Meiosis reinitiation as a model system for the study of cell division and cell differentiation

PIERRE GUERRIER*, PIERRE COLAS and ISABELLE NEANT

Laboratoire de Biologie Moléculaire et Cellulaire, UMR 49, Ecole Normale Supérieure de Lyon, Lyon and *Station Biologique de Roscoff, LP 4601, CNRS, France*

ABSTRACT In this paper, we review our findings concerning the control of meiosis reinitiation in starfish oocytes and discuss recent advances that lead to characterization of the maturation promoting factor (MPF) responsible for G_2-M transition. It is now agreed that appearance of this factor, which triggers nuclear envelope breakdown, chromosome condensation and metaphase spindle formation, corresponds to the activation of a M-phase specific H1-kinase. MPF has been shown to be constituted of equimolar amounts of a 34 kDa catalytic subunit protein homologous to the yeast cdc2/CDC28 gene product and a cyclin protein homologous to the yeast cdc13 gene product. "*In vivo*" and "*in vitro*" studies based on the use of inhibitors of protein synthesis, protein kinases, phosphoprotein phosphatases and proteases lead to a better understanding of the complex series of events which regulate activation and inactivation of MPF. In the unfertilized metaphase 2-arrested vertebrate oocyte, it has also been shown that stabilization of MPF depends on the kinase activity of the c-mos protooncogene. This review attemps to illustrate how the significant progress made in the understanding of the regulation of cell cycle trasverse directly resulted from the convergence of observations in multidisciplinary studies in yeast genetics, development and oncogenesis. It also offers a model for considering the highly integrated events which, starting at the level of the plasma membrane, may eventually result in early cell differentiation.

KEY WORDS: *cell cycle, maturation-promotin factor, protein-kinases, phosphoprotein phosphatases, protooncogenes*

Introduction

One of the most fundamental problems in developmental biology is to understand how the same genetic message can be differently translated in the various cells which constitute the embryo or the adult. In fact, we know that the state of differentiation of a cell cannot rely on its informative DNA since this DNA is integrally transmitted from the mother to the daughter cells. Thus, differentiation must depend on factors that are either introduced through differential transcriptions and translations or that are modified by post-translational mechanisms. All these processes are controlled by specific inductions or repressions which, at each step, depend on the level of organization of the surrounding cytoplasm.

Thus, the problem of the acquisition of a peculiar state of cell differentiation re-emerges in the question of how new conditions might have appeared in the cytoplasm. At this point, one can ask a few significant questions such as: do regional differences exist at the very beginning of development? How can they vary, under the influence of what factors and according to which mechanisms?

Addressing such questions naturally directs the attention towards the oocyte, i.e. that primordial female stem cell which not only carries a nucleus but also the organized cytoplasm that constitutes the natural environment for the first nuclei that would appear in the embryo.

Abbreviations used in this paper: c-AMP, adenosine 3': 5'-cyclic monophosphate; ATP-γS, adenosine 5'-0-(3-thiotriphosphate); CSF, cytostatic factor; dbc-AMP, N^6, 2'-0-dibutyryladenosine 3': 5'-cyclic monophosphate; 6-DMAP: 6-dimethylaminopurine; EGTA, ethylene glycol-bis(-amino-ethyl ether) N,N,N',N'-tetra acetic acid; GI prot, inhibitory GTP binding protein; GSS, gonad-stimulating substance; GV, germinal vesicle; GVBD, germinal vesicle breakdown; HDP, hormone dependent period; 8-HETE, 8-hydroxycicosatetraenoic acid; H1-K, histone H1-kinase; KCl, potassium chloride; 1-MeAde, 1-methyladenine; MIS, meiosis-inducing substance; MPF, M-phase or maturation-promoting factor; mRNA, messenger ribonucleic acid; α-NP, α-naphtylphosphate; OA, okadaic acid; PKI, heat stable c-AMP dependent protein kinase inhibitor; Ptase, phosphoprotein phosphatase; QUIN2/AM, 2-[(2-bis[carboxymethyl]-aminoquinolinetetrakis-[acetoxymethyl]ester; RNAase H, ribonuclease H; TMB8, 8 (N-N-diethylamino)octyl-3,4,5-trimethoxybenzoate.

***Address for reprints**: Laboratory of Molecular and Cellular Biology, UMR 49, Ecole Normale Supérieure de Lyon, 46 Allée d'Italie, 69364 Lyon Cédex 07, France

0214-6282/90

In short, the female gamete that gives rise to different cell lines through the processes of maturation, fertilization and cleavage may constitute a good model to study both the mechanisms involved in cell division and in cell differentiation. Obviously, there are two possibilities for a given cell to differ from its sister: either it has inherited a specific part of a regionally organized pattern or it has been placed in such a position that it can gain new properties. Such specific intracellular activation events as may occur downstream from the translation processes and might favor new specific transcriptions and translations are likely to merge under the influence of extracellular signals. These signals must be recognized and transformed at the level of the plasma membrane. There is evidence that such mechanisms are already at work at the oocyte level, i.e. during maturation, when specific hormones have been shown to stimulate recognition sites at the level of the plasma membrane and at fertilization, when the sperm or some of its components may activate the oocyte metabolism even if the male nucleus is prevented from entering the cytoplasm. These two significant events, maturation and fertilization, are representative of a more general situation that expresses itself during the activation of lymphocytes, the response of competent cells to an inducer or to the building up of new intercellular contacts. In all these cases, the plasma membrane must act as a relay between the extracellular signals and the cytoplasm.

Studying the mechanisms responsible for such intracellular activations leads one to address some more questions such as: How are efficient stimuli recognized by the membrane? What modifications are introduced at this level during the transduction process? What are the intimate mechanisms by which such changes may lead to a specific intracellular response? These questions are typically those one must ask in order to understand how a cell may diverge from its neighbors. Even if we have no definitive answer to these questions, it seems quite relevant that they could have been made concerning such a simple and universal system as the female gamete. Presently, however, the most important advances gained using that system concern the characterization and functional analysis of those specific gene products found to be effective in controlling the G_2-M transition of the cell cycle. For that burst of knowledge, we are greatly indebted to some of our lower relatives such as yeast, the starfish, some molluscs and frogs that so helped us to better understand the highly conserved biochemical mechanisms controling our own cell divisions. In this article, we will refer mainly to the contribution that we and others have made to this problem since we began to work on starfish oocytes at the Biological Station of Roscoff in 1972.

Normal triggers for meiosis reinitiation

It is well known that female gametes of most vertebrates are arrested at the germinal vesicle stage, after they have duplicated their DNA. Reactivation of these cells depends on various external stimuli. These may be the sperm as observed in Nematodes (Nigon *et al.*, 1960), the Echiurian *Urechis* (Holland *et al.*, 1984), the Annelid *Nereis* (Costello, 1945) and the bivalves *Spisula*, *Pholas* and *Barnea* (Guerrier, 1970; Dubé and Guerrier, 1982a, b), a superficial proteolysis as in the Annelid *Sabellaria* (Peaucellier, 1977a) or a hormonal signal as described for the Annelid *Arenicola* (Meijer, 1979), the starfish (Kanatani *et al.*, 1969; Kanatani, 1973), the mollusc *Patella* (Shirai *et al.*, 1987) and the amphibians (Masui and Clarke, 1979). While in the starfish, meiosis goes to completion, a second block may occur which takes place in metaphase 1 as observed in the Annelids *Sabellaria* and *Arenicola*, the molluscs *Mytilus*, *Dentalium* and *Patella*, or in metaphase 2, after extrusion of the first polar body as in amphibian and mammalian oocytes. Such second blocks are only released upon fertilization or activation.

The hormonal system: gonadostimulins and meiosis-inducing substances

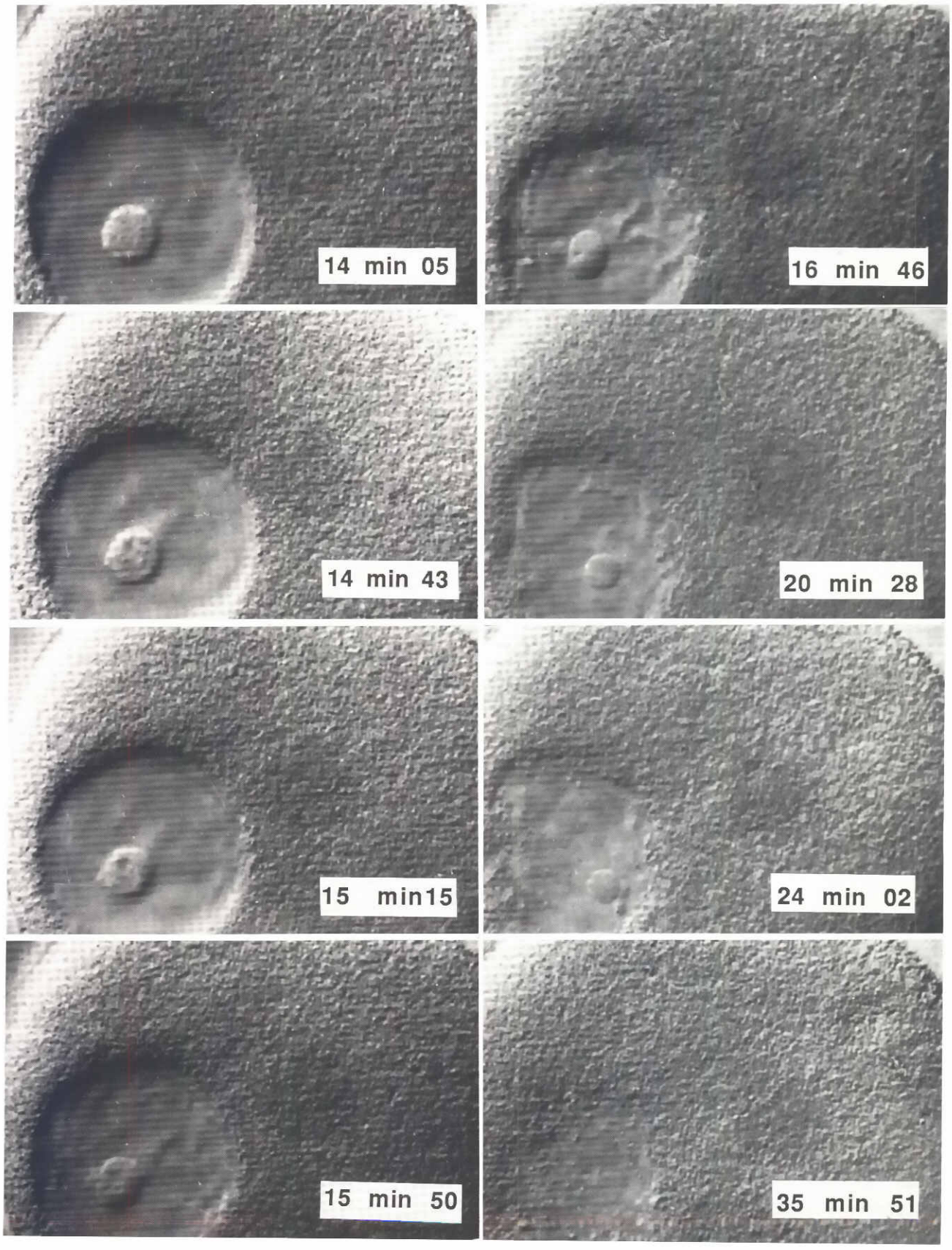
The hormonal induction of meiosis reinitiation is a multistep process. The first stimulus is a gonad-stimulating substance (GSS) of neural origin that acts on the follicle cells. These investing cells produce a relay hormone, the meiosis-inducing substance (MIS), which acts directly on bare defolliculated oocytes (reviewed by Shirai, 1986). Thus, in amphibians, gonadotropic pituitary hormones induce the follicle cells to produce progesterone (Heilbrunn *et al.*, 1939; Masui, 1967; Schuetz, 1967; Smith *et al.*, 1968; Fortune *et al.*, 1975). In the starfish, GSS from radial nerve extracts (Chaet and Mc Connaughy, 1959; Kanatani, 1964) appeared to be a small peptide with a molecular weight of about 2,100 (Kanatani *et al.*, 1971). This peptide stimulated the production of the active hormone 1-methyladenine (1-MeAde) by the follicle cells (Kanatani and Shirai, 1967; Schuetz and Biggers, 1967; Kanatani *et al.*, 1969; Hirai and Kanatani, 1971; Kanatani and Shirai, 1971). A similar system has been shown to be involved in triggering maturation of the prosobranch mollusc *Patella vulgata*. However, in this case, we still do not know the nature of the relevant GSS and MIS hormones (Guerrier *et al.*, 1986a, b; Shirai *et al.*, 1987).

The signal recognition step

It is now widely accepted that progesterone and 1-MeAde act at the level of the plasma membrane. In amphibians, the most pertinent demonstration for such a target derives from the works of Ishikawa *et al.*, (1977), Baulieu *et al.*, (1978) and Godeau *et al.*, (1978), who used non-permeant polymer steroid derivatives to trigger meiosis. It must be remembered, however, that Tso et al., (1982) showed that microinjection of progesterone dissolved in silicon oil could trigger maturation of *Xenopus* oocytes.

In the starfish, it is clear that the hormone acts on recognition sites located on the outer side of the plasma membrane. The first argument for this conclusion derived from the intracellular microinjection experiments performed by Kanatani and Hiramoto (1970). However, the negative results obtained by these authors were insufficient to dismiss the possibility that the hormone could have produced an intracellular active metabolite during its transfer across the cell membrane (Toole and Schuetz, 1974). Our finding that 1-MeAde, applied at its threshold concentration, remained able to trigger maturation when its uptake was severely and specifically inhibited by the inactive analogue 1-9-dimethyladenine definitively ruled out this eventuality (Dorée and Guerrier, 1975). The membrane localization of 1-MeAde receptors has been confirmed by the electro-physiological recordings performed by Moreau et al., (1978a),

Fig. 1. Kinetics of breakdown of the germinal vesicle as observed in an oocyte of *Asterias rubens*. *1-MeAde was added at t=0.*


14 min 05
16 min 46
14 min 43
20 min 28
15 min15
24 min 02
15 min 50
35 min 51

after 1-MeAde was applied from the internal or the external side of the plasma membrane. Moreover, we found that the hormone receptors resisted a proteolytic treatment sufficient to digest the vitelline membrane, whereas they could be extracted by a moderate treatment with Triton X-100 (Dorée *et al.*, 1976; Morisawa and Kanatani, 1978).

Studying the specificity of response of the oocytes to various structural analogues of the hormone leads us to conclude that 1-MeAde binds the receptors by its N7-N9 region (Dorée *et al.*, 1976a, b).

Besides the hormone 1-MeAde and its active structural analogues, various non-hormonal stimuli such as dithiothreitol (Kishimoto *et al.*, 1976; Guerrier *et al.*, 1977a), increased extracellular calcium (Guerrier *et al.*, 1978), phospholipase A_2 and arachidonic acid (Meijer *et al.*, 1984), methylglyoxal-bis-guanylhydrazone (Meijer and Guerrier, 1983), shaking (Guerrier *et al.*, 1983, 1988a), an ionic stress (Guerrier *et al.*, 1988b), the microinjection of nucleoplasm (Picard and Dorée, 1984), α-naphtylphosphate (Pondaven and Meijer, 1986) or okadaic acid (Picard *et al.*, 1989; Pondaven *et al.*, 1989) were found able to induce full maturation, at least in certain species of starfish. In fact, all these mimetics did produce the same intracellular mediator as observed after hormone stimulation. This effector, which causes germinal vesicle breakdown (GVBD), chromosome condensation, spindle formation and polar body extrusion when injected into a recipient GV-blocked oocyte, is known as the maturation-promoting factor or MPF. First described by Masui and Markert (1971) in the amphibian maturing oocyte, this factor was also found to appear some time before GVBD in the starfish oocyte (Kishimoto and Kanatani, 1976). It has since been further recognized as the universal, non species-specific factor which promotes the G2-M transition during both meiosis and mitosis (Kishimoto, 1988; Kishimoto *et al.*, 1982b; Meijer and Guerrier, 1984a for review). At this stage, an important question would be to understand how such a hormonal signal is transduced from the plasma membrane level to the cytoplasm.

The signal transduction step

The action of 1-MeAde (threshold: 0.1 μM) is particularly rapid since GVBD occurs within 20 min following hormone addition (Fig.1). Moreover, by eliminating 1-MeAde from the medium at various times after its addition, we were able to show that the hormone was required only during a very short period of time accounting for about 5 min at 24°C (Guerrier and Dorée, 1975). While the duration of this hormone-dependent period (HDP) could be reduced by raising the temperature, it did not change when the hormone concentration was increased (Guerrier *et al.*, 1976; Dorée *et al.*, 1976b). This showed that the HDP could not be accounted for by the time needed to load the receptors and that it must represent the time required for some biochemical process to take place, which would irreversibly commit the cell to enter meiosis. And, indeed, it was found that repeated treatments with the hormone for periods shorter than the HDP were cumulative up to the state of irreversibility (Guerrier and Dorée, 1975; Nemoto, 1982). Since GVBD always occurs normally, even in the presence of inhibitors that completely suppress RNA or protein synthesis (Guerrier and Dorée, 1975; Dorée, 1982), it thus appears clearly that the production of MPF is controlled at the post-translational level and may only result from the modification of preexisting elements. In any case, it has been shown for a long time that meiosis reinititation was always accompanied by an increased phosphorylation of endogenous proteins, as first demonstrated in the starfish (Guerrier *et al.*, 1975; 1977a) and the amphibian (Maller *et al.*, 1977).

Since most of the inhibitors of 1-MeAde action (more than 50 were listed in Meijer and Guerrier, 1984a), except nicotinamide (Sano et al., 1979) and 6-dimethylaminopurine or 6-DMAP (Néant and Guerrier, 1988a), proved effective only when added during HDP, we would here consider all those major events occurring during this period as significant prerequisites pertaining to the signal transduction process.

Evidence for the release from inhibition due to cyclic nucleotides

In amphibian (Maller, 1983; 1985a, b) and mammalian oocytes (Bornslaeger *et al.*, 1986), the existence of such an inhibitory pathway is well established, since: (1) c-AMP decreases upon hormone stimulation; (2) oocyte maturation can be induced by the microinjection of nucleotide phosphodiesterase, the regulatory subunit (R) and the heat-stable inhibitor (PKI) of c-AMP dependent protein kinase, whereas it is delayed or suppressed by cholera toxin, forskolin or the catalytic subunit (C).

In the starfish, the situation is slightly different since microinjections of the R subunit or PKI (Dorée *et al.*, 1981) or the phosphodiesterase found to be active on the amphibian oocyte (Meijer and Zarutskie, 1987) could not induce maturation. It appears, however, that 1-MeAde is able to produce a significant drop in c-AMP which is seen more clearly when the original level of intracellular c-AMP is increased by cholera toxin (Mazzei *et al.*, 1981) or forskolin (Meijer and Zarutskie, 1987). This drop is closely related to the HDP and seems necessary for the induction of maturation since microinjection of the C subunit of c-AMP-dependent protein kinase was found to block maturation when performed during HDP and provided that 1-MeAde concentration was not increased (Dorée *et al.*, 1981).

Apparently, this observation does not fit with the fact that the microinjection of dbcAMP (Moreau and Guerrier, 1979) or of the A subunit of cholera toxin (Dorée *et al.*, 1981) facilitates 1-MeAde-induced maturation. However, it is possible that under these conditions, excess cyclic nucleotides directly affect some plasma membrane structural components without involving protein phosphorylation. This is not unlikely since we observed that dbcAMP stimulated a specific 1-MeAde-dependent Ca^{2+} release both *in vivo* (Moreau and Guerrier, 1979) and *in vitro* (Moreau and Guerrier, 1980). It thus appears that cyclic nucleotides may modulate transduction of the hormonal signal across the membrane. This has been recently confirmed by the finding that 1-MeAde dependent meiosis reinitiation does involve the activation of a pertussis-sensitive G protein (Shilling *et al.*, 1989).

Evidences for the importances of a calcium releasing step

Ca^{2+} translocation from the external medium or from the plasma membrane to some sequestering structures may play an important role in triggering maturation in various systems (Guerrier et al., 1982; Moreau *et al.*, 1985). This has been clearly shown in Roscoff, using oocytes taken from the Annelids *Sabellaria* (Peaucellier, 1977 b) and *Arenicola* (Meijer, 1979, 1980) or from the bivalve *Barnea candida*. In this last species, a D-600-sensitive calcium influx, which was accompanied by a significant H^+ efflux, has been demonstrated to occur following KCl or sperm - induced activations

(Dubé and Guerrier, 1982a, b). It was also found that Ca^{2+} alone could even trigger maturation, provided that the external Mg^{2+} concentration was lowered. Both in this species and in *Spisula* (Guerrier *et al.*, 1981), we found that the pH_i change alone could not be sufficient to trigger maturation since inhibiting the calcium uptake by D-600 or cobalt, without affecting the acid release, efficiently blocked GVBD. Using the fluorescent probe Quin 2/AM, we also showed that ammonia, which is the only trigger susceptible to act in the absence of external Ca^{2+}, produced both an intracellular Ca^{2+} surge and a slight alkalinization of the internal cytoplasm (Brassard *et al.*, 1988).

At least during a part of the breeding season, we also found that oocytes of the starfish *Marthasterias glacialis* entered maturation following an increase of the external Ca^{2+} concentration and that this result could not be attributed to an indirect effect upon the hormone-releasing follicle cells (Guerrier *et al.*, 1978). Ca^{2+} acted directly on denuded oocytes and the biological response was blocked by Ca^{2+} channel blockers such as D-600, Isoptin and Lanthanum (Moreau *et al.*, 1978b). In this species, it appeared, however, that the hormone-triggered maturation could occur in the presence of La^{3+} and in the complete absence of Ca^{2+} and Mg^{2+}. This suggested that 1-MeAde could act by releasing Ca^{2+} from internal stores, a proposition that we could easily verify after loading the oocytes with the photoprotein aequorin (Guerrier *et al.*, 1978; Moreau *et al.*, 1978b). Under these conditions, we found that the hormone, its active structural analogues, an efficient increased external calcium concentration, and dithiothreitol triggered a transient surge of intracellular Ca^{2+}.

The calcium response, which begins within 1 sec of oocyte stimulation and lasts for only 30 sec, appears moreover indispensable for obtaining the biological response. We found, indeed, that efficient inhibitors of the maturation actually acted by decreasing the extent of the calcium surge below its threshold value (Dorée *et al.*, 1978; Moreau *et al.*, 1978b, c). In contrast, the intracellular injection of dbc AMP increased the Ca^{2+} surge and facilited maturation (Moreau and Guerrier, 1979, 1980). Moreover, in experiments where EGTA was pressure injected into a single aequorin loaded oocyte and the calcium response simultaneously monitored, we found that only those oocytes that did not present a normal threshold Ca^{2+} response were unable to mature (Moreau *et al.*, 1978b, c; Moreau and Guerrier, 1979). Recently, we also showed that those maturations that could be induced by shaking starfish oocytes (Guerrier *et al.*, 1983) were blocked in the presence of the intracellular Ca^{2+} antagonists Quin 2-AM and TMB8 and that these drugs were also able to inhibit hormone-dependent maturation (Guerrier *et al.*, 1988a).

Taken together, all these data strongly suggest that Ca^{2+} translocation may play an important role in the process of meiotic reinitiation. Unfortunately, this conclusion was not fairly accepted in the view of some contradictory data that arose concerning either the reality of the hormone-induced Ca^{2+} surge (Eisen and Reynolds, 1984) or the actual effect of injected EGTA (Picard and Dorée, 1983; Guerrier *et al.*, 1984; Meijer and Guerrier, 1984). Presently, however, the picture is changing since Witchel and Steinhardt (1989) were finally able to record a hormone-induced Ca^{2+} spike using the fluorescent probe fura-2 and since injection of PSTAIR, a peptide homologous to the highly conserved region of the MPF catalytic subunit, also triggered both maturation and a transient Ca^{2+} peak (Picard *et al.*, 1990).

In fact, even if the release of bound Ca^{2+} may constitute an important step in the transduction process, this event is not sufficient by itself to trigger meiosis, since the hormone, the calcium or the dithiothreitol-dependent periods exceed by far the duration of the Ca^{2+} releasing period. Similarly, we already reported that nearly all the known maturation inhibitors proved to be effective all along the HDP. It thus appears that Ca^{2+} must participate in the induction of some irreversible event that would also require that the membrane be held in its responsive state. One may conceive, for example, that the release of Ca^{2+} from the plasma membrane might activate a cAMP and Ca^{2+} inhibited kinase requiring a constant loading of the receptor to play its triggering role (Dorée and Kishimoto, 1981; Mazzei *et al.*, 1981). This would fit with the fact that Ca^{2+} is a potent inhibitor of the cortical phosphorylations as studied in *in vitro* conditions (Mazzei and Guerrier, 1981) and with the observation that the *in vivo* phosphorylation of cortical proteins reaches an irreversible steady state at the end of HDP, while endoplasmic proteins continue to increase their phosphorylation after this stage. The importance of this cortical phosphorylation step is stressed by the fact that endoplasmic phosphorylation cannot proceed further when 1-MeAde is eliminated before the end of HDP (Guerrier *et al.*, 1977a).

Finally, a more positive role for the released Ca^{2+} might also be envisaged in that it could possibly act via some Ca^{2+} or Ca^{2+} and calmodulin-dependent enzymes.

Possible involvement of Ca^{2+} activated proteases

Specific proteases have been shown to be responsible for the so-called "spontaneous maturation" which drives the oocytes of the Annelid *Sabellaria* from the prophase to the metaphase 1 stage (Peaucellier, 1977a).

Other indirect evidence for some role for proteases during starfish oocyte maturation derive from the observation that various protease inhibitors of microbial origin or their synthetic analogues can block hormone-induced meiosis reinitiation, whereas they have no effect upon MPF-induced maturations (Clark and Kanatani, 1975; Kishimoto *et al.*, 1982a; Sano and Kanatani, 1983; Sawada *et al.*, 1989). That these proteases act at an early step during transduction is confirmed by the fact that leupeptin does not interfere with binding of the hormone to the oocyte surface (Yoshikuni *et al.*, 1988). The same protease inhibitors were also found able to block progesterone but not MPF-induced maturations in *Xenopus* (Guerrier *et al.*, 1977b). We must confess that we still do not know how they can participate in MPF activation.

Possible role for Ca^{2+} calmodulin-dependent enzymes

Calmodulin has been identified in the starfish oocyte and it has also been shown that various calmodulin inhibitors, applied from the outside, inhibited 1-MeAde-induced meiosis reinitiation (Meijer and Guerrier, 1981; Dorée et al., 1982). Despite the fact that maturation cannot be induced in the starfish oocyte by injection of the calmodulin-calcium complex (Dorée *et al.*, 1981; Mazzei *et al.*, 1981; Picard and Dorée, 1982), it remains possible that this complex functions at the membrane level. Thus, oocytes exposed to trypsin for 10 min were found to lose their sensitivity to calmodulin antagonists (Meijer and Guerrier, 1984b), a situation that fits with the classical observation that various calmodulin-dependent enzymes can be irreversibly activated by moderate proteolysis (Meijer and Guerrier, 1982). Phospholipase A_2 might be

such a calmodulin-dependent and trypsin-activable enzyme. It has indeed been reported (1) that the application of exogenous arachidonic acid or phospholipase A_2 can mimic 1-MeAde in inducing maturation; (2) that this effect is facilitated in the presence of Ca^{2+} and inhibited by EGTA; (3) that two inhibitors of phospholipase A_2 activity can reversibly block hormone-induced maturation when added during the HDP (Meijer *et al.*, 1984). In addition, we found that inhibitors of the cyclooxygenase pathway did not preclude maturation, whereas this process could be efficiently blocked by inhibitors of the lipoxygenase pathway. Further studies by Meijer *et al.*, (1986a, b, c) finally presented the 8 R isomere of the hydroxyeicosatetraenoic acid (8-HETE) as the more relevant active metabolite since it could trigger maturation in the absence of Ca^{2+} and at a concentration of about 10 nM. Unfortunately, it was simultaneously observed (1) that some species of starfish could not respond to fatty acid stimulation; (2) that, even in responsive species, 1-MeAde did not significantly change the basal phospholipid metabolism. It remains, however, quite illuminating to observe that such an original metabolic pathway exists in some species where it may trigger complete maturation, including the appearance of MPF. The possibility thus remains that such a minor component as 8-HETE may play a subtle role in the transduction of the 1-MeAde signal at the level of the plasma membrane (Fig.3).

MPF, the universal intracellular effector of the G2-M transition

Relationships between MPF, protein synthesis and protein phosphorylation

In amphibians, the appearance of MPF prior to GVBD requires the synthesis of a protein initiator that has now been identified as the product of the protooncogene mos (Sagata *et al.*, 1989a). Instead, the amplification of MPF following cytoplasm microinjection into a recipient unstimulated GV-blocked oocyte does not depend on protein synthesis (Wassermann and Masui, 1975; Schuetz and Samson, 1979; Gerhart *et al.*, 1984). The same situation was found to prevail for ovine oocytes (Osborn and Moor, 1983; Moor and Crosby, 1986), whereas no new transcription or translation appears to be required for triggering GVBD and chromosome condensation in the bivalve *Spisula* (Swenson *et al.*, 1986), the mouse (Jagiello, 1969; Stern *et al.*, 1972; Golbus and Stein, 1976; Balakier and Czolowska, 1977; Crozet and Szollosi, 1980) and the starfish (Guerrier and Dorée, 1975; Dorée, 1982). This implies that oocytes of these animals contain a stockpile of MPF precursor molecules that are only activated at the post-translational level. However, after the first meiotic cycle, as after each mitotic cycle, protein synthesis inhibitors have been shown to block subsequent cleavage (Wilt *et al.*, 1967; Wagenaar, 1983) and to preclude MPF reappearance (Gerhart *et al.*, 1984; Picard *et al.*, 1985, 1987; Guerrier and Néant, 1986). Protein synthesis is thus required at the beginning of each mitotic cell cycle.

In the surf clam *Spisula*, Rosenthal *et al.* (1980, 1987) described that fertilization induced the translation of several cell cycle-related proteins from stored maternal mRNA. Two of these proteins, cyclins A and B were found to accumulate up to metaphase and to be destroyed at the end of each meiosis and mitosis (Evans *et al.*, 1983). On the basis of sequence homology, a cyclin of the B type has been also found to occur in the starfish (Standart et al., 1987; Tachibana *et al.*, 1989). When injected into recipient GV-blocked oocytes of *Xenopus*, the mRNA for the cyclins from *Spisula* (Swenson *et al.*, 1986; Westendorf *et al.*, 1989), the sea urchin (Pines and Hunt, 1987) or the starfish (Tachibana *et al.*, 1989) were found to be translated and to induce true meiosis reinitiation. It has been shown, moreover, that the first meiotic cycle is likely to be triggered in *Spisula* by a mobilization of the maternal cyclin B protein unmasked by the intracellular alkalinization step that follows sperm penetration (Westendorf *et al.*, 1989). It thus appears that cyclin synthesis and cyclin degradation may represent key events in the activation and inactivation of MPF.

For the moment, let us consider that protein synthesis is not the only process which may regulate MPF activity. It is clear, indeed, that phosphorylation must also play a fundamental role since, in the starfish, the phosphorylation burst triggered by the hormone, its active analogues and mimetics appears to share the same concentration dependence, kinetics, reversibility and sensitivity to inhibitors as the biological process itself (Guerrier *et al.*, 1977a). In addition, we also showed that the puromycin analogue 6-dimethylaminopurine or 6-DMAP (Rebhun *et al.*, 1973), which specifically affected protein kinase activity without interfering with protein synthesis, reversibly blocked meiosis reinitiation in the starfish (Néant and Guerrier, 1988a), the mollusc *Patella* (Néant and Guerrier, 1988b), the mouse (Rime *et al.*, 1989) and also suppressed the early cleavages of the sea urchin (Néant et al., 1989) and *Mytilus* (Dubé *et al.*, unpublished). In the sea urchin, the starfish and *Mytilus*, we even observed that 6-DMAP did not block the cyclic manifestation of the cyclins which were always transiently destroyed at the right time even when cell nuclei remained blocked in interphase. It is thus clear (1) that cyclin destruction may take place in conditions where protein phosphorylation is dramatically repressed; (2) that the presence of cyclin is not a sufficient condition to activate MPF; (3) that both newly formed proteins such as the cyclins or other regulatory proteins that may preexist in the oocyte must be modified by a phosphorylation process which is inhibited by 6-DMAP and appears to be necessary for the activation of MPF.

Nature of MPF

A number of analytical and experimental data strongly suggested that MPF could be a protein kinase or a phosphoprotein. First, MPF activity, whether it resulted from stimulation by the hormone or by one of its mimetics, always proved to be associated with a high level of endogenous protein phosphorylation. This could be observed during meiosis reinitiation in the starfish (Guerrier *et al.*, 1975, 1977a; Dorée *et al.*, 1983; Meijer and Guerrier, 1983; Meijer *et al.*, 1984; Picard *et al.*, 1985; Peaucellier *et al.*, 1988), the amphibians (Maller *et al.*, 1977; Karsenti *et al.*, 1987; Lohka *et al.*, 1987), mammals (Wassarman *et al.*, 1979; Crosby *et al.*, 1984) echiuroids (Meijer *et al.*, 1982), polychaets (Peaucellier *et al.*, 1982, 1984) and molluscs (Dubé *et al.*, 1987; Eckberg *et al.*, 1987; Néant and Guerrier, 1988b). This correlation between the extent of protein phosphorylation and MPF activity has also been observed following fertilization, during the early synchronous cleavages of the sea urchin (Schatt *et al.*, 1983; Meijer and Pondaven, 1988; Néant *et al.*, 1989), after activation of the amphibian oocyte (Capony *et al.*, 1986) and during divisions of somatic mammalian cells (Westwood *et al.*, 1985).

Second, partially purified MPF could be dialysed without loss of activity using buffers known to limit the activity of protein phosphatases (Drury, 1978; Wu and Gerhart, 1980; Gerhart *et al.*, 1985).

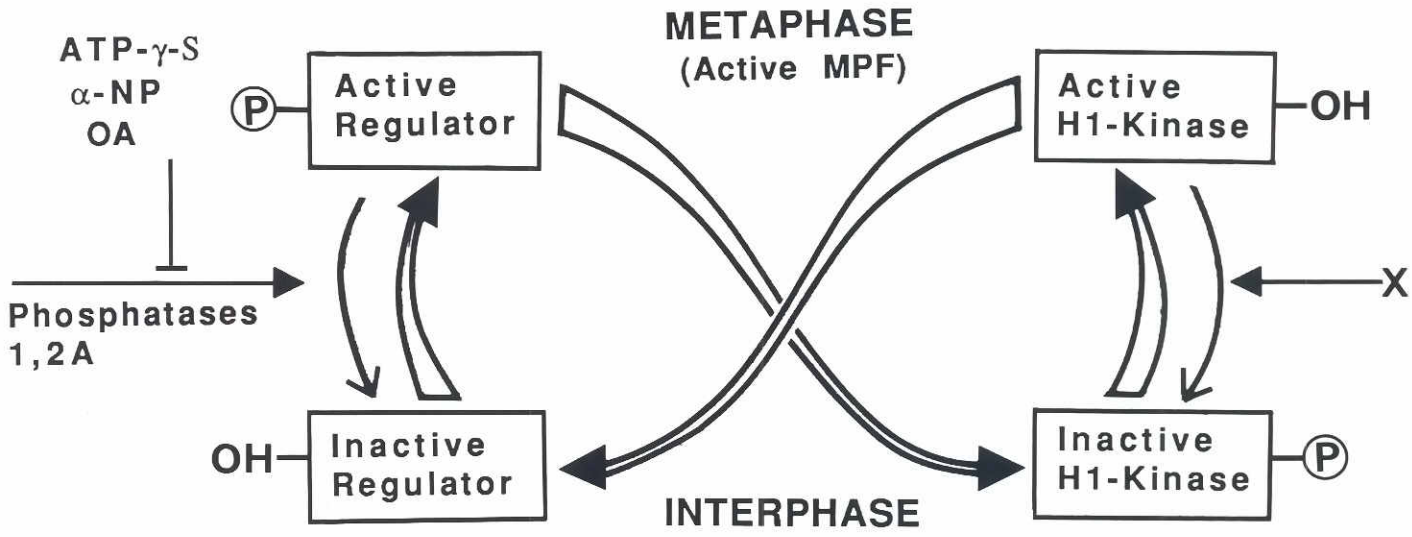


Fig. 2. Possible mechanism for MPF amplification. *This putative model is likely to fit reality since we know presently that histone H1-kinase is inactive when its catalytic subunit p34cdc2 is phosphorylated on tyrosine. The regulator thus might be the tyrosyl phosphoprotein phosphatase specific for p34 cdc2. The X element on the right would correspond to the p34 cdc2 inhibitory tyrosine kinase (see for ex. Dunphy and Newport, 1989; Morla* et al., *1989; Gould and Nurse, 1989).*

Similarly, treatment of such extracts with ATP-γ-S was found to efficiently protect MPF from inactivation (Hermann *et al.*, 1983), whereas monoclonal antibodies directed against these thio-phosphate groups immunoprecipitated this activity (Gerhart *et al.*, 1985).

Third, meiotic maturation and protein phosphorylation proved to be affected by the injection of phosphoprotein phosphatases as observed both in the starfish (Meijer *et al.*, 1986d) and the amphibian *Xenopus* (Hermann *et al.*, 1984).

Finally, Pondaven and Meijer (1986) have shown that the intracellular microinjection of α-naphtylphosphate (α-NP), a potent phosphatase inhibitor, could promote maturation of starfish oocytes and that this effect was reversed by the simultaneous injection of phosphoprotein phosphatase 2A. Starting from these data, Labbé *et al.* (1988c) showed that such a maturation could not result only from an unspecific and general effect due to the inhibition of phosphoprotein phosphatases, but rather from the actual and unexpected activation (36-fold increase) of that major Ca^{2+} and cyclic nucleotide-independent protein kinase that had been previously shown to cycle in phase with MPF activity during meiotic maturation and early embryogenesis (Sano, 1985; Picard *et al.*, 1985, 1987; Meijer *et al.*, 1987; Pelech *et al.*, 1987; Pondaven *et al.*, 1987). These authors also stressed that MPF and this histone H_1-kinase could not be considered as the same entity. Indeed, they observed (1) that cytoplasm containing H_1-kinase activity but not MPF (hormone-stimulated enucleated oocytes) could not stimulate H_1-kinase in recipient immature oocytes; (2) that cytoplasm containing MPF but low kinase activity (oocytes first injected with ATP-γ-S) was found to trigger both H_1-kinase and maturation of the same recipient oocytes. They also found, as already reported by Kishimoto *et al.* (1981), that cytoplasm containing both H_1-kinase and MPF activity could not be amplified when transferred into enucleated recipient oocytes. This last point agrees with the observation that MPF appears earlier in the nucleus than in the cytoplasm (Picard and Dorée, 1984) and argues for the compartmentalization of some important element required for MPF activation.

In summary, it thus appears that MPF alone and not H_1-kinase is capable of autocatalytic amplification, provided that some GV material is present in the recipient oocytes. Moreover, the behavior of ATP-γ-S and α-NP-injected oocytes clearly demonstrates that inhibiting the dephosphorylation of some regulatory protein would activate both MPF and the M-phase specific histone H_1-kinase. More recently, identical results have also been obtained following the injection of okadaic acid, another very potent phosphoprotein phosphatase inhibitor, in both the oocytes of *Xenopus* (Goris *et al.*, 1989; Rime *et al.*, 1990) and the starfish (Picard *et al.*, 1989; Pondaven *et al.*, 1989). The picture thus emerges of a more complex situation than previously thought, where MPF, the motor of the G_2-M transition, might be piloted by at least two different and possibly antagonistic enzyme systems (Fig.2). If one considers that the histone H_1-kinase may activate the regulatory protein by phosphorylation and that, in turn, this regulator is able to activate in some way the histone H_1-kinase, one would draw here a perfect model susceptible to account for the MPF amplification properties. One can see that such a dual system would be self perpetuating by nature, as described in some of the most famous drawings of Escher which fit the Möbius band model (Escher, 1971).

Main components of MPF

Identification of the main components of MPF has been made possible due to the convergence of the efforts displayed by the cell biologists who finally succeeded in purifying MPF (or the related M-phase specific histone H_1-kinase) and by the yeast geneticists who were able to clone and to sequence a number of genes involved in the control of the cell cycle (Lee and Nurse, 1987; Hayles and Nurse, 1989).

Among these genes, the cdc2 gene (for cell division control) has been fully characterized. Its protein product, p34 cdc2 exhibits a

histone H_1-kinase activity that oscillates periodically during the cell cycle, showing a peak during mitosis (Hindley and Phear, 1984; Booher and Beach, 1986; Simanis and Nurse, 1986; Moreno *et al.*, 1989). The other two gene products that closely interact with p34 cdc2 are the cdc13 and the suc1 gene products.

The cdc13 gene has been cloned and sequenced (Booher and Beach, 1988; Hagan *et al.*, 1988). It encodes for a protein that presents 50% identity in its central region with the cyclins which have been cloned from a variety of different organisms (Hagan *et al.*, 1988; Goebl and Byers, 1988; Solomon *et al.*, 1988). This protein, which migrates as a 62-k Da protein, binds to p34 cdc2 (Booher *et al.*, 1989) and its level is dramatically reduced just before sister chromatid separation as was also observed for the invertebrate cyclins (Moreno *et al.*, 1989). The suc1 gene product p13 suc1 (Hayles *et al.*, 1986; Hindley *et al.*, 1987) directly binds to p34 cdc2 (Brizuela *et al.*, 1987) and may negatively regulate its function.

It rapidly appeared that the cdc2 and cdc13 genes and their products have been conserved throughout the evolution as well as their capacity to bind together and to be retained by p13 suc1. Thus, the cdc2 gene from humans has been cloned by complementation of a $cdc2^{ts}$ mutant from the fission yeast *Schizosaccharomyces pombe* (Lee and Nurse, 1987). It could also replace the CDC28 homologue of cdc2 in the budding yeast *Saccharomyces cerevisiae* (Vittenberg and Reed, 1989). The MPF, which also exists from yeast (Weintraub *et al.*, 1982; Tachibana *et al.*, 1987) to humans (Sunkara *et al.*, 1979) proved, however, difficult to purify for a long time (Drury, 1978; Wu and Gerhart, 1980; Adlakha *et al.*, 1985; Kishimoto and Kondo, 1986; Nguyen-Gia *et al.*, 1986). The situation changed with the development of *in vitro* cell-free systems which allowed a more rapid estimation of the activity of the various fractions to be purified (Lohka and Masui, 1983; Newport and Kirschner, 1984; Lohka and Maller, 1985; Miake-Lye and Kirschner, 1985; Newport and Spann, 1987; Blow and Laskey, 1988).

MPF amplification, i.e. the activation of latent MPF, could be demonstrated to occur in such *Xenopus* cell-free extracts where demembranated sperm nuclei entered mitosis after the preparation was supplemented with a small amount of MPF and an ATP-regenerating system (Cyert and Kirschner, 1988). The dependency of that system to added MPF was even found to disappear after a 33% ammonium sulfate fractionation step which might have removed some kind of inhibitor. Dunphy and Newport (1988) demonstrated, after the same ammonium sulfate precipitation step, that interphase cell-free extracts also contained a latent form of MPF which could express itself in the presence of ATP-γ-S. Using a similar system to test their fractions, Lohka *et al.* (1988) were finally the first to report that the most purified fractions of MPF contained two proteins of 45 and 32 kDa, which could phosphorylate exogenous histone H1. The 32 kDa protein was further identified as the homologue of p34 cdc2 by affinity column chromatography on p13 suc1 beads or by Western blot analysis and immunoprecipitation using antibodies directed against the peptide EGVPSTAIREISLLKE, the conserved region of the cdc2 kinase (Dunphy *et al.*, 1988; Gautier *et al.*, 1988).

Another way to prepare purified MPF was suggested by the observation that the M-phase histone H_1-kinase and MPF activity followed the same cycle of activation and inactivation and depended in the same way on protein synthesis (Labbé *et al.*, 1988a; Meijer and Pondaven, 1988; Arion and Meijer, 1989). In fact, when the starfish enzyme was thus independently and simultaneously prepared, both in Roscoff (Arion *et al.*, 1988) and in Banyuls (Labbé *et al.*, 1988b), it was found to coeluate, at each step, with a 34 kDa protein which was again recognized and retained by p34 cdc2 antibodies and by p13 suc1 loaded beads.

p13 suc1 has been also identified in mammalian cell (Draetta *et al.*, 1987; Draetta and Beach, 1988; Dunphy *et al.*, 1988), where it is part of a complex including the cell-cycle regulated tyrosine kinase substrate p34 cdc2 (Draetta *et al.*, 1988) and the cyclin p62 cdc13 (Booher *et al.*, 1989; Pines and Hunter, 1989). This complex has been shown to purify simultaneously with the mammalian growth-associated histone H_1-kinase (Brizuela *et al.*, 1989; Langan *et al.*, 1989).

Dunphy *et al.* (1988) used p13 suc1 beads to remove MPF activity from a *Xenopus* egg extract and demonstrated that p13 suc1 produced in E. coli could inhibit the activation of latent MPF in activated *Xenopus* egg extracts. More recently, Dunphy and Newport (1989) showed, using the 33% ammonium sulfate fraction of Cyert and Kirschner (1988), that the ATP-dependent *in vitro* activation of latent MPF did result in a selective tyrosine dephosphorylation of p34 cdc2 and in the parallel switch-on of the H1-kinase activity. Moreover, it appeared that p13 suc1 completely blocked both events, while it had no effect once the system was set in motion. This suggests (1) that preMPF contains a latent form of tyrosine phosphatase that can be turned on when phosphatases are inhibited and ATP is given to the system; (2) that dephosphorylation of p34 cdc2 activates the M-phase histone H1-kinase (see Fig. 2). In fact, this last conclusion perfectly fits with the observation that a prokinase was present in extracts prepared from GV-blocked oocytes of *Xenopus* (Labbé *et al.*, 1988a) or the starfish (Labbé *et al.*, 1988b, 1989a), which could be activated upon ATP removal. It thus appears likely that different fractions might have been prepared in the past that were all designed as MPF but which, in fact, could correspond to quite different entities. These might have been either the M-phase cdc2 specific phosphatase which has to be activated or stabilized by phosphorylation (left part of Fig. 2) or the prokinase which needs to be dephosphorylated in order to act as a kinase (right side of Fig. 2).

The existence of an excellent correlation between the kinetics of cdc2 dephosphorylation and M-phase kinase activation has been verified both in *Xenopus* (Gautier *et al.*, 1989) and the starfish (Labbé *et al.*, 1989a; Pondaven *et al.*, 1990). Pondaven *et al.* (1990) also report that starfish H1-kinase can be activated *in vitro* using a broad spectrum potato acid phosphatase and that this activation correlates with cdc2 dephosphorylation. In mouse 3T3 cells, where p34 cdc2 becomes dephosphorylated on tyrosine upon entry in M-phase, the inhibition of this specific dephosphorylation by vanadate arrests the cell cycle in G2 (Morla *et al.*, 1989). In the fission yeast, tyrosine and threonine phosphorylation of the pp34 cdc2 kinase also decreases at mitosis (Gould and Nurse, 1989). Tyrosine phosphorylation occurs on a single Tyr 15 site located within the presumptive ATP binding domain of the protein, by which its kinase activity is efficiently repressed. The fact that substituting phenylalanine for tyrosine prematurely advances the cells in mitosis indicates that the pp34 cdc2 protein kinase activity might be regulated by a modulation of the nucleotide-binding ability of this subunit. This also suggests that the adenine analogue 6-DMAP might act in the same way to prevent or to inhibit M-phase kinase activity as was observed to occur both *in vivo* and *in vitro* (Meijer and Pondaven, 1988; Néant and Guerrier, 1988a, b; Néant *et al.*, 1989;

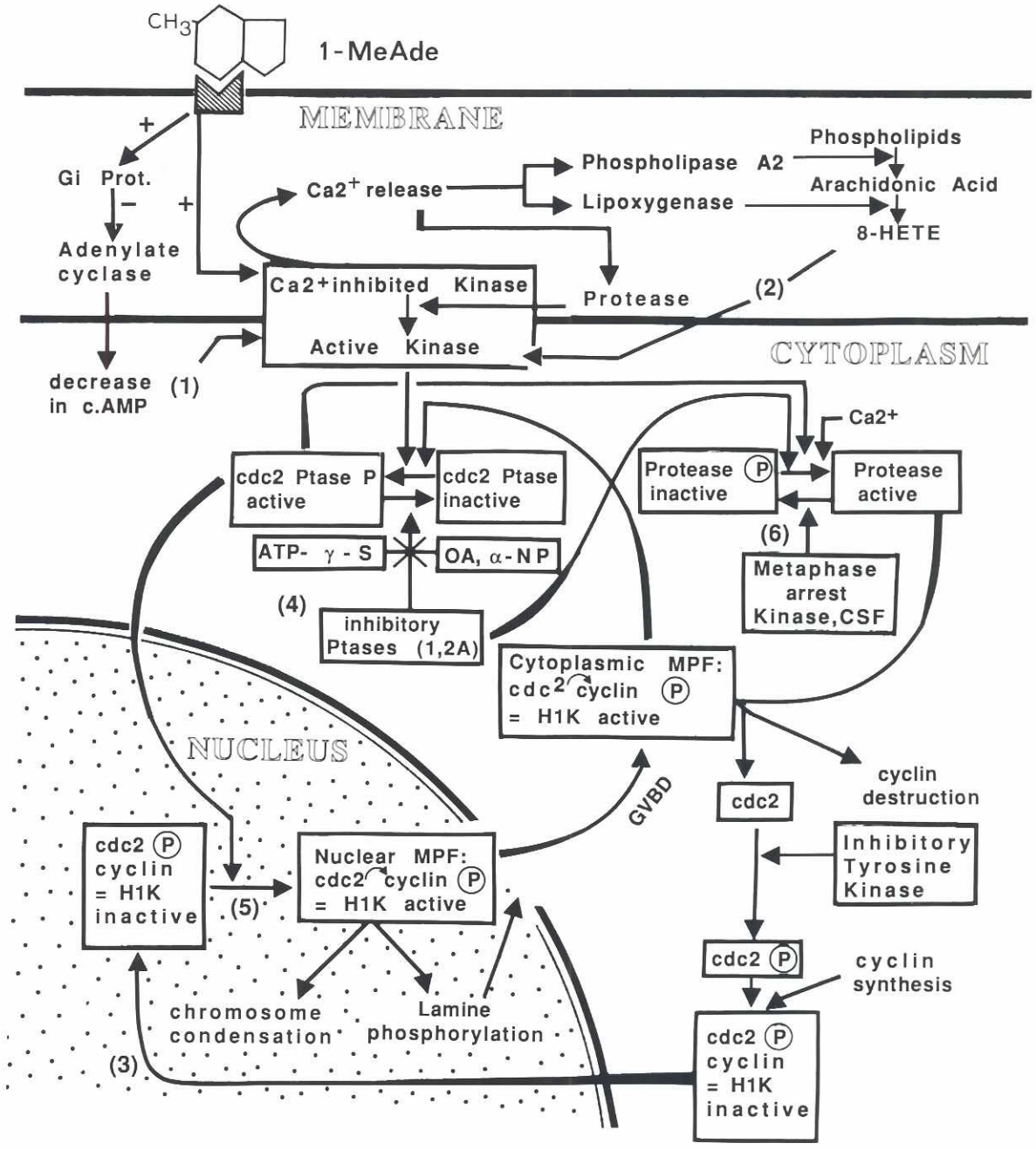


Fig. 3. A simplified model for the activation cascade set up by the hormone 1-MeAde during meiosis reinitiation of the starfish oocyte. (1) *The adenylate cyclase inhibitory pathway may act by stimulating the inhibitory kinases which phosphorylate cdc2 on tyrosine, by favoring Ca^{2+}-relocalization in the membrane or by activating the inhibitory phosphatases which dephosphorylate and inactivate the cdc2-specific phosphatases.* **(2)** *8-HETE may maintain kinase activation.* **(3)** *In S. pombe, cyclin has nuclear localization sequences and is required to drive cdc2 into the nucleus (Booher et al., 1989).* **(4)** *Phosphatase inhibitors such as ATP-γ-S, α-NP and okadaic acid protect MPF activity and can even trigger meiosis. These inhibitors may stabilize the cdc2 specific tyrosine kinase in its phosphorylated state through inhibition of the phosphatases 1 and 2A which have been shown to counteract meiosis reinitiation.* **(5)** *The complex cdc2-cyclin is present in the GV stage and represents the proMPF. It is activated through dephosphorylation of cdc2 on tyrosine. MPF appears earlier in the nucleus than in the cytoplasm. Nucleoplasm is required for MPF amplification.* **(6)** *Metaphase 2 blocked mouse oocytes depend on the synthesis of a protein which stabilizes phosphorylation even in the presence of 6-DMAP. Metaphase 2 blocked Xenopus oocytes are stable in the absence of protein synthesis and unaffected by 6-DMAP. The stable and stabilizing protein might be pp39 c-mos. It may inactivate cyclin-specific protease by phosphorylation. Cyclin degradation occurs in vivo even when the nucleus remains blocked in an interphase configuration upon the influence of 6-DMAP. Protease activation and cyclin degradation may involve the development of cyclical transient processes such as Ca^{2+} surges which may continue to be triggered by the cell oscillator set up at fertilization.*

Rime *et al.*, 1989). Presently, we still do not know if p13 suc1, which blocks cdc2 tyrosine dephosphorylation and histone H1-kinase activation (Dunphy and Newport, 1989), acts in the same way or indirectly, for example by stimulating an inhibitory tyrosine kinase or by inhibiting the phosphotyrosine phosphatase. This might help to understand why microinjection of the peptide PSTAIR could facilitate MPF activation in *Xenopus* (Gautier *et al.*, 1988) and even trigger GVBD and M-phase kinase activation when introduced in various invertebrate oocytes at a concentration of the order of 400 µM (Labbé *et al.*, 1989a). The simplest explanation for these observations is indeed to consider that this peptide is competing with p34 cdc2 for an inhibitor.

Finally, one must stress that another important subunit is obviously required to confer M-phase kinase activity to the catalytic p34 cdc2 component of MPF. This subunit, which has been shown to bind to p34 cdc2 well before its activation, does, in fact, correspond to the cyclin, i.e. the homologue of the yeast cdc 13 gene product. Thus, in mammalian cells (Brizuela *et al.*, 1989; Langan *et al.*, 1989; Pines and Hunter, 1989) as well as in *Spisula* (Draetta *et al.*, 1989) and the starfish (Labbé *et al.*, 1989b; Meijer *et al.*, 1989; Pondaven *et al.*, 1990), the use of both anticyclin antibodies and p13 suc1 beads made it possible to demonstrate: (1) that cyclin and p34 cdc2 possibly associated in stoichiometric amounts (Labbé *et al.*, 1989b; Pondaven *et al.*, 1990) and copurified with the M-phase kinase; (2) that there was a good temporal correlation between the extent of H1-kinase activity and the accumulation of the phosphorylated form of cyclin during the cell cycle; (3) that histone H1-kinase activity declined in parallel with cyclin destruction at the metaphase-anaphase transition. It is worth noting that it was possible to reduce the rate of this inactivation in the presence of phosphatase inhibitors and ATP (Labbé *et al.*, 1987; Arion and Meijer, 1989) and that the microinjection of high concentrations of antiproteases during the first meiotic cycle of starfish oocytes stabilized their MPF activity (Picard *et al.*, 1985).

Control mechanisms for the activation and inactivation of MPF

In the preceding sections, we found that protein synthesis was required at the beginning of each cell cycle except, possibly, during the first meiotic cycle which might rely on the unmasking of preformed cyclin B and p34 proteins. This absolute requirement for protein synthesis would be easily explained if one has to restore the cyclin subunit of the MPF which appears to be destroyed at each metaphase-anaphase transition, while the amount of p34 cdc2 remains constant.

That this was indeed the case, i.e. that cyclin synthesis was necessary and sufficient to drive in the cell cycle, has been examplified by two kinds of complementary experiments. First, Minshull *et al.* (1989) demonstrated that the specific destruction of the endogenous mRNA for both frog cyclins B_1 and B_2, with antisense oligonucleotides and endogenous RNA-ase H, blocked the entry in mitosis of *Xenopus* cell-free extracts, while the ablation of either cyclin alone only caused some delay in mitosis. Second, Murray and Kirschner (1989a) showed that the exclusive addition of sea urchin or *Xenopus* cyclin mRNA to a *Xenopus* cell-free extract previously deprived of endogenous mRNA was sufficient to induce multiple cell cycles. Under these conditions, as well as *in vivo*, cyclin level, H1-kinase and MPF were found to fluctuate in parallel.

Murray *et al.* (1989) also demonstrated that cyclin degradation was directly responsible for the exit from the cell cycle towards interphase. To prove that, they prepared a 90 amino acids-deleted mutant form of cyclin that was always able to activate MPF and to drive cell-free extracts into mitosis. However, when added to a cell-free extract prepared from metaphase 2-blocked oocytes, this mutant cyclin was found to resist the Ca^{2+}-induced degradation that usually affects the wild-type cyclin. This prevented MPF inactivation and produced a stable metaphase arrest. It also appeared that *in vivo* microinjection of the mRNA for this truncated cyclin arrested both activated and fertilized eggs in metaphase.

It is thus clear that entry and exit from the mitotic cell cycle depend on the synthesis and the selective proteolytic degradation of the cyclins. One knows, however, that the association of p34 cdc2 and the various forms of the cdc13 cyclins, which already exist in the immature oocyte, is not sufficient to produce MPF. In fact, this necessary association will only succeed following that crucial step that would trigger dephosphorylation of the catalytic cdc2 subunit. That cyclin synthesis might appear as the only limiting factor required to obtain MPF activation in a cell-free system thus clearly demonstrates at least one thing, i.e. that these extracts are no more than an oversimplified representation of the reality. Obviously, such models have constituted quite powerful tools that greatly helped us in catching and deciphering some fundamental steps in MPF activation (Murray and Kirschner, 1989b). However, one must keep in mind that they may also lead one to miss or skip over some subtle and highly regulated steps which may play an important role in the living system.

Such a difficulty is particularly evident when one considers the problem of how to explain clearly what factors might be involved (1) in stabilizing metaphase conditions at the end of meiosis and, (2) in inactivating the cyclin at the end of each cell cycle, which is even more difficult to appreciate.

The metaphase block

Before fertilization, amphibian oocytes that have resumed and completed meiosis appear to be arrested during the second meiotic metaphase. When cytoplasm or cytosols are prepared from such unactivated eggs and injected into a zygote, the recipient embryos or injected blastomeres will also be arrested at metaphase for a long period of time (Masui and Markert, 1971; Masui *et al.*, 1980; Masui and Shibuya, 1987; Shibuya and Masui, 1988, 1989). The Ca^{2+} sensitive factor responsible for this effect and which disappears definitively upon fertilization or activation has been called cytostatic factor or CSF by Masui. Another important property of the amphibian CSF is to be independent on protein synthesis. In this, it appears to differ from possibly related factors which also arrest the oocytes of some invertebrates in metaphase 1 as observed in *Patella* (Néant and Guerrier, 1988b) and *Mytilus* (Dubé and Dufresne, 1990) or block the mouse oocyte in metaphase 2 (Clarke and Masui, 1983, 1985; Hashimoto and Kishimoto, 1988). In these species, the inhibition of protein synthesis induces the precocious disappearance of cyclins and drives the metaphase-blocked oocytes towards interphase, inducing the reconstitution of resting nuclei. This implies that the unfertilized frog oocyte has a stabilized form of cyclin which does not need to be continuously synthesized in order to maintain MPF activity. In fact, to be quite rigorous, one must stress that it is not fully proven that the exit from the metaphase stage of the invertebrate oocyte necessarily re-

quires complete cyclin destruction since we observed: (1) that the inhibition of protein synthesis also reduced protein phosphorylation (Néant and Guerrier, 1988b); (2) that 6-DMAP, which only induced protein dephosphorylation also drove *Mytilus* and *Patella* oocytes to interphase (Guerrier *et al.*, 1990). These nuclear changes did not occur in the case of *Xenopus* and the mouse, which could maintain condensed chromosomes in the presence of the same concentrations of 6-DMAP which appeared sufficient to block MPF formation and to produce interphase nuclei when applied during metaphase 1 (Rime *et al.*, 1989 and unpublished; Charbonneau, unpublished).

Taken together, these observations suggest that a stable phosphorylated protein factor must be responsible for maintaining metaphase conditions in the amphibian oocytes, whereas the possible homologues of this protein in the other systems need to be continuously synthesized. Presently, there is persuasive evidence that the c-mos protooncogene might be such a stable protein that would thus correspond to CSF. First, Watanabe *et al.* (1989) reported that the *Xenopus* c-mos protooncogene protein pp39 mos accumulated specifically in the unfertilized egg during maturation, that it was hyperphosphorylated and exhibited a high protein kinase activity which appeared to be directed against proteins of a molecular weight close to that of the cyclins. They also showed that pp39 mos, like CSF, was rather stable in the presence of cycloheximide and underwent selective proteolysis upon activation or fertilization, an event that involved activation of the Ca^{2+}-dependent cystein protease calpaïn. Identification of pp39 mos as CSF was moreover confirmed by the observation (1) that microinjection of synthetic mos mRNA into one blastomere of a two cell embryo arrested its cleavage in metaphase; (2) that truncated forms of c-mos RNA, which produced a mos protein lacking the ATP binding domain necessary for mos-kinase activity, are unable to induce cleavage arrest; (3) that neutralization or ablation of the pp39 mos protein using specific antibodies blocked cytosolic CSF activity (Sagata *et al.*, 1989b).

In the mouse, where c-mos maternal mRNA is degraded immediately after fertilization (Goldman *et al.*, 1987; Mutter and Wolgemuth, 1987; Keshet *et al.*, 1988; Paules *et al.*, 1989), the ablation of c-mos RNA with antisense oligonucleotides was also shown to prevent meiosis 2 arrest (O'Keefe *et al.*, 1989). Under these conditions, the authors observed the same cytological aspects (resting nuclei) that we obtained when 6-DMAP was found to inhibit endogenous protein phosphorylation during the first meiotic cell cycle (Rime *et al.*, 1989). This confirms both that no stable CSF protein can exist in this species and that the required continuous synthesis of pp39 c-mos would exert its stabilizing effect through phosphorylation.

The cyclin destruction step

This last point appears the most difficult to resolve since quite divergent pieces of information have been collected on that topic from the two main sources at our disposal, i.e. the living oocytes and the cell-free systems.

Thus, in cell-free extracts, it appeared (1) that the protein kinase inhibitor 6-DMAP could stabilize both the cyclin and histone H1-kinase activity; (2) that cyclin destruction seemed to require ATP and Mg2+ (Felix *et al.*, 1989; Luca and Ruderman, 1989). This suggested that protein phosphorylation was required both for cyclin destruction and kinase inactivation and, thus, that the putative specific protease responsible for these effects must be active in its phosphorylated form.

On the contrary, the fundamental observation that pp39 mos maintains the metaphase block by phosphorylation would rather suggest that the putative cyclin-specific protease might be kept switched off by pp39 mos-mediated phosphorylation and that it might be activated by a phosphatase, when functional (Hunt, 1989). An alternative hypothesis would be that the cyclins become resistant to proteolysis, whether they are phosphorylated by pp39 mos or by the cdc2 kinase. Here again, the activation of some relevant phosphatase might eventually be an effective trigger for cyclin destruction. Under these conditions, the observation that cyclins from the cell-free extracts are stabilized in the presence of 6-DMAP might be explained by the fact that the relevant phosphatase would have been inactivated by dephosphorylation (Fig. 3). It is also possible that 6-DMAP might have dissociated the active cdc2-cyclin complex, which would confer a greater stability to the isolated cyclins.

Presently, in the absence of more precise information, we are completely unable to discriminate between these possibilities and would refer moreover to our *in vivo* studies which covered both the meiotic period in the starfish (Néant *et al.*, 1988a) and the early cleavages of the sea urchin and *Mytilus* (Néant *et al.*, 1989; Dubé *et al.*, unpublished). In fact, these experiments demonstrated that 6-DMAP did not preclude the cyclic synthesis and transient destruction of the cyclins, even when nuclear and mitotic cycles appeared to be blocked under these conditions. This indicates: (1) that the cyclins may be destroyed and that their specific proteases may function whenever they are phosphorylated or not; (2) that the cyclins continue to respond at the right time to some specific signals that must have been programmed following 1-MeAde addition or sperm penetration. However, when 6-DMAP was present before fertilization, these signals were not set up, so that the two presumptive cyclins that we observed to fluctuate when 6-DMAP was added later seemed to be stabilized under these conditions. It thus appears that an important imprinting step must take place during the first cell cycle, which is likely to require both protein synthesis and protein phosphorylation. The function of this event might be to sensitize the endogenous enzymatic systems so that they can respond to the Ca^{2+} surges which punctuate the cell cycle of the living embryo and are thought to induce nuclear envelope breakdown and to trigger the entry into anaphase (Poenie *et al.*, 1985; Steinhardt and Alderton, 1988; Schollmeyer, 1988; Twigg *et al.*, 1988; Hepler, 1989). Finally, this picture seems to fit better with the observation that pp39 mos maintains the metaphase block through phosphorylation and that this block is also released following the activation of a Ca^{2+} activated protease.

Conclusion

At the end of this review, where we tried to present most of our basic knowledge on the subject, we are still confronted with an increasing amount of experimental data that is not yet fully understood. Fig. 3 offers an oversimplified but still speculative overview of that spider web of interactive pathways which are at work in the living cell. One can see that this scheme remains silent about the highly complex integrated steps which would control spindle kinetics and cytokinesis. In turn, it contains some additional information about the intracellular localization of some factors that we had not had the opportunity to discuss beforehand. Thus, our drawing takes

into account the fact that p34 cdc2 is mainly a nuclear protein (Bailly *et al.*, 1989; Riabowol *et al.*, 1989), which must be driven in that position after its binding to the cyclins (Booher *et al.*, 1989). At this point, one must stress that the tyrosyl phosphatase specific for p34 cdc2 has not been yet characterized and that we know nothing about its possible localization. We also have yet to discover the nature of the intranuclear factors required for MPF amplification in the starfish oocyte (Kishimoto *et al.*, 1981; Picard and Dorée, 1984).

Finally, whatever may be the degree of precision or incertitude of our model, we would stress that it seems particularly stimulating to observe that an external signal, recognized by the plasma membrane, could set in motion so many integrated processes which would definitively change cell function. One can easily imagine that similar processes, which do not necessarily involve any internalization or transfer of material, may also play an important role in introducing significant differences between sister cells, during the early steps of differentiation of the young embryo.

Acknowledgments

Thanks are due to Mrs. C. Guerrier for her excellent assistance and for preparing the figures, to Mrs. M. A. Maillard for typing the text, to the fishermen of the Roscoff MBL for providing us with living animals, to our masters in the field, E.G. Conklin, T.H. Morgan, E.B. Wilson, A. Dalcq, J.J. Pasteels, J. Brachet, A. Monroy, H. Kanatani, V. Nigon and to our old associates in Roscoff, L. Meijer, M. Moreau, M. Dorée, L. Dufresne, F. Dubé, M. Charbonneau, G. Mazzei and G. Peaucellier. This work would not have been made possible without the help of the CNRS, the INSERM, the DGRST, the Fondation pour la Recherche médicale, the Centre Jacques Cartier and the Association pour la Recherche contre le Cancer. Bibliography was stopped on December 25, 1989.

References

ADLAKHA, R.C., WRIGHT, D.A., SAHASRABUDDHE, C.G., DAVIS, F.M., PRASHAD, N., BIGO, H. and RAO, P.N. (1985). Partial purification and characterization of mitotic factors from HeLa cells. *Exp. Cell Res. 160:* 471-482.

ARION, D. and MEIJER, L. (1989). M-Phase specific protein kinase from mitotic sea urchin eggs: cyclic activation depends on protein synthesis and phosphorylation but does not require DNA or RNA synthesis. *Exp. Cell Res. 183:* 361-375.

ARION, D., MEIJER, L., BRIZUELA, L. and BEACH, D. (1988). cdc2 is a component of the M-phase-specific histone H1 kinase: evidence for identity with MPF. *Cell 55:* 371-378.

BAILLY, E., DORÉE, M., NURSE, P. and BORNENS, M. (1989). p34cdc2 is located in both nucleus and cytoplasm; part is centrosomally associated in G2/M and enters vesicles at anaphase. *EMBO J. 8:* 3985-3995.

BALAKIER, H. and CZOLOWSKA, N. (1977). Cytoplasmic control of nuclear maturation in mouse oocytes. *Exp. Cell Res. 110:* 466-469.

BAULIEU, E.E., GODEAU, J.F., SCHORDERET, M. and SCHORDERERT-SLATKINE, S. (1978). Steroid induced meiotic division in *Xenopus laevis* oocytes: surface and calcium. *Nature 275:* 593-598.

BLOW, J.J. and LASKEY, R.A. (1988). A role for the nuclear envelope in controlling DNA replication within the cell cycle. *Nature 332:* 546-548.

BOOHER, R.N., ALFA, C.E., HYAMS, J.S. and BEACH, D. (1989). The fission yeast cdc2/cdc13/suc 1 protein kinase: regulation of catalytic activity and nuclear localization. *Cell 58:* 485-497.

BOOHER, R. and BEACH, D.H. (1986). Site-specific mutagenesis of cdc2, a cell cycle control gene of the fission yeast *Schizosaccharomyces pombe*. *Mol. Cell Biol. 6:* 3523-3530.

BOOHER, R. and BEACH, D. (1988). Involvement of cdc 13^{+} in *Schizosaccharomyces pombe:* possible interaction of the gene product with microtubules. *EMBO J. 7:* 2321-2327.

BORNSLAEGER, E.A., MATTEI, P. and SCHULTZ, R. M. (1986). Involvement of cAMP-dependent protein kinase and protein phosphorylation in regulation of mouse oocyte maturation. *Dev. Biol. 114:* 453-462.

BRASSAR, M., DUCLOHIER, H., MOREAU, M. and GUERRIER, P. (1988). Intracellular pH change does not appear as a prerequisite for triggering activation of *Barnea candida* (Mollusca pelecypoda) oocytes. *Gamete Res. 20:* 43-52.

BRIZUELA, L., DRAETTA, G. and BEACH, D. (1987). p13^{suc1} acts in the fission yeast cell division cycle as a component of the p34^{cdc2} protein kinase. *EMBO J. 6:* 3507-3514.

BRIZUELA, L., DRAETTA, G. and BEACH, D. (1989). Activation of human cdc2 protein as a histone H1 kinase is associated with complex formation with the p62 subunit. *Proc. Natl. Acad. Sci. USA 86:* 4362-4366.

CAPONY, J.P., PICARD, A., PEAUCELLIER, G., LABBÉ, J.C. and DORÉE, M. (1986). Changes in the activity of the maturation-promoting factor during maturation and following activation of amphibian and starfish oocytes: their correlations with protein phosphorylation. *Dev. Biol. 117:* 1-12.

CHAET, A.B. and Mc CONNAUGHY, R.A. (1959). Physiologic activity of nerve extracts. *Biol. Bull. (Woods Hole, Mass.) 117:* 407.

CLARK, T.G. and KANATANI, H. (1975). Effect of some inhibitors of proteolytic enzymes on maturation of starfish oocytes. *Biol. Bull. 149:* 423.

CLARKE, H.J. and MASUI, Y. (1983). The induction of reversible and irreversible chromosome decondensation by protein synthesis inhibition during meiotic maturation of mouse oocytes. *Dev. Biol. 97:* 291-301.

CLARKE, J.J. and MASUI, Y. (1985). Inhibition by dibutyryl cyclic AMP of the transition to metaphase of mouse oocyte nuclei and its reversal by cell fusion to metaphase oocytes. *Dev. Biol. 108:* 32-37.

COSTELLO, D.P. (1945). Experimental studies of germinal localization in Nereis. I. The development of isolated blastomeres. *J. Exp. Zool. 100:* 19-66.

CROSBY, I.M., OSBORN, J.C. and MOOR, R.M. (1984). Changes in protein phosphorylation during the maturation of mammalian oocytes *in vitro*. *J. Exp. Zool. 229:* 459-466.

CROZET, N. and SZOLLOSI, D. (1980). Effects of actinomycin D and α-amanitin on the nuclear ultrastructure of mouse oocytes. *Biol. Cellulaire 38:* 163-170.

CYERT, M.S. and KIRSCHNER, M.W. (1988). Regulation of MPF activity *in vitro*. *Cell 53:* 185-195.

DORÉE, M. (1982). Protein synthesis is not involved in initiation or amplification of the maturation-promoting factor (MPF) in starfish oocytes. *Exp. Cell Res. 139:* 127-133.

DORÉE, M. and GUERRIER, P. (1975). Site of action of 1-methyladenine in inducing oocyte maturation in starfish: kinetic evidence for receptors localized on the cell membrane. *Exp. Cell Res. 96:* 296-300.

DORÉE, M., GUERRIER, P. and LEONARD, N.J. (1976a). Hormonal control of meiosis: specificity of the 1-methyladenine receptors in starfish oocytes. *Proc. Natl. Acad. Sci. USA 73:* 1669-1673.

DORÉE, M., GUERRIER, P. and MOREAU, M. (1976b). Contrôle hormonal de la méiose des ovocytes d'Etoiles de mer. II. Etude de quelques propriétés fondamentales du récepteur de la 1-méthyladénine. In *Actualité sur les hormones d'Invertébrés* (Ed. CNRS). Coll. Intern. CNRS 251, pp. 199-206.

DORÉE, M. and KISHIMOTO, T. (1981). Calcium mediated transduction of the hormonal message in 1-methyladenine-induced meiosis reinitiation of starfish oocytes. In *Metabolism and Molecular Activities of Cytokinins* (Eds. J. Guern and C. Peaud-Lenoel). Springer-Verlag, Berlin, pp. 338-348.

DORÉE, M., KISHIMOTO, T., LE PEUCH, C., DEMAILLE, J.G. and KANATANI, H. (1981). Calcium-mediated transduction of the hormonal message in meiosis reinitiation of starfish oocytes. Modulation following injection of cholera toxin and c-AMP dependent protein kinase. *Exp. Cell Res. 135:* 237-249.

DORÉE, M., MOREAU, M. and GUERRIER, P. (1978). Hormonal control of meiosis. *In vitro* induced release of calcium ions from the plasma membrane in starfish oocytes. *Exp. Cell Res. 115:* 251-260.

DORÉE, M., PEAUCELLIER, G. and PICARD, A. (1983). Activity of the maturation-promoting factor and the extent of the protein phosphorylation oscillate simultaneously during meiotic maturation of starfish oocytes. *Dev. Biol. 99:* 489-501.

DORÉE, M., PICARD, A., CAVADORE, J.C., LE PEUCH, C. and DEMAILLE, J.G. (1982). Calmodulin antagonists and hormonal control of meiosis in starfish oocytes. *Exp. Cell Res. 139:* 135-144.

DRAETTA, G. and BEACH, D. (1988). Activation of cdc2 protein kinase during mitosis in human cells: cell cycle-dependent phosphorylation and subunit rearrangement. *Cell 54:* 17-26.

DRAETTA, G., BRIZUELA, L., POTASHKIN, J. and BEACH, D. (1987). Identification of p34 and p13, human homologs of the cell cycle regulators of fission yeast encoded by cdc2 and suc1+. *Cell 50:* 319-325.

DRAETTA, G., LUCA, F., WESTENDORF, J., BRIZUELA, L., RUDERMAN, J. and BEACH,

D. (1989). cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. *Cell 56:* 829-838.

DRAETTA, G., PIWNICA-WORMS, H., MORRISON, D., DRUKER, B., ROBERTS, T. and BEACH, D. (1988). Human cdc2 protein kinase is a major cell-cycle regulated tyrosine kinase substrate. *Nature 336:* 738-74.

DRURY, K.C. (1978). Method for the preparation of active maturation promoting factor (MPF) from *in vitro* matured oocytes of *Xenopus laevis*. *Differentiation 10:* 181-186.

DUBE, F. and DUFRESNE, L. (1990). Release of metaphase arrest by partial inhibition of protein synthesis in blue mussel oocytes. *J. Exp. Zool.* (In press).

DUBE, F., GOLSTEYN, R. and DUFRESNE, L. (1987). Protein kinase C and meiotic maturation of surf clam oocytes. *Biochem. Biophys. Res. Com. 142:* 1072-1076.

DUBE, F. and GUERRIER, P. (1982a). Activation of *Barnea candida* (Mollusca Pelecypoda) oocytes by sperm or KCl, but not by NH4Cl requires a calcium influx. *Dev. Biol. 92:* 408-417.

DUBE, F. and GUERRIER, P. (1982b). Acid release during activation of *Barnea candida* (Mollusca Pelecypoda) oocytes. *Dev. Growth Differ. 24:* 163-171.

DUNPHY, W.G., BRIZUELA, L., BEACH, D. and NEWPORT, J. (1988). The *Xenopus* homolog of cdc2 is a component of MPF, a cytoplasmic regulator of mitosis. *Cell 54:* 423-431.

DUNPHY, W.G. and NEWPORT, J.W. (1988). Mitosis-inducing factors are present in a latent form during interphase in the *Xenopus* embryo. *J. Cell Biol. 106:* 2047-2056.

DUNPHY, W.G. and NEWPORT, J.W. (1989) Fission yeast p13 blocks mitotic activation and tyrosine dephosphorylation of the *Xenopus* cdc2 protein kinase. *Cell 58:* 181-194.

ECKBERG, W.R., SZUTS, E.Z. and CARROLL, A.G. (1987). Protein kinase C activity, protein phosphorylation and germinal vesicle breakdown in *Spisula* oocytes. *Dev. Biol. 124:* 57-64.

EISEN, A. and REYNOLDS, G. (1984). Calcium transients during early development of single starfish *Asterias forbesi* oocytes. *J. Cell Biol. 99:* 1878-1882.

ESCHER, M.C.(1971). *Graphic und Zeichnungen*. Heinz Moos Verlag GmbH & Co. KG, München.

EVANS, T., ROSENTHAL, E.T., YONGBLOM, J., DISTEL, D. and HUNT, T. (1983). Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell 33:* 389-396.

FELIX, M.A., PINES, J., HUNT, T. and KARSENTI, E. (1989). A post-ribosomal supernatant from activated *Xenopus* eggs that displays post-translationally regulated oscillation of its cdc2+ mitotic kinase activity. *EMBO J. 8:* 3059-3069.

FORTUNE, J.E., CONCANNON, P.W. and HANSEL, W. (1975). Ovarian progesterone level during *in vitro* oocyte maturation and ovulation in *Xenopus laevis*. *Biol. Reprod. 13:* 561-567.

GAUTIER, J., MATSUKAWA, T., NURSE, P. and MALLER, J. (1989). Dephosphorylation and activation of *Xenopus* p34 cdc2 protein kinase during the cell cycle. *Nature 339:* 626-629.

GAUTIER, J., NORBURY, C., LOHKA, M., NURSE, P. and MALLER, J. (1988). Purified maturation-promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene cdc2+. *Cell 54:* 433-439.

GERHART, J., WU, M. and KIRSCHNER, M. (1984). Cell cycle dynamics of an M-phase-specific cytoplasmic factor in *Xenopus laevis* oocytes and eggs. *J. Cell Biol. 98:* 1247-1255.

GERHART, J., WU, M., CYERT, M. and KIRSCHNER, M. (1985). M-phase promoting factors from eggs of *Xenopus laevis*. *Cytobios 43:* 335-347.

GODEAU, F., SCHORDERET-SLATKINE, S., HUBERT, P. and BAULIEU, E.E. (1978). Induction of maturation in *Xenopus laevis* oocytes by a steroid linked to a polymer. *Proc. Natl. Acad. Sci. USA 75:* 2353-2357.

GOEBL, M. and BYERS, B. (1988). Cyclin in fission yeast. *Cell 54:* 739-740.

GOLBUS, M.S. and STEIN, M.P. (1976). Qualitative patterns of protein synthesis in the mouse oocyte. *J. Exp. Zool. 198:* 337-342.

GOLDMAN, D.S., KIESSLING, A.A., MILLETTE, C.F. and COOPER, G.M. (1987). Expression of c-mos RNA in germ cells of male and female mice. *Proc. Natl. Acad. Sci. USA 84:* 4509-4513.

GORIS, J., HERMANN, J., HENDRIX, P., OZON, R. and MERLEVEDE, W. (1989). Okadaic acid, a non-TPA tumor promoter, inhibits specifically protein phosphatases, induces maturation and MPF formation in *Xenopus laevis* oocytes. *Adv. Prot. Phosphatases 5:* 579-592.

GOULD, K.L. and NURSE, P. (1989). Tyrosine phosphorylation of the fission yeast cdc2+ protein kinase regulates entry into mitosis. *Nature 342:* 39-45.

GUERRIER, P. (1970). Les caractères de la segmentation et la détermination de la polarité dorsoventrale dans le développement de quelques Spiralia. III. Pholas dactylus et *Spisula subtruncata*. *J. Embryol. Exp. Morphol. 23:* 667-692.

GUERRIER, P., BRASSART, M., DAVID, C. and MOREAU, M. (1986a). Sequential control of meiosis reinitiation by pH and Ca2+ in oocytes of the prosobranch molusk *Patella vulgata*. *Dev. Biol.: 114* 315-324.

GUERRIER, P. and DORÉE, M. (1975). Hormonal control of reinitiation of meiosis in starfish. The requirement of 1-methyladenine during nuclear maturation. *Dev. Biol. 47:* 341-348.

GUERRIER, P., DORÉE, M. and FREYSSINET, G. (1975). Stimulation précoce des activités protéines kinases au cours du processus hormonal de réinitiation de la méiose dans les ovocytes d'Etoile de mer. *C.R. Séances Acad. Sci. (Paris) 281:* 1475-1478.

GUERRIER, P., DORÉE, M., MOREAU, M., CHEVAL, J. and FREYSSINET, G. (1976). Controle hormonal de la méiose des ovocytes d'Etoiles de mer. I. Analyse des évènements précoces induits par la 1-MeAde In *Actualité sur les hormones d'Invertébrés* (Ed. CNRS). Coll. Intern. CNRS 251, pp. 191-198.

GUERRIER, P., DUBE, F. and MOREAU, M. (1981). External calcium requirement for oocyte maturation in the surf clam *Spisula solidissima*. *Biol. Bull. (Woods Hole)* 161: 335-336.

GUERRIER, P., GUERRIER, C., NEANT, I. and MOREAU, M. (1986b). Germinal vesicle nucleoplasm and intracellular pH requirements for cytoplasmic maturity in oocytes of the prosobranch mollusk *Patella vulgata*. *Dev. Biol 116:* 92-99.

GUERRIER, P., MOREAU, M. and DORÉE, M. (1977a). Hormonal control of meiosis in starfish: stimulation of protein phosphorylation induced by 1-methyladenine. *Mol. Cell Endocrinol. 7:* 137-150.

GUERRIER, P., MOREAU, M. and DORÉE, M. (1977b). Inhibition de la reinitiation de la meiose des ovocytes de *Xenopus laevis* par trois antiprotéases naturelles, l'antipaine, la chymostatine et la leupeptine. *C.R. Séances Acad. Sci. (Paris) 284:* 317-319.

GUERRIER, P., MOREAU, M. and DORÉE, M. (1978). Control of meiosis reinitiation in starfish: calcium ion as the primary effective trigger. *Biol. Anim. Biochem. Biophys. 18:* 441-452.

GUERRIER, P., MEIJER, L., MOREAU, M. and LONGO, F.J. (1983). Hormone-independent GVBD induces cytoplasmic maturity in the starfish oocyte. *J. Exp. Zool. 226:* 303-309.

GUERRIER, P. , MEIJER, L., MOREAU, M. and RENDER, J.A. (1984). Early steps in transduction of the hormonal signal controlling starfish oocyte maturation. In *Biosynthesis, Metabolism and Mode of Action of Invertebrate hormones* (Eds. J. Hoffman and M. Porchet). Springer-Verlag, Berlin, pp. 487-496.

GUERRIER, P., MOREAU, M., MEIJER, L., MAZZEI, G., VILAIN, J.P. and DUBE, F. (1982). The role of calcium in meiosis reinitiation. In *Membranes in Growth and Development* (Eds. J.J. Hoffmann, G.H. Giebish and L. Bolis). Progress in Clinical and Biological Research, Vol. 91. Alan R. Liss, New York, pp. 139-155.

GUERRIER, P. and NEANT, I. (1986). Metabolic cooperation following fusion of starfish ootid and primary oocyte restores meiotic-phase-promoting activity. *Proc. Natl. Acad. Sci. USA 83:* 4814-4818.

GUERRIER, P., NEANT, I., CHARBONNEAU, M. and MOREAU, M. (1988a). Conditions for the appearance of maturation promoting factor (MPF) following germinal vesicle disruption of prophase-arrested starfish oocytes. *J. Exp. Zool. 246:* 23-32.

GUERRIER, P., NEANT, I. and CLEDON, P. (1988b). Urea-induced meiosis reinitiation in oocyte of the starfish *Marthasterias glacialis*. *Dev. Growth Differ. 30:* 93-103.

GUERRIER, P., NEANT, I., COLAS, P., DUFRESNE, L., SAINT PIERRE, J. and DUBE, F. (1990). Protein synthesis and protein phosphorylation as regulators of MPF activity. In *Mechanism of Fertilization, Plants to Humans* (Ed. B. Dale). NATO ARW. Springer-Verlag (In press).

HAGAN, I., HAYLES, J. and NURSE, P. (1988). Cloning and sequencing of the cyclin related cdc^{13+} gene and a cytological study of its role in fission yeast mitosis. *J. Cell Sci. 91:* 587-595.

HASHIMOTO, N. and KISHIMOTO, T. (1988). Regulation of meiotic metaphase by a cytoplasmic maturation promoting factor during mouse oocyte maturation.*Dev. Biol. 126:* 242-252.

HAYLES, J., AVES, S. and NURSE, P. (1986). suc 1 is an essential gene involved in both the cell cycle and growth in fission yeast. *EMBO J. 5:* 3373-3379.

HAYLES, J. and NURSE, P. (1989). A review of mitosis in the fission yeast *Schizosaccharomyces pombe*. *Exp. Cell Res. 184:* 273-286.

HEILBURN, L.V., DAUGHERTY, K. and WILBUR, K.M. (1939). Initiation of maturation in the frog egg. *Physiol. Zool. 12:* 97-100.

HEPLER, P.K. (1989). Calcium transients during mitosis: observations in flux.*J. Cell*

Biol. 109: 2567-2573.

HERMANN, J., BELLE, R., TSO, J. and OZON, R.(1983). Stabilization of the maturation promoting factor (MPF) from *Xenopus laevis* oocytes. Protection against calcium ions. *Cell Differ. 13:* 143-148.

HERMANN, J., MULNER, O., BELLE, R., MAROT, J., TSO, J. and OZON, R. (1984). *In vivo* effects of microinjected alkaline phosphatase and its low molecular weight substrates on the first meiotic cell division in *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci. USA 81:* 5150-5154.

HINDLEY, J. and PHEAR, G. (1984). Sequence of the cell division gene cdc2 from *Schizosaccharomyces pombe*: patterns of splicing and homology to protein kinases. *Gene 31:* 129-134.

HINDLEY, J., PHEAR, G.A., STEIN, M. and BEACH, D. (1987). Suc 1+ encodes a predicted 13-kilodalton protein that is essential for cell viability and directly involved in the division cycle of *Schizosaccharomyces pombe. Mol. Cell. Biol.:* 504-511.

HIRAI, S., KANATANI, H. (1971). Site of production of meiosis-inducing substance in ovary of starfish. *Exp. Cell Res. 67:* 224-227.

HOLLAND, L.Z., GOULD-SOMERO, M. and PAUL, M. (1984). Fertilization acid release in *Urechis* eggs. I. The nature of the acid and the dependence of acid release and egg activation on external pH. *Dev. Biol. 103:* 343-354.

HUNT, T. (1989). Under arrest in the cell cycle. *Nature 342:* 483-484.

ISHIKAWA, K., HANAOKA, Y., KONDO, Y. and IMAI, K. (1977). Primary action of steroid hormone at the surface of amphibian oocyte in the induction of germinal vesicle breakdown. *Mol. Cell. Endocrinol. 9:* 91-100.

JAGIELLO, G.M. (1969). Meiosis and inhibition of ovulation in mouse eggs treated with actinomycin D. *J. Cell Biol. 42:* 571-574.

KANATANI, H. (1964). Spawning of starfish: action of gamete-shedding substance obtained from radial nerves. *Science 146:* 1177-1179.

KANATANI, H. (1973). Maturation-inducing substances in starfishes. *Int. Rev. Cytol. 35:* 253-298.

KANATANI, H. and HIRAMOTO H. (1970). Site of action of 1-methyladenine in inducing oocyte maturation in starfish. *Exp. Cell Res. 61:* 280-284.

KANATANI, H., IKEGAMI, S., SHIRAI, H., OIDE, H. and TAMURA, S. (1971). Purification of gonad-stimulating substance obtained from radial nerves of the starfish. *Asterias amurensis. Dev. Growth Differ. 13: 151-164.*

KANATANI, H. and SHIRAI (1967). *In vitro* production of meiosis inducing substance by nerve extract in ovary of starfish. *Nature 216:* 284-286.

KANATANI, H. and SHIRAI, H. (1971). Chemical structure requirements for induction of oocyte maturation and spawning in starfishes. *Dev. Growth Differ. 13:* 53-64.

KANATANI, H., SHIRAI, H., NAKANISHI, K. and KUROKAWA, T. (1969). Isolation and identification of meiosis-inducing substance in starfish. *Nature 21:* 273-274.

KARSENTI, E., BRAVO, R. and KIRSCHNER (1987). Phosphorylation changes associated with the early cell cycle in *Xenopus* eggs. *Dev. Biol. 119: 442-453.*

KESHET, E., ROSENBERG, M.P., MERCER, J.A., PROST, I., VAN DE WOUDE, G.F., JENKIS, N.A. and COPELAND, N.G. (1988). Developmental regulation of ovarian-specific mos expression. *Oncogene 2:* 235-240.

KISHIMOTO, T. (1988). Regulation of metaphase by a maturation-promoting factor. *Dev. Growth Differ. 30:* 105-115.

KISHIMOTO, T., CAYER, M.L. and KANATANI, H. (1976). Starfish oocyte maturation and reduction of disulfide-bond on oocyte surface. *Exp. Cell Res. 101:* 104-110.

KISHIMOTO, T., CLARK, T.G., KONDO, H.K., SHIRAI, H. and KANATANI, H. (1982a). Inhibition of starfish oocyte maturation by some inhibitors of proteolytic enzymes. *Gamete Res. 5:*11-18.

KISHIMOTO, T. HIRAI, S. and KANATANI, H. (1981). Role of germinal vesicle material in producing maturation-promoting factor in starfish oocyte. *Dev. Biol. 81:*177-181.

KISHIMOTO, T. and KANATANI, H. (1976). Cytoplasmic factor responsible for germinal vesicle breakdown and meiotic maturation in starfish oocytes. *Nature 260:* 321-322.

KISHIMOTO, T. and KONDO, H. (1986). Extraction and preliminary characterization of maturation-promoting factor from starfish oocytes. *Exp. Cell Res. 163:* 445-452.

KISHIMOTO , T., KURIYAMA, R., KONDO, H. and KANATANI, H. (1982b). Generality of the action of various maturation-promoting factors. *Exp. Cell Res. 137:* 121-126.

LABBÉ, J.C., PICARD, A., KARSENTI, E. and DORÉE, M. (1988a) An M-phase-specific protein kinase of *Xenopus* oocytes: partial purification and possible mechanism of its periodic activation. *Dev. Biol. 127:* 157-169.

LABBÉ, J.C., LEE, M.G., NURSE, P., PICARD, A. and DORÉE, M. (1988b). Activation at M-phase of a protein kinase encoded by a starfish homologue of the cell cycle conrol gene cdc2+. *Nature 335:* 251-254.

LABBÉ, J.C., PICARD, A. and DORÉE, M. (1988c). Does the M-phase promoting factor (MPF) activate a major Ca^{2+} and cyclic nucleotide independent protein kinase in starfish oocytes. *Dev. Growth Differ. 30:* 197-207.

LABBÉ, J.C., PICARD, A., PEAUCELLIER, G., CAVADORE, J.C., NURSE, P. and DORÉE, M. (1989a). Purification of MPF from starfish: identification as the H1 histone kinase p34 cdc2 and a possible mechanism for its periodic activation. *Cell 57:* 253-263.

LABBEÉ, J. C., CAPONY, J. P., CAPUT, D., CAVADORE, J.C., DERANCOURT, J., KAGHAD, M., LELIAS, J.M., PICARD, A. and DORÉE, M. (1989b). MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cyclin B. *EMBO J. 8:* 3053-3058.

LANGAN, T.A., GAUTIER, J., LOHKA, M., HOLLINGSWORTH, R., MORENO, S., NURSE, P., MALLER, J.and SCLAFANI, R.A. (1989). Mammalian growth-associated H1 histone kinase: a homolog of cdc2+/CDC28 protein kinases controlling mitotic entry in yeast and frog cells. *Mol. Cell. Biol. 9:* 3860-3868.

LEE, M.G. and NURSE, P. (1987). Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2. *Nature 327:* 31-35.

LOHKA, M.J., HAYES, M.K. and MALLER, J.L. (1988). Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proc. Natl. Acad. Sci. USA 85:* 3009-3013.

LOHKA, M.J., KYES, J.L. and MALLER, J.L. (1987). Metaphase protein phosphorylation in *Xenopus laevis* eggs. *Mol. Cell. Biol. 7:* 760-768.

LOHKA, M. and MALLER, J. (1985). Induction of nuclear membrane breakdown, chromosome condensation and spindle formation in cell free extracts. *J. Cell Biol. 101:* 518-523.

LOHKA, M.J. and MASUI, Y. (1983). Formation *in vivo* of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. *Science 20:* 719-721.

LUCA, F.C. and RUDERMAN, J.V. (1989). Control of programmed cyclin destruction in a cell-free system. *J. Cell Biol. 109:* 1895-1909.

MALLER, J.L. (1983). Interaction of steroids with the cyclic nucleotide system in amphibian oocytes. *Adv. Cyclic Nucleotide Res. 15 :* 295-336.

MALLER, J.L. (1985a). Regulation of amphibian oocyte maturation. *Cell Differ. 16:* 211-221.

MALLER, J.L. (1985b). Oocyte maturation in amphibian in *Developmental Biology* (Ed. L.W. Browder). Plenum, New York, pp. 289-311.

MALLER, J.L., WU, M. and GERHART, J.C. (1977). Changes in protein phosphorylation accompanying maturation of *Xenopus laevis* oocytes. *Dev. Biol. 58:* 295-312.

MASUI, Y. (1967). Relative roles of the pituitary follicle cells and progesterone in the induction of oocyte maturation in *Rana pipiens. J. Exp. Zool. 166:* 365-376.

MASUI, Y. and CLARKE, H.J. (1979). Oocyte maturation . *Int. Rev. Cytol.57:* 185-282.

MASUI, Y. and MARKERT, C. (1971). Cytoplasmic control of nuclear behaviour during meiotic maturation of frog oocytes. *J. Exp. Zool. 177:* 129-146.

MASUI, Y., MEYERHOF, P.G. and MILLER, M.A. (1980). Cytostatic factor and chromosome behavior in early development. *Symp. Doc. Dev. Biol. 8:* 235-256.

MASUI, Y. and SHIBUYA, E.K. (1987). Development of cytoplasmic activities that control chromosome cycles during maturation of amphibian oocytes. In *Molecular Regulation of Nuclear Events in Mitosis and Meiosis* (Eds. R.A. Schlegel, H.S. Halleck, and P.N. Rao). Academic Press, San Diego, pp. 1-42.

MAZZEI, G. and GUERRIER, P. (1982). Changes in the pattern of protein phosphorylation during meiosis reinitiation in starfish oocytes. *Dev. Biol. 91:* 246-256.

MAZZEI, G., MEIJER, L., MOREAU, M. and GUERRIER, P. (1981). Role of calcium and cyclic nucleotides during meiosis reinitiation in starfish oocytes. *Cell. Differ. 10:* 139-145.

MEIJER, L. (1979). Hormonal control of oocyte maturation in *Arenicola marina* L. (Annelida, polychaeta). *Dev. Growth Differ 21:* 303- 314.

MEIJER, L. (1980). Hormonal control of oocyte maturation in *Arenicola marina* L. (Annelida, polychaeta). III. Involvement of Ca2+ and -SH groups in meiosis reinitiation. *Dev. Growth Differ. 22:* 33-38.

MEIJER, L., ARION, D., GOLSTEYN, R., PINES, J., BRIZUELA, L., HUNT, T. and BEACH, D. (1989). Cyclin is a component of the sea urchin egg M- phase-specific histone H1 kinase. *EMBO J. 8:* 2275-2282.

MEIJER, L. and GUERRIER, P. (1981). Calmodulin in starfish oocytes. I. Calmodulin antagonists inhibit meiosis reinitiation. *Dev. Biol. 88 :* 318-324.

MEIJER, L. and GUERRIER, P. (1982). Activation of calmodulin-dependent NAD $^+$ kinase by trypsin. *Biochim. Biophys. Acta* 702: 143-146.

MEIJER, L. and GUERRIER, P. (1983). Immobilized methylglyoxalbis (guanylhydrazone)

induces starfish oocyte maturation. *Dev. Biol. 100:* 308-317.

MEIJER, L. and GUERRIER, P. (1984a). Maturation and fertilization in starfish oocytes. *Int. Rev. Cytol. 86:* 129-196.

MEIJER, L. and GUERRIER, P. (1984b). Calmodulin in starfish oocytes. II. Trypsin treatment suppresses the trifluoperazine-sensitive step. *Dev. Biol. 101:* 257-262.

MEIJER, L., GUERRIER, P. and MACLOUF, J. (1984). Arachidonic acid, 12- and 15-hydroxyeicosatetraenoic acids, eicosapentaenoic acid, and phospholipase A_2 induce starfish oocyte maturation. *Dev. Biol. 106:* 368-378.

MEIJER, L., MACLOUF, J. and BRYANT, R.W. (1986a). Arachidonic acid metabolism in starfish oocytes. *Dev. Biol. 114:* 22-23.

MEIJER, L., MACLOUF, J. and BRYANT, R.W. (1986b). Contrasting effects of fatty acids on oocyte maturation in several starfish species. *Prostaglandins Leukotrienes Med. 23:* 179-184.

MEIJER, L., BRASH, A.R., BRYANT, R.W. , NG, K., MACLOUF, J. and SPRECHER, H. (1986c). Stereospecific induction of starfish oocyte maturation by 8 (R) - hydroxyeicosatetraenoic acid (8(R)-HETE). *J. Biol. Chem. 261:* 17040-17047.

MEIJER, L., PONDAVEN, P., TUNG, H.Y.L., COHEN, P. and WALLACE, R.W. (1986d). Protein phosphorylation and oocyte maturation. II. Inhibition of starfish oocyte maturation by intracellular microinjection of protein phosphatases 1 and 2A and alkaline phosphatase. *Exp. Cell Res. 163:* 489-499.

MEIJER, L., PAUL, M. and EPEL, D. (1982). Stimulation of protein phosphorylation during fertilization-induced maturation of *Urechis caupo* oocytes. *Dev. Biol. 94:* 62-70.

MEIJER, L., PELECH, S.L. and KREBS, E.G. (1987). Differential regulation of histone H_1 and ribosomal S6 kinases during sea star oocyte maturation. *Biochem. 26:* 7968-7974.

MEIJER, L. and PONDAVEN, P. (1988). Cyclic activation of histone H1 kinase during sea urchin egg mitotic divisions. *Exp. Cell Res. 174:* 116- 129.

MEIJER, L. and ZARUTSKIE, P. (1987). Starfish oocyte maturation: 1- methyladenine triggers a drop of c-AMP concentration related to the hormone-dependent period. *Dev. Biol. 121:* 306-315.

MIAKE-LYE, R. and KIRSCHNER, M. (1985). Induction of early mitotic events in a cell-free system. *Cell 41:* 165-175.

MINSHULL, J., BLOW, J.J. and HUNT, T. (1989). Translation of cyclin mRNA is necessary for extracts of activated *Xenopus* eggs to enter mitosis. *Cell 56: 947-956.*

MOREAU, M. and GUERRIER, P. (1979). Free calcium changes associated with hormone action in oocytes. In *Detection and Measurement of Free Ca^{2+} in Cells* (Eds. C. C. Ashley and A.K. Campbell). Elsevier North Holland Biomedical Press, Amsterdam, pp. 216- 226.

MOREAU, M. and GUERRIER, P. (1980). *In vitro* interaction between membrane, hormone and cyclic nuleotides as revealed with aequorin. *Dev. Biol. 79:* 488-492.

MOREAU, M., GUERRIER, P. and DOREE, M. (1978a). Hormonal control of meiosis reinitiation in starfish oocytes. New evidence for the absence of efficient intracellular receptors for 1-methyladenine recognition. *Exp. Cell. Res. 115:* 245-249.

MOREAU, M., GUERRIER, P. and DOREE, M. (1978, b). Membrane recognition and transduction during the hormone induced meiosis reinitiation in starfish oocytes. *Biol. Cellulaire 32:* 69-74.

MOREAU, M., GUERRIER, P., DOREE, M. and ASHLEY, C.C. (1978b). 1- methyladenine-induced release of intracellular calcium triggers meiosis in starfish oocytes. *Nature 272:* 251-253.

MOREAU, M., GUERRIER, P.and VILAIN J.P. (1985). Ionic regulation of oocyte maturation. In *Fertilization.* (Eds. C. Metz and A. Monroy). Acad. Press, New York, pp. 297-343.

MORENO, S., HAYLES, J. and NURSE, P. (1989). Regulation of p34 cdc2 protein kinase during mitosis. *Cell 58:* 361-372.

MORISAWA, M. and KANATANI, H. (1978). Oocyte-surface factor responsible for 1-methyladenine-induced oocyte maturation in starfish. *Gamete Res. 1:* 157-164.

MORLA, A.O., DRAETTA, G., BEACH, D. and WANG, J.Y.J. (1989) Reversible tyrosine phosphorylation during entry into mitosis. *Cell 58:* 193- 203.

MOOR, R.M. and CROSBY, I.M. (1986). Protein requirements for germinal vesicle breakdown in ovine oocytes. *J. Embryol. Exp. Morphol. 94 :* 207-220.

MURRAY, A.W. and KIRSCHNER, M.W. (1989a). Cyclin synthesis drives the early embryonic cell cycle. *Nature 339:* 275-280.

MURRAY, A.W. and KIRSCHNER, M.W. (1989b). Dominoes and clocks : the union of two views of the cell cycle. *Science 246:* 614-621.

MURRAY, A.W. , SOLOMON, M.J. and KIRSCHNER, M.W. (1989). The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature 339:* 280-286.

MUTTER, G.L. and WOLGEMUTH, D.L. (1987). Distinct developmental patterns of c-mos protooncogene expression in female and male mouse germ cells. *Proc. Natl. Acad. Sci. USA 84:* 5301-5305.

NEANT, I. and GUERRIER, P. (1988a). 6-dimethylaminopurine blocks starfish oocyte maturation by inhibiting a relevant protein kinase. *Exp. Cell Res. 176:* 68-79.

NEANT,I. and GUERRIER, P. (1988b). Meiosis reinitiation in the mollusc *Patella vulgata.* Regulation of MPF, CSF and chromosome condensation activity by intracellular pH, protein synthesis and phosphorylation. *Development 102:* 505-516.

NEANT,I., CHARBONNEAU M. and GUERRIER, P. (1989). A requirement for protein phosphorylation in regulating the meiotic and mitotic cell cycles in Echinoderms. *Dev. Biol. 132:* 304-314.

NEMOTO, S.I. (1982). Nature of the 1-methyladenine requiring phase in maturation of starfish oocytes. *Dev. Growth Differ. 24:* 429-442.

NEWPORT, J.W. and KIRSCHNER, M.W. (1984). Regulation of the cell cycle during early *Xenopus* development. *Cell. 37:* 731-742.

NEWPORT, J. and SPANN, T. (1987). Disassembly of the nucleus in mitotic extracts: membrane vesicularization, lamin disassembly and chromosome condensation are independent processes. *Cell 48:* 219-230.

NGUYEN-GIA, P., BOMSEL, M., LABROUSSE, J.P., GALLIEN, C.L. and WEINTRAUB, H. (1986). Partial purification of the maturation promoting factor MPF from unfertilized eggs of *Xenopus laevis. Eur. J. Biochem. 161:* 771-777.

NIGON, V., GUERRIER, P. and MONIN, H. (1960). L'architecture polaire de l'oeuf et les mouvements des constituants cellulaires au cours des premières étapes du développement chez quelques nématodes. *Bull. Biol. Fr. Belg.93:* 131-202.

O'KEEFE, S.J., WOLFES, H., KIESSLING, A.A. and COOPER, G.M. (1989). Microinjection of antisense c-mos oligonucleotides prevents meiosis II in the maturing mouse egg. *Proc. Natl. Acad. Sci USA 86:* 7038-7042.

OSBORN, J.C. and MOOR, R.M. (1983). Time-dependent effects of a-amanitin on nuclear maturation and protein synthesis in mammalian oocytes. *J. Embryol Exp. Morphol. 73:* 317-338.

PAULES, R.S., BUCCIONE, R., MOSCHEL, R.C., VANDE WOUDE, G.F. and EPPIG, J.J. (1989). Mouse mos protooncogene product is present and functions during oogenesis. *Proc. Natl. Acad. Sci USA 86:* 5395-5399.

PEAUCELLIER, G. (1977a). Initiation of meiotic maturation by specific proteases in oocytes of the polychaete annelid *Sabellaria alveolata. Exp. Cell Res 106:* 1-14.

PEAUCELLIER, G. (1977b). Mise en évidence du rôle du calcium dans la réinitiation de la méiose des ovocytes de *Sabellaria alveolata* (L.) (annélide polychète). *C.R. Acad. Sci. (Paris) 285:* 913-915.

PEAUCELLIER, G., DORÉE, M. and DEMAILLE, J.G. (1982). Stimulation of endogenous protein phosphorylation in oocytes of *Sabellaria alveolata* (Polychaete annelid) at meiosis reinitiation induced by protease, fertilization, or ionophore A 23187. *Gamete Res. 1:* 115-123.

PEAUCELLIER, G., DORÉE, M. and PICARD, A. (1984). The rise and fall of protein phosphorylation during meiotic maturation of oocytes of *Sabellaria alveolata* (Polychaete annelid). *Dev. Biol. 106:* 267-274.

PEAUCELLIER, G., PICARD, A., ROBERT, J.J., CAPONY, J.P. LABBÉ, J.C. and DORÉE, M. (1988). Phosphorylation of ribosomal proteins during meiotic maturation and following activation in starfish oocyte: its relationship with changes in intracellular pH. *Exp. Cell Res. 174:* 71-88.

PELECH, S.L., MEIJER, L. and KREBS, E.G. (1987). Characterization of maturation-activated histone H_1 and ribosomal S6 kinase in sea star oocytes. *Biochem. 26:* 7960-7968.

PICARD, A., CAPONY, J.P., BRAUTIGAN, D.L. and DORÉE, M. (1989). Involvement of protein phosphatases 1 and 2A in the control of M-phase promoting factor activity in starfish. *J. Cell Biol. 109:* 3347-3354.

PICARD, A., CAVADORE, J.C., LORY, P., BERNENGO, J.C., OJEDA, C. and DORÉE, M. (1990). Microinjection of a conserved peptide sequence of p34 cdc2 induces Ca^{2+} transient in oocytes. *Science 247:* 327-329.

PICARD, A. and DORÉE, M. (1982). Intracellular microinjection of anticalmo-dulin drugs does not inhibit the cortical reaction induced by fertilization, ionophore A 23187 or injection of calcium buffers in sea urchin eggs. *Dev. Growth Differ. 24:* 155-162.

PICARD, A. and DORÉE, M. (1983). Is calcium the second messenger of 1-Methyladenine in meiosis reinitiation of starfish oocytes? *Exp. Cell Res. 145:* 325-337.

PICARD, A. and DORÉE, M. (1984). The role of the germinal vesicle in producing maturation-promoting factor (MPF) as revealed by the removal and transplantation of nuclear material in starfish oocytes. *Dev. Biol.104:* 357-365.

PICARD, A., PEAUCELLIER, G., Le BOUFFANT, F. Le PEUCH, C. and DORÉE, M. (1985). Role of protein syntesis and proteases in production and inactivation of maturation-promoting activity during meiotic maturation of starfish oocytes. *Dev. Biol. 109:* 311-320.

PICARD, A., LABBÉ, J.C., PEAUCELLIER, G., Le BOUFFANT, F., Le PEUCH, C. and DOREE, M. (1987). Changes in the activity of the maturation-promoting factor are correlated with those of a major cyclic AMP and calcium- independent protein kinase during the first meiotic cell cycles in the early starfish embryo. *Dev. Growth Differ. 29:* 93-103.

PINES, J. and HUNT, T. (1987). Molecular cloning and characterization of the mRNA for cyclin from sea urchin eggs. *EMBO J. 6:* 2987-2995.

PINES, J. and HUNTER, T. (1989). Isolation of a human cyclin cDNA: evidence for cyclin m RNA and protein regulation in the cell cycle and for interaction with p34 cdc2. *Cell 58:* 833-846.

POENIE, M., ALDERTON, J., TSIEN, R.Y. and STEINHARDT, R.A. (1985). Changes of free calcium levels with stages of the cell division cycle. *Nature 315:* 147-149.

PONDAVEN, P. and MEIJER, L. (1986). Protein phosphorylation and oocyte maturation. I. Induction of starfish oocyte maturation by intracellular microinjection of a phosphatase inhibitor, α-naphtylphosphate. *Exp. Cell. Res. 163:* 477-488.

PONDAVEN, P., MEIJER, L. and BEACH, D. (1990). Activation of -specific histone H1 kynase by modification of the phosphorylation of its p34cdcz and cyclin components. *Genes and Development 4*: 9-17.

PONDAVEN, P., MEIJER, L. and BIALOJAN, C. (1989). La micro-injection d'acide okadaïque, un inhibiteur de phosphoprotéine phosphatases, induit la maturation de l'ovocyte d'étoile de mer. *C.R. Séances Acad. Sci. (Paris) :309:* 563-569.

PONDAVEN, P., MEIJER, L. and PELECH, S.L. (1987). Protein phosphorylation in starfish oocyte meiotic divisions and sea urchin egg mitotic divisions. *Adv. Prot. Phosphatases 4:* 229-251.

REBHUN, L.L., WHITE, D., SANDER, G. and IVY, N. (1973). Cleavage inhibition in marine eggs by puromycin and 6-dimethylaminopurine. *Exp. Cell. Res. 77:* 312-318.

RIABOWOL, K., DRAETTA, G., BRIZUELA, L., VANDRE, D. and BEACH, D. (1989). The cdc2 kinase is a nuclear protein that is essential for mitosis in mammalian cells. *Cell 57:* 393-401.

RIME, H., NEANT, I., GUERRIER, P. and OZON, R. (1989). 6-dimethylaminopurine (6-DMAP), a reversible inhibitor of the transition to metaphase during the first meiotic cell division of the mouse oocyte. *Dev. Biol. 133:* 169-179.

RIME, H., HUCHON, D. JESSUS, C., GORIS, J., MERLEVEDE, W. and OZON, R. (1990). Characterization of MPF activation by okadaic acid in *Xenopus* oocytes. *Cell Differ.* 29: 47-58.

ROSENTHAL, E.T., HUNT, T. and RUDERMAN, J.V. (1980). Selective translation of mRNA controls the pattern of protein synthesis during early development of the surf clam, *Spisula solidissima. Cell 20:* 487-494.

ROSENTHAL, E.T. and RUDERMAN, J.V. (1987). Widespread change in the translation and adenylation of maternal messenger RNAs following fertilization of *Spisula* oocytes. *Dev. Biol. 121:* 237-246.

SAGATA, N., DAAR, I., OSKARSSON, M., SHOWALTER, S.D. and VANDE WOUDE, G.F., (1989a). The product of the mos proto-oncogene as a candidate "initiator" for oocyte maturation. *Science 245:* 643-645.

SAGATA, N., WATANABE, N., VANDE WOUDE, G.F. and IKAWA, Y. (1989b). The c-mos proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. *Nature 342:* 512-518.

SANO, K. (1985). Calcium- and cyclic AMP-independent, labile protein kinase appearing during starfish oocyte maturation: its extraction and partial characterization. *Dev. Growth Differ. 27:* 263-275.

SANO, K. and KANATANI, H. (1983). Effect of various protease inhibitors on starfish oocyte maturation. *Biomed. Res. 4:* 139-146.

SANO, K., KISHIMOTO, T., KOIDE, S.L., KANATANI, H. and KOIDE, S.S. (1979). Inhibition of germinal vesicle breakdown and polar body formation by nicotinamide in starfish and surf clam oocytes. *Dev. Growth Differ. 21:* 457-463.

SAWADA, M.T., SOMENO, T., HOSHI, M. and SAWADA, H. (1989). Inhibition of starfish oocyte maturation by leupeptin analogs, potent trypsin inhibitors. *Dev. Biol. 133:* 609-612.

SCHATT, P., MOREAU, M. and GUERRIER, P. (1983). Variation cycliques de la phosphorylation des protéines et de l'activité MPF pendant la segmentation précoce de l'oeuf d'oursin. *C.R. Séances Acad. Sci. (Paris): 296:* 551- 554.

SCHOLLMEYER, J.E. (1988). Calpain 2 involvement in mitosis. *Science 240:* 911-913.

SCHUETZ, A.W. (1967). Action of hormones on germinal visicle breakdown in frog oocytes (*Rana pipiens*). *J. Exp. Zool. 166:* 345-347.

SCHUETZ, A.W. and BIGGERS, J.D. (1967). Regulation of germinal vesicle breakdown in starfish oocytes. *Exp. Cell Res. 46:* 624-628.

SCHUETZ, A.W. and SAMSON, D. (1979). Nuclear requirement for post- maturational cortical differentiation in amphibian oocytes: effects of cycloheximide. *J. Exp. Zool. 210:* 307-320.

SHIBUYA, E.K. and MASUI, Y. (1988). Stabilization and enhancement of primary cytostatic factor (CSF) by ATP and NaF in amphibian egg cytosols. *Dev. Biol. 129:* 253-264.

SHIBUYA, E.K. and MASUI, Y. (1989). Molecular characteristics of cytostatic factors in amphibian egg cytosols. *Development 106:* 799-808.

SHILLING, F., CHIBA, K., HOSHI, M., KISHIMOTO, T. and JAFFE, L.A. (1989). Pertussis toxin inhibits 1-methylademine induced maturation in starfish oocytes. *Dev. Biol. 133:* 605-608.

SHIRAI, H. (1986). Gonad-stimulating and maturation-inducing substance. *Methods Cell Biol. 27:* 73-88.

SHIRAI, H., NEANT, I. and GUERRIER, P. (1987). Induction of oocyte maturation by a ganglion extract in *Patella vulgata. Dev. Growth Differ. 29:* 398.

SIMANIS, V. and NURSE, P. (1986). The cell cycle control gene cdc 2$^+$ of fission yeast encodes a protein kinase potentially regulated by phosphorylation. *Cell 45:* 261-268.

SMITH, L.D., ECKER, R.E. and SUBTELNY, S. (1968). *In vitro* induction of physiological maturation in *Rana pipiens* oocytes removed from ovarian folicles. *Dev. Biol. 17:* 627-643.

SOLOMON, M., BOOHER, R., KIRSCHNER, M. and BEACH, D. (1988) Cyclin in fission yeast. *Cell 54*: 738-739.

STANDART, N., MINSHULL, J., PINES, J. and HUNT, T. (1987). Cyclin synthesis, modification and destruction during meiotic maturation of the starfish oocytes. *Dev. Biol. 124:* 248-258.

STEINHARDT, R.A. and ALDERTON, J. (1988). Intracellular free calcium rise triggers nuclear envelope breakdown in the sea urchin embryo. *Nature 332:* 364-366.

STERN, S., RAYYIS, A. and KENNEDY, J.F. (1972). Incorporation of aminoacids during maturation *in vitro* by the mouse oocyte: effect of puromycin on protein synthesis. *Biol. Reprod. 7:* 341-346.

SUNKARA, P.S., WRIGHT, D.A. and RAO, P.N. (1979). Mitotic factors from mammalian cells induce germinal vesicle breakdown and chromosome condensation in amphibian oocytes. *Proc. Natl. Acad. Sci. USA 76:* 2799-2808.

SWENSON, K.I., FARRELL, K.M. and RUDERMAN, J.V. (1986). The clam embryo protein cyclin A induces entry into M phase and the resumption of meiosis in *Xenopus* oocytes. *Cell 47:* 861-870.

TACHIBANA, K., YANAGISHIMA, N. and KISHIMOTO, T. (1987). Preliminary characterization of maturation factor from yeast *Saccharomyces cerevisiae. J. Cell Sci. 88*: 273-281.

TACHIBANA, K., ISHIURA, M., UCHIDA, T. and KISHIMOTO, T. (1989). Molecular cloning and sequencing of the starfish egg m RNA responsible for meiotic maturation. *Dev. Growth Differ. 31:* 410.

TOOLE, B.P. and SCHUETZ, A.W. (1974). Evidence for metabolism of 1- methyladenine during incubation of starfish oocyte maturation *in vitro. Dev. Biol. 39*: 177-182.

TSO, J., THIBIER, C., MULNER, O. and OZON, R. (1982). Microinjected progesterone reinitiates meiotic maturation of *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci. USA 79:* 552-556.

TWIGG, J., PATEL, R. and WHITAKER, M. (1988). Translational control of Ins P_3-induced chromatin condensation during the early cell cycles of sea urchin embryos. *Nature 332:* 366-369.

VITTENBERG, C. and REED, S.I. (1989). Conservation of function and regulation within the CDC 28/cdc 2 protein kinase family: characterization of the human cdc2 Hs protein kinase in *Saccharomyces cerevisiae. Mol. Cell. Biol. 9:* 4064-4068.

WAGENAAR, E.B. (1983). The timing of synthesis of proteins required for mitosis in the cell cycle of the sea urchin embryo. *Exp. Cell Res. 144 :* 393-403.

WASSARMAN, P.M., SCHULTZ, R.M. and LETOURNEAU, G.E. (1979). Protein synthesis during meiotic maturation of mouse oocytes *in vitro*. Synthesis and phosphorylation of a protein localized in the germinal vesicle. *Dev. Biol. 69:* 94-107.

WASSERMAN, W.J. and MASUI, Y. (1975). Effects of cycloheximide on a cytoplasmic factor initiating meiotic maturation in *Xenopus* oocytes. *Exp. Cell Res. 91:* 381-388.

WATANABE, N., VANDE WOUDE, G.F., IKAWA, Y. and SAGATA, N. (1989). Specific proteolysis of the c-mos proto-oncogene product by calpain on fertilization of *Xenopus* eggs. *Nature 342:* 505-511.

WEINTRAUB, H., BUSCAGLIA, M., FERREZ, M., WEILLER, S., BOULET, A., FABRE, F. and BAULIEU, E.E.(1982). Mise en évidence d'une activité "MPF" chez *Saccharomyces cerevisiae. C.R. Séances Acad. Sci. (Paris) (III)* 2095: 787-790.

WESTENDORF, J.M., SWENSON, K.I. and RUDERMAN, J.V. (1989). The role of cyclin B in meiosis 1. *J. Cell Biol. 108:* 1431-1444.

WESTWOOD, J.T., CHURCH, R.B. and WAGENAAR, E.B. (1985). Changes in protein phosphorylation during the cell cycle of chinese hamster ovary cells. *J. Biol. Chem. 260:* 10 308-10 313.

WILT, F.H., SAKAI, H. and MAZIA, D. (1967). Old and new protein in the formation of the mitotic apparatus in cleaving sea urchin eggs. *J. Mol. Biol. 27:* 1-7.

WITCHEL, H.J. and STEINHARDT, R.A. (1989). 1-methyladenine can consistently induce a maturation-independent transient calcium increase in oocytes of the starfish *Asterina miniata. J. Cell Biol. 109 :* 31A.

WU, M. and GERHART, J.C. (1980). Partial purification and characterization of the maturation-promoting factor from eggs of *Xenopus laevis. Dev. Biol. 79:* 465-477.

YOSHIKUNI, M., ISHIKAWA, K. and NAGAHAMA, Y. (1988). Inhibition of starfish oocyte maturation: effects on signal reception and transduction. *Zool. Sci. 5:* 1254.