

## Maturation promoting factor and cell cycle regulation

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### SUMMARY

Cell cycles in early amphibian embryos are characterized by the absence of  $G_1$  and  $G_2$  phases. The simple cycle of S phase and mitosis does show similarities with other systems, particularly in the presence of cytoplasmic components advancing nuclei into DNA synthesis and mitosis. Maturation-promoting factor induces nuclear envelope breakdown and subsequent chromosome condensation. Cytoplasmic factors appear during maturation which are capable of inducing DNA synthesis, and arrest of the nuclear division cycle in metaphase (cytostatic factor). The timing of appearance of these activities is considered and their relationship in integrating DNA synthesis during early cleavage is discussed.

### INTRODUCTION

Amphibian eggs and embryos have, for a long time, been favoured material for analysis of problems in embryology primarily because their large size makes manipulation seem easier, and external fertilization and development makes observation straightforward. The high volume ratio of cytoplasm to nucleus has focused attention on the role of the cytoplasm. Studies on early embryonic cell lineages led Wilson (1896) to propose that nucleocytoplasmic interactions were important in inducing gradual changes in cell function. The most obvious change in cell activity between the oocyte and early embryo is the resumption of the cell cycle. The purpose of this review is to consider the ability of cytoplasmic activities to integrate the two major events of the cell cycle – DNA synthesis and mitosis.

### COMPARISON OF EMBRYONIC AND ADULT CELL CYCLES

Events in the cell cycle are usually analysed in relation to the nuclear events (activities) of DNA synthesis (S-phase) and mitosis (M-phase). These two easily detectable components of nuclear activity are separated in most cells by a post-mitotic gap ( $G_1$ ) and a second gap ( $G_2$ ) between DNA synthesis and mitosis. Studies on the duration of these phases indicate that while all phases can vary in length, postembryonic cells show greatest variation in the length of  $G_1$ , both between cell types and within one population (Prescott 1976, Smith & Martin, 1973). Once a cell is committed to enter S phase, the duration of S,  $G_2$  and M is relatively constant. Adult cells are characterized by a relatively long  $G_1$  which may reflect an

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inability to complete the necessary doubling in mass within S, G<sub>2</sub> and M (Stancel, Prescott & Liskay, 1981).

The cell cycle of amphibian embryos during early cleavage is dramatically different in several ways. Firstly during the early synchronous cleavages no G<sub>1</sub> phase can be detected and G<sub>2</sub> (if it exists at all) is extremely short (Graham & Morgan, 1966). The absence of these phases presumably reflects the absence of growth in early embryos. The volume of individual blastomeres decreases during cleavage and the total protein content of an embryo remains constant until hatching (Benbow, Pestell & Ford, 1975). Secondly the cell cycle is extremely rapid, with cleavage recurring every 35 minutes (Newport & Kirschner, 1982). Correlated with this is the inheritance by the egg of large quantities of components necessary for DNA synthesis and mitosis (e.g. histones, Woodland & Adamson, 1977; DNA polymerase, Benbow *et al.* 1975, tubulin, Pestell, 1975). Thirdly a surface contraction wave recurs in each cell cycle (Hara *et al.* 1980). While these contraction waves continue in the absence of a nucleus or centriole, repeated DNA synthesis and mitosis do not occur if the contraction waves are inhibited (Newport & Kirschner, 1984). These waves have been interpreted in terms of an autonomous cytoplasmic oscillator which directs the orderly repetition of DNA synthesis and mitosis. This does not imply that cytoplasmic oscillators are not found in adult cell cycles (Klevecz, 1976). The additional regulatory inputs necessary to integrate growth with DNA synthesis and mitosis in adult cells and the consequent asynchrony may mask an underlying cyclical pattern observed in synchronous embryonic cell division.

The genetic dissection of functions necessary for the cell cycle has been particularly successful using the yeasts *Saccharomyces cerevisiae* (Hartwell, 1978) and *Schizosaccharomyces pombe* (Nurse, 1981). The isolation of cell division cycle (*cdc*) mutants has defined, not surprisingly, a large number of functions necessary for cell cycle progression. Particularly interesting are the mutants defining genes regulating commitment to DNA synthesis and to mitosis. In *S. pombe* genetic evidence strongly favours the view that *wee 1* defines a negative element involved in controlling mitosis (Nurse & Thuriaux, 1980, reviewed in Nurse, 1981). Evidence for a positive element controlling mitosis has also been obtained. This gene, *cdc2*, is intriguing in that it is required in G<sub>2</sub> for control of mitosis and also in G<sub>1</sub> for commitment to the cell cycle (Nurse & Bissett, 1981). *cdc2* defines a transition point beyond which the cell is committed to DNA synthesis and mitosis. *cdc28*, which defines an equivalent transition point (start) in *S. cerevisiae*, shows functional homology to *cdc2* (Beach *et al.* 1982). This indicates not only that this important function is conserved between the two species but that a similar mechanism may commit the cell to DNA synthesis and advance the cell into mitosis.

Another approach to the study of temporal order and control of cell cycle events has made use of cell synchronization and cell fusion procedures with vertebrate cells in culture (Johnson & Rao, 1971). Analysis of cells synchronized in different parts of the cell cycle and fused in varying proportions generated heterokaryons which

were advanced or delayed in cell cycle activities depending on the cell cycle phases of the cells fused. In particular, mitotic cells induced premature chromosome condensations (PCC) when fused with cells in G<sub>2</sub>, S or G<sub>1</sub>, implying that the metaphase cell contained signal(s) which dominated the behaviour of nuclei from interphase cells. However if one M phase cell was fused with two or three G<sub>1</sub> cells PCC rarely occurred (Johnson, Rao & Hughes, 1970) and nuclear membranes formed round the chromosomes of the mitotic cell (Obara, Chai, Weinfield & Sandberg, 1974). G<sub>1</sub> cells could inhibit the signals promoting mitosis. Similar analysis of various interphase fusions indicated that a) S phase cells advanced G<sub>1</sub> nuclei into S phase; 2) G<sub>2</sub> nuclei could not be induced to re-enter S phase following fusion with S phase cells and 3) G<sub>1</sub> nuclei were not advanced into S phase by fusion with G<sub>2</sub> cells (Johnson & Rao, 1971). These authors concluded that S phase cells contained inducers of DNA synthesis, which were not present in G<sub>2</sub> cells. G<sub>2</sub> cells contained nuclear autonomous inhibitors preventing reinitiation of DNA synthesis.

There is considerable similarity in the evidence for cytoplasmically transmissible inducers of DNA synthesis in S phase mammalian cells and cytoplasmic inducers of DNA synthesis in amphibian eggs (Graham *et al.* 1966). In both cases G<sub>1</sub> nuclei are induced to enter DNA synthesis precociously. Only one round of replication is induced in each cell cycle (Harland & Laskey, 1980). The functional similarity of factors involved in promoting entry into mitosis is even more striking since extracts from late G<sub>2</sub> or mitotic cells of yeast (Weintraub *et al.* 1982) HeLa (Sunkara, Wright & Rao, 1979) or cleavage embryos (Wasserman & Smith, 1978) can induce germinal vesicle breakdown and chromosome condensation following injection into oocytes of *X. laevis*.

#### MATURATION PROMOTING FACTOR (MPF).

Isolated amphibian oocytes respond to progesterone by initiating a complex series of events which leads to the resumption of meiotic divisions (recently reviewed by Maller, 1983). The most noticeable morphological event following hormone stimulation is the breakdown of the oocyte nucleus (germinal vesicle breakdown, GVBD) leading to the appearance of a white spot at the animal pole. Dissolution of the nuclear membrane and condensation of the chromosomes leads to the completion of 1st meiotic division and arrest of the mature unfertilized egg in metaphase of 2nd meiotic division. Shortly before GVBD, an activity appears in the cytoplasm which is able to initiate meiotic events. This maturation promoting factor (MPF, Masui & Markert, 1971) induces precocious GVBD in comparison to hormone-induced GVBD. MPF appears in hormone-treated enucleated oocytes (Masui & Markert, 1971) and is amplified in both nucleate and enucleated recipient oocytes as judged by serial transfers of cytoplasm from one recipient to the next (Reynhout & Smith, 1974; Drury & Schorderet-Slatkine, 1975; Schorderet-Slatkine & Drury, 1973). MPF has been partially purified from *Xenopus* eggs, the activity behaving as a protein with a native molecular weight of 100 kD (Wu &

Gerhart, 1980). These authors showed that the amount of activity observed was critically dependent on the amount of extract injected and the volume in which the extract was injected. They defined a unit of MPF as the amount of activity in 20 nl that induces maturation in 50 % of the recipient oocytes. MPF behaves in a highly cooperative manner providing an effective trigger for subsequent events.

MPF activity is lost extremely rapidly in the presence of  $Ca^{2+}$  and is stabilized by EGTA (Wasserman & Masui, 1976). MPF activity is also stabilized by phosphate compounds and adenosine 5'-[ $\gamma$ thio] triphosphate, which are thought to protect phosphoproteins from phosphatases (Wu & Gerhart, 1980; Gerhart, Wu & Kirschner, 1984; Hermann *et al.* 1984). It is possible that the behaviour of MPF is modified by coinjection of stabilizing components in the extraction buffers. In this regard Hermann *et al.* (1984) have shown that injection of 50 nl of 50 mM-2-glycerophosphate markedly reduces the time required to reach 50 % GVBD following progesterone stimulation.

The separation of MPF function from the early events of progesterone-induced maturation is reflected by the rapidity of MPF-induced GVBD and the occurrence of this response in the presence of protein synthesis inhibitors (reviewed by Maller, 1983 and Masui & Clarke, 1979). The appearance and amplification of MPF during maturation has been separated into two steps: the first in which a small amount of MPF is activated by an event requiring protein synthesis; the second, where MPF becomes autoactivating. Whether this second step requires protein synthesis has been reanalysed recently by Gerhart *et al.* (1984). Oocytes injected with MPF accumulate high levels of MPF activity which abruptly disappears at a time when parallel oocytes have completed 1st meiotic division (see Fig. 1). MPF abruptly reappeared as the oocyte approached second metaphase (Gerhart *et al.* 1984). Oocytes bathed in cycloheximide and injected with MPF show considerable MPF amplification, though not to the level observed in oocytes injected in the absence

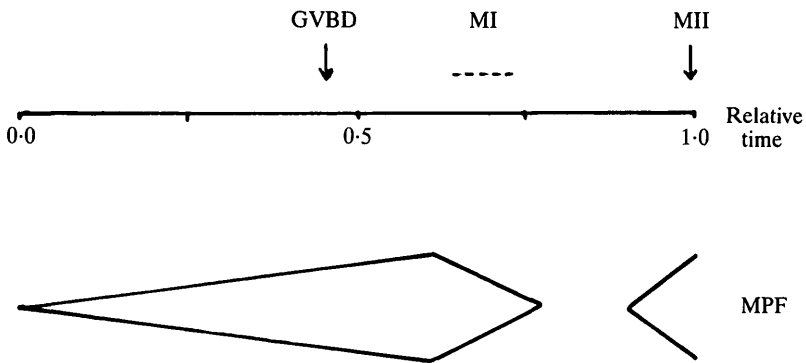


Fig. 1. Diagrammatic representation of changes in MPF activity following MPF injection into oocytes (derived from the data of Gerhart *et al.* 1984). MI, Probable time of first meiotic metaphase. MII, Second meiotic metaphase. Time indicated relative to MPF injection (0.0) and MII (1.0). MPF, < = Increasing activity > = Decreasing activity.

of protein synthesis inhibitor. In the presence of cycloheximide the second rise in MPF activity was not detected and could not be elicited by further MPF injection.

The cycling of MPF activity with the meiotic divisions is perhaps not surprising in view of Wasserman & Smith's (1978) observation of oscillation of MPF activity in activated eggs. They proposed that MPF promoted dissolution of the nuclear membrane leading to chromosome condensation in both meiotic and mitotic cells. In activated or fertilized eggs, exposure to protein synthesis inhibitors blocks reinitiation of DNA synthesis, mitosis and cleavage depending on the time at which exposure occurs (Wasserman & Smith, 1978; Harland & Laskey, 1980; Ford, Wall & Smith, 1983; Miake-Lye, Newport & Kirschner, 1983). Syncytial embryos blocked with cycloheximide contain interphase nuclei which are not synthesizing DNA (Miake-Lye *et al.* 1983). When MPF is injected into such cycloheximide-arrested embryos nuclear membrane breakdown occurs extremely rapidly and condensed chromosomes are observed. In these arrested cells MPF amplification is at least three times less than in oocytes (Miake-Lye *et al.* 1983). These data indicate that nuclear membrane dissolution occurs rapidly in the presence of MPF. The apparent differences in amplification properties between cycloheximide-treated oocytes and eggs remains to be resolved.

Evidence for the presence of inhibitors of MPF during G<sub>1</sub> in mammalian cells comes from experiments in which extracts from G<sub>1</sub> cells mixed with extracts from mitotic cells reduced the MPF activity of the mitotic cell extracts (Adlakha, Sahasrabudhe, Wright & Rao, 1983). The inhibitory factors are relatively heat stable, sensitive to low pH and apparently greater than 12 kD molecular weight. Analysis of the decay in MPF activity following injection of high levels of MPF early in the first cell cycle in *Xenopus* indicates that MPF degrading capacity (anti MPF) remains high in the first half of the first cell cycle (Gerhart *et al.* 1984). These observations support the notion from cell fusion studies that early G<sub>1</sub> cells are able to advance mitotic cells into G<sub>1</sub>.

#### CYTOSTATIC FACTOR (CSF)

In each embryonic and somatic cell cycle MPF activity disappears, perhaps through the action of anti-MPF. M phase is usually the shortest cell cycle phase. However, unfertilized eggs are arrested in 2nd meiotic metaphase and contain cytoplasmic factors capable of inducing nuclear dissolution and chromosome condensation (Gurdon, 1968). Masui & Markert found that cytoplasm, extracted carefully from unfertilized eggs so as not to induce activation, was able to arrest the embryonic cell cycle in M phase (reviewed by Masui, Meyerhoff & Miller, 1980). Brain nuclei injected into CSF-arrested blastomeres advanced into mitosis (Meyerhoff & Masui, 1979). Such nuclei were stably arrested and the surface morphology of arrested blastomeres showed striking similarity with unfertilized eggs (Masui *et al.* 1980). MPF activity is detected in CSF-arrested blastomeres, but disappears rapidly after injection of lysolecithin-treated sperm nuclei (Shibuya & Masui,

1982). They suggested that injection of sperm suspensions could induce reinitiation of cell cycle activities.

Unfertilized eggs contain both CSF and MPF. Fertilized eggs injected with CSF do not show the usual embryonic cycling of MPF activity, but instead MPF activity remains at a high level in such cells (Gerhart *et al.* 1984). The surface contraction wave is also blocked by CSF injection (Newport & Kirschner, 1984). These authors concluded that MPF is stabilized in the presence of CSF. Cycloheximide-injected embryos are also prevented from cell cycle progression but in this case MPF activity is not detected (Gerhart *et al.* 1984). This observation makes it possible to test the effect of CSF on nuclei in the absence of MPF. Newport & Kirschner (1984) found that cycloheximide-arrested syncytial embryos did not enter M phase when injected with CSF alone. Thus CSF appears to stabilize the metaphase state without inducing it. It is interesting to note that although MPF induces nuclear envelope breakdown and condensation, ordered metaphase alignment is not seen unless CSF is also present (Newport & Kirschner, 1984).

Both MPF and CSF activity appear in enucleated oocytes after exposure to progesterone (Masui & Markert, 1971). This implies that their activation does not require new transcription. However brain nuclei or sperm nuclei do not condense when injected into enucleate, progesterone-stimulated oocytes (Ziegler & Masui, 1973; Katagiri & Moriya, 1976). Injection of GV material taken from maturing oocytes was effective in recovering the capacity to induce chromosome condensation. This implies that MPF is separable from chromosome condensation activity, since appearance of MPF activity does not require a contribution from the germinal vesicle, while chromosome condensation activity does.

#### THE ONSET OF DNA REPLICATION

During oogenesis large quantities of DNA polymerase accumulate which are inherited by the egg (Benbow *et al.* 1975, Zierler, Marini, Stowers & Benbow, 1985). Efficient priming and complementary strand synthesis on a single-stranded M13 template can be obtained using egg cytoplasmic extracts (Mechali & Harland, 1982). Several other enzymes that might be expected to be involved in DNA replication can be detected in fractionated egg extracts (Benbow *et al.* 1977). Components involved in the formation of nuclei are also present at high levels in eggs (Forbes, Kirschner & Newport, 1983). The presence of high levels of these components presumably facilitates the high rates of replication observed after fertilization. However there is evidence that this replication machinery in the egg can be saturated. Incorporation stimulated by injected DNA reaches a maximum when between 1 and 10 ng DNA is injected per egg (Ford & Woodland, 1975; Newport & Kirschner, 1984). A similar saturation level is observed using *Xenopus* liver nuclei, even after template injection at two places in the egg to minimize possible compartmentalization effects (Ford and McKune, unpublished observations). The rate of synthesis stimulated by saturating amounts of DNA is much less than the rate

that must occur in a late cleavage embryo. Incubation of DNA-injected eggs in medium containing the tumour promoter TPA (4 phorbol-12-myristate-13 acetate) enhances the rate of DNA synthesis, most probably by increasing the efficiency of initiation within a cell cycle (Mechali, Mechali & Laskey, 1983). These points may be interpreted to indicate that, despite the high levels of replication components inherited by the egg, factors involved in initiation of replication are readily saturated in the egg and increase during cleavage.

When does the ability to initiate DNA replication first appear in early development?  $G_1$  nuclei injected into eggs rapidly enlarge and synthesize DNA whereas similar nuclei injected into stage VI oocytes, enlarge more slowly but do not synthesize DNA (Graham *et al.* 1966, Gurdon, 1967). Oocytes will convert single-stranded DNA to double-stranded and allow some synthesis to continue in injected S phase nuclei, but initiation of replication on double stranded DNA templates does not occur (Gurdon, 1968; Gurdon, Birnstiel & Speight, 1969; Ford & Woodland, 1975; Harland & Laskey, 1980). Nuclei injected into maturing oocytes undergo nuclear membrane breakdown and chromosome condensation if located in the animal hemisphere, but remain in interphase and synthesize DNA if located at the vegetal pole (Gurdon, 1967; Gurdon, 1968). This clearly indicates that different signals can dominate nuclear activity in different regions of the large egg. However SV40 DNA, injected into the germinal vesicles of oocytes which were subsequently matured, did not undergo DNA synthesis until the resultant eggs were activated (Harland & Laskey, 1980). These results may be reconciled if GV-injected DNA remains in the animal hemisphere after GVBD and undergoes a condensation reaction similar to nuclear chromosomes such that condensed chromatin is effectively withdrawn from the signals initiating DNA replication.

Three further pieces of evidence support this view. Firstly cultured mammalian cells, lacking  $G_1$  and  $G_2$  phases, have been shown to contain inducers of DNA synthesis during mitosis (Rao, Wilson & Sunkara, 1978). Secondly Katagiri & Moriya (1976), in an analysis of spermatozoan response to eggs matured after removal of the germinal vesicle, found that detergent-treated sperm only responded when GV material was also injected into the enucleated matured cells. In this circumstance chromosomes and multipolar spindles, and swollen nuclei that had synthesized DNA were found in different regions of the same egg. Thirdly Newport & Kirschner (1984) analysed the ability of activated eggs, arrested in M phase by injection of CSF, to induce and maintain DNA synthesis of injected plasmid (pBR322). When low concentrations (5–50 ng per egg) of pBR322 were injected into CSF-arrested eggs, DNA synthesis was detectable for the first hour after injection, but not subsequently. When very high concentrations (500 ng per egg) of DNA were injected into similarly arrested cells DNA synthesis was only partially inhibited and continued for several hours. That the M-phase arrest was maintained in this situation was indicated by the conversion of injected nuclei to metaphase figures in eggs previously injected with CSF and 500 ng plasmid DNA. M-phase arrested cells clearly retain the ability to initiate and maintain DNA replication. Newport &

Kirschner (1984) suggested that the inhibition of DNA synthesis in mitosis resulted from a modification of the DNA template, probably by stoichiometric binding of a factor, since the inhibitory effect could be titrated out by high concentrations of DNA. The maximum amount of DNA that could still be brought into M-phase inhibition was 175 ng (equivalent to the DNA content of 30,000 *Xenopus* nuclei). The *Xenopus* egg stores sufficient histones to convert about this amount of DNA into chromatin (Laskey, Mills & Morris, 1977).

These data indicate that an S phase state capable of initiating DNA synthesis appears during oocyte maturation before the unfertilized egg is activated. It may be possible to determine when this occurs by injecting large amounts of DNA into maturing oocytes.

#### INTEGRATION OF DNA SYNTHESIS AND MITOSIS

##### *Timing of cell cycle events.*

Time-lapse photomicroscopy has been used to visualize a surface contraction wave which recurs at each cell division, reflecting an underlying cell cycle oscillator (Hara *et al.* 1980). The timing of biochemical events linked to the cell cycle in *Xenopus* have been analysed in several recent studies (Wasserman & Smith, 1978; Harland & Laskey, 1980; Ford *et al.* 1983; Miake-Lye *et al.* 1983; Gerhart *et al.* 1984; Newport & Kirschner, 1984). Figure 2 represents a combination of this data on a relative time scale in which first cleavage is represented by 1.0. It would of course be preferable to have data on all these biochemical activities derived from one group of embryos grown at one temperature. However the figure illustrates several findings.

If protein synthesis is inhibited from fertilization or activation by addition of cycloheximide, DNA synthesis in second S phase ( $S_2$  Fig. 2) does not occur nor does first mitosis (Harland & Laskey, 1980; Miake-Lye *et al.* 1983). MPF activity is not detectable at the expected time in first cell cycle and the surface contraction wave does not occur (Gerhart *et al.* 1984; Newport & Kirschner, 1984). The cycloheximide-sensitive period ( $P_2$ ) for first mitosis, first cleavage and second S phase ends very abruptly over about 10 minutes, only to give way to a period of cycloheximide sensitivity ( $P_3$ ) for second mitosis and 3rd S phase (Ford *et al.* 1983; Newport & Kirschner, 1984). The periods of sensitivity ( $P_2$  and  $P_3$ ) are substantially in advance of the MPF activation associated with the affected mitosis ( $M_1$  and  $M_2$  respectively). The implication of this is that, from about 0.4 in the first cycle the presence of cycloheximide does not prevent MPF appearance at 0.8 resulting in mitosis. In an analysis of timing of protein synthesis required for mitosis in sea urchins, Wagenaar (1983) found an emetine-sensitive period well in advance of the mitosis affected. Separate sensitive periods for nuclear envelope breakdown activity and chromosome condensation activity were observed.

The timings of S phase and mitosis would suggest that the embryonic nucleus spends some time in  $G_2$  even though the results discussed above imply that the



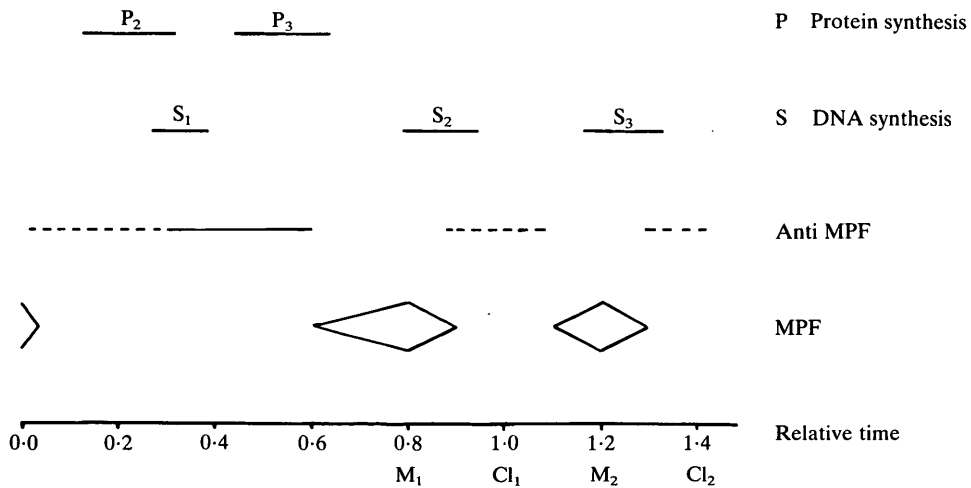


Fig. 2. Timing of cell cycle events in *Xenopus*. The figure has been compiled from data of Wasserman & Smith, 1978; Harland & Laskey, 1980; Ford *et al.* 1983; Miake-Lye *et al.* 1983; Gerhart *et al.* 1984; Newport & Kirschner, 1984. The scale is time relative to first cleavage set at 1.0. M<sub>1</sub>, M<sub>2</sub>, predicted positions of 1st and 2nd mitosis. Cl<sub>1</sub>, Cl<sub>2</sub>, 1st and 2nd cleavage. S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, 1st, 2nd and 3rd S phases (DNA synthesis). P<sub>2</sub>, P<sub>3</sub>, cycloheximide sensitive periods affecting M<sub>1</sub>S<sub>2</sub> and M<sub>2</sub>S<sub>3</sub> respectively. The transitions from P<sub>2</sub> to P<sub>3</sub> is very sharp (between 0.33 and 0.44). The latest point of P<sub>3</sub> sensitivity has not been clarified. Anti-MPF, solid line = activity detected; dotted line = expected activity. MPF, < = activity increasing; > = activity decreasing.

capacity to initiate replication is continuously present from GVBD, even in mitosis. That the embryonic nucleus does not undergo illegitimate reinitiation presumably reflects the presence of a block to reinitiation (Harland & Laskey, 1980) and an effective withdrawal from the S phase environment by chromosome condensation (Newport & Kirschner, 1984).

*Driving the cell cycle.*

Newport & Kirschner (1984), in an elegant series of experiments, have made use of the observation that eggs or syncytial embryos exposed to cycloheximide synthesize DNA once and arrest in a premitotic state with no detectable MPF activity (see section 2). MPF injection into cycloheximide-arrested syncytial embryos initially induces nuclear envelope breakdown and chromosome condensation, but at later times DNA synthesis can be detected. This indicates that MPF promotes entry into an M-phase which is not stably maintained. Instability in this situation is consistent with the observations that MPF is not detectable and apparently not amplified in cycloheximide-treated cells (Gerhart *et al.* 1984). Thus, in eggs unable to synthesize protein, injection of MPF can be used to promote repeated cycles of mitosis followed by DNA synthesis (Newport & Kirschner, 1984).

Injection of both MPF and CSF into cycloheximide-arrested syncytial embryos

generated stable metaphase chromosomes and the embryos contained detectable MPF, consistent with the idea that MPF activity is stabilized by CSF. Injection of 50 nl of 5 mM-CaCl<sub>2</sub> was enough to release the M-phase block induced by CSF. Ca<sup>2+</sup>-induced loss of M-phase lead to reinitiation of DNA synthesis and restarting of the surface contraction waves. Newport & Kirschner (1984) suggest that the embryonic cell cycle may be considered in terms of cycles of MPF leading to chromosome condensation and consequent removal from a cytoplasmic environment continuously capable of DNA synthesis. The close correlation between MPF cycles and the surface contraction waves in their sensitivity to inhibitors seems to indicate a tight link between them (Gerhart *et al.* 1984).

#### CONCLUSIONS

MPF and CSF, because of the difference in the way they have been assayed, tend to be considered separately. However the end point of each assay is an arrested cell, one in meiotic metaphase the other in mitotic metaphase. Are MPF and CSF activities really separable? They are distinguishable in function since MPF, but not CSF will induce nuclear membrane breakdown and chromosome condensation in cycloheximide-arrested embryos (Newport & Kirschner, 1984). They are distinguishable in time since MPF, but not CSF, can be detected in cleaving embryos (Wasserman & Smith, 1978; Masui & Markert, 1971).

Is MPF a trigger for the events of M-phase? This possibility (Masui & Clarke, 1979; Smith, 1981) is strongly supported by the results reviewed here. Gerhart *et al.* (1984) point out, however, that the link between MPF and the cell cycle oscillator may imply that MPF activity itself is regulated by the cell cycle oscillator. Protein synthesis is necessary for cell cycle oscillation. The temporal separation of the cycloheximide-sensitive period and the appearance of MPF (see Fig. 2) is such that appearance of MPF activity may be several steps down a dependent pathway (see Hartwell, Culotti, Pringle & Reid, 1974) from the cell cycle oscillator. Anti-MPF might be on that pathway. It should be borne in mind that MPF has been partially purified and CSF preparations are crude cell extracts. Much will be learnt from their purification.

Is initiation of DNA synthesis triggered by MPF? During oocyte maturation MPF activity reaches its first peak when the capacity to initiate DNA replication first appears (Gerhart *et al.* 1984; Gurdon, 1967). While temporal coincidence may be fortuitous, genetic analysis in yeast, if applicable to *Xenopus*, provides evidence for at least one common component involved in control of mitosis and entry into DNA synthesis. The capacity to initiate replication appears to remain throughout the cell cycles of cleavage, even during M-phase. If more initiation components were activated in each cell cycle, they would add to the existing pool in preparation for the greater demands made for such factors by the exponential increase in cell number during cleavage.

In the embryonic cell cycle, DNA synthesis would start as soon as the

decondensing chromosomes became accessible. After one round of replication, components prevent reinitiation (as also occurs in G<sub>2</sub> nuclei fused to S phase cells). Entry into mitosis is signalled by MPF and as the chromosomes condense (or subsequently decondense), components blocking reinitiation are removed. Condensed chromosomes are effectively inhibited from replication despite the presence of initiation components. The development of *in vitro* systems able to induce chromosome decondensation and recondensation (Lokha & Masui, 1983; Iwao & Katagiri, 1984) should be particularly valuable in defining these events. Comparisons with mammals and yeast suggest that further characterization of MPF and CSF will provide insights into the control of adult, as well as embryonic, cell cycles.

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## DISCUSSION

*Speaker: C. Ford*

*Question from Anne Warner (University College, London):*

It is well known that if you lower calcium, you can achieve quite a good block of cleavage which is very similar to the kind of block which you get with cyostatic factor. Might the "factor" actually be lack of calcium?

*Answer:*

Certainly I would expect calcium to be involved, but there are two reasons for thinking there may be some additional components. One is the sperm injection experiment . . . . . (interrupted)

*A. Warner:*

That could just release calcium because it is known that sperm do that.

*Answer:*

OK, but there must be a mechanism for generating that release. The other observation is the mammalian data. I agree that this may relate to MPF and not CSF, but the tendency is to think when you name a "factor" that you are looking for one molecule.

*Question from R. Laskey (Cambridge):*

Is protein synthesis required for formation of CSF in response to injection of MPF? The MPF injection must induce the formation of CSF only in the meiotic division and not in mitotic division, so do you need new protein synthesis to do it or is it modification?

*Answer:*

If you inject MPF into a cycloheximide-arrested oocyte, you get (according to Gerhart) amplification to give germinal vesicle breakdown and first meiotic division, but you do not get the re-appearance of MPF leading to the stable second meiotic stage. Now the implication of that is that you require some further protein synthesis, either to allow amplification of MPF or to allow production of CSF, or both.