DNA of the cell; (ii) revertants have lost this information from both cellular RNA and DNA; (iii) fewer copies of the MSV genome exist per cell in the RNA of a sarcoma-positive subclone than in the MSV-producing cat cell P521; and (iv) the MSV transformed cat cell 81, which has MSV and a complete sarcoma RNA copy, does not have an apparent complete MSV copy in its DNA. The proportion of MSV-specific DNA in cell DNA apparently increases after FeLV infection. This increase was not due to FeLV infection alone since only 17 percent of the CCC-FeLV cell DNA was hybridized as judged by fractionation on a hydroxylapatite column; the thermal elution profile of the hybrid was unstructured and the hybrid was 99 percent digestible by nuclease S1. An increase in copies in 81-FeLV cells may be responsible for the apparent increase in genome content (*15*).

There may be multiple mechanisms of control leading to in vitro reversion including repression of transcription, loss of chromosomes containing an integrated virus, and plasmid segregation. Revertants of hamster kidney cells transformed by Rous sarcoma virus have the provirus in the chromosomal DNA, but virus-specific RNA is reduced (*11*).

Polyoma and SV40 virus transformed cells have a high rate of spontaneous reversion with an imbalance of chromosome number and makeup and a tendency to transform back with retention of the viral genome (*17*). Both polyoma transformed and revertant hamster cells have eight viral DNA equivalents in the cell DNA (*18*). Here again reversion may be due to control, perhaps by chromosomal factors (*17*), of the expression of the viral genes concerned with the maintenance of transformation. Within the sensitivity of our methods (> 0.1 DNA copy per haploid genome and > 0.003 percent of the total cellular RNA), the cat cell system described above represents the only revertant cell that has lost the transforming viral genome. Several properties of the transformed cat cells, including the high rate of reversion and, as demonstrated, the loss of the genome from revertants, suggest that the genome may exist as a plasmid in cell line 81 (*19*). Although the mechanisms of eviction of a transforming virus genome are not known, an understanding of such phenomena may represent an alternative approach to the control of malignancy.

ARTHUR E. FRANKEL
DANIEL K. HAAPALA
RICHARD L. NEUBAUER
PETER J. FISCHINGER
Viral Leukemia and Lymphoma Branch, National Cancer Institute, Bethesda, Maryland 20014

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12. Thermal elutions were performed with the hybrids (5000 count/min) from the incubations described for Fig. 1b. The hybrid was diluted with 0.12*M* phosphate buffer and passed through a hydroxylapatite column (1 cm^3), washed with 0.12*M* phosphate buffer at 2°C increments from 50° to 100°C. The P521 and 81 hybrids had a high T_m, an 86°C component, and an unstructured component of 10 to 15 percent which melted between 50° and 70°C. The CCC and revertant hybrids had only the unstructured component.
13. We thank D. Colcher for iodination of 65*S* RNA.
14. ^{125}I-Labeled 65*S* hybridized MSV-MuLV RNA (2400 count/min equal to 0.012 μg) was boiled 10 minutes with 4 mg of sheared cell DNA (made as described in the legend of Fig. 1b) and incubated in 0.3 ml of 0.3*M* phosphate buffer at 63°C. At various times 50-μl portions (4000 count/min) were removed and divided equally into two 1-ml volumes of double-strength sodium saline citrate buffer. One portion was treated with 200 units of ribonuclease T1 (Calbiochem). Both were incubated for 30 minutes at 37°C, precipitated with trichloroacetic acid, and filtered; the radioactivity in the precipitate was counted; the proportion of radioactivity remaining with ribonuclease treatment represents the percentage of hybridization of the RNA. With no DNA, protection was 5 percent; CCC and revertants protected 9 percent; 81 protected 20 percent; P521 protected 44 percent; and 3T3 mouse cells producing MSV-MuLV protected 61 percent.
15. The lower final hybridization with 81 cell DNA may be related to the low copy number in these cells. Reconstruction experiments using nine-tenths CCC cell DNA and one-tenth P521 cell DNA revealed three copies per haploid genome. Fifty percent rather than 75 percent of the probe hybridized with the diluted P521 DNA.
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A Cytoplasmic Factor Promoting Oocyte Maturation: Its Extraction and Preliminary Characterization

Abstract. *Cytoplasm of maturing amphibian oocytes possesses a factor that induces germinal vesicle breakdown. This factor was extracted from* Rana pipiens *eggs and assayed by microinjection into* Xenopus laevis *oocytes. The activity of this factor is Mg-dependent, Ca-sensitive, and associated with heat-labile protein. Centrifugation on a sucrose density gradient revealed that the factor exists in three different molecular sizes.*

In Amphibia, fully grown ovarian oocytes that have been arrested at meiotic prophase resume meiosis when exposed to progesterone in vitro as well as when normally ovulated in vivo (*1, 2*). The resumption of meiosis, called meiotic maturation, is signaled by nuclear membrane breakdown (germinal vesicle breakdown). Since such breakdown is not induced when progesterone is injected into the oocyte (*3, 4*), a cytoplasmic factor, produced in the oocyte in response to externally applied progesterone, has been postulated as the direct cause. Indeed, cytoplasm taken from oocytes matured in the presence of progesterone induces germinal vesicle breakdown when injected into untreated ovarian oocytes (*2–8*). This cytoplasmic factor, the maturation-promoting factor (MPF), is not species-specific among amphibians (*6*), nor does it appear to be cell-type specific, in that it induces similar changes in nuclei from somatic cells when they are injected into cytoplasm of fully mature oocytes (*9*).

Table 1. Frequency of germinal vesicle breakdown (GVBD) in recipient oocytes injected with extract on the day of extraction. Each extract was injected into 15 *Xenopus* oocytes at 80 nl per oocyte.

Addition to extraction medium	Ca^{2+}		Mg^{2+}		EGTA		EDTA	
	Total	GVBD	Total	GVBD	Total	GVBD	Total	GVBD
0 m*M*	30	30	30	30	45	45	30	30
1 m*M*	30	3	15	15	30	30	15	13
2 m*M*	30	1	15	15	45	45	15	5
5 m*M*	30	0	30	30	30	30	30	0
10 m*M*	30	0	30	30	30	30	15	0

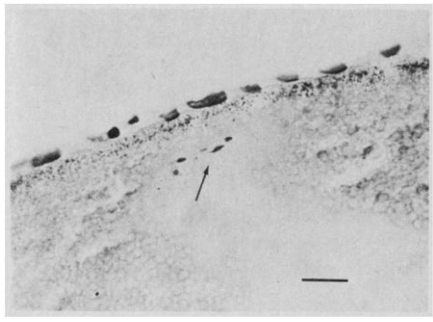

Fig. 1. Germinal vesicle migration to the animal pole, its breakdown, and condensation of the chromosomes (arrow). The *Xenopus laevis* oocyte was fixed 2 hours after the injection of 80 nl of extract made from mature *Rana pipiens* eggs. Bar represents 20 μm.

Cytoplasmic transfer experiments have revealed something of the nature of MPF. The frequency with which germinal vesicles in the recipient oocytes are broken down is proportional to the volume of injected cytoplasm (*3*). Thus, the activity of the factor is expressed as the frequency of germinal vesicle breakdown. The highest activity of MPF is found in the clear hyaline ooplasm of oocytes that are stratified by centrifugation without breaking the cells (*10*). MPF activity rises sharply before the vesicles are broken down and persists throughout the period of meiotic maturation (*3, 6, 7*) and even after fertilization, although at a low level (*3*). The development of MPF in maturing oocytes depends on protein synthesis (*7*), but its induction of germinal vesicle breakdown does not (*7, 8*). Exposing *Xenopus laevis* oocytes to divalent cations at high concentrations in the presence of ionophore A23187 initiates the production of MPF and the resultant breakdown of the germinal vesicles (*11*). These observations suggest that MPF may be a protein of the oocyte cytoplasm, and its activity may be regulated by divalent cations. Our results on extracted MPF support these hypotheses.

Uterine eggs were collected from *Rana pipiens* 48 hours after hormone injection; the jelly was removed and the eggs were extracted as described (*12*) with some modifications. A papain solution (0.035*M* Na_2HPO_4, 0.5 percent papain, 0.3 percent cysteine hydrochloride, *p*H 6.5) was used to remove the jelly. After being washed with 0.1*M* NaCl and then with 0.025*M* NaH_2PO_4, dejellied eggs were packed in a centrifuge tube containing an extraction medium (0.2*M* NaCl, 0.25*M* sucrose, 0.01*M* Na_2HPO_4–NaH_2PO_4, *p*H 6.5) under 1*g*. Excess medium above the eggs was withdrawn; the eggs were centrifuged (25,000*g* for 15 minutes, 2°C), which caused the yolk, cortex, and some lipid to form a pellet. The turbid supernatant was removed and centrifuged again (150,000*g* for 120 minutes, 2°C, Beckman SW 50.1). The final clear supernatant was assayed for MPF activity. Usually, 2.4 ml of extract was obtained from 1400 eggs that were packed in 5 ml. When the average volume of an egg was calculated to be 2.5 μl, the extract contained 1.5 ml of the extraction medium (62 percent) and 0.9 ml of egg material (38 percent). The protein concentration in this extract ranged from 25 to 27 mg/ml, as determined by the Lowry method (*13*).

Extracts were assayed for MPF activity by injecting 80 nl into each fully grown *Xenopus laevis* oocyte. These extracts exhibited MPF activity for at least 8 hours during storage on ice, although all activity was lost by 24 hours. Germinal vesicle breakdown induced by *Rana* extract in *Xenopus* oocytes occurred approximately 3 to 4 hours in advance of that induced by progesterone in sibling *Xenopus* oocytes. Histological examination revealed that the extract induced migration of the germinal vesicle toward the animal pole as well as its breakdown and chromosome condensation (Fig. 1). If the eggs were homogenized before they were centrifuged the resulting extracts did not induce breakdown but only the migration of the germinal vesicle (pseudomaturation) (*14*). The violent disruption of the egg by homogenization may cause the extensive disintegration of the cortex and cytoplasmic inclusions and seems to be responsible for the inactivation of MPF.

Table 2. Germinal vesicle breakdown in recipient oocytes injected with digested extract. Insoluble protease (Sigma) or insoluble ribonuclease (Sigma) was added to extracts and incubated in a shaking waterbath at 25°C for 1 and 2 hours. The particle-bound enzymes were removed by centrifugation at 10,000*g* for 10 minutes, and the supernatants were assayed for MPF activity. Results are given as the number of oocytes in which the vesicles broke down (GVBD) out of the number of oocytes injected.

Enzyme added to extract (units/ml)	Extract volume injected (nl)	GVBD/total	
		1 hour	2 hours
		Control	
	40	30/30	30/30
	10	15/15	15/15
		Protease	
0.2	40	15/15	11/15
1.0	40	4/15	5/15
1.0	10	0/15	1/15
		Ribonuclease	
0.3	40	15/15	15/15
1.5	40	15/15	15/15
1.5	10	15/15	15/15

The presence of Ca^{2+} in extracts obtained without homogenization inactivates MPF. When Ca^{2+} was added to the extrac-

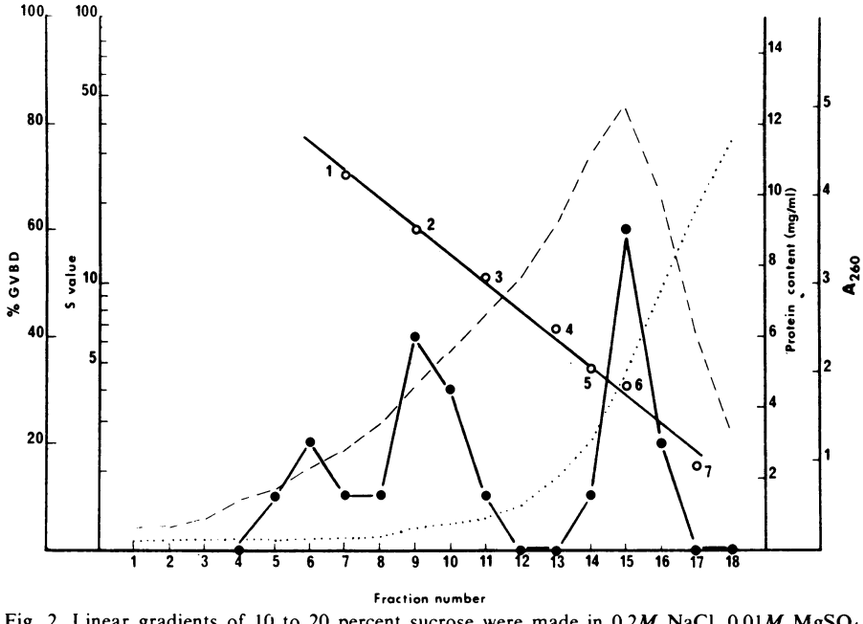

Fig. 2. Linear gradients of 10 to 20 percent sucrose were made in 0.2*M* NaCl, 0.01*M* $MgSO_4$, 0.002*M* EGTA, and 0.01*M* Na_2HPO_4/NaH_2PO_4, *p*H 6.5. Extract (0.5 ml) was layered on 5-ml gradients and spun at 85,000*g* for 17 hours or at 150,000*g* for 10 hours (Beckman SW 50.1). The tubes were punctured at the bottom and fractions (20 drops each) were collected. Each fraction was assayed for MPF activity (percent of germinal vesicles broken down) by injecting 20 *Xenopus* oocytes with 80 nl each (closed circles), each fraction was monitored for absorbancy 260 nm (dotted line), and the protein content of each fraction (dashed line) was determined by the Lowry method (*13*). Open circles refer to markers (*15*). The enzyme markers used were as follows. **1**, L-glutamate dehydrogenase, 26.6*S*; **2**, β-galactosidase, 15.9*S*; **3**, catalase, 11.3*S*; **4**, lactate dehydrogenase, 7.3*S*; **5**, alcohol dehydrogenase, 4.8*S*; and **7**, ribonuclease, 2*S*. Bovine serum albumin, 4.1*S*, point **6**, was also a marker. All were obtained from Sigma.

tion medium, the resulting extracts exhibited little if any MPF activity (Table 1). Addition of Ca^{2+} to final extracts at a concentration of $10^{-5}M$ completely inactivated MPF. Therefore, low levels of endogenous Ca^{2+} released by the eggs during centrifugation may account for the rapid loss in MPF activity during storage on ice. This possibility is supported by the observation that when ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA), a selective chelator of Ca^{2+}, was added to either the extraction medium or the final extract, MPF was still active for 24 hours.

In contrast to the effect of Ca^{2+}, the addition of Mg^{2+} to the extraction medium caused MPF activity to last significantly longer (Table 1). Extracts made with medium containing 10 m*M* $MgSO_4$ retained MPF activity for at least 24 hours, although most activity was lost after the extracts had been stored on ice for 48 hours. When ethylenediaminetetraacetic acid (EDTA), which chelates Mg^{2+} as well as Ca^{2+}, was added either to the extraction medium (Table 1) or to the final extract, MPF was immediately inactivated. Therefore, it seems that both the extractability and stability of MPF are increased in the presence of Mg^{2+} and the absence of Ca^{2+}.

Addition of Mg^{2+} to the extraction medium is indispensable when tris-maleate buffer is used instead of phosphate. Extracts made with tris-maleate buffer never exhibited MPF activity unless Mg^{2+} was added to the extraction medium at a concentration of 5 to 10 m*M*. Addition of Mg^{2+} to tris-maleate extracts made in the absence of Mg^{2+} failed to recover any MPF activity. Perhaps, maleate may chelate endogenous Mg^{2+} in the eggs as EDTA does to reduce the extractability of MPF.

The duration of this activity in both the maleate- and phosphate-buffered systems is dependent on the *p*H of the extraction medium. The optimum *p*H was between 6.4 and 6.8.

On the basis of these findings, in our later experiments we used an extraction medium that contained 0.2*M* NaCl, 0.25*M* sucrose, 0.01*M* $MgSO_4$, 0.002*M* EGTA, and 0.01*M* Na_2HPO /NaH_2PO_4, *p*H 6.5. Activity of MPF in extracts made with this improved medium has been detected for at least 48 hours and sometimes for 72 hours or more when the extracts have been kept on ice. However, activity was unstable at higher temperatures. There was no activity after 4 hours at 25°C or 15 minutes at 37°C.

MPF completely lost its ability to break down germinal vesicles when it was exposed to carboxymethyl cellulose-bound protease for 1 hour at 25°C, whereas there was no loss when it was exposed to polyacrylamide gel–bound ribonuclease (Table 2). This suggests that MPF activity is associated with protein but not with RNA.

The extracts were centrifuged on linear sucrose density gradients to determine the molecular size of MPF. After centrifugation, 18 fractions were collected, and each fraction was assayed for MPF activity. Three distinct peaks of activity (Fig. 2) were observed repeatedly after centrifugation at 150,000*g* for 10 hours or 85,000*g* for 17 hours at 2°C. The sedimentation coefficient (*S*) values for MPF in these fractions were estimated by comparing them to enzymes with known *S* values, which were centrifuged on separate gradients (*15*). The *S* values of the three MPF peaks were approximately 32*S*, 15*S*, and 4*S*. Therefore, under these in vitro conditions, MPF activity seems to be associated with heat-labile protein having three different molecular sizes. These may represent the association of MPF with other molecules or different degrees of aggregation of MPF molecules.

WILLIAM J. WASSERMAN*
YOSHIO MASUI

Department of Zoology,
University of Toronto,
Toronto, Ontario, Canada M5S 1A1

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* Present address: Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

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High Resolution of Human Chromosomes

Abstract. *G-band prophase chromosomes from synchronized cells permit a high degree of resolution not previously attained in the study of chromosome structure and birth defects in man.*

The G-band patterns of human chromosomes visualized at various stages of mitosis demonstrate that the major bands of metaphase result from the progressive coalescence of numerous smaller bands uncovered in late prophase. This phenomenon occurs by a simple process of chromosomal contraction that clarifies our understanding of chromosome structure during cell division. In this study, the use of cell synchronization with amethopterin and a brief exposure to Colcemid as mitotic inhibitor allowed the achievement of consistently large numbers of excellent quality mitoses in prophase, prometaphase, and metaphase. In addition, in late prophase chromosomes, 1256 bands per haploid set were observed, which represents four times the number of bands previously found in metaphase. Since it is believed that man has approximately 30,000 genes per cell (*1*) and prophases would therefore average out to have 23 genes per band, the examination of late prophase chromosomes in the study of gene mapping and in individuals with birth defects may facilitate the localization of phenotypic characteristics to specific minute chromosome segments, extending our understanding of the organization and function of the human genome.

Peripheral blood (0.2 ml) was cultured for 72 hours at 37°C in 5 ml of RPMI 1603 medium (Grand Island Biological) supplemented with 20 percent fetal calf serum (Grand Island Biological) and 0.2 ml of phytohemagglutinin M (Difco). Amethopterin (methotrexate, Lederle), at a concentration of $10^{-7}M$, was then added to cultures to synchronize lymphocytes by a modification of the technique used by Rueckert and Mueller (*2*) for HeLa cells. The amethopterin block was released 17 hours later by the addition of $10^{-5}M$ thymidine-rich medium, after the cells were washed three times with culture medium and centrifuged at 60*g* for 8 minutes after each washing. The cells were then allowed to grow at 37°C for 5½ to 6½ hours and treated during this period for only 15 minutes with Colcemid (0.2 μg/ml) (Grand Island Biological) at 37°C to arrest cells in metaphase and inhibit the formation of spindle fibers of late prophase and