FACTORS REGULATING MITOSIS IN EUKARYOTIC CELLS

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ABSTRACT

The orderly progression of eukaryotic cells from interphase to mitosis requires the close coordination of various nuclear and cytoplasmic events. These include the breakdown of the nuclear membrane, condensation of chromosomes, reorganization of the microtubule network, centriole duplication, formation of the mitotic spindle and cytokinesis. Mitotic chromosome replication is an important and critical event for the proper segregation of genetic material between the two daughter cells. Although the mechanisms for the control of these events are not properly understood, several recent studies utilizing a variety of different experimental approaches suggest that the presence or absence of specific proteins or enzymatic activities may be extremely important. Our laboratory has recently demonstrated the existence of two such nonhistone protein factors, one of which is only present in mitotic cells and the other only in G_1 cells. These factors seem to play an important role in the regulation of the initiation and the completion of mitosis, respectively. The properties, the physiological roles and the mechanisms by which these two factors regulate mitosis are reviewed.

INTRODUCTION

THE life cycle of a eukaryotic cell consists of a pre-DNA synthesis (G_1) period, a period of DNA synthesis (S phase), a post-DNA synthesis (G_2) period, and mitosis (M) (figure 1). In most eukaryotic cells the time required for the actual process of cell division, i.e. mitosis, is usually less than an hour, but the preparations for it may span a number of hours during interphase. As cells traverse from interphase to mitosis, various dramatic changes occur in the nucleus of a eukaryotic cell. The major changes include the condensation of chromatin into discrete chromosomes, breakdown of the nuclear membrane, disassembly of the cytoskeletal arrays and formation of the mitotic spindle[1–10]. Beginning with telophase these processes are driven in the opposite direction until the completion of DNA synthesis. The nuclear envelope re-forms and chromosomes begin to decondense. The chromatin reaches its most decondensed state by the end of G_1 period, when it becomes accessible for DNA replication. Following replication of chromosomes the whole process of major macromolecular reorganization of the nucleus associated with mitosis restarts.

In the last 15 years, studies in the fields of genetics, cell biology, biochemistry, molecular genetics and immunology have provided new insights into the life cycle of a eukaryotic cell. It has become apparent that multiple gene products are involved in the temporal sequence and control of these cell cycle events. It is extremely important to identify these gene products in order to understand better the regulation of cell cycle traverse in general and mitosis in particular. In this article, we review briefly the recent work in this field and discuss in detail studies from our laboratory about the nature of some of these gene products and how they might regulate this orderly coordination of the various biochemical events involved in the entry of cells into mitosis and their exit from it.

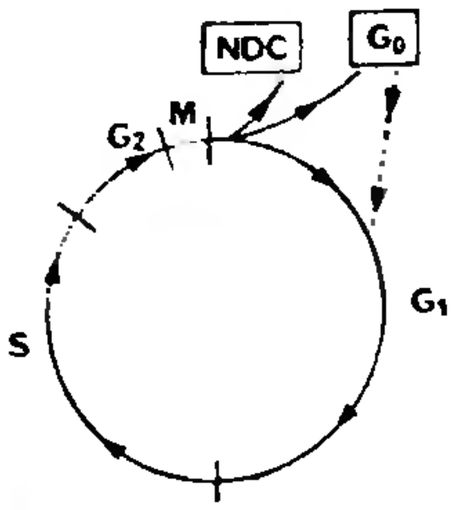
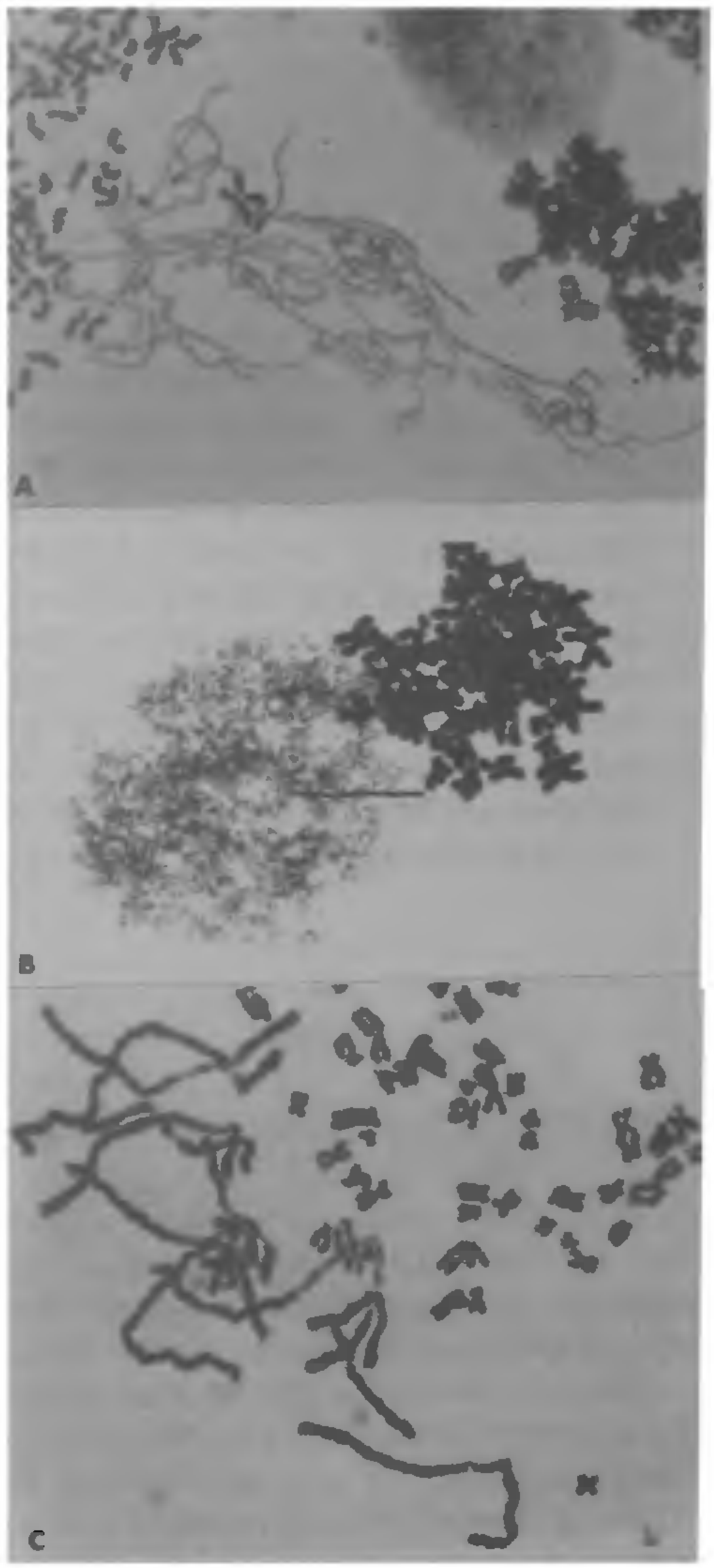


Figure 1. Various phases of the eukaryotic cell cycle. In an exponentially growing population cells proceed from mitosis (M) through interphase to mitosis again. The interphase can be subdivided into the pre-DNA synthetic period (G_1) during which a cell makes necessary preparations for the initiation of DNA synthesis, the period of DNA synthesis (S phase) and the post-DNA synthetic period (G_2) during which cell makes preparations for the initiation of mitosis. Some cell populations leave the cell cycle, never to reenter again; these are termed nondividing cells (NDC). Other cell types can be diverted from the cell cycle, under certain conditions during interphase, to become noncycling quiescent (G_0) cells; these cells may be induced to reenter the cycle (dashed line) by an appropriate stimulus. Whether a daughter cell continues to cycle or enters the G_0 state is closely controlled in normal cells by endogenous substances acting at a specific point (or points) during G_1 phase.

EXPERIMENTAL APPROACHES TO STUDY EVENTS RELATED TO MITOSIS

Most studies of the cell cycle, until recently have largely dealt with defining the temporal sequence of events during the cell cycle. Nuclear transplantation experiments conducted by Gurdon[11] clearly demonstrated that the cytoplasm is dominant over the nucleus in determining the cell cycle and developmental state of the nucleus. Subsequently, Johnson and Rao[12–14], using the technique of cell fusion—involving fusion of cells synchronized at different phases of the cell cycle—demonstrated that mitotic cells induced premature chromosome condensation (PCC) when fused with cells in G_1, S, or G_2 phases (figure 2), implying that mitotic cells contain factors that dominate the behaviour of nuclei from interphase cells. However, if one mitotic cell was fused with two or more G_1 phase cells, PCC rarely occurred[14] Some groups have even



Figures 2A-C. Prematurely condensed chromosomes of HeLa cells. The darkly stained chromosomes are of the mitotic HeLa cells. **A.** G_1 PCC with single chromatids; **B.** S-PCC exhibiting pulverized appearance; and **C.** G_2-PCC with double chromatids. (From Johnson and Rao[12]).

observed nuclear membranes formed around the chromosomes of the mitotic cells[15], indicating the presence of certain factors in G_1 cells that could inhibit the action of mitotic factors responsible for the induction of PCC. However, in fusions involving G_2 cells with G_1 cells the inhibitory effect of the G_1 component in the binucleate (G_1/G_2) cell was significantly neutralized by the addition of Mg^{2+}, which allowed the G_2 nucleus to progress into mitosis asynchronously, thus inducing PCC in the G_1 nucleus. Since Mg^{2+} is known to promote PCC it was suggested[16] that in the G_1/G_2 binucleate cell the lagging G_1 component caused the decondensation of G_2 chromatin, thus blocking the G_2 nucleus from entering mitosis[17].

Similarly, nuclear transplantation[18,19] and cell fusion[20,21] studies have clearly demonstrated the presence of factors crucial to the initiation of DNA synthesis in S phase cells and able to induce this event in G_1 cells. However, fusion of G_2 cells with S phase cells did not result in the reinitiation of DNA synthesis in G_2 cells, and the G_2 component did not inhibit the completion of DNA synthesis in S phase nuclei in the S/G_2 binucleate cells. These results suggest that even though the inducers of DNA synthesis are present, the G_2 chromatin is not accessible for another round of replication[13].

Another experimental approach to study the temporal order and control of cell cycle events has been the isolation and characterization of certain cell division cycle (*cdc*) mutants, for example of the yeast *Saccharomyces cerevisiae*[22] and *Schizosaccharomyces pompe*[23,24]. The isolation of these *cdc* mutants has helped enormously in defining a number of factors necessary for the traverse of cells through the cell cycle. These mutants manifest a defect in a specific step during the cell cycle at nonpermissive temperature. Although the genetic studies have revealed the involvement of both positive and negative elements controlling mitosis[23,24], the approach has resulted in the identification of only a very few gene products that are specifically involved in mitosis. The paucity is due at least in part to the following limitations:

(i) It may be difficult to isolate conditional lethal alleles for many genes because many gene products are not as susceptible to thermolability[25,26]. (ii) Many genes may exist in more than one copy per haploid genome[27,28], and only one copy may be necessary for viability[29,30]. It would, therefore, seem impossible to isolate redundant genes by recessive mutation. (iii) Some gene products may contribute to the high fidelity of chromosome replication and segregation but may not be essential for cell division (for examples see[31,32]). (iv) The detection of a majority of *cdc* mutants (except when the gene product was known) has been completely dependent on morphological criteria. Some mutations may fail to satisfy a morphological criterion but may perform cell-cycle specific functions (for examples see[33],[34]). Nevertheless, Russell and Nurse[35] have recently been able to clone the *cdc* 25^+ gene in *S. pompe*. They demonstrated that the *cdc* 25^+ gene product (protein of M.W. 67,000) acts as a dosage-dependent inducer in mitotic control. They have also shown that *cdc* 25^+ functions to counteract the mitotic inhibition activity of *wee* 1^+ and that *wee* 1^+ and *cdc* 25^+ independently control mitosis.

Very recently, three new methods have been developed to isolate genes that regulate mitotic events in yeast (for review see 36). The first of these has used the approach of reverse genetics. Two different laboratories[33,34] have isolated yeast strains containing lethal mutations in the gene encoding topoisomerase II, an enzyme that catalyzes the one-step passage of one double-stranded DNA molecule through a second double-stranded molecule. This enzyme has recently been shown to be an important constituent of the scaffold proteins from mitotic chromosomes[37]. In these studies it was demonstrated that the inactivation of topoisomerase II was lethal to the cell, but this inactivation did not result in the arrest of mutant cells with homogeneous morphology

when cells were shifted to the nonpermissive temperature. It was shown that topoisomerase II was required by the cell only during mitosis. Since the loss of viability was not immediately manifested, cells failed to accumulate with a homogeneous terminal morphology. However, these studies do not rule out the possibility that topoisomerase II may have additional functions in the cell[36].

The second approach involves the isolation cold-sensitive (CS) mutants to study mitosis. One of these mutants, recently described by Thomas and Botstein[38], is *ndc*–1. At the nonpermissive temperature, chromosomes fail to separate at mitosis in this mutant strain but cell cycle traverse is not blocked. This defect resulted in an asymmetric cell division in which one daughter cell received all the chromosomes and doubled in ploidy whereas the other received none. The spindle poles segregated properly to the two daughter cells. The phenotype of *ndc*–1 strains suggests that the newly synthesized chromosomes do not attach to a separate spindle pole body prior to mitosis. They either remain attached to their sister chromatids or both chromatids become attached to the same spindle pole. One possible explanation is that *ndc*–1 strains fail to synthesize a mitotic spindle pole body capable of anchoring or projecting chromosome-linked microtubules. Another explanation is that kinetochores are not duplicated following chromosome replication. A third is that if they are duplicated, the newly synthesized kinetochores cannot attach to the microtubules emanating from the poles[36]. Furthermore, meiosis I, in which homologous pairs separate, is not affected by this mutation. However, meiosis II, in which chromatids segregate to opposite poles as in mitosis, is affected by the *ndc*–1 mutation.

The third approach recently described by Hartwell and colleagues[39,40] utilized the concept of disruption of mitosis by overexpression of certain genes. This approach is based on the assumption that formation of mitotic structures might require stoichiometric amounts of individual components: an imbalance of one component could disrupt assembly of different structures. They have tested this idea by measuring the frequency of chromosome nondisjunction in *S. cerevisiae* strains in which specific components of the mitotic components are overproduced (by propagation of genes on high-copy plasmids). They have observed that overproduction of either pair of histone genes, H_2A and H_2B or H_3 and H_4, in yeast yielded an increased frequency of loss of chromosomes V and VII. In contrast, in strains in which all four genes are carried on a high-copy plasmid or when histone genes were present in single-copy plasmids, there was no increase in the rate of chromosome loss. Using this technique they have isolated two DNA sequences, designated MIF-1 and MIF-2, that reduce the fidelity of chromosome transmission during mitosis. MIF-1, a unique sequence located on the right arm of chromosome XII, stimulated loss and recombination of both chromosomes V and VII when present in a high-copy-number plasmid. This gene was shown to be not essential for cell division but necessary for the normal fidelity of chromosome transmission. MIF-2, a unique sequence located on 15 cM distal to HIS_6 on chromosome IX, if present in high copy number, induces loss of chromosomes V and VII with low and high frequency, respectively. This gene had no effect on mitotic recombination. Disruption of the MIF-2 locus was shown to be lethal: cells lacking this function failed to divide and exhibited a phenotype characteristic of a block in DNA replication or nuclear division[40]. These approaches have been very helpful in broadening our understanding of the basic mechanisms that regulate mitosis.

Our present knowledge of the regulation of cell-cycle specific events also improved greatly from the studies of oocyte maturation and the early embryonic development in *Xenopus laevis*. The cell cycle in the early cleavage stages of *Xenopus* embryos is much simpler as it has no G_1 phase or any detectable G_2 phase[41]. The absence of these phases probably

reflects the absence of growth in size in the early embryonic cells. The volume of individual blastomeres decreases during cleavage, and the total protein content of an embryo remains constant until hatching[42]. The unfertilized *Xenopus* egg is naturally arrested in metaphase of second meiotic division. When stimulated by fertilization, the egg proceeds in a rapid, nearly synchronous series of cell divisions, with cleavage occurring every 35 min[43–45]. The egg has also been shown to contain large quantities of components necessary for DNA synthesis and mitosis (histones, tubulin, DNA polymerase, deoxyribonucleotides, and nuclear membrane components) in reserve for the first 12 cell divisions, which proceed synchronously without measurable RNA synthesis[46–49]. It was recently shown[50] that some events of the cell cycle in *Xenopus* could proceed even in the absence of nuclei and centrioles. Fertilized or activated (by pricking) eggs that were subsequently enucleated failed to divide but underwent periodic contractions of their cortex, producing surface-contraction waves corresponding to the time of cleavage in the controls. Furthermore, replication of DNA and centrioles injected into these enucleated eggs come under cytoplasmic control[5,51]. The manifestation of these surface contraction waves has been interpreted in terms of an autonomous cytoplasmic oscillator (for a review see[52]).

Immature oocytes of *Xenopus laevis*, which are naturally arrested in the G_2 phase before first meiotic division and because of their large size (1.3 mm in diameter), are convenient for needle microinjection, have also been extremely helpful in the study of mitosis and meiosis. Hormomes such as progesterone or insulin stimulate the oocytes to progress through meiosis to metaphase of the second meiotic division. These features of *X. laevis* oocytes have been exploited to study fundamental questions about the control of cell proliferation and the regulation of the cell cycle. When amphibian oocytes are exposed to progesterone, oocytes synchronously complete meiotic maturation i.e. germinal vesicle breakdown (GVBD), chromosome condensation, spindle formation, and extrusion of the first polar body. These oocytes then proceed to meiosis II, arresting finally at metaphase as an unfertilized egg (reviewed in[53,54]). The cytoplasmic control of nuclear behaviour in amphibian oocytes was demonstrated several years ago by cytoplasmic transfer. Injection of cytoplasm from maturing oocytes into immature oocytes induced GVBD and other nuclear events associated with meiotic maturation[55–57] The activity responsible for inducing these changes was called maturation-promoting factor (MPF). A similar activity has also been reported in starfish[58] and mouse[59] oocytes undergoing meiotic maturation. MPF-induced maturation occurs in 2–3 hr even in the presence of protein synthesis inhibitors, whereas progesterone-induced maturation takes 7–9 hr and is dependent on new protein synthesis[53–54] MPF activity has also been found in mitotic stages of early cleaving embryos of *X. laevis*, whereas it was undetectable during S phase[60,61]. More recently, an MPF-like activity has also been found in cells arrested in mitosis from a variety of eukaryotic organisms ranging from mammalian cells in culture to *cdc* mutants of yeast[58,62–65] (for details see the next section on mitotic factors).

Studies with unfertilized eggs have also revealed the presence of another cell cycle-specific cytoplasmic regulatory factor called the cytostatic factor[66–68] (CSF). CSF has been suggested to be responsible for the metaphase arrest of the unfertilized egg because injection of CSF into one blastomere of a two-cell embryo leads to metaphase arrest of the blastula nucleus at the next mitosis. Newport and Kirschner[69] have recently shown that CSF stabilizes MPF, presumably by inactivating anti-MPF or deregulating the synthesis of anti-MPF (for details refer to the section on inhibitors of the mitotic factors).

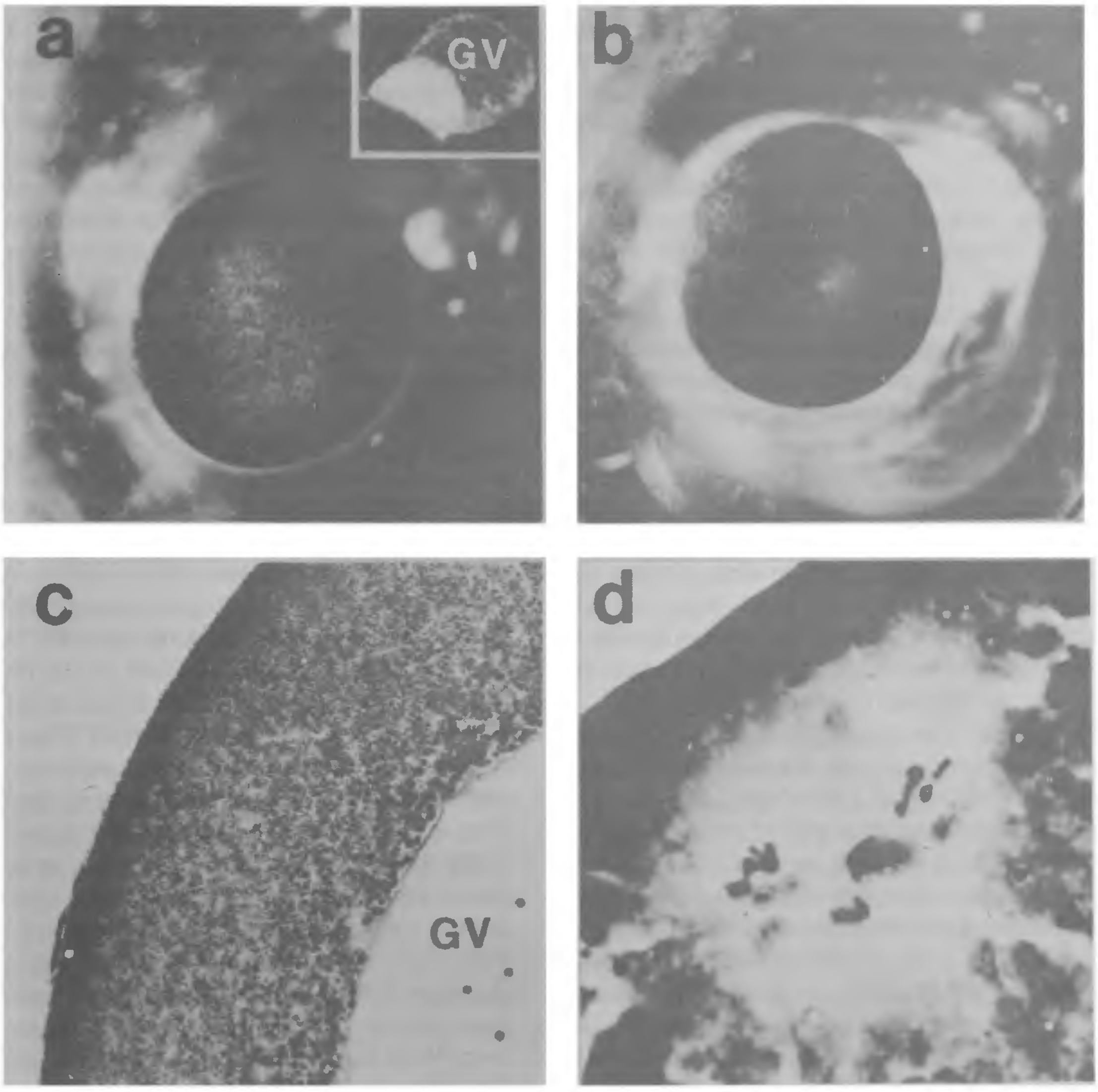
Another experimental approach to study the temporal order and control of cell cycle-specific events has been to raise monoclonal

antibodies to cell cycle specific gene products. Our laboratory has recently raised a number of monoclonal antibodies, using extracts of synchronized mitotic HeLa cells as immunogen, that exhibit greater specificity to mitotic cells than to interphase cells as judged by either indirect immunofluorescence or by enzyme-linked immunosorbent assay (ELISA). These antibodies specifically react with different structural components of the cells in mitosis. Some of these antibodies exhibit species specificity whereas others have been shown to react with mitotic or meiotic cells of every species tested[70,71]. Two of these antibodies, designated MPM-1 and MPM-2, have been studied extensively. These antibodies react specifically with structural components of the microtubule-organizing structures, including centrosomes, kinetochores, and midbodies[72]. These antibodies recognized a family of phosphoprotein antigens with major bands at 70,000 daltons, 118,000 daltons, and 182,000 daltons. Although these antigens were shown to be synthesized as polypeptides, primarily during the S phase, they are probably not phosphorylated until the cells enter mitosis, since antibodies recognize cells only during mitosis. The antigenicity of these proteins was destroyed when they were treated with alkaline phosphatase. These studies suggest that the phosphorylation of these antigens may play a prominent functional role in mitosis. This proposition is discussed in detail in the last part of this review. Attempts have also been made to raise monoclonal antibodies to *Xenopus* MPF in Kirschner's laboratory[73]. They were able to raise several MPF-binding antibodies, each one of which recognized more than 20 protein bands. It was subsequently discovered that the MPF-binding antibodies were directed against the thiophosphate group on many proteins, including probably MPF. When MPF was prepared in the absence of γ-thio-ATP, it did not bind to the antibodies. These studies suggest that MPF may be a thiophosphoprotein.

MITOTIC FACTORS

As mentioned earlier, the presence of specific factors in mitotic cells and their ability to induce events characteristic of mitosis, (i.e. nuclear envelope breakdown and condensation of chromosomes) in an interphase cell was clearly demonstrated in 1970 by the cell fusion experiments of Johnson and Rao[12–14]. These mitotic factors were shown to have no species-specificity. These studies also revealed that, during the induction of PCC, prelabelled proteins from mitotic cells became associated with the PCC of the interphase cell (for review see[74]). However, the progress on identification and characterization of the mitotic factors was hampered due to the lack of a suitable *in vitro* biological assay. In 1979, Sunkara *et al*[64] discovered the amphibian oocytes, because of the specialized features mentioned earlier, could be ideal for this purpose. They showed that injection of extracts from mitotic HeLa cells into *Xenopus laevis* oocytes induced meiotic maturation (figure 3). Since the major events of meiotic maturation, (i.e. GVBD and chromosome condensation) are similar to mitotic events, this system was assumed to be useful for purifying and characterizing mitotic factors. MPA was not present in G_1 and S phase cells. The mitotic factors accumulated gradually during G_2, reached a peak in mitosis, and were undetectable in early G_1 phase.

The entry of a G_2 cell into mitosis is dependent on new RNA and protein synthesis: if any one of them is inhibited during G_2, cells do not enter mitosis[75–77]. These observations indicate that both the mRNA for the mitotic factors and the mitotic factors themselves must be synthesized during G_2 phase only, and their sythesis seems critical for the G_2–mitosis transition. Like MPF, the mitotic factors induced maturation in about 2–3 hr independent of new protein synthesis[78]. Subsequently, it was shown[63] that extracts from mitotic CHO, human D98/AH$_2$ cells[62] and *cdc* mutants of



Figures 3a-d. GVBD and chromosome condensation in *Xenopus* oocytes injected with mitotic HeLa cell extracts. **a.** Oocyte animal hemisphere 3 hr after injection with 65 nl of extraction medium (× 26), (inset). Clear germinal vesicle (GV) dissected from buffer-injected living oocyte. **b.** Appearance of an oocyte animal hemisphere at 3 hr after injection with mitotic HeLa cell extract (228 ng of protein in 65 nl) (× 26). Note the bright spot indicating the depigmented area caused by GVBD. **c.** Histological section of an oocyte 4 hr after injection with S phase HeLa cell extract (406 ng of protein in 65 nl) (stained with Feulgen/fast green) (× 236). Note intact germinal vesicle. **d.** Condensed chromosomes on meiotic spindle near oocyte surface 1.5 hr after injection with HeLa mitotic cell extract (309 ng of protein in 65 nl). (Stained with Feulgen/fast green) (×1750). (From Sunkara *et al*[64] 1979).

Saccharomyces cerevisiae arrested in late G_2 or mitosis[65] also induced maturation in 2–3 hr if injected into *Xenopus* oocytes. Although meiotic maturation in fully grown *X. laevis* oocytes can be induced by a number of agents, such as progesterone and insulin[55,57,79–81], surface-acting agents like lanthanum[82], local anaesthetics, cationic drugs[83], sulphydryl reagents[84], calcium-calmodulin complex[85], regulatory subunit, or a specific heat-stable protein inhibitor of a cAMP-dependent protein kinase[86,87], and more recently the product of human H-*ras* gene[88], it is different from the one induced by mitotic cell extracts or MPF in many respects, especially in the time course of maturation induction and dependence on new protein synthesis.

These studies suggest that MPF and the mitotic factors most likely belong to the same class of proteins. Moreover, it was recently shown that MPF could induce mitosis-like events when injected into frog embryos arrested in a G_2-like state by inhibition of protein synthesis[89]. Similarly, injection of mitotic factors from either D98/AH_2 cells[90] or HeLa cells[75] into these cycloheximide-arrested multinucleated embryos resulted in the induction of PCC. Thus, both the MPF and the mitotic factors induce mitosis or meiosis-like events in a very similar manner. These results support the notion that factors involved in the initiation of mitosis, meiosis and in the induction of PCC are very similar, if not identical.

Since both the induction of maturation in *Xenopus* oocytes and induction of PCC in multinucleated embryos by MPF or the mitotic factors occur even in the presence of cycloheximide, it is reasonable to expect that these factors either induce maturation directly or indirectly by activating a series of similar biochemical events. The possible molecular basis of action of these factors in inducing maturation is discussed in detail in the final part of this review. A four-step scheme has been proposed for the meiotic maturation of *Xenopus* oocytes induced by progesterone[91]. According to this scheme incubation of oocytes with progesterone leads to rapid inhibition of adenylate cyclase activity[92,93] followed by a decrease in cAMP levels and then a decrease in the level of the catalytic subunit of cAMP-dependent protein kinase, resulting in the dephosphorylation of a putative maturation protein (Mp-P) by phosphoprotein phosphatases. The dephosphorylation of Mp-P triggers the synthesis of MPF in a cycloheximide-sensitive step. How MPF triggers maturation in steps insensitive to cycloheximide remains to be elucidated. However, recent studies (to be discussed later) indicate that a protein phosphorylation-dephosphorylation cascade may be crucial to its action.

Purification and characterization of mitotic factors

Several investigators have been working to purify MPF from mature frog oocytes for a number of years with limited success[61,94–96]. Only a 50-fold purification of MPF has been reported[95]. Since the availability of the bioassay, our laboratory has been trying to purify and characterize the mitotic factors from synchronized mitotic HeLa cells. Initially, we were also unable to obtain anything greater than a 50-fold enrichment of the mitotic factors using virtually every mode of chromatography. Based upon our observations that mitotic factors were preferentially localized on chromosomes and could be released by mild digestion with endonucleases[97,98] we recently used affinity chromatography on DNA-cellulose. With this technique we have obtained a 500-fold purification of the mitotic factors in a single step[75,99]. Very recently by using a combination of DNA-affinity chromatography and high-performance liquid chromatography (HPLC) we have obtained a 1600 to 2000-fold purification of these factors[100].

Purification of the factors has been very difficult. The major reasons for the limited success are: (i) the instability of the mitotic factors even at −70°, especially if the protein content is reduced below 50 μg/ml; (ii) the

small proportion of the total cellular proteins these factors compose in the mitotic cells; (iii) extensive dilution during fractionation; (iv) extremely low recovery; and (v) the non-linearity of the bioassay. However, we have surmounted most of these difficulties by using chromatographic methods that are rapid and only minimally dilute the sample.

By using these partially purified preparations of MPF or the mitotic factors, it has been shown that both these factors are Ca^{2+}-sensitive, Mg^{2+}-dependent, heat-labile nonhistone proteins with a molecular weight of about 100,000. The activity of these factors is greatly stabilized by the presence of phosphatase inhibitors such as sodium fluoride, sodium β glycerol phosphate, ATP, and thio-ATP and the addition of bovine serum albumin as a carrier protein[95,97,101]. These studies suggest that genes encoding these factors should be very similar, if not identical. A protein kinase activity that is independent of cyclic AMP, Ca^{2+}, or calmodulin and is neither inhibited by heparin nor stimulated by spermine appears to be associated with the purified mitotic factors[75,99,100]. However, purification to homogeneity would be necessary in order to conclusively establish whether the mitotic factors themselves act as a kinase or as activators of an *in vivo* kinase responsible for the GVBD and chromosome condensation in *Xenopus* oocytes. Using this 1600 to 2000-fold purified mitotic factor preparations, we are now attempting to raise monoclonal antibodies and to identify and clone the genes encoding these factors.

THE INHIBITORS OF THE MITOTIC FACTORS OR THE G_1 FACTORS

The existence of factors in interphase cells that are antagonistic to the action of the mitotic factors was initially suggested by the cell fusion studies of Johnson and Rao[12–14] (for a review see[74]) referred to earlier. However, attempts to isolate these factors were until recently hampered by the nonavailability of a suitable *in vitro* bioassay. In our attempts to understand what happens to the mitotic factors at the end of mitosis, we recently discovered the presence of certain protein factors in HeLa cells during G_1 phase that could neutralize the action of the mitotic factors. When mitotic cell extracts were mixed with extracts of G_1 cells in various proportions and the mixtures injected into *Xenopus* oocytes, the extracts of G_1 cells neutralized the maturation-promoting activity (MPA) of the mitotic factors in a dose-dependent manner. These factors could be detected as early as telophase of mitosis and were present throughout the G_1 period, suggesting that these factors are either activated or newly synthesized at the end of mitosis. Kinetic studies using inhibitor of protein synthesis revealed that these factors are activated rather than newly synthesized. These studies also showed that the activity of these inhibitory factors fluctuates in a cyclical manner during the cell cycle. Very little or no activity was observed in S phase cells and extracts of G_2 cells also did not show any activity. These data are not surprising since during G_2 phase mitotic factors have been shown to accumulate. Furthermore, extracts from noncycling G_0 cells contained little or no activity.

These data indicated that the activity of the inhibitory factors present in G_1 cells coincided well with the process of chromosome decondensation, which is known to begin at telophase and continue until the beginning of S phase when chromatin reaches it most decondensed state (for reviews see[100,102]). These factors have been called either inhibitors of the mitotic factors (IMF) or chromosome decondensation factors[100,103]. The results suggest that the activation of IMF or CDF at telophase may lead to a rapid inactivation of the mitotic factors and consequently may result in the decondensation of chromosomes. This proposition is further strengthened by our recent studies in which we observed that the activity of IMF could be induced in G_0 cells by UV irradiation, which has been shown to cause chromosome decondensation and unscheduled

DNA synthesis[104–107]. The induction of IMF activity in G_0 cells was further enhanced if hydroxyurea and arabinosylcytosine (ara C) were present during incubation following UV irradiation. Furthermore, this induction of IMF was independent of new protein synthesis, as it occurred even in the presence of cycloheximide[103]. Very recently, we have observed that UV irradiation of mitotic HeLa cells resulted in the decondensation of chromosomes, inactivation of mitotic factors, and the activation of IMF[108]. These studies also indicated that IMF inactivated the mitotic factors by directly binding to them and forming an inert complex. In V79-8 cells which lack G_1 and G_2 periods in their cell cycle, IMF were manifest in early S phase only[102]. These results suggest that IMF may play an important role in the regulation of chromosome decondensation and nuclear envelope re-formation. However, to establish whether the activation of IMF is the cause or the effect of chromosome decondensation must await further investigations.

Recently, reports from several laboratories have described factors, similar to the IMF or CDF, that (like IMF or CDF) inactivate MPF at the end of meiosis and have called these factors anti-M factors[95,109–112]. These investigators have also shown that addition of partially-purified MPF could cause nuclear envelope breakdown, chromosome condensation, spindle formation, and subsequently nuclear envelope re-formation and chromosome decondensation due to the inactivation of MPF by anti-M factors in a cell-free system containing nuclei from a variety of sources. These results indicate that the IMF, like the mitotic factors, do not exhibit any species-specificity.

Using the inactivation of mitotic factors as the bioassay we have been attempting to purify and characterize the IMF. These studies have revealed that IMF are nondialyzable, nonhistone proteins with a molecular weight of greater than 12,000. Unlike the mitotic factors, they are heat stable and extremely sensitive to low pH, becoming either inactive or less active. In addition to inducing inactivation of the mitotic factors, IMF, when incubated with the mitotic extracts, induced the dephosphorylation of mitotic nonhistone proteins[113] and mitosis-specific phosphoprotein antigens[1,71] and specifically decreased the activity of a mitosis-specific kinase[114,115]. It is not yet certain whether IMF also possess a phoshatase activity, since only crude preparations of IMF have been used in these studies. More than one mechanism may be involved.

ROLE OF PROTEIN PHOSPHORYLATION DURING MEIOTIC MATURATION OF *X. LAEVIS* OOCYTES

The postsynthetic modification, especially phosphorylation, of proteins has been shown to be a mechanism of paramount importance in the regulation of numerous intracellular events, including mitosis and meiosis (for reviews see[1,73,75,100,115–123]). During meiotic maturation of *Xenopus* oocytes, the incorporation of ^{32}P into proteins was shown to be maximal prior to GVBD and chromosome condensation[124]. Subsequently, it was demonstrated that the microinjection of the catalytic subunit of a cyclic AMP–dependent protein kinase inhibited progesterone-induced maturation, whereas microinjection of the regulatory subunit or a specific heat-stable protein inhibitor of the catalytic subunit of cyclic AMP-dependent kinase induced maturation[86,87]. More recently, it was reported that the microinjection of the specific inhibitors of protein phosphatase-1 blocked progesterone-induced maturation of oocytes, but not the maturation of oocytes induced by MPF[125,126]. These results suggest that activation of protein phosphatase-1 may be responsible for the dephosphorylation event, which is known to precede the activation of MPF that is responsible for triggering maturation[91]. Several laboratories have recently reported[127–130] an increase in the phosphorylation of ribosomal protein S_6. An S_6–specific protein kinase from *Xenopus laevis* eggs was recently purified to homogeneity[131]. Boyer *et al*[132] reported an

increase in the phosphorylation of a 105 kDa protein during maturation. Phosphorylation of lamins A and C has also been shown to occur during MPF–induced maturation of oocytes[69,112]. These results clearly demonstrate that protein phosphorylation may represent a crucial regulatory mechanism in the control of meiotic maturation. However, whether MPF is a kinase by itself or activates a series of kinases remains to be established.

ROLE OF PROTEIN PHOSPHORYLATION IN MITOSIS

There is conclusive evidence that phosphorylation of several different proteins is much higher during mitosis than in interphase (for reviews see[71,75,100,116,119]). Histone phosphorylation has been shown to correlate strongly with the entry of cells into mitosis (for review see[116,119]), and induction of PCC in interphase cells also resulted in an increased phosphorylation of their histones H1 and H3[133–135]. However, it has been demonstrated that superphosphorylation of histone H1 by itself is not sufficient for chromosome condensation and entry of cells into mitosis[133–138].

In recent years, compelling evidence in studies from our laboratory and others suggests that phosphorylation of nonhistone proteins (NHP) may play an equally important role in the control of events associated with mitosis and meiosis. Increased phosphorylation of nuclear matrix[139], nuclear lamina[140–142], intermediate filaments[143–146], nucleolar proteins[147] and high mobility group (HMG) proteins[148,149] has been observed during G_2 to mitosis transition. Our recent studies suggest that phosphorylation of specific subset of NHP (proteins extractable in 0.2 M NaCl) may be causally related to the entry of cells into mitosis and their dephosphorylation to the exit from mitosis[150]. Phosphorylation and dephosphorylation of NHP appeared to be dynamic processes, with equilibrium shifting to phosphorylation during G_2–M and dephosphorylation during M-G_1 transition (figure 4). NHP phosphorylation was completely blocked when

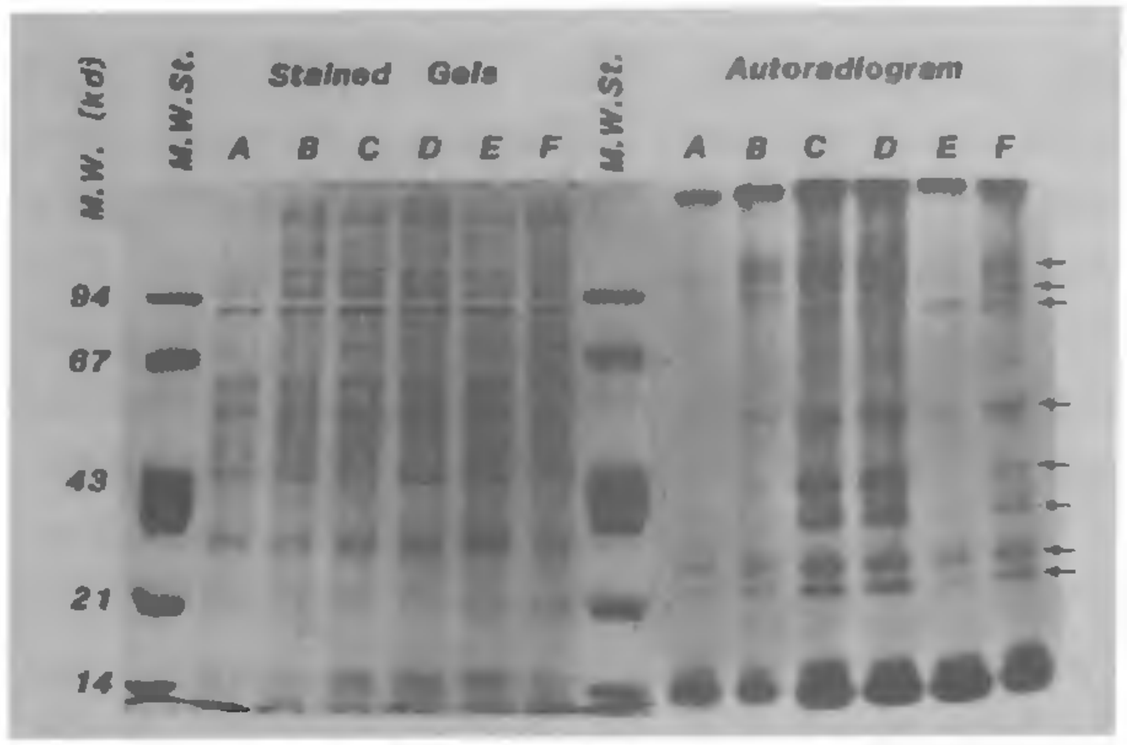


Figure 4. Identification of the proteins phosphorylated during G_2-M and dephosphorylated during M-G_1 transitions. HeLa cells synchronized in S phase by double-thymidine block were continuously labelled with ^{32}P beginning at the end of S phase. Cells were collected in mid G_2, mitosis, and early G_1, and extracts prepared as described previously[150]. Proteins were separated by SDS–polyacrylamide gel electrophoresis and the gels stained by Coomassie Blue. For radioautography Kodak XAR-5 film was exposed to dried gels. Lanes: A, G_2 cytoplasmic; B, G_2 nuclear; C, mitotic cytoplasmic; D, mitotic chromosomal; E, G_1 cytoplasmic; F, G_1 nuclear. The migration of molecular weight standards (MW St.) is shown. Arrows indicate the eight major protein bands phosphorylated during mitosis. Note the decrease in the intensity of labelling of these eight bands in early G_1 (lanes E and F) as compared to mitotic (lanes C and D) extracts. (from Sahasrabuddhe *et al*[150]).

HeLa cells were arrested in G_2 by cis-acid (cis-4-[[[(2-chloroethyl)-nitroso-amino] carbonyl] amino] cyclohexane carboxylic acid)[150]. These G_2-arrested cells have previously been shown to lack certain G_2-specific proteins[151]. X-ray induced mitotic delay in a synchronized population of G_2 cells resulted in a corresponding delay in NHP phosphorylation. Kinetic studies on the entry of cells into mitosis in the presence of cycloheximide revealed that commitment of a cell to enter mitosis was dependent not only on continued protein synthesis but also on their immediate phosphorylation. Similar changes in the NHP phosphorylation have also been reported during the cell cycle of CHO cells[152]. However, Song and Adolph[153],

also working with HeLa cells, reported that NHP from isolated metaphase chromosomes were strikingly dephosphorylated in comparison with those of S-phase chromatin. The differences between these studies are most likely the result of different experimental protocols used[150] for the extraction of NHP.

UV-induced chromosome decondensation, which is associated with the inactivation of mitotic factor activity, resulted in a significant dephosphorylation of mitosis-specific NHP. These results suggest that UV may induce or activate phosphatases that specifically dephosphorylate these mitosis-related NHP. A similar dephosphorylation of these mitotic NHP was also observed when partially purified prelabelled mitotic factors were mixed with G_1 cell extracts (but not with mid-S phase cell extracts), indicating the presence of similar phosphatases in G_1 cells[113]. These findings suggest that phosphorylation of this subset of NHP, in addition to H1 phosphorylation, may represent a crucial mechanism for the regulation of mitosis.

The additional evidence for the role of NHP phosphorylation-dephosphorylation in mitosis is also provided by an independent immunological study from our laboratory. Using a homogenate of mitotic HeLa cells as immunogen a number of monoclonal antibodies have been raised to proteins present in mitotic cells but not in interphase cells. Two of these hybridoma clones that have been studied extensively, MPM-1 and MPM-2, react with a family of phosphorylated polypeptides. Because the antigens recognized by these antibodies are all phosphoproteins and the antigenic reactivity is lost when the phosphate groups are removed by alkaline phosphatase digestion, it is likely that this family of polypeptides share a common or similar phosphorylated site[70]. Although these antigens were distributed throughout mitotic cells, they were preferentially localized on chromosomes, on the spindle, and particularly on microtubule-organizing centres. including the centrosomes, centromeres, kinetochores, and midbodies[72].

Microinjection of these antibodies into mammalian cells inhibited the completion of the process of mitosis, but not its initiation. Microinjection of antibodies into one of the cells of a 2-cell stage embryo of *Xenopus* or *Rana pipiens* completely blocked the cleavage in the injected cell but had no effect on the uninjected sister cell. Since these antibodies do not recognize the mitotic factors or inhibit either the progresterone—or the mitotic factor-induced maturation of oocytes—it seems likely that the activation of the mitotic factors and the phosphorylation of the mitosis-specific antigens represent different steps in the initiation of mitosis (for review see[71]).

MOLECULAR BASIS FOR THE ACTION OF MITOTIC FACTORS AND THE IMF

The mechanisms by which the mitotic factors act to induce the meiotic maturation in *Xenopus laevis* oocytes are not yet completely clear; however, in the light of studies presented here, protein phosphorylation appears to be the most likely possibility. To establish more conclusively the role of protein phosphorylation in the activity associated with the mitotic factors, we have performed several experiments with the purified preparations of mitotic factors referred to earlier. In one of these experiments, we observed that microinjection of alkaline phosphatase (APase) into oocytes preinjected with the mitotic factors resulted in a complete block of meiotic maturation in a time- and dose-dependent manner. No inhibition was observed when APase was injected within an hour prior to maturation[101]. Similarly, Hermann *et al*[154] have shown that microinjection of APase totally blocked the maturation of oocytes induced either by progesterone or MPF. These results clearly demonstrate a crucial role for protein phosphorylation in the meiotic maturation induced by the mitotic factors or the MPF. However, we have also observed that pretreatment of the mitotic factors with APase, attached to beaded agar-

ose, had no effect on the activity of mitotic factors. These results indicate that the activity of the mitotic factors does not necessarily depend on their being phosphorylated. These studies have also revealed that the phosphatase inhibitors greatly stabilize the activity of the mitotic factors by inactivating the phosphatases or by deregulating their synthesis or activation in oocytes.

As mentioned earlier we have observed a protein kinase activity associated with the 1600-fold purified mitotic factors. These findings taken together with the observations that the purified mitotic factors induce maturation even in the presence of cycloheximide reinforces the notion that the mitotic factors either act as a kinase *in vivo* or as activators of an *in vivo* protein kinase cascade responsible for the induction of GVBD and chromosome condensation. Our demonstration that a major portion of the mitotic factors is localized on metaphase chromosomes suggest that the mitotic factors may also play a direct role in the condensation of chromosomes, but unequivocal data are not yet available.

Our studies of the mitotic factors and those of others of MPF suggest that both these factors, besides inducing GVBD and chromosome condensation, induce autoamplification of MPF, as judged by serial injections, even in the presence of cycloheximide[97,155,156]. Thus, immature oocytes probably contain MPF in an inactive form that can be activated when MPF or a small amount of the mitotic factors are injected into oocytes. Autophosphorylation is a possible mechanism by which the autoamplication could occur. However, more work is needed to determine whether the MPF or the mitotic factors are the putative activated form of MPF *in vivo* or just the trigger for the activation of MPF. In light of the observations summarized here, we speculate that a protein kinase cascade mechanism is involed in the breakdown of the nuclear envelope and condensation of chromosomes at mitosis or meiosis and a protein phosphatase cascade mechanism in the re-formation of the nuclear envelope and

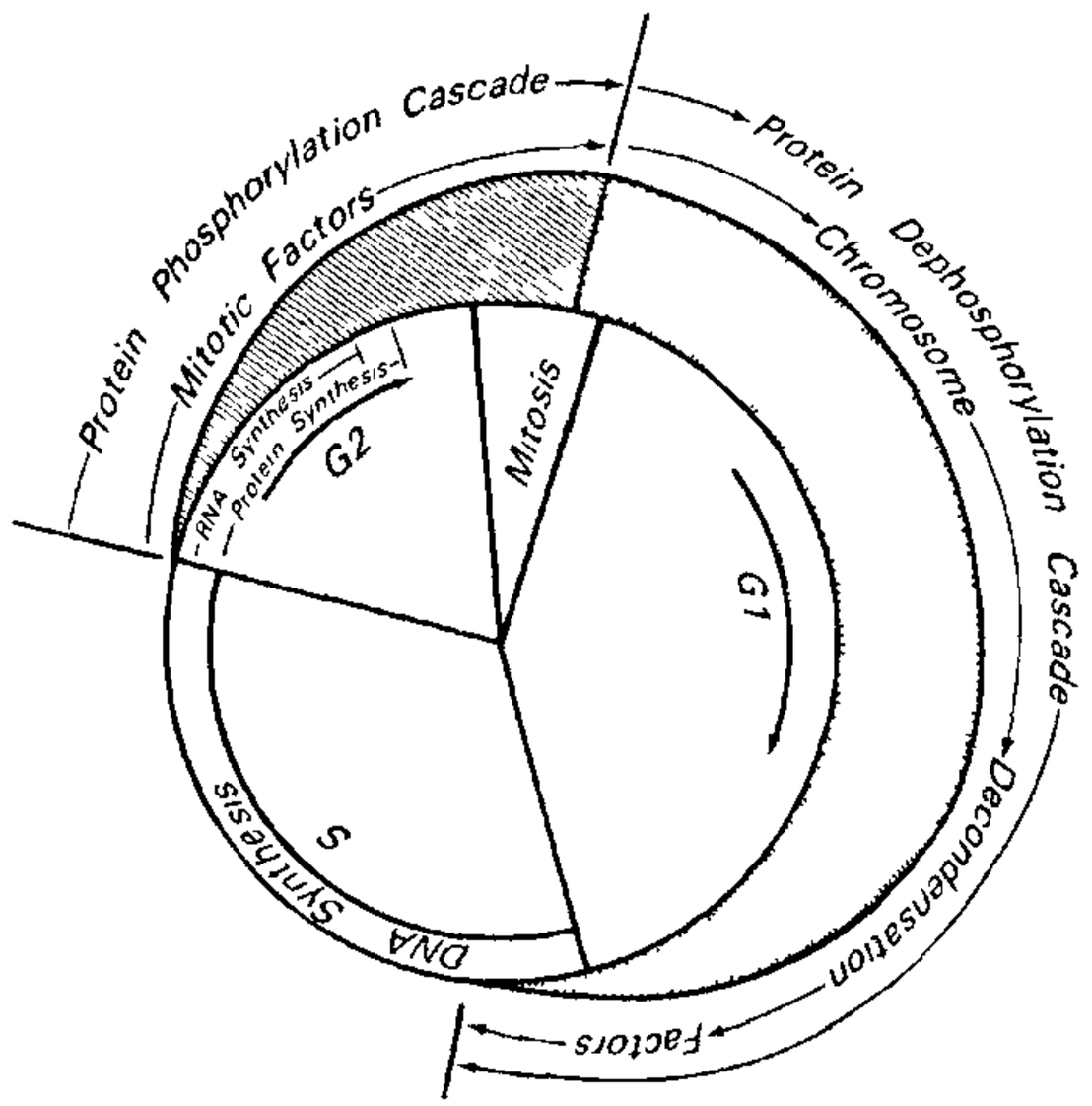


Figure 5. A schematic presentation of the relative activities of the mitotic factors and the chromosome decondensation factors during the mammalian cell cycle. Hatched area indicates the relative activity of the mitotic (or chromosome condensation) factors; the stippled area indicates that of CDF. RNA synthesis as indicated is necessary until about 2 hr before mitosis and protein synthesis until 1 hr before mitosis. Protein phosphorylation (histones, NHP, lamins etc.) occurs during the G_2-to-M-transition. When mitotic factors are synthesized or activated during G_2 phase, chromosomes condense and the nuclear envelope breaks down. Protein dephosphorylation occurs during the M to G_1 transition. When mitotic factors are inactivated at telophase by CDF, the nuclear envelope re-forms and chromosomes begin to decondense. The decondensation of chromosomes continues throughout the G_1 period up to a critical point (late-G_1), at which time the chromatin becomes accessible for replication. (from Adlakha and Rao[100]).

decondensation of chromosomes during the mitosis-to-G_1 transition (figure 5).

Recently, Kirschener and his colleagues[69,89,95,112], through an elegant series of studies using partially purified MPF, developed a model similar to the one proposed in figure 5 to explain how the frog egg (embryonic) cell cycle consisting of only M and S phases is regulated. They have suggested that

M phase results from the presence of MPF and S phase from the absence of MPF or the presence of anti-MPF. Addition of cytostatic factor (CSF) maintains MPF at high levels, presumably by stabilizing MPF by either protecting it from anti-MPF or deregulating the synthesis or activation of anti-MPF. Based on their observation that lamins A and C are phosphorylated in response to MPF (though not immediately) they suggested that MPF may be a trigger for the initiation of a phosphorylation cascade. A mammalian somatic cell, which is functionally quite different from an embryonic cell, might require not only the synthesis or activation of the trigger, (i.e. the mitotic factors) but also that of other proteins involved in the cascade mechanism.

CONCLUDING REMARKS

The advances of the past decade have revolutionized our understanding of the regulation of various events associated with the entry of a eukaryotic cell into mitosis. In this brief review we have tried to place in perspective the various investigations on the different elements controlling mitosis. Several novel approaches listed in this review have already provided invaluable information about the mechanisms of mitosis and will further facilitate the characterization of the various gene products involved in the temporal sequence and control of these events. We have presented evidence that two gene products, one present mainly in mitotic cells and the other exclusively in G_1 cells, play a pivotal role in the regulation of initiation and completion of mitosis, respetively. Partial purification and preliminary characterization of these gene products have been achieved. In spite of our recent advancements we still have a long way before we achieve a complete understanding of the various mechanisms involved in the regulation of cell division. What are the signals that dictate the synthesis or activation of these factors? What is the nature of these factors and how do they work? We are now beginning to obtain some insights into these questions. Delineation of these signals and characterization of these factors is a major challenge facing the investigators in this field. Thus, future efforts should be focussed on the purification and characterization of these gene products, cloning the genes encoding these factors, and characterization of the proteins phosphorylated during mitosis.

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1. Adlakha, R. C., Davis, F. M. and Rao, P. N., In: *Control of animal cell proliferation*, (eds) A. L. Boynton and H. L. Leffert, Academic Press, New York, 1985, Vol. 1, p. 485.
2. Baserga, R., *Multiplication and division of mammalian cells*, Marcel Dekker, New York, 1976.
3. Baserga, R., *New Eng J. Med.*, 1981, **304**, 453.
4. Brinkley, B. R., Fuller, G. M. and Highfield, D. P., *Proc. Natl. Acad. Sci. USA*, 1975, **72**, 4981.
5. Karsenti, E., Newport, J., Hubble, R. and Kirschner, M. W., *J. Cell. Biol.*, 1984, **98**, 1730.
6. Mazia, D., *Sci. Am.*, 1974, **1**, 55.
7. Osborn, M. and Weber, K., *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 867.
8. Pardee, A. B., Dubrow, R., Hamlin, J. L. and Kletzien, R. F., *Annu. Rev. Biochem.*, 1978, **47**, 715.

9. Prescott, D. M., *Reproduction of eukaryotic cells*, Academic Press, New York, 1976.
10. Robbins, E. and Gonatas, N. K., *J. Cell. Biol.*, 1964, **21,** 429.
11. Gurdon, J. B., *J. Embroyl. Exp. Morphol.*, 1968, **20,** 401.
12. Johnson, R. T. and Rao, P. N., *Nature (London)*, 1970, **226,** 717.
13. Johnson, R. T. and Rao, P. N., *Biol. Rev.*, 1971, **46,** 97.
14. Johnson, R. T., Rao, P. N. and Hughes, S. D., *J. Cell. Physiol.*, 1971, **77,** 151.
15. Obara, Y., Chai, L. S., Weinfeld, H. and Sandberg, A. A., *J. Cell Biol.*, 1974, **62,** 104.
16. Pederson, T. and Robbins, E., *J. Cell. Biol.*, 1972, **55,** 322.
17. Rao, P. N., Hittelman, W. N. and Wilson, B. A., *Expt. Cell Res.*, 1975, **90,** 40.
18. DeTerra, N., *Proc. Natl. Acad. Sci. USA*, 1967, **57,** 607.
19. Gurdon, J.B., *Proc. Natl. Acad. Sci. USA*, 1967, **58,** 545.
20. Graves, J. A. M., *Exp. Cell Res.*, 1972, **72,** 393.
21. Rao, P. N. and Johnson, R. T., *Nature (London)*, 1970, **225,** 159.
22. Hartwell, L. H., *J. Cell Biol.*, 1978, **77,** 627.
23. Nurse, P. N. and Thuriaux, P., *Genetics*, 1980, **96,** 627.
24. Nurse, P. N. and Bissett, Y., *Nature (London)*, 1981, **292,** 558.
25. Hartwell, L. H., Culotti, J., Pringle, J. R. and Rein, B. J., *Science*, 1974, **183,** 46.
26. Reed, S. I., *Genetics*, 1980, **95,** 561.
27. Hereford, L., Fahrner, K., Woolford, J. Jr., Rosbash, M. and Kaback, D. B., *Cell*, 1979, **18,** 1261.
28. Smith, M. M. and Murray, K., *J. Mol. Biol.*, 1983, **169,** 641.
29. Kolodrubetz, D., Rykowski, M. C. and Grunstein, M., *Proc. Natl. Acad. Sci. USA*, 1982, **79,** 7814.
30. Rykowski, M. C., Wallis, J. W., Choe, J. and Grunstein, M., *Cell*, 1981, **25,** 477.
31. Echols, H., Lu, C. and Burgers, P. M. J., *Proc. Natl. Acad. Sci. USA*, 1983, **80,** 2189.
32. McHenry, C. and Kornberg, A., In: *The enzymes*, (ed.) P. D. Boyer, Academic Press, New York, 1981, **14,** 39.
33. DiNardo, S., Voelkel, K. and Sternglanz, R., *Proc. Natl. Acad. Sci. USA*, 1984, **81,** 2616.
34. Holm, C., Goto, T., Wang, J. C. and Botstein, D., *Cell*, 1985, **41,** 553.
35. Russell, P. and Nurse, P., *Cell*, 1986, **45,** 145.
36. Broach, J. R., *Cell*, 1986, **44,** 3.
37. Earnshaw, W. C., Halligan, B., Cooke, C. A., Heck, M. M. S. and Liu, L. F., *J. Cell Biol.*, 1985, **100,** 1706.
38. Thomas, J. H. and Botstein, D., *Cell*, 1986, **44,** 65.
39. Meeks-Wagner, D. and Hartwell, L. H., *Cell*, 1986, **44,** 43.
40. Meeks-Wagner, D., Wood, J. S., Garvic, B. and Hartwell, L. H., *Cell*, 1986, **44,** 53.
41. Graham, C. F. and Morgan, R. W., *Dev. Biol.*, 1966, **14,** 439.
42. Benbow, R. M., Pestell, R. Q. W. and Ford, C. C., *Dev. Biol.*, 1975, **43,** 159.
43. Forbes, D. J., Kirschner, M. W. and Newport, J. W., *Cell*, 1983, **34,** 13.
44. Laskey, R. A., Mills, A. D. and Morris, N. R., *Cell*, 1977, **10,** 237.
45. Newport, J. W. and Kirschner, M. W., *Cell*, 1982, **30,** 675.
46. Ford, C. C., *J. Embryol. Exp. Morphol.*, 1985, **89,** 271.
47. Newport, J. W. and Kirschner, M. W., *Cell*, 1982, **30,** 687.
48. Pestell, R. Q. W., *Biochem. J.*, 1975, **145,** 527.
49. Woodland, H. R. and Adamson, E. D., *Dev. Biol.*, 1977, **57,** 118.
50. Hara, K., Tydeman, P. and Kirschner, M. W. *Proc. Natl. Acad. Sci. USA*, 1980, **77,** 462.
51. Harland, R. M. and Laskey, R. A., *Cell*, 1980, **21,** 761.
52. Kirschner, M., Butner, K., Newport, J., Black, S., Scharf, S. and Gerhart, J., *Neth. J. Zool.*, 1981, **31,** 50.
53. Maller, J. L., *Cell Diff.*, 1985, **16,** 211.
54. Masui, Y. and Clarke, H., *Int. Rev. Cytol.*, 1979, **57,** 185.
55. Masui, Y. and Markert, C. L., *J. Exp. Zool.*, 1971, **177,** 129.
56. Reynhout, J. K. and Smith, L. D., *Dev. Biol.*, 1974, **38,** 394.
57. Smith, L. D. and Ecker, R. E., *Dev. Biol.*, 1971, **25,** 233.
58. Kishimoto, T., Kuriyaman, R., Kondo, H. and Kanatani, H., *Exp. Cell Res.*, 1982, **137,** 121.
59. Sorensen, F., Cyert, M. and Pederson, R., *J. Cell Biol.*, 1985, **100,** 1637.

60. Wasserman, W. J. and Smith, L. D., *J. Cell Biol.*, 1978, **78**, R15.
61. Wu, M. and Gerhart, J. C., *Dev. Biol.*, 1980, **79**, 465.
62. Halleck, M. S., Lumley-Sapansky, K., Reed, J. A., Iyer, A. P., Mastro, A. M. and Schlegel, R. A., *FEBS Lett.*, 1984, **167**, 193.
63. Nelkin, B., Nichols, C. and Vogelstein, B., *FEBS Lett.*, 1980, **109**, 233.
64. Sunkara, P. S. Wright, D. A. and Rao, P. N., *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2799.
65. Weintraub H., Buscaglia, M., Ferrez, M., Weiller, S., Boulet, A., Fabre, F. and Baulieu, E. E., *C. R. Hebd. Senac. Acad. Sci. Paris III*, 1982, **295**, 787.
66. Masui, Y., In: *Molecular regulation of nuclear events in mitosis and meiosis*, (eds) R. A. Schlegel, M. S. Halleck and P. N. Rao, Academic Press, New York, 1986 (in press).
67. Meyerhoff, P. G. and Masui, Y., *Exp. Cell Res.*, 1979, **123**, 345.
68. Meyerhof, P. G. and Masui, Y., *Dev. Biol.*, 1979, **72**, 182.
69. Newport, J. W. and Kirschner, M. W., *Cell*, 1984, **37**, 731.
70. Davis, F. M. Tsao, T. Y., Fowler, S. K. and Rao, P. N. *Proc. Natl. Acad. Sci, USA*, 1983, **80**, 2926.
71. Davis, F. M. and Rao, P. N., In: *Molecular regulation of nuclear events in mitosis and meiosis*, (eds) R. A. Schlegel, M. S. Halleck and P. N. Rao, Academic Press, New York, 1986 (in press).
72. Vandre, D. D., Davis, F. M., Rao, P. N. and Borisy, G. G., *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 4439.
73. Gerhart, J. C., Wu, M., Cyert, M. and Kirschner, M. W., *Cytobios*, 1985, **43**, 335.
74. Rao, P. N., In: *Premature chromosome condensation: Applications in basic clinical and mutation research*, (eds) P. N. Rao, R. T. Johnson and K. Sperling, Academic Press, New York, 1982, p. 1.
75. Adlakha, R. C. and Rao, P. N., In: *Molecular regulation of nuclear events in mitosis and meiosis*, (eds) R. A. Schlegel, M. S. Halleck and P. N. Rao, Academic Press, New York, 1986, (in press).
76. Kishimoto, S. and Lieberman, I., *Exp. Cell Res.*, 1964, **36**, 92.
77. Tobey, R. A., Petersen, D. F., Anderson, E. C. and Puck, T. T., *Biophysics J.*, 1966, **6**, 567.
78. Sunkara, P. S., Wright, D. A., Adlakha, R. C., Sahasrabuddhe, C. G. and Rao, P. N., In: *Premature chromosome condensation: Application in basic, clinical and mutation research*, (eds) P. N. Rao, R. T. Johnson and K. Sperling, Academic Press, New York, 1982, p. 233.
79. El-Etr, M., Schorderet-Slatkine, M. and Baulieu, E. E., *Science*, 1979, **205**, 1397.
80. Maller, J. L. and Koontz, J. W., *Dev. Biol.*, 1981, **85**, 309.
81. Masui, Y., *J. Exp. Zool.*, 1967, **166**, 365.
82. Schorderet-Slatkine, S., Schorderet, M. and Baulieu, E. E., *Nature* (*London*), 1976, **262**, 289.
83. Baulieu, E. E., Godeau, F., Schorederet, M. and Schorderet-Slatkine, S., *Nature* (*London*), 1976, 275, 593.
84. Brachet, J., Baltus, E., Pays-DeSchutter, A., Hanocq-Quertier, J., Hubert, E. and Steinest, G., *Proc. Natl. Acad. Sci. USA*, 1975, **72**, 1574.
85. Wasserman, W. J. and Smith, L. D., *J. Cell Biol.*, 1981, **89**, 389.
86. Maller, J. L. and Krebs, E. G., *J. Biol. Chem.*, 1977, **252**, 1712.
87. Ozon, R., Belle, R., Huchon, D., Marot, J., *Annu. Biol. Anim. Biochim. Biophys.*, 1978, **18**, 483.
88. Birchmeier, C., Borek, D. and Wigler, M., *Cell*, 1985, **43**, 615.
89. Miake-Lye, R., Newport, J. W. and Kirschner, M. W., *J. Cell Biol.*, 1983, **97**, 81.
90. Halleck, M. S., Reed, J. A., Lumley-Sapanski, K. and Schlegal, R. A., *Exp. Cell Res.*, 1984, **153**, 561.
91. Huchon, D., Ozon, R., Fischer, E. H. and Demaille, J. G., *Mol. Cell Endicrinol.*, 1981, **22**, 211.
92. Finidori-Lepicard, J., Schorderet-Slatkine, S., Hanoune, J. and Baulieu, E. E., *Nature* (*London*), 1981, **292**, 255.
93. Sadler, S. E. and Maller, J. L., *J. Biol. Chem.*, 1981, **257**, 355.
94. Drury, K. C., *Differentiation*, 1978, **10**, 181.
95. Gerhart, J. C., Wu, M. and Kirschner, M. W., *J. Cell Biol.*, 1984, **98**, 1247.
96. Wasserman, W. J. and Masui, Y., *Science*, 1976, **191**, 1266.
97. Adlakha, R. C., Sahasrabuddhe, C. G.,

Wright, D. A., Lindsey, W. F. and Rao. P. N., *J. Cell Sci.*, 1982, **54,** 193.
98. Adlakha, R. C., Sahasrabuddhe, C. G., Wright, D. A., Lindsey, W. F., Smith, M. L. and Rao, P. N., *Nucl. Acids Res.*, 1982, **10,** 4107.
99. Adlakha, R. C., Prashad, N., Wright, D. A., Rasmus, M. and Rao, P. N., *J. Cell Biol.*, 1985, **101,** 142.
100. Adlakha, R. C. and Rao, P. N., *Bio Essays*, 1986, **5,** 100.
101. Adlakha, R. C., Wright, D. A., Sahasrabuddhe, C. G., Davis, F. M., Prashad, N., Bigo, H. and Rao, P. N., *Exp. Cell Res.*, 1985, **160,** 471.
102. Rao, P. N. and Adlakha, R. C., In: *Mediators in cell growth and differentiation*, (eds) R. J. Ford and A. L. Maizel, Raven Press, New York, 1985, p. 45.
103. Adlakha, R. C., Sahasrabuddhe, C. G., Wright, D. A. and Rao, P. N., *J. Cell Biol.*, 1983, **97,** 1707.
104. Hittleman, W. N. and Pollard, M., *Radiat. Res.*, 1982, **92,** 497.
105. Hittelman, W. N. and Pollard, M., *Carcinogenesis*, 1984, **5,** 1277.
106. Mullinger, A. M. and Johnson, R. T., *J. Cell Sci.*, 1985, **73,** 159.
107. Schor, S. L., Johnson, R. T. and Waldren, C. G., *J. Cell Sci.*, 1975, **17,** 539.
108. Adlakha, R. C., Wang, Y. C., Wright, D. A., Sahasrabuddhe, C. G., Bigo, H. and Rao, P. N., *J. Cell Sci.*, 1984, **65,** 279.
109. Burke, B. and Gerace, L., *Cell*, 1986, **44,** 639.
110. Lohka, M. J. and Masui, Y., *Science*, 1983, **220,** 719.
111. Lohka, M. J. and Maller, J. L., *J. Cell Biol.*, 1985, **101,** 518.
112. Miake-Lye, R. and Kirschner, M. W., *Cell*, 1985, **41,** 165.
113. Adlakha, R. C. Sahasrabuddhe, C. G., Wright, D. A., Bigo, H. and Rao, P. N., In: *Growth, cancer and the cell cycle*, (eds) P. Skehan and S. J. Friedman, Humana Press, New Jersey, 1984, p. 59.
114. Halleck, M. S., Reed, J. A., Lumley-Sapanski, K. and Schlegel, R. A., In: *Growth, cancer and the cell cycle*, (eds) P. Skehan and S. J. Friedman, Humana Press, New Jersey, 1984, p. 143.
115. Halleck, M. S. and Schlegel, R. A., In: *Molecular regulation of nuclear events in mitosis and meiosis*, (eds) R. A. Schlegel, M. S. Halleck and P. N. Rao, Academic Press, New York, 1986 (in press).
116. Bradbury, E. M., In: *Molecular regulation of nuclear events in mitosis and meiosis*, (eds) R. A. Schlegel, M. S. Halleck and P. N. Rao, Academic Press, New York, 1986 (in press).
117. Cohen, P., *Nature (London)*, 1982, **296,** 613.
118. Fischer, E. H., *Bull. Inst. Pasteur (Paris)*, 1983, **81,** 7.
119. Gurley, L. R., Tobey, R. A., Walters, R. A., Hildebrand, C. E., Hohmann, P. D., D'Anna, J. A., Barham, S. S. and Deaven, L. L., In: *Cell cycle regulation*, (eds) J. R. Jeter, I. L. Cameron, G. M. Padila and A. M. Zimmerman, Academic Press, New York, 1978, p. 37.
120. Ingebritsen, J. S. and Cohen, P., *Science*, 1983, **221,** 331.
121. Krebs, E. G. and Beavo, J. A., *Annu. Rev. Biochem.*, 1979, **48,** 923.
122. Nestler, E. J. and Greengard, P., *Nature (London)*, 1983, **305,** 583.
123. Nishimoto, T., Ajiro, K., Davis, F. M., Kamashita, K., Kai, R., Rao, P. N. and Sekiguchi, M., In: *Molecular regulation of nuclear events in mitosis and meiosis*, (eds) R. A. Schlegel, M. S. Halleck and P. N. Rao, Academic Press, New York, 1986 (in press)
124. Maller, J. L., Wu, M. and Gerhard, J. C., *Dev. Biol.*, 1977, **58,** 295.
125. Foulkes, J. G. and Maller, J. L., *FEBS Lett.*, 1982, **150,** 155.
126. Huchon, D., Ozon, R. and Demaille, J. G., *Nature (London)*, 1981, **294,** 358.
127. Hanocq-Quertier, J. and Baltus, E., *Eur. J. Biochem.*, 1981, **120,** 351.
128. Kalthoff, H., Darmer, D., Towbin, H., Gordon, J., Amons, R., Moller, W. and Richter, D., *Eur. J. Biochem.*, 1982, **122,** 439.
129. Nielsen, P. J., Thomas, G. and Maller, J. L., *Proc. Natl. Acad. Sci. USA*, 1982, **79,** 2937.
130. Wasserman, W. J. and Houle, J. G., *Dev. Biol.*, 1984, **101,** 436.
131. Erikson, E. and Maller, J. L., *J. Biol. Chem.*, 1986, **261,** 350.
132. Boyer, J., Belle, R., Capony, J. P. and Ozon, R., *Biochimie*, 1983, **65,** 15.
133. Ajiro, K., Nishimoto, T. and Takahashi, T., *J. Biol. Chem.*, 1983, **258,** 4534.

134. Hanks, S. K., Rodriguez, L. V. and Rao, P. N. *Exp. Cell Res.*, 1983, **148**, 293.
135. Krystal, G. W. and Poccia, D. L., *Exp. Cell Res.*, 1981, **134**, 41.
136. Allis, C. D. and Gorovsky, M. A. *Biochemistry*, 1981, **20**, 3828.
137. Gorovsky, M. A. and Keevert, J. B., *Proc. Natl. Acad. Sci. USA*, 1975, **72**, 2672.
138. Tanphaichitr, N., Moore, K. C., Granner, D. K. and Chalkley, R., *J. Cell Biol.*, 1976, **69**, 43.
139. Henry, S. M. and Hodge, L. D., *Eur. J. Biochem.*, 1983, **133**, 23.
140. Gerace, L. and Blobel, G., *Cell*, 1980, **19**, 277.
141. Jost, E. and Johnson, R. T., *J. Cell Sci.*, 1981, **47**, 25.
142. Ottoviano, Y. and Gerace, L., *J. Biol. Chem.*, 1985, **260**, 624.
143. Bravo, R., Fey, S. J., Moselarsen, P. and Celis, J. E., *Cold Spring Harbor Symp. Quant. Biol.*, 1982, **46**, 379.
144. Evans, R. M. and Fink, L. M., *Cell*, 1982, **29**, 43.
145. Evans, R. M., *J. Biol. Chem.*, 1984, **259**, 5372.
146. Robinson, S. I., Nelkin, B., Kaufmann, S. and Vogelstein, B., *Exp. Cell Res.*, 1981, **133**, 445.
147. Shibayama, T., Nakaya, K., Matsumoto, S. and Nakamura, Y., *FEBS Lett.*, 1982, **139**, **214**.
148. Bhorjee, J. S., *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 6944.
149. Paulson, J. R. and Taylor, S. S., *J. Biol. Chem.*, 1982, **257**, 6064.
150. Sahasrabuddhe, C. G., Adlakha, R. C. and Rao, P. N., *Exp. Cell Res.*, 1984, **133**, 439.
151. Al-Bader, A. A., Orengo, A. and Rao, P. N., *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 6064.
152. Westwood, J. T., Church, R. B. and Wagenaar, E. B., *J. Biol. Chem.*, 1985, **260**, 10308.
153. Song, M. K. H. and Adolph, K. W., *J. Biol. Chem.*, 1983, **258**, 3309.
154. Hermann, J., Mulner, O., Belle, R., Marot, J., Tso, H. and Ozon, R., *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 5150.
155. Drury, K. C. and Schorderet-Slatkione, S., *Cell*, 1975, **4**, 269.
156. Schorderet-Slatkine, S., *Cell Different.*, 1972, **1**, 179.

NEWS

SIR C. V. RAMAN AWARD FOR RESEARCH IN PHYSICAL SCIENCES FOR 1983 AND 1984

Prof. R. Vijayaraghavan and Prof. S. Biswas, both of the Tata Institute of Fundamental Research, Bombay received the Sir C. V. Raman award for research in Physical Sciences, for the year 1983 and 1984 respectively. The Award given by the Hari Om Ashram Trust is of the value of Rs. 10,000/– each.