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Protein Kinases and Protooncogenes: Biochemical Regulators of the Eukaryotic Cell Cycle†

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Cell division is a fundamental biological process that begins with the fertilized egg and continues throughout an organism's life span until individual cells terminally differentiate or die. The most simplified cell cycle, characterized in nature by the rapid early embryonic cell divisions, consists of a round of DNA replication followed by the splitting of the cell and its duplicated genetic material into two daughter cells, each identical with the parent. Mitosis is defined as the series of steps that result in the formation of two new nuclei, each having the same number of chromosomes as the parental nucleus. Following mitosis, the cytoplasm is partitioned around the two nuclei giving rise to the two daughter cells. During the cell cycle of most somatic cells, DNA synthesis (S-phase) and mitosis (M-phase) are separated by two "growth" stages (G_1 and G_2) of varying duration. Thus, a typical eukaryotic cell passes through G_1, S, G_2, and M and back into G_1 during a single cycle (Figure 1). The early embryonic cell cycle, with G_1 and G_2 phases that are virtually nonexistent, has recently become the system of choice for studying the biochemistry of mitosis [see Cross et al. (1989) for a review]. In this paper, we will review the current ideas of how entry into mitosis is controlled in eukaryotic cells, paying particular attention to the roles for protein phosphorylation and dephosphorylation in this process. [For reviews on G_1 and S-phase control of the cell cycle, see Pardee (1989) and Laskey et al. (1989).]

MPF: Promoter of Mitosis and Meiosis

MPF in Mitosis. Over the past 20 years, scientists have identified several of the biochemical players that act during mitosis, including a cytoplasmic activity that is capable of driving interphase cells into mitosis (Table I). This cell cycle promoter, called M-phase promoting factor (MPF), has been subjected to much experimental scrutiny, but only recently has its role been defined. During the cell cycle, MPF activity oscillates in parallel with the coming and going of M-phase. It first appears during G_2 and rises as the cell enters prophase, the first stage of mitosis. MPF activity peaks during mitotic metaphase and then rapidly vanishes near the end of mitosis at the anaphase/telophase transition. The significance of MPF is exemplified by its ability to induce mitosis in a variety of cells and by its ability to promote M-phase in interphase extracts in the absence of protein synthesis [see Pines and Hunter (1990b) for a review].

MPF in Meiosis. Much of what is known about MPF was learned through the use of concentrated egg extracts that, when activated, simulate the mitotic cleavages of the early embryo. In fact, MPF (originally called maturation promoting factor) was first identified as an activity present in unfertilized frog eggs that was capable of promoting the resumption of meiosis when injected into prophase-arrested oocytes (Masui & Markert, 1971; Smith & Ecker, 1971). Meiosis, the process in which haploid germ cells arise from diploid progenators, involves two consecutive nuclear divisions: a reduction division followed by a mitotic division in which half the normal chromosome content is partitioned into daughter cells. In females, the nonrenewing population of fully grown oocytes is arrested at the earliest stage of the first meiotic division (prophase I). Hormones such as progesterone stimulate the release from prophase I arrest and the resumption of meiosis in a series of events known as meiotic maturation. The end product of meiotic maturation is the unfertilized egg, which is arrested in metaphase of the second meiotic division in all vertebrates. Fertilization triggers the release from the metaphase II block and allows the final steps of meiosis [see Maller (1985) for a review]. Thus, meiotic maturation can be thought of as consecutive mitotic divisions, and the dual role of MPF as "maturation promoting factor" in oocytes and "M-phase promoting factor" in somatic cells suggests that many of the

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Table I: Regulators and Substrates of the M-Phase Machinery

component	biochemical activity	rel molecular mass (kDa)	postulated M-phase function
MPF	serine/threonine protein kinase	~100	promoter of mitosis and meiosis
CSF	serine/threonine protein kinase?	?	inducer of mitotic arrest; stabilizer of MPF
$p34^{cdc2}$	serine/threonine protein kinase	32–34	catalytic subunit of MPF; also controls G_1/S transition in yeast
cyclins	?	45–63	regulatory subunit of MPF; substrate of $p34^{cdc2}$; product of fission yeast *cdc13* gene
INH	serine/threonine type 2A phosphatase	?	maintains pre-MPF in an inactive state in oocytes
type 1 phosphatase	serine/threonine phosphatase	37	activation of pre-MPF; needed for proper chromosome separation; inactivation of CSF?
p13	?	13	maintains MPF in an inactive state; product of fission yeast *suc1* gene
nim1	serine/threonine protein kinase	50[a]	activator of mitosis; inhibitor of *wee1* function
wee1	serine/threonine protein kinase	112[a]	inhibitor of mitosis; negative regulator of *cdc2* function
cdc25	?	80	regulates timing of MPF activation
histone H1	DNA binding protein	21[a]	in vitro substrate of $p34^{cdc2}$; H1 phosphorylation may be important for chromosome condensation
nucleolin	?	92	in vitro substrate $p34^{cdc2}$; associated with chromosomes in mitosis
N038	?	38	in vitro substrate of $p34^{cdc2}$; nucleolar protein
MAP-2	microtubule-associated protein	>200	in vitro substrate of $p34^{cdc2}$
caldesmon	actin, calmodulin binding protein	83	in vitro substrate of $p34^{cdc2}$; implicated in microfilament disassembly during mitosis
src	tyrosine protein kinase	60	in vitro substrate of $p34^{cdc2}$; phosphorylated $p34^{cdc2}$ in vitro
lamins	component of nuclear membrane	65–75	putative in vivo substrate of $p34^{cdc2}$; lamin phosphorylation promotes lamina disassembly
mos	serine/threonine protein kinase	39	activator of MPF in meiosis; putative component of CSF
met	receptor-like tyrosine protein kinase	157[a]	oncogene induces GVBD in oocytes
ras	GDP/GTP-binding protein; hydrolyzes GTP	21	oncogene induces GVBD in oocytes; functions upstream of *mos* in meiosis

[a] Predicted molecular mass.

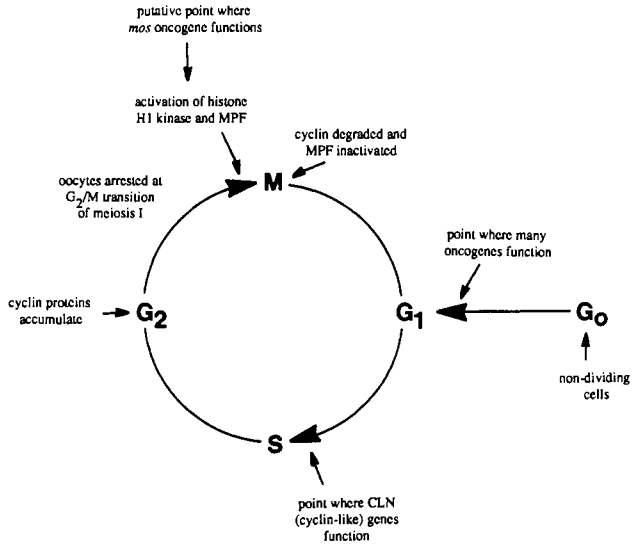

FIGURE 1: Location of regulatory events in the eukaryotic cell cycle. Quiescent somatic cells are arrested in a nongrowth state known as G_0. Various mitogenic agents and numerous oncogenes can stimulate cells to enter the cell cycle at G_1. In contrast, oocytes are arrested at the G_2/M-phase border. Hormones such as progesterone as well as several oncogenes stimulate oocytes to undergo meiotic maturation, which involves two successive nuclear divisions.

biochemical mechanisms of meiosis and mitosis will be shared.

Meiotic maturation of oocytes from the South African clawed frog *Xenopus laevis* can be stimulated in vitro by treatment of isolated oocytes with progesterone. A telltale sign of maturation is the appearance of a white spot in the pigmented animal hemisphere of the oocyte that occurs as a result of germinal vesicle, or nuclear envelope, breakdown (GVBD). GVBD is a sign of the first nuclear division, and as such, the timing of many of the events that precede GVBD is determined with respect to this phenomenon. MPF activity rises during hormone-stimulated meiotic maturation in a manner analogous to the increase in activity that occurs as somatic cells pass from G_2 into M-phase. MPF activity is first detected at approximately the halfway point between hormonal stimulation and GVBD. It then increases as oocytes undergo GVBD until it peaks at metaphase I (Gerhart et al., 1984). Activation of MPF during progesterone-induced maturation is dependent upon protein synthesis, and this protein synthesis requirement is alleviated at about the same time that MPF activity first appears (Wasserman & Masui, 1975). As the first meiotic division is completed, MPF activity transiently decreases only to rise again with the onset of the second nuclear division. In unfertilized eggs, MPF activity peaks and is stabilized until fertilization, when it once again rapidly disappears as the newly formed zygote enters interphase (Gerhart et al., 1984). Thus, for the purpose of characterizing meiotic as well as mitotic events, the use of in vitro oocyte maturation systems offers the advantage of a synchronized population of cells that can be readily manipulated in the laboratory.

Experiments utilizing cell cycle staged somatic cells, as well as cell-free extracts that are capable of oscillating between interphase and mitosis, have defined some of the criteria for MPF activation and inactivation that are likely to be important for both mitosis and meiosis. However, the stabilization of MPF in unfertilized eggs, arrested at metaphase II, is a property unique to meiosis. At the time MPF was discovered, an additional activity present in unfertilized eggs was shown to be capable of arresting cleaving embryos in mitosis (Masui & Markert, 1971). This activity, called cytostatic factor (CSF), is believed to be responsible for the stabilization of MPF in unfertilized eggs. Unlike MPF, CSF has thus far evaded attempts at biochemical purification, although a clue to its composition has recently emerged in the surprising form of an oncogenic protein kinase (Sagata et al., 1989b).

Regulation of M-Phase by Phosphorylation. Several observations have underscored an essential role for protein phosphorylation and dephosphorylation in regulating MPF and the events associated with the meiotic and mitotic divisions. During oocyte maturation, a general increase in protein phosphorylation and the appearance of MPF activity are

coincident (Maller et al., 1977). Similarly, an overall increase in phosphorylation occurs as somatic cells enter M-phase, and this is followed by a corresponding decrease in protein phosphorylation when cells exit mitosis. Structural proteins such as the nuclear lamins and caldesmon, which play intricate roles in the rearrangement of the nuclear matrix during mitosis, are phosphorylated in a mitotic-specific manner (Gerace & Blobel, 1980; Yamashiro et al., 1990). In addition, histone H1 has long been known to undergo mitotic-specific phosphorylations (Bradbury et al., 1974). Indeed, a major obstacle in the purification of MPF was overcome by the inclusion of several nonspecific protein phosphatase inhibitors, such as sodium β-glycerophosphate, NaF, and α-naphthyl phosphate, in the extraction buffers (Lohka et al., 1988). Hints for a kinase activity in MPF came from studies showing that MPF activity could be inhibited by a nonhydrolyzable ATP analogue but not by ATP or a hydrolyzable analogue, γ-thio-ATP (Lohka et al., 1987). Other experiments revealed an MPF-associated protein kinase activity as evidenced by the ability of partially purified MPF to phosphorylate one of its components (Lohka et al., 1988). Further evidence that MPF contained phosphorylated subunits came from the ability of monoclonal antibodies specific for thiophosphorylated proteins to immunoadsorb and inhibit MPF activity (Cyert et al., 1988). These observations provided hints for the further characterization of MPF as a major cell cycle regulator that functions by altering an intricate balance between phosphorylation and dephosphorylation.

The genetic characterization of cell division control (*cdc*) mutants in yeast has dramatically demonstrated the significance of protein kinases for mitosis [see Lee and Nurse (1988) for a review]. The defective genes of several mutants that are unable to progress through mitosis normally encode protein kinases. One such gene in fission yeast, the *cdc2* gene, encodes an ~34-kDa phosphoprotein that possesses serine/threonine protein kinase activity (Hindley & Phear, 1984). In addition to being required for the initiation of mitosis, fission yeast p34^{cdc2} kinase activity has been shown to oscillate during the cell cycle, peaking at mitosis (Draetta & Beach, 1988). The affected genes in two other cell cycle mutants, *nim1* and *wee1*, encode putative protein kinases. Genetic evidence has implicated the products of the *wee1* and *nim1* genes in a pathway involved in the regulation of the p34^{cdc2} kinase (Russell & Nurse, 1987a,b). Highly conserved *cdc2* homologues have been isolated from several organisms, including humans, by their ability to complement the *cdc2* mutation in yeast (Lee & Nurse, 1987). Thus, the *cdc2* genes from the most distant branches of the eukaryotic evolutionary tree are able to control entry into mitosis when expressed in yeast.

p34^{cdc2} Is the Protein Kinase Component of MPF. The development of an MPF-dependent cell-free system that mimics the early events of mitosis led directly to the purification of MPF from frog eggs and the identification of two major polypeptide components. The purified MPF complex possessed a serine/threonine protein kinase activity that could phosphorylate histone H1, casein, and phosphatase inhibitor 1 (Lohka et al., 1988). Soon after the identification of a 32-kDa component of MPF, Dunphy et al. (1988) and Gautier et al. (1988) linked the work of many geneticists and biochemists by demonstrating that the 32-kDa polypeptide purified from frog eggs was a homologue of the yeast p34^{cdc2} protein. The observation that immunoprecipitates of *Xenopus* p34^{cdc2} possessed histone H1 kinase activity (Gautier et al., 1988; Lohka et al., 1988) led to another important discovery. A mitotic-specific histone H1 kinase activity present in starfish eggs and embryos, and known to oscillate during meiosis and mitosis in a manner analogous to MPF activity, was shown to contain a homologue of p34^{cdc2} (Labbe et al., 1988; Arion et al., 1988). Together, these findings culminated in the discovery that the major cell cycle oscillators responsible for entry into mitosis in eukaryotes ranging from yeast to man (yeast p34^{cdc2} protein kinase activity, starfish M-phase-specific histone H1 kinase, and amphibian MPF) all contain the p34^{cdc2} protein kinase.

Cyclins Are the Regulatory Subunit of MPF. The latest chapter in the characterization of MPF has been the identification of a second subunit. As was the case for *cdc2*, the cooperation of developmental biologists, geneticists, and biochemists was required to demonstrate that a family of proteins, known as cyclins, constituted the second component of MPF. Cyclins were originally named as a result of their unusual pattern of protein expression during the early embryonic cell cycle of sea urchins and clams (Evans et al., 1983). Cyclin protein accumulates during interphase and maximum levels are achieved in mitosis. As cells exit mitosis, the accumulated cyclin protein is rapidly degraded. Thus, the fluctuation in cyclin protein levels strikingly parallels the oscillation of MPF activity. Surprisingly, microinjection of RNA encoding a clam cyclin protein into frog oocytes caused a resumption of meiosis and induced GVBD in the absence of hormone stimulation, very similar to the induction of GVBD by MPF (Swenson et al., 1986). Microinjection of sea urchin cyclin RNA also induced GVBD in frog oocytes (Pines & Hunt, 1987). Thus, for the first time, a direct link was forged between the phenomenological accumulation and destruction of cyclin protein and the machinery responsible for driving cells into M-phase.

In fission yeast, another *cdc* mutant, *cdc13*, was found to encode a protein with characteristics similar to the cyclin proteins identified in sea urchins and that shared a limited degree of amino acid sequence identity with known cyclins (Solomon et al., 1988; Goebl & Byers, 1988). (Cyclins have since been classified into two groups, cyclin A and cyclin B, on the basis of specific regions of sequence identity.) These results led to the cloning of two cyclin B proteins from *Xenopus* and the demonstration that cyclin translation is necessary for entry into mitosis in cell-free egg extracts (Minshull et al., 1989). The relationship between cyclins and MPF was cemented by the identification of the cyclin B proteins as the second component of frog MPF (Gautier et al., 1990). In clams, both cyclin A and cyclin B proteins were found to associate with p34^{cdc2}, apparently in separate cyclin/p34^{cdc2} complexes (Draetta et al., 1989). Additional cyclin B/p34^{cdc2} complexes were identified in sea urchin eggs (Meijer et al., 1989), starfish oocytes (Labbe et al., 1989a), and human HeLa cells (Pines & Hunter, 1989). Recently, a complex consisting of cyclin A and a protein related to, but distinct from, p34^{cdc2} was identified in human HeLa cells (Pines & Hunter, 1990a). All these complexes possess M-phase-specific histone H1 kinase activity that can be detected in either anti-p34^{cdc2} immunoprecipitates or anti-cyclin immunoprecipitates. All known cyclin proteins have a predicted molecular mass of ~45–55 kDa, although they often migrate on SDS–polyacrylamide gels more slowly than expected (~45–63 kDa). On the basis of the molecular mass of active MPF (~100 kDa), MPF is likely to be a heterodimer of p34^{cdc2} and cyclin (Labbe et al., 1989a; Pondaven et al., 1990).

Phosphorylation and Dephosphorylation Combine To Regulate MPF

Unlike the level of cyclin protein, p34^{cdc2} protein levels remain constant throughout the cell cycle of continuously

proliferating cells (Simanis & Nurse, 1986; Draetta & Beach, 1988). However, the kinase activity and substrate specificity of p34^{cdc2} change during the cell cycle. These changes have been correlated with both the phosphorylation state of p34^{cdc2} and its association with other proteins. Just how p34^{cdc2} kinase activity is regulated by its phosphorylation state is not completely understood. Early studies suggested a temporal relationship between p34^{cdc2} phosphorylation and activation of its histone H1 kinase activity. In addition, p34^{cdc2} from G_1 cells is dephosphorylated and devoid of histone H1 kinase activity (Draetta & Beach, 1988). However, further deciphering of the phosphorylation state of p34^{cdc2} in starfish, frog, and mammalian cells revealed that although overall phosphorylation of p34^{cdc2} increased during G_2, phosphorylation of p34^{cdc2} dramatically decreased as cells entered mitosis (Labbe et al., 1989b; Gautier et al., 1989; Morla et al., 1989). There now exists a strong correlation between overall dephosphorylation of p34^{cdc2} and activation of histone H1 kinase activity and entry into M-phase. Moreover, inactivation of frog MPF in vitro is accompanied by increased p34^{cdc2} phosphorylation and a decline in histone H1 kinase activity (Gautier et al., 1989).

Tyrosine Dephosphorylation Activates p34^{cdc2} Kinase. Phosphoamino acid analysis of in vivo phosphorylated p34^{cdc2} from mammalian, frog, and, surprisingly, yeast cells revealed mostly phosphotyrosine and phosphothreonine with lower levels of phosphoserine (Draetta et al., 1988; Morla et al., 1989; Dunphy & Newport, 1989; Gould & Nurse, 1989). The results of Gould and Nurse (1989) provided the first definitive demonstration of tyrosine phosphorylation of a yeast protein. In human HeLa cells, a site of tyrosine phosphorylation on p34^{cdc2} in vivo can be phosphorylated by the *src* protooncogene product in vitro (Draetta et al., 1988). Whether the *src* protein phosphorylates p34^{cdc2} in vivo remains to be determined, but an intriguing relationship exists between the *src* protein and MPF. The transforming *src* oncogene (which is a more active kinase than the *src* protooncogene but shares similar substrate specificity) can accelerate oocyte maturation induced by progesterone (Spivack et al., 1984). Recent studies have demonstrated that tyrosine phosphorylation of p34^{cdc2} varies with the cell cycle and that tyrosine dephosphorylation of p34^{cdc2} is required for activation of its kinase activity. Thus, it is unlikely that the acceleration of maturation by *src* is a result of its potential ability to directly phosphorylate p34^{cdc2}. In frogs, tyrosine phosphorylation of p34^{cdc2} was greatest during interphase but absent during mitosis. Furthermore, activation of MPF in vitro was associated with tyrosine dephosphorylation and activation of histone H1 kinase activity (Dunphy & Newport, 1989). During the cell cycle of mouse fibroblasts, tyrosine phosphorylation of p34^{cdc2} was first observed during S-phase and was greatest during G_2. Tyrosine dephosphorylation occurred as cells entered M-phase, and once again, this correlated with maximal histone H1 kinase activity. When tyrosine dephosphorylation was blocked with sodium vanadate, cells became arrested in G_2. This arrest could be reversed by the removal of vanadate. However, removal of the majority of phosphotyrosine from p34^{cdc2} in vitro was insufficient to activate its histone H1 kinase activity, suggesting that tyrosine dephosphorylation alone is not the mechanism by which p34^{cdc2} is activated (Morla et al., 1989). It is not clear if the low levels of phosphoserine and phosphothreonine found in M-phase p34^{cdc2} play a role in fully activating the kinase.

In fission yeast, the relationship between the phosphorylation state of p34^{cdc2} and its kinase activity is identical with that in higher eukaryotes (Gould & Nurse, 1989). The cell cycle specific changes in phosphoserine and phosphothreonine in p34^{cdc2} are more subtle than the change in phosphotyrosine. As cells enter mitosis, the low level of phosphoserine in p34^{cdc2} appears to remain relatively constant, whereas the level of phosphothreonine drops to a barely detectable amount. The site of tyrosine phosphorylation on yeast p34^{cdc2} has been mapped to a single tyrosine residue located close to the N-terminus. Interestingly, this tyrosine is situated within a highly conserved protein kinase motif, and it has been postulated that phosphorylation of this residue may impede kinase activity by interfering with ATP binding (Gould & Nurse, 1989). All known *cdc2* homologues encode a tyrosine residue at this position, suggesting that this mechanism of p34^{cdc2} regulation has been conserved throughout evolution. The expression of a mutated *cdc2* gene, encoding a tyrosine-to-phenylalanine substitution, rescued a temperature-sensitive allele of *cdc2* and resulted in cells entering mitosis prematurely (Gould & Nurse, 1989). This phenotype is similar to that observed for yeast strains containing a mutation in the *wee1* gene and is likely to be the result of a constitutively active p34^{cdc2} kinase. From genetic experiments, the protein encoded by the *wee1* gene is believed to function as an inhibitor of p34^{cdc2} activity (Russell & Nurse, 1987a). The tyrosine-to-phenylalanine mutated *cdc2* gene can also rescue a temperature-sensitive allele of another cell division control gene, the *cdc25* gene. The protein encoded by this gene appears to act independently of the *wee1* gene product and is believed to be an activator of p34^{cdc2} (Russell & Nurse, 1986). Although expression of a constitutively active *cdc2* mutant can induce cells to enter mitosis, thus bypassing the normal inhibitory timing mechanisms, the recipient cells display several mitotic abnormalities (Gould & Nurse, 1989). Apparently, the prevention of tyrosine phosphorylation on p34^{cdc2} is not enough to completely deregulate its activity. It is possible that transient rephosphorylation of this tyrosine is crucial for events leading to the next cell cycle or for exit from mitosis.

Phosphorylation of Cyclin May Lead to Formation of MPF. As discussed above, p34^{cdc2} and an associated cyclin protein constitute both active MPF and M-phase-specific histone H1 kinase. The histone H1 kinase activity of p34^{cdc2} and MPF is only detected when p34^{cdc2} is complexed with a cyclin protein. In clams, histone H1 kinase activity is present in immune complexes precipitated with either anti-cyclin A or anti-cyclin B antibodies (Draetta et al., 1989). Furthermore, the two frog cyclin B proteins are associated with histone H1 kinase activity and are present in MPF. Both frog cyclin B proteins can be phosphorylated when purified MPF is incubated with ATP, and both can act as exogenous substrates for p34^{cdc2} in vitro. The protein kinase activity directed toward frog cyclins oscillates during the cell cycle coincident with histone H1 kinase activity and MPF activity (Gautier et al., 1990). Human cyclin can also be phosphorylated in p34^{cdc2}/cyclin immune complexes, and in vitro phosphorylation of human cyclin parallels histone H1 kinase activity. Phosphoamino acid analysis of phosphorylated human cyclin revealed phosphoserine and phosphothreonine (Pines & Hunter, 1989). In sea urchin and starfish oocytes, a temporal relationship exists between the accumulation of phosphorylated cyclin and the appearance of histone H1 kinase activity (Meijer et al., 1989; Pondaven et al., 1990). Thus, cyclin phosphorylation by p34^{cdc2} may be necessary to further activate the p34^{cdc2} kinase or possibly to alter its substrate specificity. Current models portray cyclin as a regulatory subunit for the p34^{cdc2} kinase, but binding of cyclin to p34^{cdc2} is, by itself, not enough to fully activate the complex (Moreno et al., 1989;

Pondaven et al., 1990). This is further supported by the association of cyclin with the most highly phosphorylated form of p34^{cdc2} during G_2 in mammalian cells (Pines & Hunter, 1989). Although tyrosine phosphorylation of p34^{cdc2} correlates with cyclin/p34^{cdc2} complex formation in these cells, only complexes containing p34^{cdc2} that lacks phosphotyrosine have histone H1 kinase activity (Morla et al., 1989). Thus, phosphorylation of cyclin on serine and threonine residues, formation of the cyclin/p34^{cdc2} complex, and tyrosine dephosphorylation of p34^{cdc2} all appear to contribute to the activation of MPF.

An obvious, but still unproven, mechanism for the regulation of p34^{cdc2} kinase activity would depend upon the specific association of the different cyclin proteins with certain forms of p34^{cdc2}. Specific cyclin/p34^{cdc2} complexes could confer unique substrate specificities upon the kinase, alter the intracellular localization of the complex, or lead to activation of the kinase at varying times in the cell cycle. There is now some evidence for these methods of regulation. In *Drosophila*, there are dramatic differences in the intracellular distribution of cyclin A and cyclin B mRNAs both in developing embryos and in adult somatic cells (Whitfield et al., 1989; Lehner & O'Farrell, 1989, 1990). Expression of the clam cyclin A and cyclin B proteins occurs at different times during oocyte maturation (Luca & Ruderman, 1989). In both clam and *Drosophila*, the timing of cyclin A and B degradation varies, with proteolysis of cyclin A initiating prior to cyclin B (Luca & Ruderman, 1989; Lehner & O'Farrell, 1990). Moreover, several studies have shown that cyclin and p34^{cdc2} are localized to the nucleus at some point in the cell cycle (Riabowol et al., 1989; Booher et al., 1989; Lehner & O'Farrell, 1990). However, just how and when translocation to the nucleus occurs are not clear. A putative nuclear targeting signal in a yeast cyclin protein has been observed and suggested to function in the transfer of the cyclin/p34^{cdc2} complex into the nucleus (Booher et al., 1989). Temporal regulation of p34^{cdc2} activity occurs in fission yeast and budding yeast where *cdc2* homologues are known to regulate the cell cycle at both the G_1/S-phase and G_2/M-phase transitions. Loss of function of these genes correlates with loss of kinase activity of the p34^{cdc2} homologues (Richardson et al., 1989). Three cyclin homologues (*CLN* genes) have been identified in budding yeast and shown to comprise a set of functionally redundant genes that are essential for the G_1/S-phase transition (Hadwiger et al., 1989; Richardson et al., 1989). Recently, the *CLN2* protein was shown to associate with the budding yeast p34^{cdc2} homologue to form a kinase-active complex (Wittenberg et al., 1990). However, in higher eukaryotes, *cdc2* regulation of the G_1/S-phase transition has not yet been observed.

Destruction of Cyclin Protein at the End of M-Phase Inactivates MPF

The disappearance of MPF activity that occurs upon fertilization of frog eggs and at the metaphase/anaphase transition of mitosis in somatic cells strictly correlates with the rapid degradation of cyclin protein. CSF-arrested egg extracts that are arrested in metaphase II of meiosis and contain high levels of MPF activity can be released from metaphase arrest and induced to enter interphase by the addition of calcium. Upon addition of calcium to CSF-arrested extracts, cyclin levels dramatically decrease within 10 min, in parallel with a decrease in MPF and histone H1 kinase activities (Murray et al., 1989). Furthermore, degradation of exogenously added cyclin to interphase extracts does not occur unless sufficient cyclin is added to induce mitosis. Deletion mutagenesis of cyclin B has defined a domain required for cyclin proteolysis. Addition of calcium to CSF-arrested extracts containing the proteolysis-resistant cyclin does not result in release from metaphase arrest, decrease in MPF and histone H1 kinase activities, or degradation of cyclin (Murray et al., 1989). These studies demonstrate that cyclin must be destroyed for cells to exit M-phase and suggest that cyclin degradation leads directly to MPF inactivation. There are little data concerning the mechanism of cyclin degradation. Events such as changes in cyclin phosphorylation, the accessibility of a cyclin protease, or the cell cycle specific activation or translation of a protease could be involved in the control of MPF inactivation.

Experiments in which in vitro transcribed clam, sea urchin, and frog cyclin RNAs were injected into oocytes demonstrated that cyclin protein synthesis can induce meiotic maturation in the absence of hormonal stimulation (Swenson et al., 1986; Pines & Hunt, 1987). In mRNA ablation experiments, the cyclin B proteins were shown to be the only proteins whose synthesis was required for the induction of mitosis in interphase egg extracts (Minshull et al., 1989). In mRNA-dependent frog egg extracts, the translation of exogenous cyclin B RNA was sufficient to drive these extracts into M-phase and through multiple cell cycles (Murray & Kirschner, 1989). However, recent experiments with mutant *Drosophila* embryos deficient in cyclin A demonstrated that the accumulation of cyclin B protein is insufficient to induce entry into mitosis (Lehner & O'Farrell, 1990). Together, these results suggest that the synthesis of cyclin proteins is the sole translational requirement for activating MPF in mitotic cells.

Phosphatases as Regulators of MPF

Pre-MPF and INH. MPF has been shown to exist as an inactive "pre-MPF" complex in frog oocytes (Cyert & Kirschner, 1988). This is consistent with the observation that both p34^{cdc2} and cyclin B proteins are already present in resting oocytes (Westendorf et al., 1989; Gautier et al., 1990). Crude preparations of pre-MPF can be fractionated into a component containing ATP-dependent MPF activity and an additional fraction, called INH, that contains an activity capable of inhibiting MPF (Cyert & Kirschner, 1988). Thus, the mere presence of an MPF complex is not an indicator of MPF activity. INH contains a protein that is immunologically related to the catalytic subunit of protein phosphatase 2A (Solomon et al., 1990). The importance of phosphatase activity in regulating MPF has recently been demonstrated in several ways [see Cyert & Thorner (1989) for a review]. For example, INH activity in oocytes may itself be regulated by a type 1 protein phosphatase as demonstrated by the ability of phosphatase inhibitor 1, which is specific for type 1 phosphatase, to delay the activation of pre-MPF (Cyert & Kirschner, 1988). Similarly, microinjection of frog oocytes with either of two type 1 phosphatase inhibitors, inhibitor 1 or inhibitor 2, delays progesterone-induced maturation but not maturation induced by MPF (Huchon et al., 1981; Foulkes & Maller, 1982). This suggests that dephosphorylation of one or more proteins by type 1 phosphatases may regulate maturation at a point upstream of MPF activation and that type 2A phosphatases may be required for the maintenance of pre-MPF.

Further involvement of type 1 and 2A phosphatases in the control of MPF activity has been seen in both starfish oocytes and *Xenopus* oocytes. Microinjection of okadaic acid, a tumor promotor that specifically inhibits type 1 and 2A phosphatases, induces both MPF activation and GVBD (Picard et al., 1989; Rime et al., 1990). Once activated, MPF can be stabilized for several hours by okadiac acid or, surprisingly, antibodies that neutralize type 1 phosphatase (Picard et al., 1989). Thus, inhibition of both type 1 and 2A phosphatases by okadaic acid

results in the activation and stabilization of MPF activity. However, inhibition of only type 1 phosphatase has the apparently conflicting effects of delaying MPF activation in oocytes but stabilizing active MPF in eggs. If type 2A phosphatase activity is homologous with the MPF inhibitor INH, then the effects of okadaic acid described above could be accounted for.

A type 1 phosphatase may play a role in negatively regulating INH, which would be consistent with the retardation of MPF activation when type 1 phosphatase is inhibited in *Xenopus* oocytes. In rat fibroblasts, protein levels and activity of a type 1 phosphatase inhibitor, inhibitor 2, oscillate during the cell cycle peaking at both S-phase and M-phase. Overexpression of $p34^{cdc2}$ in these cells during G_1 results in several morphological changes characteristic of mitosis; however, $p34^{cdc2}$ overexpression during S-phase has no effect. When $p34^{cdc2}$ is overexpressed in S-phase cells in the presence of neutralizing antibodies against inhibitor 2, morphological effects are observed that are similar to those occurring in G_1-cells overexpressing $p34^{cdc2}$ (Brautigan et al., 1990). These results suggest that type 1 phosphatase may modulate $p34^{cdc2}$ activity at points in the cell cycle other than the G_2/M-phase transition. In yeast and *Drosophila*, mutations in genes encoding type 1 phosphatase homologues result in a block in mitosis and a defect in chromosome separation (Doonan & Morris, 1989; Ohkura et al., 1989; Booher & Beach, 1989; Cohen & Cohen, 1989). Thus, type 1 phosphatases may also be important in the coupling of MPF activation to other mitotic events such as chromosome separation.

What Are the Substrates for These Phosphatases? Is $p34^{cdc2}$ itself a substrate for these phosphatases? In interphase extracts prepared from early *Xenopus* embryos, specific inhibition of type 2A phosphatase, but not type 1 phosphatase, induces the histone H1 kinase activity associated with $p34^{cdc2}$. In prophase extracts, activation of the $p34^{cdc2}$ kinase appears to involve a protein phosphatase distinct from types 1 and 2A (Felix et al., 1990). Similarly, MPF activation induced by okadaic acid in starfish oocytes is associated with the dephosphorylation of serine and threonine residues in $p34^{cdc2}$, again implicating an additional phosphatase that is not subject to inhibition by okadaic acid. In addition, okadaic acid does not induce MPF activation in starfish oocytes in the absence of protein synthesis, suggesting that at least some of the effects of okadaic acid are not directed toward $p34^{cdc2}$ (Picard et al., 1989). Finally, nothing is known about the tyrosine phosphatase that presumably is required for activation of $p34^{cdc2}$ kinase activity.

Could the cyclin proteins be direct substrates for the type 1 or 2A phosphatases implicated above? Phosphorylation of the cyclin B proteins in sea urchin eggs correlates with activation of the $p34^{cdc2}$ kinase, but whether this is required is not known (Meijer et al., 1989). The effect of phosphatases on cyclin stability is also unclear: in *Xenopus* eggs, okadaic acid stimulates the degradation of cyclin (Felix et al., 1990), whereas in starfish oocytes, okadaic acid inhibits the degradation of cyclin that normally occurs following mitosis (Picard et al., 1989). These results demonstrate that regulated dephosphorylations by type 1 and 2A phosphatases are significant events in the control of MPF (with both positive and negative effects) but that other unidentified phosphatases also have a role in regulating MPF.

Other M-Phase Regulators

The suc1 Gene Product, p13. Several proteins in yeast and human cells other than cyclins can interact with $p34^{cdc2}$ to regulate its activity. Overexpression of the fission yeast *suc1* gene can suppress the mitotic block induced by certain temperature-sensitive alleles of *cdc2* (Hayles et al., 1986). Moreover, the 13-kDa protein encoded by *suc1*, p13, can bind the cyclin/$p34^{cdc2}$ complex, although only a fraction of total $p34^{cdc2}$ is bound (Draetta et al., 1987). While p13 has not been identified as a component of MPF, yeast p13 can directly bind frog MPF, inhibit interphase egg extracts from entering mitosis, inhibit activation of histone H1 kinase, and inhibit tyrosine dephosphorylation of $p34^{cdc2}$. However, p13 has no effect on the $p34^{cdc2}$ histone H1 kinase once it is activated (Dunphy & Newport, 1989). These results suggest a possible role for p13 in the maintenance of pre-MPF, and two homologues of the *suc1* gene have recently been cloned from human cells (Richardson et al., 1990). A p13/$p34^{cdc2}$/cyclin complex may be refractory to activation due to an inability of $p34^{cdc2}$ tyrosine to be dephosphorylated.

nim1, wee1, and cdc25. Three other mitotic control genes have been isolated in fission yeast. The products of two of these genes, *nim1* and *wee1*, encode protein kinases whereas the activity of the third gene, *cdc25*, is unknown (Russell & Nurse, 1986, 1987a,b). In genetic experiments, the *wee1* product functions as a dose-dependent inhibitor of mitosis that acts upstream of $p34^{cdc2}$ (Russell & Nurse, 1987a). The *nim1* protein is an indirect activator of $p34^{cdc2}$ because it appears to regulate mitosis by inhibiting the *wee1* mitotic inhibitor (Russell & Nurse, 1987b). The substrates for these protein kinases are unknown, and *nim1* and *wee1* homologues have not yet been found in other eukaryotes. The *cdc25* protein competes with the *wee1* inhibitor for control over the activation of *cdc2* (Russell & Nurse, 1986). Interestingly, the tyrosine-to-phenylalanine mutation in $p34^{cdc2}$ that results in a constitutively active kinase is sufficient to bypass the function of *cdc25*, suggesting that the normal function of *cdc25* involves activation of a tyrosine-specific phosphatase or inhibition of a tyrosine protein kinase (Gould & Nurse, 1989). However, the sequence of the *cdc25* protein does not resemble any known phosphatase subunits. Unlike *nim1* and *wee1*, homologues of *cdc25* have been identified in other organisms, suggesting that its mechanism of activation is highly conserved (Edgar & O'Farrell, 1989; Russell et al., 1989). In other studies, the activation of *cdc25* was shown to be dependent upon completion of DNA replication, thus linking the timing of $p34^{cdc2}$ activation with earlier cell cycle events in S-phase (Enoch & Nurse, 1990). Recent experiments show that the yeast *cdc25* protein is phosphorylated, and its level of expression increases during interphase and peaks at mitosis (Moreno et al., 1990). In addition, the level of *cdc25* protein has been shown to have a direct effect on the rate at which cells pass through G_2 and enter into mitosis (Russell & Nurse, 1986). These results have led to the hypothesis that *cdc25* acts as a cell division initiator by regulating the timing of M-phase via the activation of MPF.

A Multitude of Substrates for $p34^{cdc2}$

Although many proteins have recently been shown to be in vitro substrates for $p34^{cdc2}$ kinase activity, histone H1 remains one of the best. In fact, $p34^{cdc2}$ is responsible for the majority of histone H1 kinase activity in vivo. MPF activity, during both meiotic maturation and mitosis, correlates with histone H1 kinase activity and, as a result, phosphorylation of histone H1 has become the first biochemical assay for MPF activity. Histone H1 is phosphorylated at specific sites during M-phase, and these same sites are phosphorylated by $p34^{cdc2}$ in vitro (Langan et al., 1989). However, no biological consequences of these M-phase-specific phosphorylations have been observed, although it has been postulated that they may play a role in chromosome condensation (Mueller et al., 1985). Identifi-

cation of the p34^{cdc2} phosphorylation sites in histone H1 has revealed the sequence Ser/Thr-Pro-X-Lys/Arg as a consensus substrate for p34^{cdc2} (Langan et al., 1989). This information has proven useful in identifying specific residues in other proteins that are the target for p34^{cdc2} phosphorylation. Additional proteins that act as in vitro substrates for p34^{cdc2} include casein (Simanis & Nurse, 1986), SV40 large T antigen (McVey et al., 1989), RNA polymerase II (Cisek & Corden, 1989), the nucleolar proteins nucleolin and NO38 (Peter et al., 1990b), MAP-2 (Shenoy et al., 1989), caldesmon (Yamashiro et al., 1990), the *src* (Shenoy et al., 1989; Morgan et al., 1989) and *abl* protooncogene products (Kipreos & Wang, 1990), the antioncogene product p53 (Bischoff et al., 1990; Addison et al., 1990), elongation factors EF-1γ and EF-1β (Belle et al., 1989), the B-type cyclins (Pines & Hunter, 1989; Gautier et al., 1990), and the nuclear lamins (Ward & Kirschner, 1990; Peter et al., 1990a). This broad spectrum of potential targets for p34^{cdc2} kinase activity supports a pleiotropic model for MPF's influence on the cell cycle [see Moreno and Nurse (1990) for a review].

Many of these proteins are localized within the nucleus and are structural proteins or DNA-binding proteins. Some of these, such as the lamins, nucleolin and NO38, *src*, *abl*, p53, and SV40 large T antigen, undergo mitotic-specific phosphorylations in vivo at residues that correspond to those phosphorylated in vitro by p34^{cdc2}. However, the nuclear lamins are currently the only p34^{cdc2} substrates in which mitotic phosphorylation has a known biological function. Phosphorylation of lamin B by p34^{cdc2} results in solubilization of lamin and disassembly of the nuclear lamina in vitro (Peter et al., 1990a). Mutations of p34^{cdc2} phosphorylation sites in lamin A block nuclear lamina disassembly during mitosis, a process that normally leads to nuclear envelope breakdown (Heald & McKeon, 1990). A structural role for p34^{cdc2} phosphorylation of lamins has been proposed on the basis of the proximity of two of these phosphorylation sites to sequences involved in lamin dimer formation; presumably, phosphorylation of these sites interferes with lamin polymerization. Other structural consequences of p34^{cdc2} phosphorylation have been suggested on the basis of the proximity of phosphorylation sites in RNA polymerase II, histone H1, nucleolin, and NO38 to DNA binding domains (Peter et al., 1990b; Moreno & Nurse, 1990; Suzuki, 1990). Phosphorylation of these sites may possibly facilitate chromosome condensation during mitosis by weakening the DNA binding properties of these proteins. Residues phosphorylated by p34^{cdc2} in SV40 large T antigen and p53 are adjacent to nuclear targeting sequences within these proteins although the effect of phosphorylation at these sites on localization is unknown (Addison et al., 1990; Bischoff et al., 1990). p34^{cdc2} phosphorylation may play a regulatory role in the activation of *src* tyrosine kinase activity. Mitotic phosphorylation of certain serine and threonine residues in *src* results in an increase in *src* kinase activity (Chackalaparampil & Shalloway, 1988). The same residues are phosphorylated by p34^{cdc2} in vitro (Shenoy et al., 1989; Morgan et al., 1989). Interestingly, the *src* kinase activity has been speculated to have an effect on microfilament arrangements and cytoskeletal alterations, events that occur during cell division.

It should be noted that the source of p34^{cdc2} kinases used in the above studies varied greatly but each consisted of a complex between p34^{cdc2} and a cyclin protein [or, in one case, an unidentified cyclin-sized protein (Cisek & Corden, 1989)]. Systematic use of some of these substrates should provide important information regarding the activity and substrate specificity of various p34^{cdc2}/cyclin complexes. However, the lessons learned from previous attempts at characterizing relevant substrates for protein kinases should cause us to exercise some caution in assigning roles for p34^{cdc2} in the regulation of the potential substrates described above (Kamps et al., 1986). The phosphorylation of many of these proteins by p34^{cdc2} may not occur in vivo, and some that do may not have biological significance.

Activation of MPF by the *mos* Protooncogene Product, a Meiotic MPF Stabilizer

Translation of the mos Protein Kinase Is Required for Meiosis. As mentioned earlier, translation of cyclins appears to be the only protein synthesis requirement for activating MPF in mitotic cells. However, the meiotic maturation of oocytes requires the translation of at least one additional protein. Translation of the protein kinase encoded by the *mos* protooncogene is required for normal progesterone-induced maturation of frog oocytes (Sagata et al., 1988), and recently several other experiments have demonstrated an important role for *mos* in MPF activation. Microinjection of *Xenopus* oocytes with *mos*-specific antisense oligonucleotides prevents hormone-induced GVBD and MPF activation. The activation of p34^{cdc2} histone H1 kinase and an S6 kinase (which phosphorylates the S6 protein of the 40S ribosomal subunit and is activated in response to progesterone) is also blocked by *mos* antisense oligonucleotides (Barrett et al., 1990). In addition, oocytes can be induced to undergo GVBD in the absence of progesterone by microinjection of *mos* RNA, demonstrating that expression of the *mos* protein is sufficient for activation of MPF and progression through meiosis (Freeman et al., 1989; Sagata et al., 1989a). The *Xenopus mos* protein is a 39-kDa phosphoprotein that is a member of the serine/threonine protein kinase family. *mos* mRNA is present throughout oocyte growth and maturation and also during the early stages of embryonic development. However, the *mos* protein is only detected after stimulation of oocyte maturation, accumulating to maximal levels prior to MPF activation, and is rapidly degraded shortly after fertilization (Watanabe et al., 1989). Thus, *mos* expression is not only required for the activation of MPF but its degradation at the end of meiosis correlates temporally with the degradation of cyclin and the inactivation of MPF. In *Xenopus*, the appearance of MPF activity during normal maturation requires protein synthesis and, as discussed above, a complex of pre-MPF exists in resting oocytes that seems to be activated by phosphorylation. These results suggest that synthesis of the *mos* protein kinase may be the translational prerequisite for activation of MPF during oocyte maturation.

The timing of *mos* activity during maturation of mouse oocytes is somewhat different than in *Xenopus*. Microinjection of mouse oocytes with *mos*-specific antisense oligonucleotides does not block GVBD but does inhibit meiosis at a later stage prior to the initiation of meiosis II (Paules et al., 1989; O'Keefe et al., 1989). The phenotype of maturing mouse oocytes that results from loss of *mos* function (decondensed chromosomes and a re-formed nucleus) is similar to the phenotype predicted to result from inactivation of MPF (O'Keefe et al., 1989). Unlike *Xenopus mos*, the murine *mos* protein is present in prophase-arrested oocytes, and if this pool of *mos* protein were stable, it might be sufficient to stimulate GVBD even when de novo synthesis of *mos* protein is inhibited. In contrast to *Xenopus* oocytes, protein synthesis is not required until after GVBD during normal mouse oocyte maturation (Wassarman et al., 1976). Consequently, in both *Xenopus* and mouse oocytes, the microinjection of *mos*-specific antisense oligonucleotides blocks meiosis at the stage where protein synthesis

is required. This suggests the possibility that *mos* may be required for the reactivation of MPF that occurs between meiosis I and II.

mos as CSF. *mos* protein synthesized during maturation is much more stable in the unfertilized egg than in the maturing oocyte. However, the stability of *mos* in unfertilized eggs is rapidly and completely compromised in the presence of added calcium. This is, at least in part, the result of the calcium-dependent protease calpain, which is activated by the increase in cytoplasmic free calcium that occurs upon fertilization or after treatment of eggs with a calcium ionophore (Watanabe et al., 1989). These properties of *mos* are very similar to those of CSF (Meyerhof & Masui, 1979; Shibuya & Masui, 1988, 1989). CSF activity was first identified in the cytoplasm of unfertilized frog eggs and is now believed to be ubiquitous among vertebrates. CSF can induce M-phase arrest when injected into a dividing zygote and is believed to maintain unfertilized eggs in a state of M-phase arrest, presumably by stabilizing MPF (Masui & Markert, 1971).

A function for the *mos* protein, apparently distinct from its role in inducing GVBD, was demonstrated by experiments in which *mos* RNA was microinjected into cleaving frog embryos. Microinjection of *mos* RNA into half of a two-cell embryo resulted in mitotic arrest of the injected blastomere. The same result is observed when cytoplasm from CSF-arrested eggs is injected into embryos. Moreover, the use of *mos*-specific antibodies to neutralize or immunodeplete the *mos* protein from egg extracts abolished CSF activity (Sagata et al., 1989b). The ability of *mos* to induce M-phase arrest (as well as activate MPF and induce GVBD in the experiments described above) is dependent upon an intact ATP-binding domain as demonstrated by the lack of activity associated with a *mos* protein containing a lysine-to-arginine mutation in the ATP-binding domain (Freeman et al., 1990). These results suggest that the *mos* protein kinase is an integral component of CSF and that *mos* may be able to arrest mitotic cleavage by preventing the inactivation of MPF that normally accompanies the exit from M-phase.

How Does mos both Stimulate and Stabilize MPF Activity? The activation and stabilization of MPF by *mos* may be the result of phosphorylation events that shift the pre-MPF/MPF equilibrium toward active MPF. This could be accomplished by the removal of an inhibitor of MPF activation or by preventing cyclin degradation. Recent evidence has emerged suggesting that *mos* may activate MPF through interactions with the cyclin proteins. *mos* immunoprecipitates contain an activity that can phosphorylate *Xenopus* cyclin B2 in vitro (Roy et al., 1990). In addition, co-overexpression of *mos* and cyclin B in oocytes dramatically accelerates GVBD as compared with the kinetics of GVBD induced by either *mos* or cyclin B alone. However, no increase in in vivo phosphorylation of cyclin B2 is detected when *mos* and cyclin B2 are overexpressed in oocytes (Freeman et al., in press). Thus, the ability of *mos* to accelerate cyclin B induced GVBD does not seem to be accomplished by *mos* phosphorylating cyclin directly. It is not yet known whether *mos* exists in a complex with pre-MPF prior to MPF activation.

Interestingly, the role of *mos* as a protein kinase activator of MPF is reminiscent of the role of the yeast protein kinase *nim1*. *nim1* indirectly activates $p34^{cdc2}$ by inhibiting the *wee1* protein kinase, an inhibitor of M-phase. No homologue of *nim1* has been identified in *Xenopus*, and a *mos* gene in yeast has not been found. A speculative role for *mos* as a functional equivalent of *nim1* could place *mos* as an indirect inhibitor of INH. For example, *mos* phosphorylation of an unknown protein kinase could result in inactivation of a phosphatase such as INH. Alternatively, *mos* could function to inactivate a protein kinase (perhaps a tyrosine protein kinase) that negatively regulates $p34^{cdc2}$ activation. Of course *mos* phosphorylation could also directly regulate one of the phosphatases or phosphatase inhibitors that have been implicated in the activation of MPF.

Oncogenic Transformation as a Consequence of Inappropriate Activation of MPF

Many models for oncogenic transformation involve abnormal progression into the early stages of the cell cycle, such as entry into G_1. However, there are oncogenic proteins, in addition to *mos*, that are capable of functioning at the G_2/M-phase transition, as demonstrated by their ability to induce or accelerate oocyte maturation. Activated forms of the *met* oncogene (I. Daar and G. F. Vande Woude, personal communication) and the oncogenic *ras* protein (Birchmeier et al., 1985) are capable of inducing maturation in the absence of progesterone stimulation. Moreover, microinjection of oocytes with an activated form of the *src* oncogene accelerates the rate of progesterone-induced maturation (Spivack et al., 1984). The *met* protooncogene encodes a transmembrane receptor like protein with intrinsic tyrosine protein kinase activity (Park et al., 1987). The oncogenic version of this protein is thought to induce a mitogenic response at the cell surface of transformed cells in a ligand-independent manner. The *ras* protooncogene encodes a cell membrane associated GTP-binding protein with an intrinsic GTPase activity (McGrath et al., 1984; Gibbs et al., 1984; Sweet et al., 1984). Therefore, the normal functions of *met* and *ras* involve the coupling of external stimuli at the cell surface to proteins more directly linked with cell cycle control and eventually with MPF. In cells transformed by the oncogenic counterparts of these genes, constitutive activation of the transforming protein removes the requirement for an external mitogenic signal. The conventional view of how these oncogenes transform cells involves promoting quiescent, nondividing cells to enter the cell cycle at G_1. On the basis of the ability of these oncogenes to activate MPF and induce progression through mitosis in prophase-arrested oocytes, the oncogenic *met* and *ras* proteins may also function late in the cell cycle during the G_2/M-phase transition, in addition to promoting entry into the cell cycle at G_1.

In *mos* transformed cells, the endogenous *ras* protein has been postulated to function in a pathway separate from, or prior to, *mos* (Smith et al., 1986). In oocytes, microinjection of *mos*-specific antisense oligonucleotides inhibits GVBD induced by the oncogenic *ras* protein (Barrett et al., 1990). The *mos* protein is localized in the cytoplasm of *mos* transformed cells and in oocytes and has no known association with the cell membrane (Papkoff et al., 1983; Watanabe et al., 1989). These results, together with those described above, predict that *mos* acts downstream of *ras*, as well as *met*, and more proximal to MPF during oocyte maturation.

Surprisingly, expression of *mos* mRNA and protein has been difficult to detect in somatic cells. The only known functions for the *mos* protooncogene are its induction of MPF activity during meiosis and its CSF-like ability to induce mitotic arrest. Transient CSF activity has been postulated to be present in somatic cells as a stabilizer of cyclin during M-phase (Murray & Kirschner, 1989). Conceivably, a low level of *mos* protein could be transiently expressed during the cell cycle and function to stabilize MPF until the completion of mitotic metaphase. Transformation by *mos* could then be a consequence of *mos* expression at earlier points in the cell cycle leading to the inappropriate activation of MPF.

From the results described above, it is clear that altering the expression or activity of certain protooncogenes can result in the activation of MPF. However, mutant forms of the *cdc2* and cyclin genes have not been identified as transforming genes. In addition, the yeast genes identified as activators (*nim1* and *cdc25*) and inhibitors (*wee1* and *suc1*) of MPF do not as yet have oncogenic homologues. Consequently, the mutational activation of MPF, or proteins intimately associated with MPF, has not been observed to lead to oncogenic transformation. If changes in the activity or expression of genes that are closely associated with MPF are lethal to the cell due to the widespread effects of MPF phosphorylation, then this could explain their absence from the list of known oncogenes. It may well be that a correctly regulated MPF kinase activity is essential for the ability of transformed cells to continuously divide, just as the correct regulation of MPF activity is obviously essential for cell cycle control in normal cells.

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