated nuclear breakdown following such washing thus suggests that cycloheximide prevented the formation rather than the effects of MPF.

Involvement of ions, especially calcium, cyclic nucleotides, adenyl cyclase, protein phosphorylation and cAMP-dependent protein kinase, have also been implicated in the maintenance of meiotic arrest and meiosis reinitiation [31, 32, 33, 34, 35, 37, 38, 39, 26]. Steroid induced maturation is inhibited by removal or complexing of external and internal stores of calcium [40, 8]. Exposure of *Xenopus* and *Rana* oocytes to lanthanum ions, which presumably displace calcium ions, also induced maturation and MPF production [41, 42]. A marked and rapid drop in the levels of cAMP in *Rana* and *Xenopus* oocytes occurs following exposure to progesterone [34, 38, 26]. Inhibition of maturation, protein synthesis and the cAMP decrease was observed following treatment of oocytes with theophylline, a cyclic nucleotide phosphodiesterase inhibitor. Purified regulatory subunit of cAMP-dependent protein kinase was shown to induce nuclear maturation, whereas the catalytic subunit of this enzyme inhibited steroid induced maturation when injected into oocytes [36]. In most cases, these factors or treatments which mimic progesterone require protein (MPF) synthesis for oocyte maturation; they presumably act during some early stage of the induction process. In many instances, it has not been established whether oocytes matured under these conditions undergo a normal sequence of maturation and are capable of embryonic development.

Mediation of steroid induced maturation in the various amphibian species (primarily *Xenopus* and *Rana*) is considered to occur by common mechanisms. Oocytes of both species respond to the same steroid. Additionally, MPF produced in either species effectively induces nuclear breakdown when injected into

oocytes of the other species [43]. Recent experiments, however, indicate that there are differences in the nature and/or activity of the MPF generated in the oocytes of these two species. Following injection of steroid-induced *Rana* MPF into other *Rana* oocytes maturation was induced; however, this effect was invariably inhibited when injected oocytes were also incubated with cycloheximide [42]. MPF induced by lanthanum treatment also exhibited the same sensitivity to cycloheximide inhibition. Nuclear disintegration, therefore, does not occur directly in response to MPF, but only following additional protein synthesis. In contrast, *Xenopus* MPF injected into *Xenopus* oocytes induces nuclear breakdown, even in the presence of cycloheximide [44, 29]. The basis for this difference in sensitivity to cycloheximide remains unresolved, but may indicate that more than one protein is required for nuclear disintegration.

SITE OF PROGESTERONE ACTION

Particular attention has been given to defining where within the oocyte the steroid signal is transduced such that cytoplasmic MPF and maturation are initiated. The cell surface has been implicated strongly in these reactions, although in most cases it is not clear whether cell surface refers to the plasmalemma or the cell membrane and its closely associated cytoplasm. Injection of progesterone into oocyte cytoplasm does not lead to maturation and this result has been interpreted to suggest that the effects of the steroid are on the oocyte surface [10]. Similar conclusions have been reached concerning the site of action of other maturation initiating factors. Other data suggest a possible alternative explanation for the inactivity of injected steroids. Experiments involving the localized external application of steroids to the follicle and oocyte surface show that incorporated steroid remains primarily localized in the region of application and does not diffuse rapidly through the cytoplasm [45, 46]. The proportion as well as the specific region of the external gamete surface exposed to the steroid also markedly alters the biological activity of the steroid. The animal pole was found to be much more sensitive to the steroid than the vegetal pole. Thus, failure of injected hormone to induce maturation may be linked to a slow rate of steroid diffusion within or out of the oocyte rather than be due to a lack of interaction with the surface. Clearly a more uniform rate of steroid access to the "activation" site can be achieved when the hormone is present in the surrounding culture media. A surface effect of steroids has also been postulated as a result of studies involving the use of hormone linked to a polymer or agarose beads [47, 48].

OOCYTE GROWTH AND MATURATION

The growth process in oocytes plays a key, but poorly defined, role in controlling the maturation events within the oocyte. This is suggested by the fact that steroid induced maturation typically occurs only in the larger "fully" grown oocytes, whereas the previtellogenic and small and intermediate-sized vitellogenic oocytes do not respond to such treatment. Failure of smaller oocytes to undergo maturation has been linked to their inability to respond to the hormone and produce MPF, rather than to an inability of the oocyte nucleus to undergo disintegration. This conclusion is suggested by the results of cytoplasmic transfer studies [49]. Germinal vesicle breakdown and chromosome condensation were both seen when small (Stage III) and medium-sized (Stage IV), hormone insensitive *Xenopus* oocytes were injected with MPF. Furthermore, gradations in the response of smaller oocytes to MPF were observed, including failure of nuclear breakdown, migration to the animal pole and maturation spindle formation. Interruption of oocyte growth is also produced by certain steroids and this activity is closely associated with the size of the oocyte and the maturation inducing activity of the hormones [50]. The cellular mechanisms for transducing the steroid trigger, as well as those interrupting growth, thus appear or complete their differentiation in association with growth of the oocyte. These changes may also be linked to cell surface functions.

THE CELL SURFACE: MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES

Despite the lack of precise information concerning the cellular site of progesterone action, considerable experimental evidence demonstrates that the cell surface undergoes a dramatic series of morphological and physiological changes in association with the processes of follicular and oocyte maturation and establishment of conditions for activation [1, 2, 13]. Particularly striking are those changes involving dissociation of the microvillus connections between the oocyte plasmalemma and vitelline membrane and the macrovillus connections between the follicle cells and the oocyte plasmalemma. These morphological changes are readily induced *in vitro* in the ovarian follicle of defolliculated oocytes following exposure to steroid or pituitary hormone stimulation and are closely coordinated with germinal vesicle breakdown and ovulation. The capacity of the oocyte cell membrane to transport proteins and other molecules is also altered during the course of these maturational events. Fully grown, immature oocytes retain a residual capacity to incorporate the cytoplasmic yolk platelet precursor vitellogenin via the cell membrane [50]. Exposure to steroid hormones, which induce maturation, suppresses this incorporation, presumably by inhibiting micropinocytosis. Marked changes in the surface area of the oocyte, however, also occur in relation to these events and may play a role in inhibition of vitellogenin incorporation. Cytoplasmic transfer experiments further demonstrate that cytoplasm taken from mature, but not immature, oocytes effectively suppresses oocytic incorporation of

vitellogenin [51]. Regulation of the cell membrane activity thus appears to be mediated in part by an intracellular cytoplasmic factor rather than directly by the steroid hormone.

Other studies also demonstrate that steroids induce alterations in the capacity of the oocyte cell membrane to transport sodium and potassium (Schuetz, unpublished, 1). Depolarization of the oocyte cell membrane has been observed, even in the absence of the nucleus, following maturation induction and shown to be dependent upon prior protein synthesis [52]. Separation of the vitelline membrane from the oocyte plasmalemma and cortical granule rupture occur at the time of egg activation and are both dependent upon prior initiation of maturation [1, 13]. On the basis of such studies, it appears that the cell membrane plays an important role in controlling initiation and mediation of the oocyte maturation process.

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