ciliary fragments and other material (*2*). time of the open state in the present system after treatment with diethyl sulfide is very similar to the behavior of the glutamate-gated channel (*11*).

Although channel open times are long in comparison with those from patch-clamped living cells (*12*), they are in general accord with results of others (*13, 14*) for reconstituted single ion channels in planar bilayers. Benz *et al.* (*13*) reported analogous behavior of open channel states from the functional reconstitution of a porin from *Salmonella typhimurium*. Channels formed from a sea anemone neurotoxin (*15*) have a tendency to remain open, although bistable fluctuations have been reported for the same material (*16*).

Our data suggest the presence of a discrete chemosensitive ion channel in rat olfactory epithelium which can be functionally transferred into an artificial, essentially solvent-free lipid bimolecular membrane. This channel is activated by odorous compounds such as diethyl sulfide and (−)-carvone. We suggest that occupancy of this channel is associated with the initial events of chemoreception.

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## Formation in vitro of Sperm Pronuclei and Mitotic Chromosomes Induced by Amphibian Ooplasmic Components

Abstract. *A cell-free preparation of the cytoplasm from activated eggs of* Rana pipiens *induces, in demembranated sperm nuclei of* Xenopus laevis, *formation of a nuclear envelope, chromatin decondensation, initiation of DNA synthesis, and chromosome condensation. Both soluble and particulate cytoplasmic constituents are required to initiate these processes in vitro. The observed changes resemble processes occurring during fertilization and the mitotic cycle in early amphibian embryos. Therefore, this cell-free system may be useful in biochemical analysis of the interactions of nucleus and cytoplasm that control nuclear behavior.*

In the cytoplasm of the fertilized egg, the sperm nucleus undergoes changes; its nuclear envelope breaks down, its chromatin disperses, and a new nuclear envelope is assembled around it to form the male pronucleus, which enlarges, synthesizes DNA, and enters mitosis (*1*). Similarly, sperm or somatic cell nuclei injected into the cytoplasm of activated amphibian eggs decondense, swell, synthesize DNA, and enter mitosis (*2–3*). Cytoplasmic preparations from *Xenopus laevis* eggs have been shown to induce decondensation of the chromatin of red blood cell nuclei (*4*) and DNA synthesis in liver nuclei (*5*). Swelling of sperm nuclei in cytoplasmic preparations from eggs has been observed in sea urchins (*6*). However, only certain aspects of the process of pronuclear formation appeared to be reproduced in these earlier cytoplasmic preparations. We report that when demembranated sperm nuclei from *X. laevis* were incubated in a cytoplasmic preparation from activated *Rana pipiens* eggs, the sperm chromatin underwent changes similar to those in activated eggs.

Lysolecithin-treated sperm (*7*) from *X. laevis* were incubated with a cytoplasmic fraction prepared from activated *R. pipiens* eggs with the buffer and procedures described (*8*). Sperm incubated in this buffer did not show any morphological change. Instead, the cytoplasmic fraction prepared in this buffer induced a series of changes in sperm nuclear morphology (Fig. 1). The sperm nuclei were highly condensed when added to the cytoplasmic preparations (Fig. 1A), but during incubation they became partially decondensed (Fig. 1B). These nuclei continued to decondense to form small pronuclei, which later enlarged (Fig. 1C). The decondensing sperm nuclei synthesized DNA, as evidenced by the incorporation of [methyl-$^3$H]thymidine 5′-triphosphate (*9*) ([$^3$H]dTTP; Fig. 1D). This incorporation was inhibited by aphidicolin (*9*), a specific inhibitor of DNA

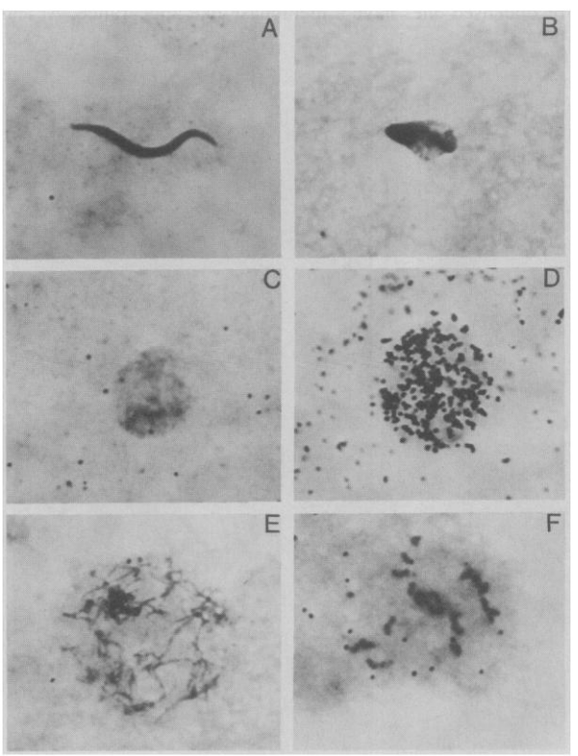


Fig. 1. Morphology of *Xenopus laevis* sperm nuclei during incubation in a cell-free cytoplasmic preparation (*14*). (A) Intact sperm nucleus. (B) Partially decondensed sperm nucleus. (C) Completely decondensed sperm nucleus. (D) Autoradiograph of decondensed sperm nucleus that was incubated for 3 hours in the cytoplasmic preparation containing [$^3$H]dTTP (40 μCi/ml). (E) Chromosomes with a prophase-like appearance. (F) Chromosomes with a metaphase-like appearance. Magnification, ×1100.

polymerase α (*10*) at a concentration of 5 μg/ml. Since aphidicolin inhibits DNA replication (*10*), this result suggests that DNA replication could be initiated by the decondensing sperm nuclei, although it may not continue to completion. Later, the nuclei formed chromosomes resembling those seen at prophase (Fig. 1E) and at metaphase (Fig. 1F) of mitosis. These changes in nuclear morphology were similar to those observed in lysolecithin-treated *X. laevis* sperm injected into activated *R. pipiens* eggs (*11*) and those seen following fertilization of *R. pipiens* eggs (*2*).

The nuclear changes observed during a 6-hour incubation of lysolecithin-treated sperm in a cytoplasmic preparation are shown in Fig. 2. At 1 hour, some partially decondensed sperm nuclei were observed. Between 1 and 3 hours, the percentage of sperm nuclei that decondensed and enlarged to form swollen interphase nuclei steadily increased with incubation time (Fig. 2A). After 3 hours, however, the percentage of fully decondensed nuclei decreased as prophase chromosomes were formed (Fig. 2B). Later, these chromosomes condensed further to form metaphase chromosomes (Fig. 2B). At 6 hours, the chromosomes appeared to decondense again to form interphase nuclei. The chromosome condensation observed in these preparations could have resulted from the synthesis, during incubation, of proteins necessary for mitotic chromosome condensation (*12*). Alternatively, the modification of proteins already present in the activated egg may have created the required conditions for chromosome condensation. Variation was observed in the timing of chromosome condensation, in the percentage of nuclei that formed metaphase chromosomes, and in the ability of chromosomes to decondense again. These variations may be accounted for by differences in the ability of individual preparations to synthesize or modify the putative proteins required for chromosome condensation. Nevertheless, metaphase chromosomes formed during a 6-hour incubation in seven of ten experiments.

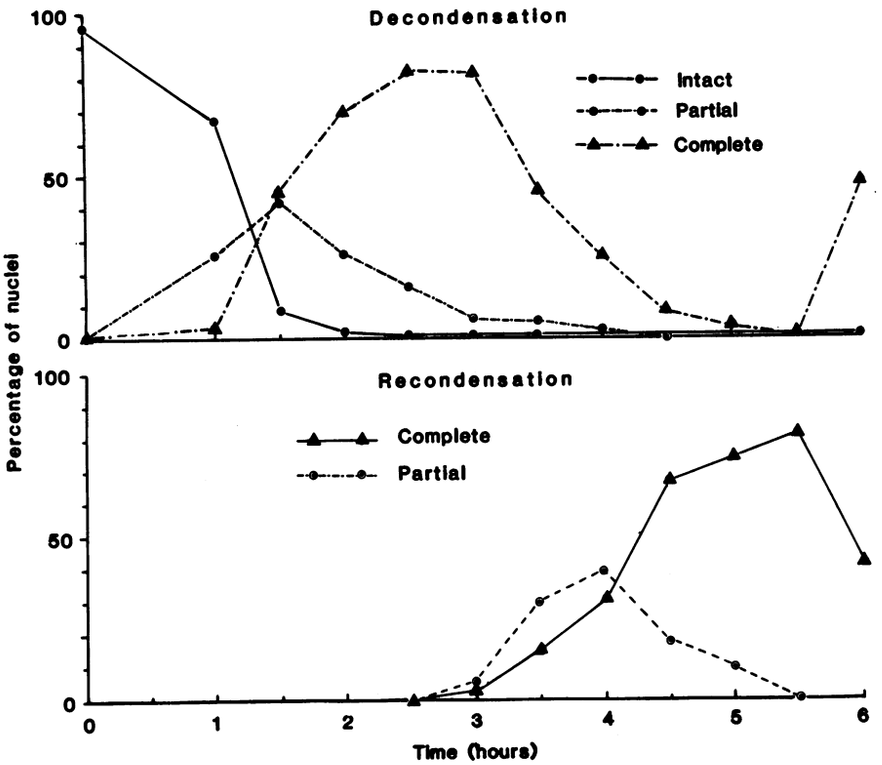


Fig. 2. Time course of the changes in sperm nuclear morphology in cell-free preparations. (A) Decondensation of sperm nuclei. (B) Chromosome condensation by decondensed sperm nuclei. Partially recondensed chromosomes have a prophase-like appearance, whereas completely recondensed ones have a metaphase-like appearance. At least 500 nuclei were examined each time.

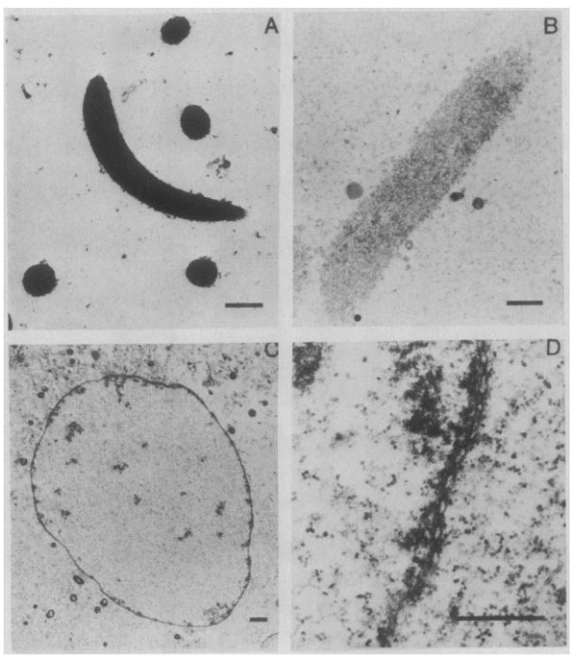


Fig. 3. Ultrastructure of *Xenopus laevis* sperm nuclei in cytoplasmic preparations. Lysolecithin-treated sperm nucleus (A) before addition to cytoplasmic preparation (scale bar, 1.0 μm); (B) 5 minutes after addition to cytoplasmic preparation (scale bar, 1.0 μm); (C) 180 minutes after addition to cytoplasmic preparation (scale bar, 1.0 μm); (D) nuclear envelope surrounding decondensed chromatin at 120 minutes of incubation (scale bar, 0.5 μm).

The process of sperm nuclear decondensation, described above, was examined at the ultrastructural level (*13*). Lysolecithin treatment of sperm removed most of the plasma membrane and nuclear envelope, leaving the chromatin highly condensed (Fig. 3A). The sperm nuclei remained unchanged during a 3-hour incubation in the buffer alone. However, within 5 minutes after being mixed with the cytoplasmic preparation, the sperm chromatin dispersed (Fig. 3B). During incubation, cytoplasmic vesicles contributed to the assembly of a nuclear envelope at the periphery of the sperm chromatin (*14*). Other vesicles appeared to fuse with the nuclear envelope during the period of nuclear enlargement, which resulted in completely decondensed sperm nuclei (Fig. 3C). The nuclear envelope surrounding decondensed sperm chromatin (Fig. 3D) appeared to have the usual structure of a double membrane containing pores. The supernatant prepared after centrifugation of the cytoplasmic preparation at 150,000*g* for 2 hours did not induce nuclear envelope formation or decondense sperm nuclei during a 3-hour incubation unless it was

mixed with the fluffy part of the sediment, although the initial dispersion of chromatin occurred. These observations suggest that the decondensation of sperm nuclei involves a rapid dispersion of chromatin by soluble cytoplasmic components, but formation of a nuclear envelope and swelling of the nuclei requires the presence of cytoplasmic vesicles.

Thus cell-free preparations of amphibian egg cytoplasm induce nuclear envelope formation, nuclear decondensation, initiation of DNA synthesis, and chromosome condensation in lysolecithin-treated sperm. These changes in nuclear morphology and activity, observed in vitro may represent the behavior of the sperm nucleus during fertilization and the mitotic cycle in the early amphibian embryo. Therefore, the biochemical basis for the cytoplasmic control of nuclear behavior may be examined with this cell-free system.

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7. Testes were dissected from sexually mature *X. laevis* males 1 hour after injection of 100 I.U. of human chorionic gonadotropin (hCG), washed, and incubated overnight at 18°C in Ringer solution containing antibiotics and hCG (10 I.U./ml). Sperm, released by gently squeezing the testes, were centrifuged, treated for 5 minutes at 22°C with nuclear isolation medium (NIM) [D. H. Ziegler and Y. Masui, *Dev. Biol.* **35**, 283 (1973)] containing 0.05 percent lysolecithin and soybean trypsin inhibitor (1 μg/ml), and washed once in NIM and 3 percent bovine serum albumin (BSA) and three times with NIM and 0.4 percent BSA. For some experiments lysolecithin-treated sperm were stored at −80°C in 30 percent (by volume) glycerol in NIM. Lysolecithin-treated sperm were washed thoroughly with buffer (see below) before use.
8. *Rana pipiens* eggs from which the jelly was removed enzymatically were placed in Ringer solution and activated by electric shock (80 V for 200 msec), incubated in 10 percent Ringer solution at 19° ± 1°C for 1 hour and washed in an ice-cold buffer consisting of 250 m*M* sucrose, 200 m*M* KCl, 1.5 m*M* $MgCl_2$, 2.0 m*M* β-mercaptoethanol, and 10 m*M* tris-HCl at *p*H 7.5. Eggs were transferred to 5-ml centrifuge tubes containing buffer on ice and crushed by centrifugation at 9000*g* for 15 minutes. The band of heavier supernatant above the packed pigment and yolk was transferred to a 0.6-ml centrifuge tube and centrifuged at 9000*g* for 15 or 30 minutes to remove most of the pigment. Lysolecithin-treated sperm were incubated at 18°C in 200 μl of this supernatant to give a concentration of $5 \times 10^4$ to $1 \times 10^5$ sperm per milliliter.
9. The cytoplasmic preparation (100 μl) was mixed with 100 μl of 33 percent buffer containing [$^3$H]dTTP (44 Ci/mmole; Amersham) at a concentration of 80 μCi/ml. In some experiments, aphidicolin [from a stock solution dissolved at 5 mg/ml in dimethyl sulfoxide (DMSO)] was added to the 33 percent buffer to obtain a concentration of 10 μg/ml. Addition of DMSO alone had no effect on incorporation. Squash preparations were washed with cold 5 percent trichloroacetic acid, coated with Kodak NTB2 emulsion, and exposed for 17 days at 4°C.
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13. Portions of the incubation mixture were fixed overnight on ice in 2 percent glutaraldehyde in 0.05*M* phosphate buffer at *p*H 7.4, washed three times with the same buffer, postfixed in 1 percent osmium tetroxide for 2 hours at room temperature, dehydrated through a graded series of ethanol concentrations to propylene oxide, and embedded in Epon 812.
14. M. J. Lohka and Y. Masui, in preparation.
15. Portions of the incubation mixture were fixed in a mixture of ethanol and acetic acid (3:1) and stained by the Feulgen procedure. Squash preparations [D. H. Ziegler, P. G. Meyerhof, Y. Masui, *J. Ster. Biochem.* **11**, 715 (1979)] were stained with 2 percent Giemsa.
16. We thank J. Rossant for the gift of aphidicolin; R. Valladiego for sectioning material for electron microscopy; and H. Clarke, M. Miller, and E. Shibuya for encouragement and helpful discussions. Supported by a grant from the Natural Science and Engineering Research Council of Canada (to Y.M.).

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## Ectopic Pro-Opiolipomelanocortin: Sequence of cDNA Coding for β-Melanocyte–Stimulating Hormone and β-Endorphin

Abstract. *A recombinant bacterial plasmid, pMS1, was constructed that contains 318 nucleotides complementary to a portion of pro-opiolipomelanocortin (proOLMC) messenger RNA from an ectopic adrenocorticotropin-producing tumor. The cloned complementary DNA insert, which contains the sequence that codes for all of the β-melanocyte–stimulating hormone and β-endorphin portions of proOLMC, as well as the 3′ nontranslated section, is identical to the genomic sequence. Hybridization of tumor proOLMC complementary DNA to RNA subjected to electrophoresis and transferred to a nitrocellulose filter revealed two proOLMC messenger RNA species in the tumor polyadenylated RNA, but only one in pituitary polyadenylated RNA. At least one of the tumor proOLMC messenger RNA's is similar, if not identical, to human pituitary proOLMC messenger RNA.*

The syndrome later to be associated with nonpituitary, or "ectopic," secretion of adrenocorticotropin (ACTH) was reported by Brown in 1928 (*1*) and was the first of the ectopic humoral syndromes to be described. The ectopic ACTH syndrome was also the first to be satisfactorily explained, with the discovery of ACTH-like biologic activity in the plasma and tumor tissue of patients with this disorder (*2–5*). Since that time, ectopic production of a variety of peptide hormones has been reported [see (*6*) for review].

Extensive studies of ectopically produced hormones indicate that they are similar if not identical to the corresponding eutopic hormones, but the amino acid sequence of an ectopic hormone has yet to be determined and compared with that of the eutopic molecule. The nearest approximation was based on amino acid composition and suggested that an ACTH-like peptide lacking the COOH-terminal and $NH_2$-terminal amino acids was the major product of a thymic tumor (*7*). Ectopic ACTH-producing tumors also produce peptides similar to β-lipotropin (β-LPH), γ-LPH, β-endorphin (β-End), and γ-melanocyte–stimulating hormone (γ-MSH) (*8–10*), suggesting that they arise from a common precursor, pro-opiolipomelanocortin (proOLMC), as they do in the pituitary gland (*11, 12*). RNA from an ACTH-producing tumor was shown to direct the synthesis, in a cell-free translation system, of a large immunoreactive ACTH protein with a size similar to that of the pituitary proOLMC precursor protein (*13*). Hybridization of bovine pituitary proOLMC complementary DNA (cDNA) to tumor RNA revealed two species of messenger RNA (mRNA): a predominant species that appeared to be similar in size to the single species of human pituitary proOLMC mRNA detected, and a minor species of higher molecular weight (*13*).

The RNA used in our experiments was isolated from a malignant ACTH-producing carcinoid tumor removed from the pancreas but thought to be a metastasis from a preexisting thymic carcinoid tumor causing Cushing's syndrome in a 64-year-old man. The tumor was thinly sliced shortly after removal, frozen immediately, and stored at −56°C until extraction. RNA was extracted by the sodium dodecyl sulfate–phenol procedure (*14*), and polyadenylated [poly(A)] RNA was isolated by oligodeoxythymidylate-cellulose chromatography (*15*); 273 μg of poly(A) RNA was prepared from 10.8 g of tumor tissue. From 100 μg of tumor poly(A) RNA, 595 ng of S1 nuclease–treated double-stranded cDNA