CONTROL OF CHROMOSOME BEHAVIOR

IN AMPHIBIAN OOCYTES

II. The Effect of Inhibitors of RNA and Protein

Synthesis on the Induction of Chromosome Condensation

in Transplanted Brain Nuclei by Oocyte Cytoplasm

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ABSTRACT

We studied the effects of actinomycin D, α-amanitin, puromycin, and cyclohexi-mide on the cytoplasmic activity of maturing *Rana pipiens* oocytes that induces chromosome condensation in transplanted brain nuclei. Treatment of oocytes with each inhibitor suppressed the chromosome condensation induced by metaphase I oocytes to varying degrees depending upon the dose of inhibitor, despite the fact that untreated metaphase I oocytes already possessed chromosome condensation activity (CCA). Treatment of brain nuclei before injection completely suppressed condensation at all doses used. Chromosome condensation induced by metaphase II oocyte cytoplasm, however, was insensitive to all the inhibitors, even when the brain nuclei were pretreated. Oocytes treated with α-amanitin throughout matura-tion induced chromosome condensation when tested at metaphase II. Removal of the oocyte chromosomes after the germinal vesicle (GV) broke down did not prevent the development of CCA, whereas removal of the entire GV before initiation of maturation deprived oocytes of CCA. The results suggest that metaphase I oocyte cytoplasm stimulates synthesis of brain nuclear RNAs that are translated into proteins necessary for chromosome condensation, whereas meta-phase II oocytes possess all the factors for chromosome condensation. In both cases, GV nucleoplasm appears indispensable for the development of CCA, whereas immediate activity of the oocyte genome is not required.

Chromosome condensation is induced in inter-phase nuclei exposed to the cytoplasm of cells undergoing mitosis or meiosis. Such premature chromosome condensation was induced in inter-phase cells fused to colchicine-arrested mitotic cells in culture (13). In amphibian oocytes, Batail-lon (2) and Bataillon and Tchou-Su (3) observed metaphase-like condensation of sperm chromo-somes when *Hyla* or *Triton* metaphase I oocytes were inseminated. Gurdon (11, 12) found that chromosomes of isolated interphase nuclei of so-matic cells condensed to metaphase when injected into *Xenopus laevis* oocytes shortly after germinal vesicle breakdown (GVBD). The results of these

studies show that the cytoplasm of a cell in mitosis or meiotic maturation is pervaded by factor(s) that induce chromosome condensation.

In a previous study (20) we determined the time course of the cytoplasmic activity that induces chromosome condensation in brain nuclei transplanted into *Rana pipiens* maturing oocytes. It was found that the chromosome condensation activity was first detectable shortly after GVBD, about 20 h after progesterone treatment of the ovarian oocytes, and persisted until the time when the fully mature eggs were activated. In this system, there were two potentially active genomes—viz., the genome of the oocyte and that of the injected brain nuclei. The present study was undertaken to examine, with RNA and protein synthesis inhibitors, the possible involvement of these nuclear genomes in the production and action of the chromosome condensation factors.

MATERIALS AND METHODS

Hibernating, sexually mature *Rana pipiens*, obtained from dealers in Ontario, Quebec, and Vermont in the fall and winter and then maintained in the cold, were used as oocyte donors. Fully grown oocytes (1.6–1.8 mm in diameter) were used for experiments. Defolliculation and progesterone treatment of ovarian oocytes for inducing maturation have been described in previous papers (14, 20). All the operations and subsequent incubation were carried out in a frog Ringer's solution containing antibiotics, designated as the standard solution (14). The maturation age of oocytes refers to the time (hours) in culture at 18°C after progesterone treatment and is indicated by the number subscribed to the letter T. Synchronously maturing oocytes undergoing GVBD between T_{13} and T_{17} were used in all the experiments. These oocytes usually reside in the first metaphase (M I) at T_{24} and the second metaphase (M II) at T_{48}.

Brain nuclei were isolated from adult *Rana pipiens* by the method described in a previous paper (20). A nuclear suspension containing 20–200 nuclei was injected into the animal hemisphere of a recipient oocyte immersed in the standard solution without causing perceptible swelling of the recipient. To avoid clumping of the nuclei and to facilitate their exposure to the oocyte cytoplasm, the injected nuclear suspension was mixed with the cytoplasm by several pumpings with the injection pipette before withdrawal of the pipette from the recipient.

For enucleation of ovarian oocytes, the entire germinal vesicle (GV) was squeezed out according to the method of Dettlaff et al. (8). In some experiments, chromosomes were removed from the oocytes at or near M I without loss of the bulk of the GV contents. Since at M I the oocyte chromosomes are located below a black dot on the animal pole, the chromosomes were removed

by squeezing a small quantity of underlying ooplasm through a slit across the black dot made with a sharp glass needle. Cytological examination of these operated oocytes revealed complete absence of chromosomes in 95% of the cases whereas their presence was confirmed in all unoperated oocytes.

Actinomycin D, α-amanitin, puromycin, and cycloheximide were purchased from Calbiochem, San Diego, Calif. These chemicals were dissolved in the standard solution. To inhibit RNA or protein synthesis, 40 nl of the inhibitor solution was injected into an oocyte. Concentration within the oocyte was estimated on the assumption that the injected inhibitor distributed uniformly throughout the oocyte whose volume was calculated from measurements of the diameter. This dosage is designated as the "internal dose." In order to prevent diffusion of the inhibitor, the injection was always carried out in the standard solution containing the inhibitor at the same concentration as the "internal dose," and the recipient oocytes were further incubated in the same inhibitor-containing standard solution until fixed.

When wholly mature oocytes were injected with nuclei or inhibitors, they were immersed in 0.05 M NaH_2PO_4 solution for 30 min before injection and for 15 min after injection to prevent activation caused by injury. These eggs were found to retain the ability to activate when pricked after being returned to the standard solution.

Treatment of isolated brain nuclei with inhibitor was carried out by suspending nuclei in nuclear isolation medium containing the inhibitor for 2 h at 4°C. The nuclei were then washed by centrifugation in inhibitor-free medium and resuspended in the inhibitor-free medium.

The cytological procedures used for preparing sectioned specimens were the same as those employed in a previous study (20). In addition, squash preparations were made. To prepare the squashes, oocytes were fixed in acetic alcohol (1:3) for 13 h, Feulgen stained, broken into small pieces in 50% glacial acetic acid, and squashed under a cover slip after 5 min of exposure to the acetic acid.

Chromosome condensation activity (CCA) in any group of oocytes is expressed as the percentage of oocytes in which condensed brain chromosomes were found. Also, the efficiency of chromosome condensation within a single oocyte was estimated by determining the percent of injected nuclei that had formed clusters of condensed chromosomes.

RESULTS

Effects of RNA Synthesis Inhibitors on Induction of Brain Chromosome Condensation in Maturing Oocytes (M I Oocytes)

The RNA synthesis inhibitor actinomycin D or α-amanitin was injected into defolliculated, ovar-

ian oocytes to various internal doses at T_0. At T_{24} the oocytes were injected with brain nuclei and 3 h later the specimens were fixed. Untreated oocytes induced the chromosomes in brain nuclei to condense in all cases. These brain chromosomes appeared much like the prometaphase chromatids of mitotic cells (Fig. 1). In Table I it can be seen that the induction of brain chromosome condensation was progressively suppressed with increasing doses of inhibitors and was completely inhibited by actinomycin D and by α-amanitin at concentrations above 5.5 $\mu g/ml$ and 20 $\mu g/ml$, respectively. On the other hand, complete inhibition was brought about at all doses if both brain nuclei and oocytes were treated with α-amanitin before injection.

The brain nuclei whose chromosomes failed to condense in the actinomycin D-treated oocytes often appeared pycnotic, stained much more densely than did brain nuclei *in situ*, and remained scattered in the ooplasm with no sign of swelling or dissolution (Fig. 2), whereas the brain nuclei which failed to undergo chromosome condensation in α-amanitin-treated oocytes remained as interphase nuclei without any recognizable abnormalities.

In order to answer the question of whether the RNA synthesis inhibitors prevented chromosome condensation through suppression of the development of chromosome condensation activity (CCA) in oocyte cytoplasm or through inhibition of the induction process by CCA, oocytes that already possessed CCA were treated with α-amanitin. Since CCA is known to exist at T_{20} (20), oocytes were injected with α-amanitin at T_{22} to an internal dose of 10 $\mu g/ml$ and the brain nuclei were introduced at T_{24}. All the oocytes were fixed 3 h later. To ensure that CCA was really present in the recipient oocytes, brain nuclei were injected at T_{22} into untreated oocytes obtained from the same batch as those used for the experiments. In all of these 65 untreated oocytes, many clusters of condensed brain chromosomes were found. In contrast, two-thirds of the 36 oocytes treated with α-amanitin completely failed to induce chromosome condensation and, even in the oocytes in which the brain chromosomes did condense, a large majority of the injected nuclei remained at interphase. Moreover, when the brain nuclei that were to be injected were pretreated with the inhibitor (10 $\mu g/ml$), chromosome condensation was completely suppressed in all 37 cases.

The same experiment was repeated with 0.05 M NaH_2PO_4 as the incubation medium which pre-

vents oocytes from activation. Of 30 α-amanitin-treated oocytes, none induced chromosome condensation, whereas all 30 untreated control oocytes were unaffected in their ability to induce chromosome condensation. These results suggest that the inhibitors affect the induction process of chromosome condensation rather than the development of CCA.

Effects of RNA Synthesis Inhibitors on Induction of Brain Chromosome Condensation in Fully Mature Oocytes (M II Oocytes)

The RNA synthesis inhibitor actinomycin D or α-amanitin was injected into fully mature oocytes at T_{48} to various internal doses after 30 min of pretreatment of the oocytes with NaH_2PO_4 solution containing inhibitor at the same concentration as the internal dose. 2 h later, brain nuclei, untreated or pretreated for 2 h with the inhibitor, were introduced. After 3 h of incubation the oocytes were fixed. When the inhibitor-treated specimens were compared with the untreated controls, no difference in the frequency of chromosome condensation was found (Table II). Furthermore, in both control and treated specimens, a majority of the injected brain nuclei, estimated as between 80% and 90%, underwent condensation within any one of these oocytes.

However, in the actinomycin D-treated oocytes, the condensed brain chromosomes were considerably longer and thinner and more closely resembled early prophase than the prometaphase-type chromatids that are found in α-amanitin-treated and untreated specimens (Fig. 3 A,B).

Effects of Inactivation of the Oocyte Chromosomes on Brain Chromosome Condensation

Since the foregoing experiments were performed with oocytes whose chromosomes were present, it was desirable to determine if the oocyte genome was actively involved in condensation of the brain chromosomes. Thus, the metaphase I chromosomes of prospective recipients were manually removed at T_{22} with the aid of a glass needle. To test the effectiveness of chromosome removal, 40 oocytes were fixed for cytological examination immediately after the operation without the insertion of brain nuclei. In 38 (95%) of these oocytes, no chromosomes were found, while 20 unoperated
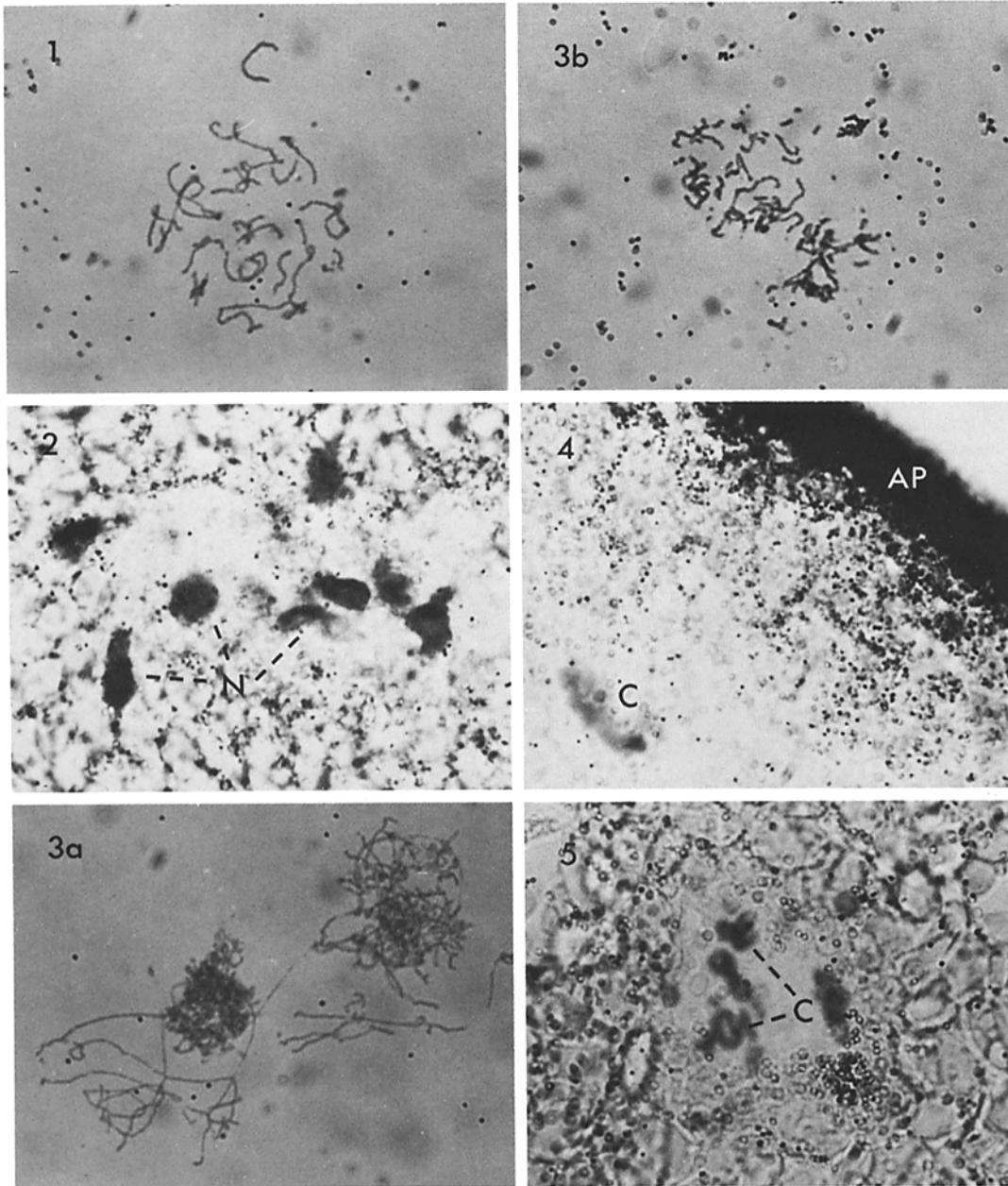
FIGURE 1 Typical prometaphase type chromosomes derived from brain nuclei exposed for 3 h to metaphase I oocyte cytoplasm. × 870. Squash preparation with Feulgen staining.

FIGURE 2 Pycnotic brain nuclei that have been exposed to metaphase I cytoplasm for 3 h. The oocytes were injected with actinomycin D to an internal dose of 5.5 μg/ml just before progesterone treatment. × 870. Specimen sectioned at 8 μm. Feulgen, light green staining. N, brain nuclei.

FIGURE 3 Brain chromosomes induced to condense after a 3-h exposure to metaphase II oocyte cytoplasm. (A) Prophase type of brain chromosomes condensed in the presence of Actinomycin D at a concentration of 5 μg/ml. × 870. (B) Typical prometaphase type of brain chromosomes induced to condense by untreated metaphase II cytoplasm. × 870. Squash preparations with Feulgen staining.

FIGURE 4 Abnormal oocyte chromosomes found in fully mature oocytes treated with actinomycin D throughout the maturation period. The "chromosomes" stained very diffusely in the Feulgen reaction and were located well below the egg cortex. Two such globules were found in this specimen. × 870. The 8-μm section was stained with feulgen, light green. C, oocyte chromosome; AP, animal pole.

FIGURE 5 The oocyte chromosomes as they appear in fully mature eggs treated with α-amanitin throughout the maturation period. Condensation is normal but chromosomes are located well below the cortex and still appear to be bivalents indicating anaphase I failure. × 870. The 8-μm section was stained with Feulgen, light green. C, oocyte chromosomes.

TABLE I

Effect of RNA Synthesis Inhibitors on the Induction of Brain Chromosome Condensation in Maturing Oocytes
(M I Oocytes)

| | Actinomycin D Brain nuclei untreated | | | α-Amanitin | | | |
| | | | | Brain nuclei untreated | | Brain nuclei treated | |
Doses	Oocytes examined	Oocytes with condensed brain chromosomes	Doses	Oocytes examined	Oocytes with condensed brain chromosomes	Oocytes examined	Oocytes with condensed brain chromosomes
$\mu g/ml$		%	$\mu g/ml$		%		%
0	40	100	0	20	100	20	100
0.75	30	60	5	22	64	35	0
1.5	24	50	10	34	29	30	0
3.0	25	4	15	38	21	30	0
5.5	25	0	20	35	0	31	0
6.0	30	0					

Defolliculated ovarian oocytes were submerged in the standard solution containing inhibitor at the indicated doses and injected with inhibitor so that the same doses were reached internally. Maturation was then induced with progesterone and, at metaphase I, isolated brain nuclei, either untreated or pretreated with the inhibitor, were injected. 3 h after nuclear injection, the recipient oocytes were fixed for cytological examination. If an oocyte contained one or more clusters of condensed brain chromosomes, it was considered a positive case.

TABLE II

The Effect of RNA Synthesis Inhibitors on the Induction of Brain Chromosome Condensation in Mature Oocytes (M II Oocytes)

| | Brain nuclei untreated | | Brain nuclei treated | |
Inhibitor	Oocytes examined	Oocytes with condensed brain chromosomes	Oocytes examined	Oocytes with condensed brain chromosomes
$\mu g/ml$		%		%
α-Amanitin				
0	20	100	20	100
5	40	100	40	100
10	40	100	40	100
15	35	100	35	100
Actinomycin D				
0	20	100	40	100
5	40	100	40	100

Defolliculated ovarian oocytes were induced to mature with progesterone. At full maturity, when the oocytes had reached metaphase II, they were submerged in 0.05 M NaH_2PO_4 solution containing inhibitor and then injected with inhibitor so that the indicated concentrations were reached internally. 2 h later, isolated brain nuclei, either untreated or pretreated with inhibitor, were injected. 3 h after nuclear injection the oocytes were fixed for cytological examination. If an oocyte contained one or more clusters of condensed brain chromosomes, it was considered a positive case.

oocytes examined were found to contain chromosomes.

In the first experiment, oocytes were injected with brain nuclei at T_{24}, 2 h after removal of chromosomes when the wounds had healed, and 3

h later the specimens were fixed. Cytological examination revealed that condensed brain chromosomes were present in 50 of 52 oocytes (98%) in which no host chromosomes were found. In the second experiment, oocyte chromosomes were

removed at T_{24} and brain nuclei were injected at T_{48}. These oocytes were fixed 3 h later and examined cytologically. It was found that all 25 oocytes which were lacking their own chromosomes had induced brain chromosomes to condense.

The requirement of oocyte genomic activity for the induction of brain chromosome condensation was also tested by chemical inactivation with α-amanitin. To accomplish these tests the inhibitor was injected to an internal dose of 10 $\mu g/ml$ into 35 oocytes at T_0 and into 30 oocytes at T_{24}. They were then allowed to mature until T_{48} when brain nuclei which had been pretreated with α-amanitin at a dose of 10 $\mu g/ml$ were injected. All the specimens were fixed 3 h later. It was found that chromosome condensation was induced in all of these oocytes. In neither instance was the percentage of chromosome condensation reduced, as compared with untreated controls in which brain chromosome condensation was found in all 42 specimens.

The role of the germinal vesicle was also studied by enucleation at T_0. The enucleated oocytes were allowed to develop after progesterone treatment until the brain nuclei were injected at T_{48}. These oocytes were fixed 3 h after nuclear injection. It was found that none of the 30 oocytes examined contained condensed brain chromosomes, but a few interphase nuclei were found to have remained.

Effects of Protein Synthesis Inhibitors

In order to determine whether suppression by RNA synthesis inhibitors of the brain chromosome condensation in M I oocytes was caused by RNA synthesis inhibition itself or was mediated through the resulting deficiency in protein synthesis, oocytes at T_{22} were injected with puromycin or cycloheximide to the internal doses indicated in Table III. At T_{24}, brain nuclei were introduced and the recipient oocytes were fixed after an additional 3 h of incubation. Condensation of the brain chromosomes was completely suppressed in all treated oocytes, whereas in most untreated controls it was induced.

The effect of cycloheximide on the induction of chromosome condensation at T_{48} was also tested with 0.05 M NaH_2PO_4 as an incubation medium

TABLE III

Effects of Protein Synthesis Inhibitors on the Induction of Brain Chromosome Condensation

| Inhibitor | Time after progesterone | | Oocytes examined | Oocytes with condensed brain chromosomes |
	Inhibitor injected	Brain nuclei injected		
$\mu g/ml$	h	h		%
Untreated	—	22	40	95
	—	24	40	100
	—	50	30	100
Puromycin				
120	22	24	30	0
Cycloheximide				
1	22	24	37	0
10	22	24	40	0
Cycloheximide				
1	48	50	25	100
10	48	50	32	100
50	48	50	30	100

Defolliculated ovarian oocytes were induced to mature with progesterone. At the indicated times after progesterone treatment, the oocytes were submerged in inhibitor-containing solution and injected with inhibitor to the indicated internal concentration. 2 h later isolated brain nuclei were introduced, and 3 h after this the oocytes were fixed. If an oocyte contained one or more clusters of condensed brain chromosomes, it was considered a positive case.

that prevents activation. 2 h later, brain nuclei were introduced. The oocytes were fixed 3 h after nuclear injection. It was found that all of the cycloheximide-treated oocytes contained condensed brain chromosomes (Table III). In both cycloheximide-treated and untreated oocytes, 80%–90% of the nuclei underwent chromosome condensation within any particular oocyte, except those treated with cycloheximide at 50 µg/ml. These latter oocytes showed symptoms of degeneration, and many fewer brain nuclei underwent chromosome condensation than in the untreated control. These results demonstrate that chromosome condensation can be induced in fully mature M II oocytes in spite of the presence of protein synthesis inhibitors, whereas it is suppressed by these inhibitors in M I oocytes.

Effects of RNA Synthesis Inhibitors on Oocyte Chromosome Condensation

Oocytes treated with actinomycin D throughout the maturation period were unable to develop chromosomes typical of metaphase II. The chromosomes in these oocytes, though more condensed than those before GVBD, appeared as globules which stained diffusely in the Feulgen reaction and were located far below the animal hemisphere cortex of the oocyte. In typical cases, the chromosome complements were found to coalesce into two to five such globules (Fig. 4). On the other hand, chromosome condensation always occurred normally in α-amanitin-treated oocytes, whether the oocytes were treated continuously or temporarily. However, it was noted that chromosomes at either M I or M II were frequently located far below the animal pole cortex (Fig. 5), while those in untreated oocytes always were found within it.

DISCUSSION

In Rana pipiens oocytes approaching metaphase I the cytoplasm becomes capable of inducing chromosome condensation in transplanted brain nuclei. This cytoplasmic activity has been designated chromosome condensation activity (CCA) in this paper. At 18°C, CCA first appears approximately 20 h after progesterone treatment, shortly after GVBD, and does not disappear until the oocytes are activated (20). The results of the present study show that treatments with RNA or protein synthesis inhibitors suppress the induction of brain chromosome condensation in M I oocytes,

whereas they have no effect on the condensation induced in fully mature M II oocytes. It seems likely that the different efficacies of the inhibitors reflect changes in the cytoplasmic state of oocytes that occur as the oocytes progress through meiotic maturation.

Actinomycin D and α-amanitin have been proved to be potent inhibitors of RNA synthesis at concentrations similar to those used here. It was reported that actinomycin D at 5 µg/ml suppresses the RNA synthesis in R. pipiens maturing oocytes by 90% (17). α-Amanitin, when used at doses within the range used in the present study, suppresses non-nucleolar RNA synthesis in Titurus lampbrush stage oocytes (7), and inhibits RNA synthesis in Xenopus cleaving embryos by 99.3% (18).

We observed that α-amanitin even at concentrations as high as 15 µg/ml inhibited neither meiotic maturation of oocytes nor cleavage of fertilized eggs in Rana pipiens (unpublished observations). Chromosome behavior in these eggs during cleavage was found to be normal at least up to the gastrula stage when the embryos always ceased development. It was noted that α-amanitin treatment did not cause recognizable abnormalities in the transplanted brain nuclei that failed to undergo chromosome condensation or in the oocyte chromosomes, though the latter were prevented from migrating towards the animal cortex. Moreover, the brain chromosomes induced to condense in M II oocytes in the presence of the inhibitor were not morphologically different from those induced in its absence. These observations permit us to assume that α-amanitin suppresses RNA synthesis but does not interfere with any other metabolic processes associated with chromosome condensation in oocytes in the range of doses used in the present study.

On the other hand, actinomycin D not only suppressed the condensation of brain chromosomes in M I oocytes, but also brought about abnormalities in both the interphase brain nuclei and the oocyte chromosomes. We confirmed the observations of Brachet (4) and Baltus et al. (1) that the chromosomes of actinomycin D-treated oocytes either underwent pycnotic degeneration or swelled, forming one or more euchromatic "globules." In addition, brain nuclei when inhibited from undergoing chromosome condensation often showed considerable pycnosis, and chromosomes, if induced to condense, were found to remain as prophase-type chromatids being prevented from

further condensation. These chromosomal abnormalities could result from physical interference with DNA coiling processes since, as Waring (19) has demonstrated, circular $\emptyset$X174 RF supercoiled DNA was unwound at certain actinomycin D concentrations and rewound in the opposite direction at higher concentrations. If physical interference of DNA coiling is the general mechanism whereby chromosome condensation is suppressed, condensation should not be induced in either M I or M II oocytes in the presence of actinomycin D. Since condensation did occur in actinomycin D-treated M II oocytes, its suppression in M I oocytes may be explained on the basis of inhibition of RNA synthesis as in the case of α-amanitin. Thus, it may be concluded that RNA synthesis is required for brain chromosome condensation to occur in M I oocytes, but not in M II oocytes.

It is worth noting that the inhibition of brain chromosome condensation by RNA synthesis inhibitors in M I oocytes was brought about in oocytes which already possessed CCA. This suggests that the inhibition was caused through interference of the inhibitors with the induction processes of chromosome condensation. Since the cytoplasm of the oocytes into which α-amanitin had been administered before progesterone treatment was found to become capable of inducing brain chromosome condensation when the oocytes reached full maturity, it is likely that the RNA synthesis inhibition does not suppress the development of CCA.

If oocyte chromosomes were removed at M I, earlier than the time of transplanting brain nuclei, there was no suppression of the induction of brain chromosome condensation. Apparently no genomic activity of the oocyte chromosomes is involved in the induction process of brain chromosome condensation. Therefore, it is highly probable that the suppression of chromosome condensation by RNA synthesis inhibitors in M I oocytes is due to the inhibition of brain nuclear RNA synthesis. This contention becomes more plausible when it is noted that complete inhibition was always caused in M I oocytes when the brain nuclei were pretreated with α-amanitin regardless of the dose of inhibitor, whereas when the oocytes alone were treated only the higher doses were effective.

In view of the fact that protein synthesis inhibitors also suppress brain chromosome condensation induced by M I oocytes, it appears likely that CCA in M I oocytes induces brain chromosome conden-

sation by eliciting synthesis of brain nuclear RNAs that are then translated into chromosomal proteins. It has been shown that puromycin and cycloheximide administered to *Rana* and *Xenopus* oocytes at doses similar to those used here suppress protein synthesis to a significant extent (16, 1). On the other hand, the fact that chromosome condensation induced by M II oocytes is resistant to both RNA and protein synthesis inhibitors suggests that fully mature oocytes are provided with the full complement of proteins required for promoting chromosome condensation. If fully mature oocytes possess all the gene products required for chromosome condensation, RNA synthesis inhibitors should not prevent the zygote and blastomere nuclei from undergoing chromosome condensation during cleavage, and this is the case (15, 5, 6).

During meiotic maturation the protein synthesis activity of *Rana pipiens* oocytes is elevated, reaching its highest point after the onset of GVBD (9). This protein synthesis appears to contribute to the development of CCA. It does not require immediate activity of the oocyte genome. Indeed, newly synthesized oocyte proteins during maturation in the presence of the GV are indistinguishable in quantity and quality from those synthesized in the absence of the GV (10). However, since removal of the entire GV from oocytes caused a total loss of CCA (20), some GV factor is necessary for the development of CCA in addition to the proteins synthesized in maturing oocyte cytoplasm. This GV factor is nucleoplasmic because the oocyte chromosomes appear not to be involved in the development of CCA as discussed above. This notion is supported by a recent finding that reintroduction of the nuclear sap prepared from isolated GVs after removing chromosomes by centrifugation restores the CCA in the oocytes whose GVs had been removed (unpublished observations).

REFERENCES

1. BALTUS, E., J. BRACHET, J. HANOCQ-QUERTIER, and E. HUBERT. 1973. Cytochemical and biochemical studies on progesterone induced maturation in amphibian oocytes (effects of inhibitors and of a

"maturation promoting factor"). *Differentiation.* 1:127-143.

2. BATAILLON, E. 1928. Études analytiques sur la maturation des oeufs de batraciens. *C. R. Hebd. Seances Acad. Sci.* 187:520-523.

3. BATAILLON, E., and TCHOU-SU. 1934. L'analyse experimentale de la fecondation et sa definition par les processus cinetiques. *Ann. Sci. Nat. Zool.* 17:9-36.

4. BRACHET, J. 1967. Effects of actinomycin, puromycin and cycloheximide upon the maturation of amphibian oocytes. *Exp. Cell Res.* 48:233-236.

5. BRACHET, J., and H. DENIS. 1963. Effects of actinomycin D on morphogenesis. *Nature (Lond.).* 198:205-206.

6. BRACHET, J., H. DENIS, and F. DE VITRY. 1964. The effects of actinomycin D and puromycin on morphogenesis in amphibian eggs and *Acetabularia mediterranea. Dev. Biol.* 9:398-434.

7. BUCCI, S., I. NARDI, G. MANCINO, and L. FIUME. 1971. Incorporation of tritiated uridine in nuclei of *Triturus* oocytes treated with α-amanitin. *Exp. Cell Res.* 69:462-465.

8. DETTLAFF, T. A., L. A. NIKITINA, and O. G. STROEVA. 1964. The role of the germinal vesicle in oocyte maturation in anurans as revealed by the removal and transplantation of nuclei. *J. Embryol. Exp. Morphol.* 12:851-872.

9. ECKER, R. E., and L. D. SMITH. 1968. Protein synthesis in amphibian oocytes and early embryos. *Dev. Biol.* 18:232-249.

10. ECKER, R. E., and L. D. SMITH. 1971. The nature and fate of *Rana pipiens* proteins synthesized during maturation and early cleavage. *Dev. Biol.* 24:559-576.

11. GURDON, J. B. 1967. On the origin and persistence of a cytoplasmic state inducing DNA synthesis in frogs'
eggs. *Proc. Natl. Acad. Sci. U. S. A.* 58:545-552.

12. GURDON, J. B. 1968. Changes in somatic cell nuclei inserted into growing and maturing amphibian oocytes. *J. Embryol. Exp. Morphol.* 20:401-414.

13. JOHNSON, R. T., and P. N. RAO. 1970. Mammalian cell fusion. II. Induction of premature chromosome condensation in interphase nuclei. *Nature (Lond.).* 226:717-722.

14. MASUI, Y. 1967. Relative roles of the pituitary, follicle cells and progesterone in the induction of oocyte maturation in *Rana pipiens. J. Exp. Zool.* 166:365-376.

15. MELTON, C. G. 1963. Effect of Actinomycin D and of protein extracts from differentiated tissue on gene action during amphibian development. *Genetics.* 48:901.

16. SMITH, L. D., and R. E. ECKER. 1969. Role of the oocyte nucleus in physiological maturation in *Rana pipiens. Dev. Biol.* 19:281-309.

17. SMITH, L. D., and R. E. ECKER. 1970. Regulatory processes in the maturation and early cleavage of amphibian eggs. *In* Current Topics in Developmental Biology. A. A. Moscona and A. Monroy, editors. Academic Press, Inc., New York. 5:1-38.

18. TOCCHINI-VALENTINI, G. P., and M. CRIPPA. 1970. Ribosomal RNA synthesis and RNA polymerase. *Nature (Lond.).* 228:993-995.

19. WARING, M. 1970. Variation of the supercoils in closed circular DNA by binding of antibiotics and drugs: Evidence for molecular models involving intercalation. *J. Mol. Biol.* 54:247-279.

20. ZIEGLER, D. H., and Y. MASUI. 1973. Control of chromosome behavior in amphibian oocytes. I. The activity of maturing oocytes inducing chromosome condensation in transplanted brain nuclei. *Dev. Biol.* 35:283-292.