COMMENTARY

Cycling of intracellular free calcium and intracellular pH in *Xenopus* embryos: a possible role in the control of the cell cycle

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Introduction

The scope of this commentary is to propose the incorporation of recent data, obtained from *Xenopus* eggs and embryos, into the models of cell cycle regulation by MPF, a universal M-phase Promoting Factor operating in most, if not all, mitotic cells, from yeast to human. These new data are: (1) the cycling activity of MPF in *Xenopus* eggs is temporally and functionally related to the cycling activity of intracellular pH (pHi) (Grandin and Charbonneau, 1990*a*); (2) cell division in *Xenopus* embryos is accompanied by oscillations of the intracellular free calcium activity ($[Ca^{2+}]_i$) (Grandin and Charbonneau, 1991). There is now definitive evidence that one of the molecular components of the cell cycle, the 'master oscillator' (or cytoplasmic clock), is represented by MPF (Masui and Markert, 1971) and its correlated cdc2 kinase activity and cyclin level (see, for instance, Draetta and Beach, 1989; Murray, 1989; Murray and Kirschner, 1989). In addition, a variety of different systems have revealed a direct implication of $[Ca^{2+}]_i$ variations in mitotic events (reviewed by Berridge and Irvine, 1989; Hepler, 1989). Although *Xenopus* embryonic cells have become, in addition to yeast, one of the most important systems for studying the molecular biology of the cell division cycle, almost no attention has been paid to the possible involvement of ionic messengers, particularly Ca^{2+}, in the control of mitosis in *Xenopus* embryos. This lack of attention to the possible role of $[Ca^{2+}]_i$ variations in the cell division cycle of *Xenopus* embryos was due to their repeatedly noted absence. However, the recent demonstration of Ca^{2+} oscillations occurring with a periodicity equal to that of the cell division cycle in *Xenopus* embryos (Grandin and Charbonneau, 1991) now offers an opportunity of re-evaluating the already proposed models of cell division.

MPF: the cdc2 kinase/cyclin complex

MPF was initially described as a Maturation-Promoting Factor in frog oocytes (Masui and Markert, 1971; Smith and Ecker, 1971). In amphibians, full-grown immature oocytes are arrested in first prophase of meiosis and, following stimulation with progesterone or microinjection of MPF, are released from this meiotic blockade to attain the second metaphase of meiosis (reviewed by Masui and Clarke, 1979; Maller, 1985). Following egg laying, fertilization triggers a number of metabolic reactions (reviewed by Charbonneau and Grandin, 1989), including a second release of the meiotic arrest, which drive the newly fertilized egg into the early embryonic cell cycle, also under the control of MPF. MPF also exists in various cells, always associated with the entry into mitosis (reviewed by Kishimoto and Kanatani, 1977; Meijer and Guerrier, 1984; Kishimoto, 1988). It is now reasonable to conceive that MPF is a universal factor, referred to as M-phase Promoting Factor rather than simply a Maturation-Promoting Factor (reviewed by Hunt, 1989; Lohka, 1989; Dorée, 1990; Maller, 1990; Nurse, 1990). MPF consists of the protein kinase $p34^{cdc2}$ and cyclin, the two becoming associated to trigger M-phase (see Nurse, 1990). The activity of $p34^{cdc2}$ is partly controlled by the successive synthesis and destruction of cyclin (see Minshull *et al.* 1989; Murray and Kirschner, 1989; Murray *et al.* 1989), probably *via* phosphorylation, since $p34^{cdc2}$ is activated by dephosphorylation and rephosphorylates upon inactivation (Dorée *et al.* 1989). Activation of the cdc2 kinase, leading to a cycling activity, appears to involve the participation of a serine/threonine phosphatase and a tyrosine phosphatase (Dunphy and Newport, 1989; Gautier *et al.*, 1989; Gould and Nurse, 1989; Morla *et al.* 1989; Félix *et al.* 1990*a*). In addition, at the end of mitosis, cyclin degradation is under the control of the cdc2 kinase (Félix *et al.* 1990*b*). The cdc2 kinase would not simply control mitotic events *via* the regulation of other enzymes, but also *via* physiological substrates (histone H1, lamins, nucleolar proteins, vimentin,...) that are readily implicated in the structural events of mitosis (reviewed by Lewin, 1990; Moreno and Nurse, 1990; see also Chou *et al.* 1990; Peter *et al.* 1990*a*,*b*).

Relations between the cdc2 kinase oscillations and pHi oscillations in *Xenopus* eggs

Oscillations of the intracellular pH (pHi) level in *Xenopus* embryos were first described by Webb and Nuccitelli (1981). It is interesting to note that *Xenopus* eggs do not

Key words: MPF activity; pHi cycling; Ca^{2+} oscillations.

possess any of the classical plasma membrane pHi-regulating systems (Na^+–H^+, Na^+–$HCO3^-$–Cl^- or H^+ pumps) existing in most cell types (Grandin and Charbonneau, 1990*b*). We have recently demonstrated that the pHi oscillations in *Xenopus* eggs represented a component of the basic cell cycle (Grandin and Charbonneau, 1990*a*). Indeed, pHi cycling, a cytoplasmic activity, was found to be suppressed by treatments that also abolished the cycling activity of MPF, while treatments that blocked cell division without affecting MPF activity cycling did not suppress the pHi oscillations (Grandin and Charbonneau, 1990*a*). Experiments using another amphibian system, *Pleurodeles waltlii*, confirm the view that physiological pHi changes are in tight relation with MPF activity changes. Indeed, the first cell cycle in *Pleurodeles* eggs is 6 h long (*versus* 1.5 h in *Xenopus*), followed by 90 min cell cycles (*versus* 30 min in *Xenopus*). In *Pleurodeles* eggs, both the kinetics of the activation-induced increase in pHi and those of MPF inactivation can be superimposed, both activities changing around 45 min after the triggering of egg activation (N. Grandin, J. P. Rolland and M. Charbonneau, unpublished). As in *Xenopus* eggs, pHi oscillations in *Pleurodeles* eggs subsequently occur in phase with the embryonic cell cycle, MPF activity cycling and the surface contraction waves (Grandin *et al.*, unpublished).

The connection between pHi and other metabolic reactions is still unclear (reviewed by Cohen and Iles, 1975; Gevers, 1977; Roos and Boron, 1981; Busa and Nuccitelli, 1984). However, it is well established that general metabolic reactions consume or produce H^+, or produce CO_2 (reviewed by Gevers, 1977). Indeed, in heart cells, ATP hydrolysis during glycolysis is the principal direct means by which protons are generated in the cytoplasm (Gevers, 1977). Therefore, the cascade of phosphorylations–dephosphorylations occurring during the control of the *Xenopus* cell cycle by the cdc2 kinase might generate pHi oscillations.

A necessary approach to studying the significance of pHi oscillations in *Xenopus* eggs was to determine the hierarchy of control between the cdc2 kinase and pHi oscillations. Three types of experiments convinced us that pHi cycling was probably a consequence of MPF activity cycling rather than the converse. First, we had noted that treatment of unactivated *Xenopus* eggs with weak bases (NH_4Cl or procaine), in a manner that induced a cytoplasmic alkalinization of a similar amplitude to that of the alkalinization induced by sperm during egg activation, did not release the metaphase block and, hence, did not induce the inactivation of MPF normally taking place during egg activation (Grandin and Charbonneau, 1989). In addition, treatments that decrease MPF activity in eggs (unactivated) are activating agents, which, accordingly, always produce the egg activation-associated increase in pHi. In other words, decreasing MPF activity by means of an activating stimulus always results in an increase in pHi. However, increasing pHi in unactivated eggs never produces changes in MPF activity. A second approach consisted in preventing the egg activation-associated increase in pHi in *Xenopus* embryos using CO_2 in the external solution to artificially change pHi, under conditions that were controled with intracellular pH microelectrodes (Grandin and Charbonneau, 1990*b*). CO_2-induced suppression of that physiological increase in pHi did not prevent the embryos from reaching the first cell division, suggesting that MPF had correctly been inactivated following egg activation and been reactivated at the first mitotic metaphase even in the absence of the increase in pHi (Grandin and Charbonneau, 1990*b*). A third line of evidence suggesting that pHi cycling is a consequence of MPF activity cycling is provided by experiments using cycloheximide, an inhibitor of protein synthesis. Cycloheximide prevents the cycling of both MPF activity (Gerhart *et al.* 1984) and pHi (Grandin and Charbonneau, 1990*a*) in activated *Xenopus* eggs. The first cyclic increase in MPF and pHi occurs 1 h after egg activation, the time of the first mitotic metaphase. However, cycloheximide does not affect the egg activation-associated increase in pHi, as shown in Fig. 1, or the pHi level in unactivated *Xenopus* eggs, but starts having an effect on pHi only at the time corresponding to the synthesis of cyclin, around 50 min after egg activation (Grandin and Charbonneau, 1990*a*). Thus, the suppression of pHi oscillations, 1 h after egg activation, by cycloheximide (Grandin and Charbonneau, 1990*a*) cannot result from a direct effect on pHi, but is rather mediated by the suppression of MPF activity cycling resulting from

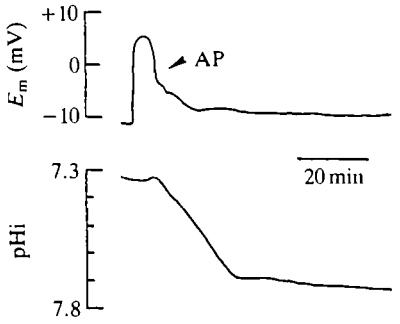

Fig. 1. Effects of cycloheximide on the pHi response to egg activation in *Xenopus*. Intracellular (pHi) was measured with H^+-selective microelectrodes made and calibrated as described by Grandin and Charbonneau (1990*a*). Each egg was impaled with a potential microelectrode, measuring the membrane potential (E_m; top trace), and a pH microelectrode, measuring E_m+pHi. E_m recorded by the potential microelectrode was subtracted at the pen recorder input from the total value (E_m+pHi) recorded by the pH microelectrode, to give the pHi value (pHi; bottom trace). Unactivated eggs were dejellied with 2% cysteine (in the physiological F1 solution, see Grandin and Charbonneau, 1990*a*) and immersed in F1 solution with or without 200 $\mu g\,ml^{-1}$ cycloheximide. In the example shown here, the egg was incubated in the presence of cycloheximide 40 min before impalement with the microelectrodes (beginning of the trace), always in the presence of cycloheximide, and activated by pricking a few minutes later. Successful egg activation was attested by the occurrence of the activation potential (AP), a Cl^--dependent plasma membrane depolarization, followed 6 min later by a typical increase in pHi, from pH 7.34 to pH 7.70. The levels of pHi in cycloheximide-treated eggs before egg activation, as well as the kinetics of the egg activation-associated increase in pHi and the elevated pHi level attained 20–30 min after egg activation, were exactly similar to those in untreated eggs. Other criteria of egg activation, observed under a stereomicroscope during electrical recording in cycloheximide-treated eggs, were normal and identical to those in untreated eggs. These morphological criteria were: the elevation of the vitelline envelope, a consequence of cortical granule exocytosis; the cortical contraction; the disappearence of the maturation spot, a consequence of meiosis resumption, reflecting the migration of the egg nucleus deeper in the cytoplasm during the passage from the metaphase II-arrested stage of meiosis to the pronucleus stage.

inhibition of the synthesis of one of the components of MPF, probably cyclin.

Temporal relationship between pHi oscillations and Ca^{2+} oscillations in *Xenopus* embryos

Monitoring the intracellular free calcium level ($[Ca^{2+}]_i$) with microelectrodes implanted in *Xenopus* embryos, we observed Ca^{2+} oscillations, which had a period equal to that of the cell division cycle (Grandin and Charbonneau, 1991). These Ca^{2+} oscillations did not occur in artificially activated eggs or nocodazole-treated embryos, in both of which the basic cell cycle (for instance, cdc2 kinase activation and inactivation) persists (Grandin and Charbonneau, 1991). This demonstrated that the Ca^{2+} oscillations are not required for the basic cell cycle in *Xenopus* embryos, and suggested that they might rather be linked to the metabolic events occurring only when both nuclear divisions and cleavage take place correctly. Both the opacity of *Xenopus* embryos and their very rapid period of cell division prevented us, at that time, from establishing a precise relationship between $[Ca^{2+}]_i$ and mitotic stages. We have now measured pHi and $[Ca^{2+}]_i$ simultaneously in the same embryo, the pHi oscillations serving as a reference mark to determine the corresponding mitotic stages (Fig. 2). Initial attempts, which relied on the observation of nuclei with the light microscope, gave erroneous results because of the asynchrony between blastomeres at advanced stages (256-cell) of development (Grandin and Charbonneau, 1990*a*). In fact, the acidic peaks of the pHi oscillations correspond to the peaks of MPF activity (metaphase), as seen both in *Pleurodeles* after direct measurement of MPF activity, and in *Xenopus*, indirectly, by correlating the cleavage-associated membrane hyperpolarizations (telophase) and the surface contraction waves that occur at the metaphase–anaphase transition, immediately before the onset of cleavage (N. Grandin, J. P. Rolland and M. Charbonneau, unpublished data). Fig. 2 shows that Ca^{2+} oscillations occur out of phase with pHi oscillations. Since the period of the cell division cycle, measured as the interval of time between two pHi or Ca^{2+} oscillations, varied slightly from one embryo to the other, we will indicate for each of the nine impaled embryos the delay between Ca^{2+} and pHi oscillations, as well as the period of the cell cycle. The period between the acidic peak of a pHi oscillation and the peak of the following Ca^{2+} oscillation, and the period of the cell cycle in the corresponding embryo were, respectively (in min): 9, 21; 13, 23; 16, 24; 10, 22; 9, 21; 6, 25; 9 20; 10, 21; 13, 24; at 26–27 °C. One can see that, in most cases, the peak level of the Ca^{2+} oscillation occurs slightly before or slightly after the alkaline peak of the pHi oscillation, that is approximately in the opposite phase with respect to the acidic peak of the pHi oscillation (Fig. 2). Since the acidic peak of the pHi oscillation corresponds to the metaphase stage of mitosis, as seen above, it follows that $[Ca^{2+}]_i$ would begin to increase between anaphase or telophase and interphase and would be at its maximal level during interphase or the next prophase. However, we are aware of the fact that the precision of the relation should be improved in the future. For the moment, the two main limitations are: (1) the opacity of the egg, which prevents visual observation of the corresponding mitotic stages; (2) the necessity of recording Ca^{2+} and pHi oscillations using a low chart speed, which increases the imprecision while measuring the time between the two types of oscillation.

A model of cell division in *Xenopus* embryos that integrates the existence of Ca^{2+} and pHi oscillations

As stated above, pHi oscillations always take place in

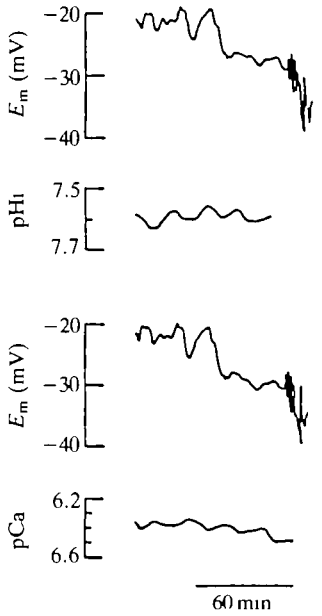

Fig. 2. Simultaneous measurements of pHi and Ca^{2+} oscillations in a single embryo of *Xenopus*. Since *Xenopus* embryos display pHi oscillations and Ca^{2+} oscillations, both with a period equal to that of the cell cycle (see text), the present experiments were conducted in order to determine the delay, if any, between these two types of oscillations. *Xenopus* embryos were dejellied and impaled each with two potential microelectrodes, a Ca^{2+}-selective microelectrode (made and calibrated as described by Grandin and Charbonneau, 1991) and a pH microelectrode. Each trace of ion activity measurement has its corresponding membrane potential trace (the one subtracted from the total signal recorded by the ion-selective microelectrode) represented above it. In the example shown here, the embryo was impaled at the 8-cell stage. Both $[Ca^{2+}]_i$ and pHi oscillated around their basal levels, 0.31–0.50 μM (pCa 6.5–6.3) and pH 7.55–7.65, respectively, for 4 or 5 cell cycles, the amplitude of the oscillations being around 50–100 nM $[Ca^{2+}]_i$ and 0.04–0.06 pH unit. In this embryo, the duration of the cell cycle was 21–24 min. The peaks of the Ca^{2+} oscillations were found to occur 9–12 min after the acidic peaks of the pHi oscillations. That delay increased as the cell cycle lengthened (12 min delay during a 24 min cell cycle), and conversely (9 min delay during a 21 min cell cycle). The great difficulty of recording ion activity changes in dividing embryos (see Grandin and Charbonneau, 1991), was enhanced by the fact that, in the present situation, all four microlectrodes had to remain correctly inserted for several hours. However, such experiments are worthwhile because of the great selectivity and sensitivity allowed by ion-selective microelectrodes. The results of the present experiments provide one more argument (in addition to those developed by Grandin and Charbonneau, 1991) against the existence of artifacts during measurement of intracellular ion activity with microelectrodes. Indeed, the findings that Ca^{2+} oscillations did not have the same shape as pHi oscillations, and that they were recorded with a delay between them, argue against the existence of 'mirror-image artifacts' and 'motion artifacts', respectively (see Grandin and Charbonneau, 1991).

activated eggs, even in the absence of cleavage, whereas Ca^{2+} oscillations do not proceed in the absence of cell division. Therefore, we suspect that the existence of these Ca^{2+} oscillations in *Xenopus* embryos might be associated with the presence of dividing nuclei. Accumulation of endoplasmic reticulum (ER) in the perinuclear region of cells might control $[Ca^{2+}]_i$ in relation to specific mitotic events. Experimental evidence of this type exists, for instance, in sea-urchin embryos, in which the ER contains a calsequestrin-like protein, which is a Ca^{2+}-binding storage protein (Henson *et al.* 1989). It is very likely that such organized ER networks, with Ca^{2+} storage properties, are present around the nuclei in *Xenopus* embryos. However, definitive evidence, even in sea-urchin embryos, that the mitosis-associated intracellular Ca^{2+} transients originate from the Ca^{2+} stored in the ER located in the perinuclear region is still missing. In addition to such possible control of Ca^{2+} oscillations in *Xenopus* embryos by internally stored Ca^{2+}, another possibility is control of these oscillations by the entry of extracellular Ca^{2+}. However, there is apparently no influx of extracellular Ca^{2+} in *Xenopus* embryos, since immersion of embryos in Ca^{2+}-free medium containing EGTA has no effect on cleavage (Baker and Warner, 1972). This suggests that intracellular Ca^{2+} oscillations in *Xenopus* embryos are not driven by the entry of extracellular Ca^{2+}, but would instead depend on internally stored Ca^{2+}. Unfortunately, this could not be directly tested on *Xenopus* embryos impaled with Ca^{2+} microelectrodes, since extracellular Ca^{2+} is needed for healing at the site of microelectrode penetration.

In fact, most of our knowledge on the Ca^{2+}-binding and -accumulation properties of the ER comes from studies on isolated vesicles from muscle cells. The Ca^{2+}-binding ability of isolated sarcoplasmic reticulum (SR) vesicles from skeletal and cardiac muscle is markedly pH-dependent (Nakamaru and Schwartz, 1970); it decreased as extravesicular pH increased, from 6.3 to 7.5, and Ca^{2+} was released from these vesicles as a consequence (Nakamaru and Schwartz, 1970). These experiments were performed *in vitro*, and, therefore, a smaller alkalinization *in vivo* might have the same consequence on release of Ca^{2+} into the cytoplasm. In addition, dissipation of the pH gradient between the external and internal sides of the SR, following an increase in the extravesicular pH, elicited Ca^{2+} release by these vesicles, possibly *via* a Ca^{2+}-release channel in the SR membrane (Shoshan *et al.* 1981). Experiments on skinned muscle fibers and intact muscle fibers led to quite variable, sometimes opposite, results concerning the relationships between $[Ca^{2+}]_i$ and pHi (see references quoted by Pressler, 1989; Kaila and Voipio, 1990), perhaps as a result of the diversity of the experimental conditions adopted, as well as of the possibility that the drugs used to change $[Ca^{2+}]_i$ or pHi may have effects other than those postulated. The experiments on isolated SR vesicles (Nakamaru and Schwartz, 1970; Shoshan *et al.* 1981) agree nicely with our experiments in *Xenopus* embryos, in which the peak level of the Ca^{2+} oscillations is, roughly, in the opposite phase with respect to the acidic peak of the pHi oscillations (Fig. 2). According to the hypothesis for an active role of pHi oscillations in the generation of Ca^{2+} oscillations, the periodical alteration of a pH gradient between the cytoplasm and an internal compartment specifically located around the nuclei would produce a periodical influx of Ca^{2+} from that internal compartment into the cytoplasm. As seen above, artificially activated (non-dividing) eggs display pHi oscillations without giving rise to subsequent Ca^{2+} oscillations. This is apparently inconsistent with our hypothesis that pHi oscillations are the driving force for Ca^{2+} oscillations. However, this contradiction can be explained, considering that pHi oscillations might generate Ca^{2+} oscillations only when compartments specifically located around the nuclei and specialized in the storage of Ca^{2+} are functional, as is the case in dividing embryos. On the other hand, artificially activated eggs do not possess such functional perinuclear compartments, since they do not divide. In other words, pHi oscillations might generate Ca^{2+} oscillations in embryos, but nevertheless exist without generating Ca^{2+} oscillations if the structures involved in the generation of the latter are not functional, which is the case in activated non-dividing eggs.

Although most of the terms of the proposed cascade of reactions are still hypothetical, none of them is impossible. It is now well established that membranes, particularly the ER and the Golgi apparatus, constitute an important component of the mitotic apparatus, and probably regulate $[Ca^{2+}]_i$ in order to control the formation and function of spindle fibers and the separation of chromosomes (reviewed by Hepler and Wolniak, 1984). For instance, in sea-urchin embryos, intracellular Ca^{2+} transients have been measured in association with events of mitosis, such as the metaphase–anaphase transition and nuclear envelope breakdown (Poenie *et al.* 1985; Steinhardt and Alderton, 1988). In addition, artificial increases in $[Ca^{2+}]_i$ cause premature chromatin condensation and breakdown of the nuclear envelope in sea-urchin embryos (Twigg *et al.* 1988). Evidence for a close relationship between $[Ca^{2+}]_i$ variations and specific stages of mitosis also exists in other cell types, such as cultured mammalian cells and plant cells (reviewed by Hepler, 1989; see also Kao *et al.* 1990; Zhang *et al.* 1990). In *Xenopus* embryos, to date there has been no direct evidence for a relationship between $[Ca^{2+}]_i$ variations and specific stages of mitosis. However, early experiments, showing that microinjection of Ca–EGTA buffers into *Xenopus* blastomeres slowed down or even arrested cleavage by specifically lowering the intracellular concentration of Ca^{2+} (Baker and Warner, 1972), support the suggestion that Ca^{2+} oscillations in *Xenopus* embryos may play a regulatory role in cell division.

We propose an improved model of control of the cell division cycle in *Xenopus* embryos (Fig. 3), taking into account our recent findings on pHi and Ca^{2+} oscillations (Grandin and Charbonneau, 1990*a*, 1991), the present results and the recently proposed models of control by the cdc2 kinase/cyclin complex. In Fig. 3, we represent the gradual accumulation of cyclin in the egg. At some point in that accumulation, the $p34^{cdc2}$ kinase is activated following its binding to cyclin. Mitosis is thought to be directly triggered by the activation of the $p34^{cdc2}$ kinase/cyclin complex (MPF). The cdc2 kinase activity, measured *in vitro* as a histone H1 kinase activity or *in vivo*, in *Xenopus* oocytes, as a maturation-promoting factor activity, increases at the onset of mitosis and reaches its maximal level during metaphase (indicated as 'high cdc2 kinase activity' in Fig. 3). The association between cyclin and the cdc2 kinase ultimately leads, after a few intermediate steps, to the dephosphorylation of the cdc2 kinase (its active form). Recent studies have demonstrated the existence of a lag period between the addition of cyclin (*in vitro*) and the appearance of H1 kinase activity, even in the presence of a large excess of cyclin (Solomon *et al.* 1990). The threshold concentration of cyclin required to

activate the cdc2 kinase/cyclin complex and the length of that lag period are regulated by an inhibitor of MPF activation, a type 2A protein phosphatase (not indicated in Fig. 3) (Solomon *et al.* 1990). It is also suggested that the abrupt transition into mitosis is due to an inhibition (by $p34^{cdc2}$) of the initial phosphorylation, on tyrosine, of $p34^{cdc2}$ by cyclin (during the lag period), which leads to a stimulation of $p34^{cdc2}$ dephosphorylation (Solomon *et al.* 1990), one of the last steps prior to the entry into mitosis. At the end of metaphase, cyclin is abruptly degraded, probably *via* specific proteolysis (Murray and Kirschner, 1989). Recent work shows that, *in vitro*, cyclin proteolysis is directly triggered by the cdc2 kinase itself, probably *via* the phosphorylation of an unknown protein that activates the cyclin-specific protease (Félix *et al.* 1990*b*). Following cyclin degradation, the cdc2 kinase is rapidly rephosphorylated (its inactive form) or combines with an inhibitor (Félix *et al.* 1990*b*). Inactivation of the cdc2 kinase/cyclin complex (indicated as 'low cdc2 kinase activity' in Fig. 3) permits exit from mitosis and entry into interphase. The alternating activated and inactivated forms of the cdc2 kinase/cyclin complex (MPF) represent, together with other activators or inhibitors mentioned above, a sort of autonomous oscillator, which regulates the basic cell cycle. In *Xenopus* embryos, each of the early cell cycles is 30 min long, with the exception of the first cell cycle, which lasts 90 min. Previous studies (Grandin and Charbonneau, 1990), as well as the present results, strongly suggest that the MPF activity oscillations control – and give rise to – pHi oscillations. In addition, we have shown that the acidic peak of the pHi oscillations corresponds to the metaphase stage of mitosis (Grandin and Charbonneau, 1991; present results), while the alkaline peak corresponds to interphase, when the activity of the cdc2 kinase/cyclin complex is at its lowest level (Fig. 3). However, the molecular mechanisms at the origin of the temporal and functional relationships between MPF activity and pHi remain to be discovered. Taking into account the existence of Ca^{2+} oscillations, which are in the opposite phase to the pHi oscillations, and assuming the existence of perinuclear ER that possibly regulates the internal store of Ca^{2+} at the origin of the Ca^{2+} oscillations, we suggest that Ca^{2+} oscillations could be driven by pHi oscillations, as described above. In such a scheme, internal compartments located around the dividing nuclei (perinuclear ER?) would possess in their membrane a pH-dependent Ca^{2+}-release channel or a Ca^{2+}–H^+ exchanger. During the passage between the acidic peak of the pHi oscillation and the alkaline peak of the pHi oscillation, there is a gradual alkalinization of the cytoplasm (pHi is the cytosolic pH). This might lead to the abolition of the pH gradient existing between the cytosol and the internal compartment (perinuclear ER). As explained above, dissipation of that pH gradient might lead to the release of Ca^{2+} by the ER vesicles through pH-dependent Ca^{2+}-release channels contained in the membrane of these vesicles, a situation that exists in muscle cells (see references above). Since pHi continuously cycles during early cell division, the associated cycling of opening and closure of such pH-dependent Ca^{2+}-release channels might result in a cycling of

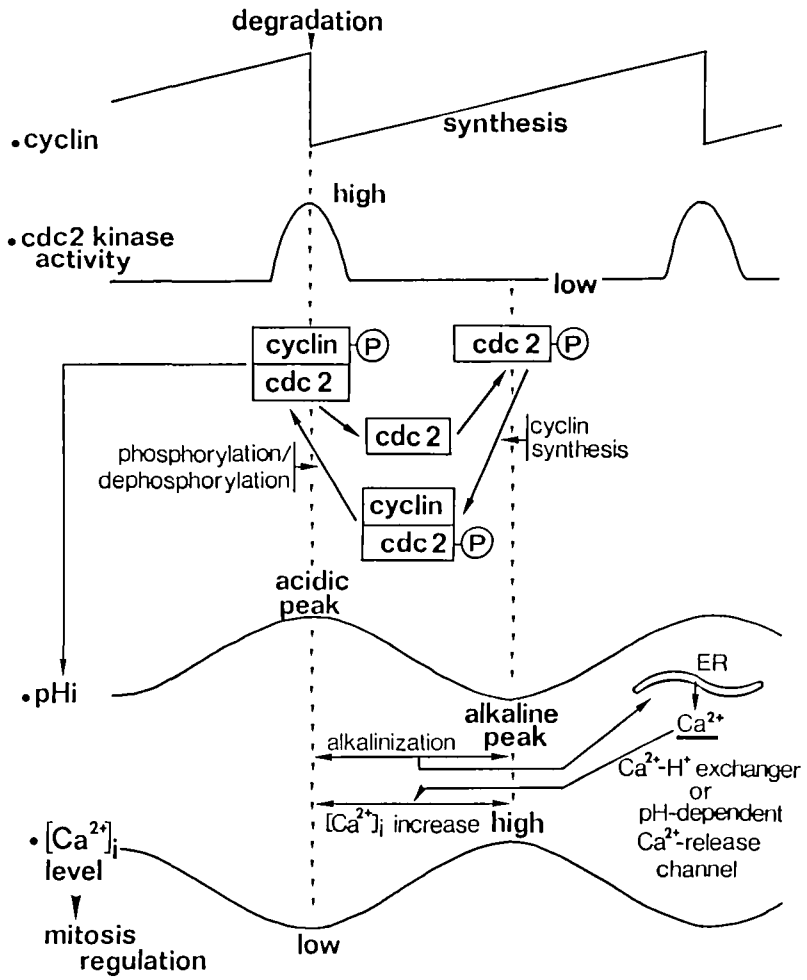

Fig. 3. Improved model of control of the cell cycle in *Xenopus* embryos, made on the basis of the existence of Ca^{2+} and pHi oscillations. All the authors cited in the section *MPF: the cdc2 kinase/cyclin complex* of the present paper, as well as many of the studies cited by these authors, have contributed by their results to the construction of the present scheme. However, some of these authors have provided general schemes of the control of the cell cycle by the cdc2 kinase and cyclins, so it might be useful for the reader to compare them with the present scheme (Draetta and Beach, 1989; Murray, 1989; Félix *et al.* 1990*b*; Minshull *et al.* 1990; Maller, 1990; Nurse, 1990). It should noted that the activity of the cdc2 kinase builds up and subsequently drops faster than the parallel changes in H^+ activity. This is not due to a technical problem, since pH microelectrodes have a response time of a few seconds only. That delay suggests that the coupling between MPF activity oscillations and pHi oscilations involves either slow metabolic reactions or a succession of several coupled reactions.

cytosolic Ca^{2+} (the Ca^{2+} oscillations) around the dividing nuclei. It should be noted that a different mechanism, not involving the participation of some intracellular compartment responding to the periodical alteration of a pH gradient between it and the cytosol, might be considered. Indeed, Picard *et al.* (1990) have recently shown that microinjection of a PSTAIR peptide, which is a conserved sequence of $p34^{cdc2}$, into *Xenopus* oocytes produced an increase in $[Ca^{2+}]_i$, independently of its histone H1 kinase activity. If such a Ca^{2+}-mobilizing activity of the cdc2 kinase was also present in *Xenopus* embryos, it might explain the generation of Ca^{2+} oscillations in response to MPF activity oscillations, directly, using a pathway independent of the pHi oscillations.

A major problem with *Xenopus* embryos is that cells are totally opaque. Therefore, the relations of $[Ca^{2+}]_i$ variations to specific mitotic events is much less clear than in other systems, for instance in sea-urchin embryos. However, experiments to try and improve our comprehension of the *Xenopus* system will be certainly worthwhile. We believe that the two systems, *Xenopus* and sea-urchin, are different from each other and that new information using the *Xenopus* system can be complementary to that provided by the study of the sea-urchin system. The pattern of $[Ca^{2+}]_i$ variations during embryonic cell division in *Xenopus* appears to be different from that in sea-urchin embryos, in which intracellular Ca^{2+} transients have been recorded in association with pronuclear migration, streak stage, nuclear envelope breakdown, chromatin condensation, onset of anaphase and cytokinesis (Poenie *et al.* 1985; Steinhardt and Alderton, 1988; Twigg *et al.* 1988). To date, however, in sea-urchin embryos, nuclear envelope breakdown and chromatin condensation are the only two mitotic events in which there is some substantial evidence for a role for $[Ca^{2+}]_i$ (Twigg *et al.* 1988; Whitaker and Patel, 1990).

A major part of our future work will be directed at consolidating our hypothesis, combining $[Ca^{2+}]_i$ measurements *in vivo* and the study of the capacity of perinuclear ER to store Ca^{2+} by using antibodies directed against ubiquitous Ca^{2+}-binding proteins. An additional requirement will be to uncover some specific targets of the Ca^{2+} generated by the Ca^{2+} oscillations. For the moment, no such $[Ca^{2+}]_i$-regulated target, capable of playing a role in the control of mitosis, has been identified by us or others.

Finally, it is important to stress that our scheme (Fig. 3) does not mean that Ca^{2+} oscillations represent a solitary endpoint signal for regulation of mitosis. This is particularly evident, considering that to date there has been no mitotic event identified as responding to intracellular Ca^{2+} signals in *Xenopus* embryos. On the contrary, we believe that most of the mitotic targets are under the control of the cdc2 kinase/cyclin complex. Indeed, several mitosis-associated substrates of cdc2 kinase have been recently identified in various systems: histone H1 (Arion *et al.* 1988; Labbé *et al.* 1988; Langan *et al.* 1989), major nucleolar proteins (Peter *et al.* 1990*a*), lamins (Peter *et al.* 1990*b*), RNA polymerase II (Cisek and Corden, 1989), elongation factor EF-1τ (Bellé *et al.* 1989) and vimentin (Chou *et al.* 1990) (see also the model described by Minshull *et al.* 1990). We suspect that Ca^{2+} oscillations in *Xenopus* embryos might act in co-operation with the MPF complex to trigger limited and specific mitotic events that have not been identified.

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