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From oocyte maturation to the in vitro cell cycle: the history of discoveries of Maturation-Promoting Factor (MPF) and Cytostatic Factor (CSF)

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Abstract This article briefly reviews the classical cell cycle studies using oocytes and zygotes of mainly amphibians in the past century. The discussions are focused on the investigations into the cytoplasmic factors that regulate meiosis during oocyte maturation and the initiation of mitosis during fertilisation, which were carried out in the author's lab between 1967 and 1987. This chronicle traces the development of the problems and the direction in which their solutions were attempted in the course of these investigations. The author tries to answer the following questions: why he decided to study oocyte maturation, how he discovered progesterone as a maturation-inducing hormone, how he discovered and characterised the cytoplasmic regulators of the cell cycle, Maturation-Promoting Factor (MPF) and Cyto-Static Factor (CSF), and how he invented the method of observing cell cycle processes in a cytoplasmic extract in vitro.

Key words cell-cycle · amphibian · oocyte · zygote · meiosis · mitosis · Maturation-Promoting Factor (MPF) · Cyto-Static Factor (CSF)

Introduction

It was half a century ago, when I was in my senior year as a student of zoology, that animal development, cell differentiation in particular, began to capture my imagination by its mysterious features and became the most fascinating subject among those I was studying. I learned from Hans Spemann's book (Spemann, 1938; p. 202) that development is the expression of gene activities in embryonic cells. However, in those days, geneticists were spending most of their time looking for genes that control enzyme activities in metabolism. Cell biologists were also busy describing fine structures and biochemical activities of the cell, being enchanted by powerful techniques of cell analysis such as phase- and electron-microscopy and cytochemistry. Embryologists were trying to understand the mechanism of development, but still followed the 19th century tradition Entwicklungsmechanik (developmental mechanics) proposed by Roux. One of their major problems was that of embryonic induction by the organizer in amphibian embryos, which they were investigating using simple microsurgical techniques. This approach to development seemed to better suit my taste for science than genetic analysis of metabolic activities and descriptions of fine structure and chemistry of the cell. I chose the problem of embryonic induction for my graduate research project.

Then, one day, I came across the paper by Markert published in 1958. In this article, he stated, that “the preferred viewpoint in this presentation has pictured a cell, when subjected to differentiating stimuli, as responding through the activation of a gene which then initiates a new reaction” (Markert, 1958; p. 11). Reading this article, I was impressed by his insight, namely differential gene activation in development, and was strongly convinced that the essential process of development involved nucleocytoplasmic interactions in the cell. At that time, most people in his lab were analyzing lactic acid dehydrogenase (LDH) isoenzymes in various animals and their changes during development. I asked Professor Markert, who was busy as Chairman of the Department of Biology at Yale University, to teach me how to approach the problem of cell differentiation through isoenzyme analysis. Thus, I joined his lab in 1966 on sabbatical leave.

He gave me packs of deep-frozen penguin embryos...
sent from the Antarctic by an expeditionary team and suggested a project to clarify developmental changes in penguin LDH isoenzyme pattern. After a half year of hard work, I found that the penguin has 3 different LDH subunits, generating 15 different tetramer isoenzymes, while mammals have 2 different subunits and 5 isoenzymes, but their developmental changes were too complicated to deduce any rule of their differentiation (Markert and Masui, 1969). This taught me that in order to develop even a single enzyme, embryonic cells must undergo complex changes in gene expression. At this point, Markert said “That’s enough”, and suggested that I should work on a project in which I am really interested, but which would be inexpensive so that I could continue in Japan.

The experience with penguin LDH led me to the pessimistic view that it was impossible to study cell differentiation at the molecular level using the 1960s’ technology. I was not convinced that all biochemical methods available at that time were sensitive enough to analyse such subtle biological processes as embryonic induction. Many embryologists also seemed to have lost confidence in discovering the mechanism of cell differentiation for other reasons. First, the discovery that a variety of chemically unrelated substances could act as the inducer of embryonic induction made us suspicious about the requirement for its specificity as the inducer of cell differentiation. Secondly, being influenced by the immunologists’ idea of clonal selection as the cause of cell differentiation (Burnet, 1959), embryologists also began to question the nature of the process of cell differentiation, whether it is cell transformation or clonal selection by the inducer. To skirt around this question I needed a cell system in which I could observe the differentiation behaviour of a single cell under the influence of a well-defined inducer. I thought that oocyte maturation could satisfy these requirements.

**Oocyte maturation**

It was in 1962 that I learned for the first time about oocyte maturation, when Professor Nace of the University of Michigan visited my laboratory on his sabbatical leave and showed me Heilbrunn’s classical experiment (Heilbrunn et al., 1939). This experiment demonstrated that a piece of frog ovary suspended in Ringer solution could be induced to ovulate oocytes *in vitro* when a macerated pituitary suspension was added to the solution. I also read Brachet’s comment on oocyte maturation in his book “Chemical Embryology” published in 1951: “This whole field, so interesting both from the point of view of embryology and of cellular physiology, remains to be explored” (p. 146). I surveyed the literature, reviewed previous work on oocyte maturation (Masui, 1967a), and learned the general features of oocyte maturation in the animal kingdom.

In all animals, oocytes growing in the ovary are arrested in the prophase of the first meiosis (P I), the G2 phase of the cell cycle. Fully grown oocytes resume meiosis when oocyte maturation begins, leading to the formation of an egg which can be fertilised (Fig. 1). In some species, such as the surf clam, *Spisula solidissima* and the echiuroid, *Urechis caupo*, oocyte maturation is initiated by fertilisation after the oocytes are spawned. However, in most animals oocyte maturation is initiated prior to ovulation when the ovary is stimulated by gonadotropin secreted from the pituitary in vertebrates or from nerves in invertebrates. Therefore, in the frog, oocyte maturation is induced by the special signal, pituitary gonadotropin, and has a well-defined endpoint, egg maturity. In addition, all changes during oocyte maturation can be observed in a single cell, the oocyte.

The first visible sign of oocyte maturation is “germinal vesicle breakdown (GVBD)”, when the large nucleus of the oocyte, called the germinal vesicle (GV), breaks down to release its nucleoplasm into the cytoplasm. This is followed by chromosome condensation to a metaphase state before the first meiotic division. If oocyte maturation is initiated by fertilisation, as in some invertebrate species as mentioned above, the oocyte completes two meiotic divisions without interruption, which is immediately followed by mitosis. If oocyte maturation is induced by gonadotropin before fertilisation, three different cases are distinguished: mature oocytes arrest 1) at metaphase of the first meiosis (M I) as in the insect and tunicate, 2) at metaphase of the second meiosis (M II) as in vertebrates, and 3) in G1 phase at the pronuclear stage as in sea urchins and jelly fish. In all these cases, for the eggs to be relieved from the arrest, complete meiosis, and proceed to mitosis they must be activated by fertilisation or artificial stimuli (Fig. 1).

Markert often talked about the economic value of cloning homozygous animals from diploid oocytes of a good strain by suppressing meiotic divisions followed by parthenogenesis. To this end, he suggested that I might try to find a way to suppress meiosis in frog oocytes. However, I thought that in order to suppress meiotic divisions, I should know how oocyte cell cycles are regulated. Thus, I realised that frog oocyte maturation would be a highly advantageous system over other cell systems for studying control of cell cycle events, because I could use stimuli that induce oocyte maturation and egg activation as separate signals to drive the cell cycle from G2 to M and then from M to G1, respectively. This could eliminate the problems of synchronizing cell cycles, always involved when we study the cell cycle in multicellular systems and therefore allow us to handle cells at a specific cell cycle stage with high accuracy.

For my study, I chose oocytes of *Rana pipiens*, the most common frog in North America and easily available from local dealers. The fully grown oocytes are easy to culture in a simple salt solution such as Ringer’s, and their large size ranging from 1.5 to 2.0 mm in diameter.
and resistance to injuries allow us to operate microsurgically as demonstrated by successful nuclear transplantation experiments (DiBerardino, 1997 for a review). Therefore, I hoped to develop a subcellular Entwicklungsmechanik of nucleocytoplasmic interactions in an inexpensive way, but also I thought that it would not be difficult to perform biochemical analysis as well, because a large quantity of oocytes at a same phase of the cell cycle can easily be obtained.

**Nucleocytoplasmic relations in oocyte maturation**

The analysis of relations between the nucleus and cytoplasm in development has a long history. It was Hertwig (1903 and 1908) who first brought to our attention the importance of nucleocytoplasmic relations for cell physiology. He interpreted alternation of cell division and cell growth as a homeostatic mechanism that maintains nucleocytoplasmic ratio; but he also pointed out exceptions, such as the oocyte which grows without division and the zygote which divides without growth. There had been accumulated many classical observations on oocytes and zygotes in various animals which showed essential roles of nucleocytoplasmic relations in cell division and differentiation (Gurdon and Woodland, 1968, for a review).

It was at the beginning of the 20th century that embryologists discovered the important role of the nucleoplasm of the GV in cell divisions of the zygote as reviewed by Wilson (1925). They fertilised oocyte fragments lacking the GV which had been separated from an oocyte before GVBD. These cytoplasmic fragments prevented from receiving nucleoplasm from the GV failed to initiate mitosis. On the other hand, fragments containing the GV or those separated after GVBD which had received nucleoplasm could undergo mitosis and develop (Fig. 2). In the sea urchin and frog, it was also found that when prematurely inseminated eggs failed to be activated, egg chromosomes remain at metaphase and sperm chromosomes incorporated into the egg cytoplasm are also condensed to a metaphase state (Gurdon and Woodland, 1968, for a review). This observation suggested that the cell cycle activity of the nucleus, either of the egg or of the sperm, conforms to that of maturing oocyte cytoplasm.

However, no further studies on this problem had been carried out until the early 1960s. Even in 1967 when I began my study of oocyte maturation, I could find no more than a few papers which dealt with the nucleocytoplasmic interaction during maturing oocytes. In 1961, Subtelny and Bradt showed that somatic nuclei transplanted into Rana pipiens oocytes at M II underwent mitosis if the recipient oocytes were activated, whereas those transplanted before M II did not. In 1964, Dettlaff and her associates, using toad oocytes, demonstrated that while oocytes maturing with the GV could develop the cell surface contractility to become capable of cell division, those whose GV had been removed beforehand fail to do so. Thus, they confirmed the discovery made 60 years ago on the role of GV nucleoplasm in cell divisions in marine invertebrate oocytes as mentioned before.

In 1966, Iwamatsu, using the medaka fish, found that the GV failed to break down in gonadotropin-stimulated oocytes if it had been displaced from the hyaline cytoplasm into yolk cytoplasm by a centrifugal force. This suggested that the factor which could cause GVBD was not the hormone itself but the hyaline cytoplasm stimulated by the hormone.
Fig. 2 Experimental scheme for testing the role of nucleoplasmic substances (dots) derived from the germinal vesicle (GV) in promoting cell division of fertilised eggs of marine invertebrates (Cerebratulus and starfish) (Wilson, 1925).

In the latter half of the 1960s, more studies on cytoplasmic control of nuclear activities appeared (Gurdon and Woodland, 1968; De Terra, 1969; Johnson and Rao, 1971 for reviews). Gurdon, Graham, and their associates using Xenopus laevis demonstrated that somatic nuclei transplanted into a zygote are induced to synthesize DNA and enter mitosis synchronously with the host cell; those put into ovarian oocytes cease DNA synthesis and initiate RNA synthesis, and those put into maturing oocytes to condense chromosomes to a metaphase state (Gurdon, 1968). Clearly, activities of transplanted nuclei always conform to those of the host cells regardless of their original activities in the donor cells, indicating the strong control of the cytoplasm over the nucleus. Similar evidence for the cytoplasmic control over the cell cycle activity of the nucleus had also been provided by exchanging the nucleus or cytoplasmic fragments in protozoa (De Terra, 1969) or by fusing cultured mammalian cells (Johnson and Rao, 1971) in different cell cycle phases. However, all these studies provided us with no clue to the nature of the factor involved in the cytoplasmic control – whether it is the chemical activity of a specific molecule in the cytoplasm or a physiological function of the cytoplasm as a whole.

**Hormonal regulation of oocyte maturation**

Although the oocytes of marine invertebrates, which mature and can be fertilised in sea water, had been excellent materials for embryological as well as cytological studies since the late 19th century, the natural cause of oocyte maturation in these animals was unknown for a long time, except for cases of maturation induced by fertilisation. On the other hand, in 1935, Pincus and Enzman found in mammals that fully grown oocytes isolated from the ovarian follicle could mature spontaneously when cultured in vitro. This suggested that removal of the oocyte from the maturation-inhibiting effect of the follicular environment by ovulation is the cause of oocyte maturation. However, in 1939, Heilbrunn et al., using the frog as mentioned before, demonstrated for the first time that ovulation and oocyte maturation could be induced by pituitary gonadotropin. Later, it was also found that the effect of gonadotropin could be augmented by progesterone (Zwarenstein, 1937; Burgers and Li, 1960; Wright, 1961). It was not until the late 1950s that gonadotropin was discovered in the invertebrates, when gonad-stimulating substance was extracted from radial nerves of the starfish and shown to induce ovulation and oocyte maturation (Chaet and McConnaughty, 1959; Kanatani, 1964).

However, even in the early 1960s it was unclear how these hormones act on the oocyte. Therefore, the study published in 1964 by Dettlaff and her colleagues bore the most important significance to answering this question. They isolated toad oocytes from the ovary by removing the surrounding follicular epithelium with forceps and cultured them in Ringer solution containing a suspension of macerated pituitary. These oocytes were found to acquire “maturation inertia” just before GVBD and produce the nucleoplasmic substance in the
Fig. 3 Experiments showing relative roles of pituitary gonadotropin, follicle cells, and progesterone in the induction of maturation of frog oocytes (Masui, 1967b).

GV that the authors showed to have the ability to induce maturation when injected into other oocytes without hormonal stimulation. Thus, once oocytes acquire maturation inertia they no longer require the presence of the hormone in the medium to continue the subsequent maturation process. From these results it was concluded that the hormone directly acts on the oocyte nucleus to induce new gene activities. This conclusion seemed to conform to the view of hormonal actions on the cell widely popular in those days, that is, gene activation by hormones (Davidson, 1965).

In early 1967, I repeated these experiments using Rana pipiens. I observed that when intact ovarian fragments were treated with gonadotropin, fully grown oocytes undergoing GVBD were shed from follicles (Fig. 3a). However, when I isolated oocytes manually from the ovary by removing follicular epithelium with forceps, I found under a dissection microscope at high magnification that many cells still adhered to the oocyte surface. It was these oocytes that remained responsive to the hormone (Fig. 3b). To obtain oocytes completely free of follicle cells, oocytes had to be washed in Ca-Mg-free Ringer solution to which EDTA was added. These clean oocytes were found to be no longer responsive to the hormone (Fig. 3c). However, they were capable of maturation when incubated with follicle cells in the presence of pituitary extract (Fig. 3d). Therefore, I concluded that the effect of gonadotropin must be mediated by follicle cells to induce frog oocytes to initiate maturation.

Since progesterone had been known to have the effect on the ovary that facilitates ovulation in the frog as mentioned before (Zwarenstein, 1937; Burgers and Li, 1960; Wright, 1961), I tested the effect of progesterone on oocytes free of follicle cells as well as on those enclosed in the follicular epithelium, either in the presence or in the absence of gonadotropin. The results showed that progesterone could induce oocytes to mature in all these cases (Fig. 3e). These mature oocytes proved to be capable of cleavage when a blastula nucleus was transplanted into them. Therefore, I concluded that “pituitary gonadotropin acts on the follicle cells to stimulate them to release a hormone that directly acts on the oocyte” (Masui, 1967b; p. 365), and that “the follicle cells secrete a progesterone-like substance that causes maturation of oocytes (Masui, 1967b; p. 374).

Progesterone action on frog oocyte maturation was
also reported independently by Schuertz (1967) and Smith et al. (1968). In 1967, the production of a substance in the starfish ovary stimulated by neural gonadotropin that causes oocyte maturation was also reported by Kanatani and Shirai and by Schuertz and Biggers. In 1969, this substance was identified as 1-methyl-adenine (1-MA) by Kanatani et al. However, in the frog, it was 1975 when radio-immunoassay became commonly available that progesterone was identified as the natural inducer of oocyte maturation by Fortune et al. (1975).

To know how progesterone acts upon the oocyte, I injected a progesterone solution into Rana pipiens oocytes to give an internal concentration of 0.5 mg/ml, but none of the oocytes were induced to mature (Fig. 3f), while those treated by applying externally a less concentrated progesterone solution all matured (Masui and Markert, 1971). Similar observations were reported by Smith and Ecker (1969) in frog oocytes treated with progesterone and by Kanatani and Hiramoto (1970) in starfish oocytes treated with 1-MA. All these observations indicated that the hormone becomes effective in inducing oocyte maturation only when its action is exerted from outside of the oocyte or on the oocyte surface. Although this conclusion was contrary to the above-mentioned notion of hormone action at that time, it gained strong support several years later from the experiments with polymer-conjugated progesterone derivatives (Ishikawa et al., 1977; Godeau et al., 1978). These experiments in Xenopus showed that the steroid applied externally could induce oocyte maturation, even though prevented from entering the oocyte because of the conjugated polymer, but it failed to induce oocyte maturation if injected into oocytes.

However, the action of progesterone as maturation inducer in amphibian oocytes did not seem to be as highly specific as I initially expected. In fact, various steroid hormones and non-steroid substances were found to be capable of inducing oocyte maturation at varying efficiency (Masui and Clarke, 1979 for a review). Interestingly, all these agents were found to be effective only when they were applied to the oocyte from the outside. When various chemical agents which could specifically affect cell physiology, such as ion channel blockers, ionophores, and chelators, became available between 1975 and 1980, these substances were tested for the ability to induce or inhibit oocyte maturation in various species. Treatment of oocytes with Ca ionophore A23187 in the presence of Ca and Mg ions at a high concentration could induce oocyte maturation in various animals. Many Ca-releasing agents were known as maturation inducers, including ionophores of Ca ions, β-adrenergic blockers, and lanthanum ions. In Xenopus, insulin and insulin-like growth factor were later found to induce oocyte maturation (El Etr et al., 1980; Maller and Koontz, 1981).

Conversely, it was shown that oocyte maturation could reversibly be inhibited by removal of Ca ions in external medium or by treatment with Ca-channel blockers as well as with Ca ion chelators such as EGTA (Masui and Clarke, 1979 for a review). When Ca metabolism in oocytes was examined using radioactive Ca and Ca-activated chemiluminescent protein, aequorin, Ca ion release from oocytes was observed at the beginning of oocyte maturation in Xenopus, Urechis caupo, and starfish (Masui and Clarke, 1979 for a review). These results strongly suggested the involvement of Ca ions in the initiation of oocyte maturation. However, later experiments with new techniques detected no significant changes in Ca ion levels in the beginning of oocyte maturation in starfish (Eisen and Reynolds, 1984) and Xenopus (Robinson, 1985). Since then, the role of Ca ions in the initiation of oocyte maturation has no longer attracted serious attention. Nevertheless, our recent re-investigation of the Ca problem confirmed some of the old observations mentioned above (Duesbury and Masui, 1996). Thus, it may be suggested that there should be further investigation into the role of Ca ions in oocyte maturation.

**Discovery of Maturation-Promoting Factor (MPF)**

As mentioned before, progesterone could induce oocyte maturation only when applied externally, but it failed to do so if injected into the oocyte. This result suggested that only the cytoplasm near the oocyte surface could receive the hormonal signal. Therefore, it was assumed that the oocyte must then create a signal in the cytoplasm that reaches the GV to induce meiotic divisions (Masui and Markert, 1971). This implies that nuclear activities involved with the initiation of oocyte maturation are under the control of cytoplasmic activity, but not vice versa. To test this assumption, I sucked cytoplasm from progesterone-treated oocytes into a calibrated graduated micropipette with the aid of a micromanipulator and injected a measured volume of the cytoplasm into an untreated immature oocyte. The recipient oocytes were indeed induced to mature without hormone treatment when they were injected with more than 5 nl of cytoplasm taken from the donor oocytes which had been kept for more than 6 h at 21°C after hormone treatment (Fig. 4A). This indicated that the frog oocyte, having received the progesterone signal near the cell surface, produced a factor in the cytoplasm that causes oocyte maturation, and this cytoplasmic factor was named "Maturation Promoting Factor (MPF)". These results were published in 1971. In the same year, Smith and Ecker also reported a similar result in the discussion of their paper that investigated the action of progesterone. They suggested, based on their preliminary experiment, that "maturing oocytes acquire an intracellular component which in itself is capable of inducing maturation" (Smith and Ecker, 1971; p. 245).

I found that if the cytoplasm taken from progester-
one-treated oocytes at a same stage of maturation was injected into untreated oocytes, the frequency with which recipient oocytes were induced to mature increased almost linearly with the volume of the injected cytoplasm. Based on this dose-dependent relationship, the relative activity of MPF in oocyte cytoplasm, being expressed as the percentage of oocytes induced to mature by injection with a constant volume of cytoplasm, was measured at various times after the initiation of maturation by progesterone treatment. Thus, it was found that MPF appeared 6 hours after progesterone treatment, 3 hours before GVBD, stayed at high levels, and then declined after egg activation by fertilisation or pricking with a glass needle (Fig. 5). However, low MPF activity could still be detected in cleaving blastomeres of zygotes (Masui and Markert, 1971).

Since the surface action of progesterone caused the appearance of MPF and surface injuries of activated eggs caused its disappearance, it was supposed that both appearance as well as disappearance of MPF must be initiated by cytoplasmic events near the cell surface without involvement of the nucleus. This was proved by experiments with oocytes whose GV had been removed prior to progesterone treatment (Fig. 4B). When treated with progesterone, the cytoplasm of these enucleated oocytes produced MPF, but when pricked with a glass needle, the oocytes were activated and their cytoplasm lost MPF activity (Masui and Markert, 1971).

On the basis of these results, it was supposed that MPF must reach the nucleus from the oocyte surface by propagating itself in the oocyte cytoplasm by its “auto-catalytic” amplification. This was corroborated by a serial transfer of cytoplasm in Rana pipiens oocytes. In this experiment, 30 to 40 nl (1.5 to 2.0% of oocyte volume) of cytoplasm were transferred from progesterone-treated donor oocytes to untreated recipient oocytes, and then from these first recipients to the second recipients and from the second to the third, successively at one day intervals (Fig. 6). The cytoplasm thus transferred was able to induce maturation in the recipients after every transfer with similar frequency (70 to 90%), showing no decrease in MPF activity in the serially transferred cytoplasm, despite the fact that the cytoplasm of the original progesterone-treated donors was extensively diluted through the serial transfers (Masui and Markert, 1971).

I also examined actual propagation of MPF activity through oocyte cytoplasm from the animal to the vegetal half of the oocyte. It was found that in progesterone-treated oocytes MPF developed earlier and its activity increased faster and became higher in the animal half than in the vegetal half. If the GV in an oocyte was displaced by centrifugation to the vegetal half and the oocyte was then constricted by thin thread along the equatorial line and treated with progesterone, there was a significant delay in GVBD and the frequency with which such oocytes underwent GVBD was decreased in proportion to the tightness of constriction (Masui, 1972).

A few years later, the presence of MPF and its autoca-
Protein synthesis and phosphorylation during oocyte maturation

In 1966 Smith et al. observed that oocytes significantly increase protein synthesis during maturation, and catalytic amplification were reported in *Xenopus* oocytes, but it was not until 1976 that MPF was found in oocytes of a variety of non-amphibian species, including the starfish, mouse and *Spisula* (Masui and Clarke, 1979, for a review).

Dettlaff (1966) showed that gonadotropin-induced maturation of follicle-enclosed oocytes is inhibited by both RNA and protein synthesis inhibitors. However, in 1967 Schuetz found that progesterone-induced oocyte maturation was not inhibited by RNA synthesis inhibitors but only by protein synthesis inhibitors, suggesting that oocytes require only translation of stored mRNA to initiate maturation.

In 1975, William Wasserman, who was working towards his PhD in my lab, defined a biological unit of time for progression of oocyte maturation, referring to the duration between the beginning of progesterone...
treatment and the time when 50% of the oocytes initiate GVBD as GVBD_50. Using *Xenopus* oocytes he showed that GVBD could be inhibited only in the oocytes injected with protein synthesis inhibitors no later than 65% of the GVBD_50, which coincided with the time of the initial appearance of MPF in 50% of the oocytes. It was also found that protein synthesis inhibition could not stop the oocyte maturation induced by MPF injection, unlike that induced by progesterone. Furthermore, protein synthesis-inhibited oocytes when injected with cytoplasm containing MPF could produce as much MPF as that produced in uninhibited oocytes. These results indicated that once MPF appears in the cytoplasm both the amplification process as well as the action of MPF to promote oocyte maturation are no longer dependent on protein synthesis. Therefore, it was hypothesized that the initiation of oocyte maturation by progesterone requires synthesis of a new protein, “initiator”, and this leads to activation of the MPF precursor protein stored in the oocyte, so-called pre-MPF. Therefore, once active MPF appears, it is autocatalytically amplified by further activation of pre-MPF with no protein synthesis required to continue the subsequent process of maturation (Wasserman and Masui, 1975).

In 1975, Drury and Schorderet-Slatkine also independently reported an experiment similar to ours. However, their serial cytoplasmic transfer experiment showed progressive decreases in MPF activity in the cytoplasm successively transferred from donor to recipient oocytes when the oocytes were treated with protein synthesis inhibitor. Therefore, they suggested protein synthesis dependency of MPF amplification. However, our results with *Xenopus* oocytes were later confirmed by other laboratories (Detlaff et al., 1977; Gerhart et al., 1984). In 1988, Sagata and his associates in Vand Woude’s lab identified “initiator” protein as Mos, the product of the proto-oncogene c-mos, and showed that this is the only protein to be synthesised in *Xenopus* oocytes to produce MPF.

In the mid 1970s, it became known in many animals, including mammals and starfish, that the induction of GVBD does not require protein synthesis at all, not even an “initiator” (Masui and Clarke, 1979, for a review). Clearly, in these animals, it is not the synthesis of a new protein, but a modification of a pre-existing protein that is required for the appearance of active MPF. Then, a rapid increase in protein phosphorylation was observed in the initial phase of maturation of starfish (Guerrier et al., 1977) and *Xenopus* oocytes (Maller et al., 1977). In the frog, this protein phosphorylation surge could be induced by injection of MPF as well as by hormonal stimulation (Maller et al., 1977), but it was preceded by a rapid decrease in cAMP levels (Speaker and Butcher, 1977). In addition, it was found that GVBD could be induced by injection of a specific inhibitor of cAMP-dependent protein kinase (PKA) (Maller and Krebs, 1977). From these observations, it was inferred that it is a switch of protein phosphorylation from that catalysed by PKA to the one catalysed by other protein kinases that is required for activation and amplification of MPF (Masui and Clarke, 1979, for a review).

However, in the late 1970s, the existence of pre-MPF was questioned in *Rana pipiens* (Schuetz and Samson, 1979) and *Acipenser stellata*, the sturgeon (Detlaff et al., 1977). In these animals, the appearance as well as amplification of MPF do require protein synthesis. In fact, these classical observations have been corroborated by recent molecular studies by Yamashita and his associates in a variety of fish and amphibian species. They found that oocytes of most species do not have pre-MPF, and must synthesize MPF de novo following hormonal stimulation to initiate maturation (Yamashita, 1998, for a review).

### Discovery of Cytostatic Factor (CSF)

Having detected low MPF activities in cleaving blastomeres as mentioned before, I became suspicious about the specificity of MPF as promoter of oocyte meiosis and curious about a possible role in mitosis. To explore this possibility, cytoplasm from oocytes which had completed GVBD was injected into blastomeres of 2-cell embryos. To my surprise, “the injected blastomere frequently stopped cleaving. This result was unexpected” (Masui and Markert, 1971; p. 135) (Fig. 4A). On the other hand, cytoplasm taken from oocytes before GVBD or fertilised eggs did not inhibit cleavage when it was injected into blastomeres of 2-cell embryos. This indicated that only the oocytes that have passed MI and of those arrested at M II possess an inhibitory factor in their cytoplasm that stops cleavage of blastomeres. Thus, I hypothesized that maturing oocytes produce in the cytoplasm a factor responsible for the arrest of oocyte meiosis at M II, and its removal by fertilization allows zygotes to cleave. This hypothetical factor I called “Cyto-Static Factor (CSF)” in 1971 (Masui and Markert, 1971).

Relative activities of CSF in oocyte cytoplasm could also be assayed in the same way as MPF based on the proportionality between the volume of the cytoplasm injected into a blastomere and the frequency with which blastomeres were arrested with the injected cytoplasm. Thus, it was found that CSF activity appears after MI, gradually increases, and remains high as oocytes mature, but abruptly decreases shortly after fertilization. However, unlike MPF, it never appears again in cleaving zygotes (Fig. 5). These changes of CSF, both its production during oocyte maturation as well as its destruction during egg activation, were found to occur independently of nuclear activities, since CSF appeared in enucleated oocytes when they were treated with progesterone (Fig. 4B) and disappeared when they were activated by prickling with a glass needle (Masui and Markert, 1971).
In 1980, we proposed the criteria for CSF to be the genuine inhibitor of the cell cycle (Masui et al., 1980; Masui, 1991; 2000 for reviews). First, as shown above, CSF should be found only in oocytes arrested at M II. Secondly, the zygotes arrested by CSF should have the same cytological as well as physiological properties as those of the unfertilised egg. Indeed, both CSF-arrested blastomeres and unfertilised eggs were found to contain a mitotic apparatus equipped with a well-formed spindle and condensed chromosomes aligned at the equator, but associated with no polar asters, showing characteristics of meiotic metaphase. As well, the cytoplasm of both CSF-arrested zygotes and unfertilised eggs were found to have MPF and chromosome-condensation activity that causes brain or sperm nuclei injected into these cells to condense chromosomes to a metaphase state. Also, the surface morphology of CSF-arrested blastomeres becomes similar to that of unfertilized eggs, forming microvilli which are absent in blastomeres undergoing mitosis (Masui et al., 1980).

Next, CSF must be inactivated during the process of egg activation. In *Rana pipiens*, this process occurs in the first 45 min following fertilisation (Meyerhof and Masui, 1977). Zygotes injected with unfertilized egg cytoplasm during this period continued to cleave, whereas those injected 60 min after fertilisation, or later, stopped cleaving. It appears that injected CSF is inactivated during the early period of egg activation together with the endogenous CSF of the recipient zygote. As seen in the following section, CSF can also be destroyed *in vitro* under the physiological condition that brings about egg activation.

Finally, inhibition of the cell cycle by CSF should be reversible: CSF-arrested blastomeres must resume cell cycles when artificially activated as unfertilized eggs do. Indeed, CSF-arrested blastomeres initiated DNA synthesis and mitosis as well as exhibited oscillation of MPF activity in the cytoplasm when the blastomeres were injected with a large number of sperm nuclei or Ca ions or treated with ionophore A23187. These observations ruled out the suspicion that CSF is a non-specific toxic substance (Masui, 1991; 2000 for reviews).

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**Extraction and chemical characterization of MPF and CSF**

MPF was found to remain active for a short while in cytoplasmic homogenates prepared from mature oocytes after brief homogenization with a glass needle in a polyethylene tube (Masui and Markert, 1971). However, when the homogenates were centrifuged, neither MPF nor CSF activity was detected in the supernatant even under the conditions that minimise protein denaturation. After two years of unsuccessful trials to extract these cytoplasmic factors, I suspected that they might be structural elements of cytoplasm or a substance firmly bound to them. In 1972, I carried out an experiment to determine the subcellular localization of MPF. In this experiment, mature oocytes floated over a Ficoll solution were gently centrifuged, and each layer of cytoplasm stratified in an unbroken oocyte (Fig. 7) was tested for MPF activity by injecting its content into immature oocytes. The strongest MPF and CSF activities were detected in the cytosol and hyaline cytoplasm (Masui, 1972; 1974).

I then realised that “homogenisation” followed by centrifugation was detrimental to MPF and CSF activities. This was quite understandable in view of the fact that mature eggs activated by even a slight injury such as pricking with a glass needle lose both MPF and CSF activities. Therefore, dejellied eggs packed in a tube were compressed by centrifugal force without homogenisation and cytosols were extracted. In this way, in 1974, I succeeded in extracting CSF without losing its activity in cytosols (Masui, 1974). However, the activity was still short-lived in the cytosols kept in a refrigerator.

In order to protect MPF and CSF further from the effect of egg activation, which might have occurred during the process of extraction, cytosols were extracted in a Ca-free buffer containing a Ca-chelator, EGTA, since a release of intracellular Ca$^{2+}$ was known to be a universal cause of egg activation (Jaffe, 1985, for a review). The cytosols extracted in this way were found to retain high MPF and CSF activities for at least three days in a refrigerator. This allowed us to perform chemical tests on these factors. Bill Wasserman, working for his PhD research in my lab, showed that MPF activity in cytosols extracted from *Rana pipiens* oocytes was quickly lost when EDTA, a Ca-Mg chelator, as well as Ca were added and that it could be inactivated by proteolytic enzymes, but not by RNAse, and sedimented into 3 different fractions with sedimentation constants 3S, 13S, and 30S. From these observations, he concluded that MPF

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**Fig. 7** Stratified oocyte of *Rana pipiens*. Progesterone-treated oocytes were centrifuged in a Ficoll solution at 4080 × g for 2 h (Masui, 1972).
is a protein existing as, or associated with, molecules of different sizes and that its activity is sensitive to Ca and dependent on Mg ions. These results were published in 1976 (Wasserman and Masui, 1976). In 1978, Drury succeeded in further stabilising MPF by adding ATP and protein phosphatase inhibitors to *Xenopus* egg extracts. Thus, in 1979, Masui and Clarke speculated that MPF must be a phosphoprotein, and “MPF activity is maintained by phosphorylation of its molecules” (Masui and Clarke, 1979; p. 246). In 1980, Wu and Gerhart succeeded in partial purification of *Xenopus* MPF using ammonium sulphate precipitation and column chromatography and found that the MPF samples purified 30- to 50-fold, containing 70 to 250 kD polypeptides, became less Ca-sensitive and were constantly phosphorylated and dephosphorylated in the presence of ATP.

Peter Meyerhof, carrying out his PhD research in my lab, found that addition of Ca²⁺ to or removal of Mg²⁺ from the cytosols quickly causes the loss of CSF activity. These Ca-containing cytosols in a vial kept on ice lost their activity completely within one hour of Ca addition to the level of 10⁻⁵ M. From these results, we hypothesised that the natural cause of CSF inactivation during egg activation is the release of Ca ions from cytoplasm. However, in the process of these studies, we noticed the curious fact that CSF activity reappeared in Ca-containing cytosols during storage in a refrigerator. Thus, we discovered to our surprise that there were two kinds of CSF, the Ca-sensitive one and a Ca-insensitive one which could only develop in stored egg cytosols after the former was inactivated by addition of Ca to the cytosols.

To distinguish these two CSFs, we labelled the former “primary CSF (1⁰CSF)” and the latter “secondary CSF (2⁰CSF)”. Their effects were cytologically indistinguishable; both bringing about metaphase arrest of the same feature as described before. However, we observed an essential difference between them: while 1⁰CSF injected into eggs shortly after fertilization failed to arrest cleavage of the recipient eggs as I mentioned before, Ca-resistant 2⁰CSF could arrest cleavage regardless of the time of its injection. This strengthened our hypothesis proposed above that Ca ion released from the egg early in fertilisation is responsible for inactivation of Ca-sensitive 1⁰CSF. We published these results in 1977 (Meyerhof and Masui, 1977).

In 1988, Ellen Shibuya, working for her PhD research in my lab, succeeded in further stabilising 1⁰CSF by adding a phosphatase inhibitor, NaF, and ATP to egg cytosols, but she also found that these chemicals could enhance its activity (Shibuya and Masui, 1988). This suggested that the activity of 1⁰CSF is dependent on protein phosphorylation. 1⁰CSF was precipitated from *Rana pipiens* egg cytosols by ammonium sulphate and sedimented in fresh cytosols by centrifugation at 150,000 × g for 6 h (Shibuya and Masui, 1988) and through a sucrose density gradient with a sedimentation coefficient of 3S in the presence of ATP and NaF (Shibuya and Masui, 1989). 1⁰CSF was partially purified eight fold through these procedures. When fresh egg cytosols were incubated with proteases, the activity of 1⁰CSF was lost, but when incubated with RNAses it was not (Shibuya and Masui, 1989). Therefore, we concluded that 1⁰CSF is a labile phosphoprotein and, like MPF, activated by its Mg²⁺-dependent phosphorylation but inactivated by Ca²⁺ ions.

On the other hand, Ca-resistant 2⁰CSF was found to be a very stable molecule, which could be precipitated with ammonium sulphate without loss of its activity in the absence of ATP, and could not be inactivated even in 8 M urea, 4 M LiCl, and alkaline solution (pH 11.5) (Shibuya and Masui, 1989). 2⁰CSF purified 40-fold by gel filtration equilibrated with 8 M urea was found to be a very large molecule, perhaps larger than 2,000 kD. It was found that 2⁰CSF could be digested by proteolytic enzymes but not by RNAses (Shibuya and Masui, 1989). Our recent study showed that egg cytosols failed to form 2⁰CSF when transglutaminase inhibitors or anti-transglutaminase antibodies were added to the cytosols (Zhang and Masui, 1997). In all probability, 2⁰CSF is an artefactual product of protein cross-linking reaction catalysed by Ca-activated transglutaminase that could occur in egg cytosols during storage.

The ubiquitous role of MPF in the control of nuclear activity

In the late 1960s, Gurdon (1968) using *Xenopus* oocytes showed that somatic cell nuclei injected into maturing oocytes were transformed into metaphase chromosomes. In 1973, this was confirmed in *Rana pipiens* oocytes by David Ziegler, who was carrying out his graduate research in my lab. He found that oocytes acquire the ability to transform the nuclei transplanted into them to metaphase chromosomes as MPF activity appeared in their cytoplasm (Ziegler and Masui, 1973). In these oocytes, brain and sperm nuclei in the G1 phase were directly transformed to metaphase chromosomes consisting of a single chromatid (Fig. 8) (Ziegler and Masui, 1976a). These results strongly suggested that oocyte cytoplasmic effects on nuclear activities are not tissue-specific. Neither did MPF seem to be a meiosis-specific factor, since MPF activities were found to persist in blastomeres at the cleavage stage as mentioned before (Masui and Markert, 1971). We were discussing the possible role of MPF in mitotic chromosome condensation. In 1978, Bill Wasserman, who left my lab to join Smith’s lab at Purdue University as a postdoc in 1976, demonstrated for the first time the periodic appearance of MPF during mitotic cycles using blastomeres of amphibian embryos (Wasserman and Smith, 1978). His finding was later re-examined and confirmed by Gerhart et al. (1984). By the end of the 1970s, it had also been established
that MPF from the oocyte of one species could induce oocyte maturation in the other species, demonstrating its species-nonspecificity (Kishimoto et al., 1982). Similarly, CSF from one amphibian species was found to arrest cleavage of blastomeres of another species (Meyerhof and Masui, 1979) (Fig. 9). Therefore, in 1979, we concluded that “the nuclear events in oocytes undergoing meiotic divisions, and those in somatic cells undergoing mitotic divisions, are under the control of the same cytoplasmic factors” (Masui and Clarke, 1979; p. 270). In the same year, the appearance of MPF during mitosis was reported in HeLa cells by Sunkara et al. (1979) and within a few years, in CHO cells by Nelkin et al. (1980), in sea urchin embryos by Schatt et al. (1983), and in yeast cells by Weintraub et al. (1982). These results proved that MPF is a ubiquitous cytoplasmic factor that causes the cell division in eukaryotes.

In the late 1970s, by labelling proteins with radioactive amino acids, we investigated the interactions of the nuclei transplanted into frog oocytes with proteins accumulated in the GV of host oocytes as well as with proteins synthesized during their maturation. It was found that these labelled proteins preferentially accumulated over the chromosomes that had been induced to condense in maturing oocytes. On the other hand, chromosomes condensed in maturing oocytes lacking the GV disintegrated in less than 3 h. From these results, we concluded that proteins synthesized in oocyte cytoplasm during maturation interact with the nucleus to induce chromosome condensation, but proteins of GV nucleoplasm are required for stabilisation of the condensed chromosomes (Ziegler and Masui, 1976b; Masui et al., 1979).

The dose-dependent effect of M phase cytoplasm on chromosome condensation of interphase nuclei was first observed in a cell fusion experiment by Johnson and Rao (1971) using cultured mammalian cells. In their experiments, chromosome condensation was induced with increasing frequency as the ratio of M phase cells fused to interphase cells was increased. However, it remained unclear whether this dose-dependent effect of M phase cytoplasm was brought about by changes in its concentration after mixing with the interphase cytoplasm or by changes in the ratio between M phase cytoplasm and nuclei. Therefore, we began our investigation into the effect of nucleocytoplasmic ratio on chromosome condensation induced by M phase cytoplasm in the early 1980s.

In 1982, Ellen Shibuya in her M.Sc. research observed that sperm nuclei injected into a single CSF-arrested blastomere of 2-cell frog embryos could be transformed into metaphase chromosomes if their number per blastomere was kept below 100, while they formed pronuclei and began DNA synthesis if the number exceeded 200 (Shibuya and Masui, 1982). Later, Clarke in his PhD research investigated the behaviour of sperm nuclei in polyspermic mouse oocytes arrested at M II following premature insemination. He found that sperm nuclei could be transformed into metaphase chromosomes if the egg received no more than 3 sperm, but if the egg received more than 3 sperm, they all form a pronucleus; this nucleocytoplasmic ratio (3 nuclei/one oocyte cytoplasm) critical for sperm chromosome condensation remained unchanged if oocytes were bisected or fused. From these results, we concluded that “the oocyte cytoplasmic factor which forms the sperm nucleus into metaphase chromosomes may be stoichiometrically titrated by sperm nuclear material” (Clarke and Masui, 1987; p. 838).

**Cell cycles in vitro**

In the late 1970s, I thought that in order to study the nucleocytoplasmic interactions involved in the cell cycle...
at the molecular level, we must investigate the behaviour of nuclei in cytoplasmic environments better chemically defined than the cytoplasm of intact oocytes and eggs. Previously, such studies were attempted by incubating *Xenopus* erythrocyte nuclei (Barry and Merriam, 1972) and sea urchin sperm nuclei (Kunkel et al., 1978; Eng and Metz, 1980) with egg homogenates or cytosols of the respective species *in vitro*. However, they did not succeed in inducing significant changes in nuclear morphology associated with the cell cycle.

In 1978, I was trying to stabilize MPF in cytosols extracted from *Rana pipiens* eggs. So far, cytosols prepared by high speed centrifugation (150,000 × g for 2 h) always had lost MPF activity on day 3 of cold storage on ice. However, when I accidentally assayed MPF activity in the cytosols on day 4, I found, to my surprise, that there was still activity. When I continued to assay MPF activity every day, the activity was found until it disappeared on day 8, but then curiously it reappeared on day 9 and disappeared on day 13 and so on. That is, MPF activity actually oscillated with fairly regular periods of 4 to 6 days. Oocyte maturation induced by these oscillating MPF activities appeared to be genuine in all respects (Fig. 10). I repeated the experiment and confirmed that MPF activity in egg extracts oscillated with the average period of 5.05 ± 1.25 day during storage at 2 °C. In 1982, I published these results and concluded that “a homogeneous cell-free system can exhibit cyclic behaviour of a cytoplasmic factor controlling nuclear activities”, and suggested “the possibility that a modification of the system may allow MPF to oscillate at a higher temperature with a periodicity similar to that of zygotes” (Masui, 1982; p. 397).

However, when I incubated frog brain nuclei in clear egg cytosols obtained by high speed centrifugation in which MPF activity cycled, although a high MPF activity was found in the cytosols, no chromosome condensation was induced. On the other hand, Manfred Lohka, who chose this project for his PhD research, prepared egg extracts which contained cytoplasmic granules by low speed centrifugation (9,000 × g, for 15–30 min) and incubated demembranated sperm nuclei in the extracts. Sperm nuclei seemed to be a better choice than brain nuclei, since in nature only the sperm nuclei have been designed to interact with egg cytoplasm. Thus, Lohka succeeded in making pronuclei from sperm nuclei *in vitro* in the extract prepared from eggs which had been activated by electric shock. These pronuclei replicated DNA and subsequently formed metaphase chromosomes in the extract. Thus, he demonstrated, for the first time, that the cell cycle events could take place *in vitro*. In 1983, we published these results and concluded that “this cell-free system may be useful in biochemical analysis of the interactions of nucleus and cytoplasm that control nuclear behavior” (Lohka and Masui, 1983; p. 719). A year later, a similar cell-free experiment using toad eggs and sperm nuclei was also reported by Iwao and Katagiri (1984).

Lohka also found that sperm nuclei were immediately transformed into metaphase chromosomes, instead of pronuclei, when they were incubated in extracts of CSF-arrested unfertilized eggs prepared in a way similar to that described above but to which the Ca-chelating agent, EGTA, had been added to prevent egg activation. However, when Ca ions were added to these extracts to inactivate MPF and CSF, chromosomes were transformed into interphase nuclei. These results of *in vitro* experiments demonstrated that MPF and CSF are essential for the formation and maintenance of metaphase chromosomes (Lohka and Masui, 1984a). He also confirmed the conclusion from *in vivo* experiments that activation and inactivation of MPF cause the transition from interphase to metaphase, and metaphase to interphase, respectively.

However, when sperm nuclei were incubated in the activated egg cytosols prepared by high speed centrifugation, therefore having cytoplasmic membrane vesicles removed, they failed to form a pronucleus and never underwent cell cycle changes (Lohka and Masui, 1983; 1984b). Similarly, nuclei incubated in CSF-arrested egg extracts lacking membrane vesicles prepared by high speed centrifugation also failed to condense chromosomes to a metaphase state even though high MPF and CSF activities were detected in the extracts as mentioned before. Thus, sperm nuclear transformations, both chromosome condensation and pronuclear formation, were found to be regulated not only by changing MPF activities in the cytosol, but also by the activities of cytoplasmic membranes.

In 1985, the cell-free system was developed by Lohka using *Xenopus* eggs, after he left Toronto in 1984 to join Mallar's lab at the University of Colorado as a postdoc. With this system, they showed that sperm pronuclei formed in activated egg extracts could be immediately transformed into metaphase chromosomes when par-
tially purified MPF was added (Lohka and Maller, 1985). This procedure was later used for rapid MPF assay for its purification (Lohka et al., 1988). This cell-free system was further refined by Murray in Kirschner’s lab at the University of California at San Francisco, and in 1989, they reported that sperm pronuclei formed in this new cell-free system, called “cycling extract”, could perform up to a few more complete cell cycles (Murray and Kirschner, 1989).

The molecular nature of MPF and CSF

In 1980, Rosenthal et al. found novel proteins that appear during meiotic and mitotic cycles in eggs of the clam, Spisula, and in 1983, Evans et al., observing that some of them were synthesized during interphase and were rapidly degraded at the onset of cell division, named these proteins “cyclins”. The observation that the cyclin cycle is closely correlated with the chromosome cycle (Cornall et al., 1983) suggested a close correlation between MPF activity and the level of cyclin. In 1986, cyclin genes were cloned from Spisula and sea urchin eggs, and cyclin A mRNA was injected into Xenopus oocytes by Swenson et al. (1986). They showed that cyclin mRNA did induce GVBD and chromosome condensation to a metaphase state in the Xenopus oocytes.

By 1986, the gene required for completion of mitosis in the yeast cell, cdc2, was cloned and sequenced, and its product was identified as a 34 kD protein kinase (Hindley and Phear, 1984; Simanis and Nurse, 1986). In the area of oocyte maturation studies, efforts had been made to purify MPF. Finally, in 1988, Manfred Lohka and his associates succeeded in purifying MPF 3000-fold using in vitro sperm chromosome condensation as the assay for MPF in Xenopus egg cytosol fractions. They found that MPF was a phosphoprotein molecule consisting of 32 and 45 kD peptides and capable of phosphorylating its own 45 kD peptide as well as histone H1 in the presence of ATP. In the following few years, it was found that the 32 kD protein and 45 kD peptide of the purified MPF cross-reacted with anti-cdc 2 antibody and the anti-cyclin B antibody, respectively. Thus, MPF was identified as a protein kinase consisting of two subunits, cdc2 and cyclin B (Nurse, 1990, for a review).

Unfortunately, purification of 1°C SF has not been successful to date because of the extreme instability of its activity in frog-egg extracts. However, its identity was revealed from a different angle. In 1989, Sagata and his associates found striking similarities in behaviour between 1°C SF and Mos, the protein kinase coded by the proto-oncogene c-mos of 39 kD designated pp39mos.Xe. In Xenopus oocytes, Mos protein rapidly increases during maturation, reaching a maximum level at M II, but it rapidly disappears following fertilisation. Mos mRNA, like 1°C SF, when injected into cleaving blastomeres of 2-cell embryos arrests the recipient blastomeres at metaphase. It was also shown that immuno-precipitation of proteins in unfertilised egg cytosols with Mos-specific antibodies deprived the cytosol of CSF activity, and that centrifugation of the cytosols at 150,000 × g for 6 h precipitated both Mos protein and CSF activity. Further, both CSF and Mos were destroyed when Ca ions were added to the cytosols as well as when Xenopus eggs were activated by Ca ionophore A23187, indicating that Mos is as sensitive as 1°C SF to Ca ions and is destroyed under the same cytoplasmic conditions as those brought about by egg activation (Watanabe et al., 1991).

Thus, they concluded that “the c-mos proto-oncogene product, pp39mos (Mos), is either the long-known endogenous meiotic inhibitor of amphibian eggs, the cytosolic factor, or at least a catalytic component of it” (Sagata et al., 1989; p. 516).

In 1989, Murray and Kirschner demonstrated that cyclin B and MPF levels both cycled in Xenopus cycling extracts and that the appearance of MPF and cyclin B were suppressed when protein synthesis in the extracts was inhibited or mRNAs were depleted. However, MPF could appear and cell cycle events could continue if cyclin mRNA was translated even in extracts from which all endogenous mRNAs had been depleted. If cyclin B mRNA lacking protease-reactive sequence was translated in extracts, MPF and cyclin B levels stayed high and failed to cycle. These results clearly proved that progression of cell cycle events in frog egg extracts requires only synthesis and proteolytic degradation of cyclin B to cause a rise and fall of MPF activity.

These experiments opened the gate for the molecular biological approach to the cell cycle control, which has grown at an amazing speed in the past decade, increasing our knowledge of the regulatory mechanisms of the cell cycle at the molecular level tremendously. However, we must note that the old questions about oocyte maturation and egg activation raised in the early years of our study are still waiting for answers. We have not fully understood the mechanisms of how maturation-inducing hormones, such as progesterone and 1-MA, act on the oocyte to give rise to MPF. We still do not know about the receptor of these hormones in the oocyte or protein phosphorylation cascades that lead to the appearance of MPF and roles of ions and cyclic nucleotides in the regulation of these processes. In addition, we have little knowledge about the mechanisms of how MPF could lead condensation of chromosomes to a metaphase state and how CSF maintains M phase (Masui and Clarke, 1979; Masui, 1992; 2000; Maller, 1998 for reviews). History tells us that learning from oocyte maturation and egg activation would help us again to gain new insight into the regulation of the cell cycle.

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