Proc. Nat. Acad. Sci. USA
Vol. 71, No. 3, pp. 690–693, March 1974

Chromosome Cycles Turned on in Unfertilized Sea Urchin Eggs Exposed to NH_4OH

(DNA synthesis/chromosome condensation)

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Contributed by Daniel Mazia, November 11, 1973

ABSTRACT In unfertilized sea urchin eggs treated with NH_4OH–sea water, the chromosomes condense after a first round of DNA synthesis and go through a chromosome cycle. The chromosomes split visibly, but sister chromosomes are not further separated. They regress into an interphase nucleus. These cycles repeat, producing eggs with large numbers of chromosomes. No mitotic apparatus is seen and the eggs do not divide. There is some microscopic evidence of limited chromosome movement, interpreted as centrifugal movement of the condensed chromosomes before they split and a centripetal movement as the split chromosomes decondense to reconstitute the nucleus. The eggs so treated with NH_4OH are considered to be unfertilized eggs and can be fertilized later. Such later fertilization permits the introduction of paternal nuclei after the maternal nuclei have progressed some way toward the above-described chromosome condensation. The paternal chromosomes condense prematurely at the time when the maternal chromosomes condense. At the same time, premature with respect to the time of fertilization, mitotic apparatus form.

The unfertilized sea urchin egg is considered to be a nearly inert cell in which chromosome replication, successive mitotic cell divisions, and the cascade of events of development are released by the act of fertilization. Recent work has shown that some of the important changes that ordinarily follow fertilization are provoked in unfertilized eggs by exposing them to NH_4OH. The eggs so treated are still regarded as unfertilized eggs because they do not show the visible or electrobiological signs of the fertilization reaction and because they can later be fertilized. In the studies carried out so far, the activities that are turned on by the treatment with NH_4OH are those that normally begin some minutes after fertilization: development of K-conductance and membrane potential (1); acceleration of protein synthesis (2); polyadenylation of RNA (3); and increased permeation of thymidine and initiation of DNA synthesis (4). In the present communication, I describe the subsequent condensation of the chromosomes and successive chromosome cycles without cell division in the unfertilized eggs.

MATERIAL AND METHODS

Eggs of two species of sea urchin *Strongylocentrotus purpuratus* and *Lytechinus pictus* were used. "NH_4OH–sea water" was prepared by bringing ordinary sea water to pH 9.0–9.1 with ammonium hydroxide. Unfertilized eggs were suspended in NH_4OH–sea water and stirred gently at 16–17° during the experiment. Occasionally the pH had to be readjusted to 9.0–9.1 by a small addition of NH_4OH. In all the experiments described the eggs remained in the NH_4OH–sea water for 1 hr, after which they were allowed to settle and were resuspended in ordinary sea water, pH 8.1–8.2.

For microscopic observation, samples were gently concentrated on a hand centrifuge and fixed in ethanol–acetic acid (3:1). After overnight fixation, the eggs could be prepared for observation by suspending them in 45 or 75% acetic acid. The commonly used 45% acetic acid produces maximum swelling and clearing of the cytoplasm. The higher concentration of acetic acid is preferable for observation of the whole egg, flattened under coverslip pressure but not disrupted by squashing. Direct phase-contrast observation is satisfactory but can be improved for photographic purposes by staining the eggs in 1% orcein in 75% acetic acid before the squashes are prepared.

OBSERVATIONS AND INTERPRETATIONS

Chromosome Cycles. It has been shown that treatment of unfertilized eggs with NH_4OH turns on DNA synthesis (4). In fertilized eggs, the first round of DNA synthesis, beginning at 20–30 min and completed by 50–60 min after fertilization, is followed shortly by the condensation of the chromosomes. In the unfertilized eggs, condensation of the chromosomes is seen by 60 min after the beginning of exposure to NH_4OH–sea water. Figs. 1*A* and *B* show the prophase figures at that time. The condensation proceeds and the nuclear membrane breaks down as in normal mitosis. At this stage, equivalent to "prometaphase" in the fertilized egg, the chromosomes are not yet fully condensed (Fig. 1*C*). The number of chromosomes is haploid, as would be expected. The number is tentatively reported as 22. The prometaphase-equivalent stage seems favorable, not only for counting the chromosomes but for recognition of individual members of the set. By the next stage, the equivalent of metaphase, the chromosomes are very much more condensed (Fig. 1*D*). At this metaphase-equivalent stage, the chromosomes lie in a small clear zone in the egg and are not oriented with respect to the poles. No poles are discerned in contrast to fertilized or parthenogenetically activated eggs in which poles are clearly indicated by asters at this stage of chromosome condensation. After the metaphase-equivalent condensation of the chromosomes, they appear to move centrifugally outward on the periphery of the clear zone in which they lie. Then they are seen as definitely split into sister pairs, as is shown in Fig. 1*E*. The chromosomes now begin to decondense, becoming clearly longer. Sister chromosomes tend to remain side by side (Fig. 1*F*). As they are decondensing, the chromosomes seem to be moving centripetally. In the progress of decondensation, the chromosomes

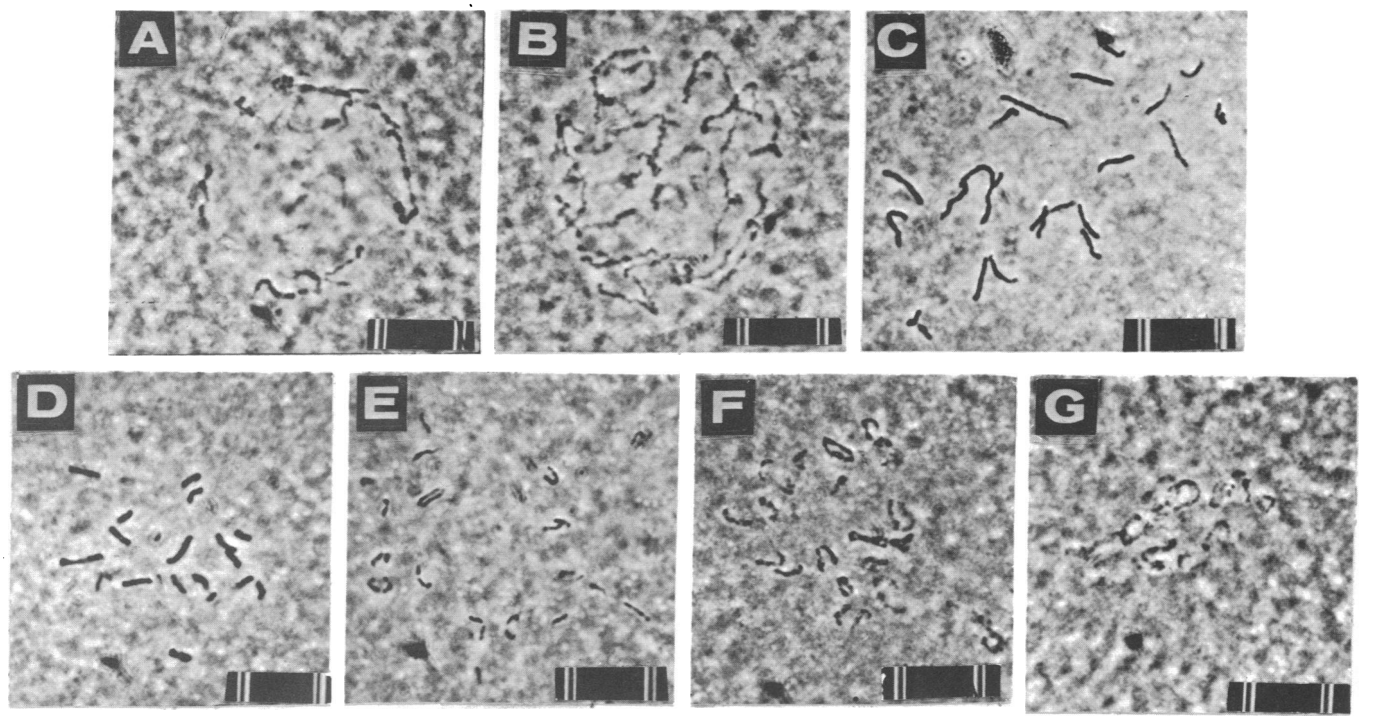

FIG. 1. The first chromosome cycle of unfertilized eggs of *Strongylocentrotus purpuratus* exposed for 1 hr to NH_4OH–sea water, then transferred to sea water. Time is given in minutes from the beginning of NH_4OH treatment. Synchrony is imperfect and different stages are seen at the same time. Scale division = 10 μm. (*A*) Midprophase (60 min). (*B*) Late prophase (60 min). (*C*) Prometaphase-equivalent (60 min). Nuclear envelope has broken down. Chromosomes spread well at this stage. (*D*) Metaphase-equivalent (95 min). Chromosomes much more condensed, compared with (*C*). Chromosomes unsplit. (*E*) Chromosomes clearly split (105 min); condensed sister chromosomes seldom separate farther than is seen in this example. (*F*) Chromosomes decondensing (120 min). Sister chromosomes still adjacent. (*G*) Later telophase equivalent (120 min). Chromosomes forming vesicles but condensed regions are still seen. The vesicles will fuse into an interphase nucleus.

appear to be forming vesicles in which condensed regions of chromatin are completing their decondensation. Finally these vesicles fuse to restore the interphase nucleus; sometimes round, sometimes lobed, sometimes subdivided into several karyomeres (Fig. 1*G*).

A note of caution: while the observations of the chromosomes themselves are straightforward, the description of apparent centrifugal and centripetal movement of the chromosomes is only a tentative interpretation of what is seen in fixed images. Since there are no poles, the only frame of reference is the clear zone in which the chromosomes lie, a zone made visible by the relative absence of large particles which occupy the rest of the cytoplasm. A useful terminology may be borrowed from the historic description of mitosis by Flemming in 1880 (5). The events up to the splitting of the chromosomes can be designated as the *progressive* phase. In the present interpretation, the chromosomes move outward as the clear zone expands in the progressive phase. They split and gather inward during the *regressive* phase, reforming a single interphase nucleus. The impression of chromosome movement during the progressive phase becomes more evident in later cycles (Fig. 2).

Otherwise there is no sign of a mitotic apparatus during the chromosome cycle of the eggs treated with NH_4OH, although

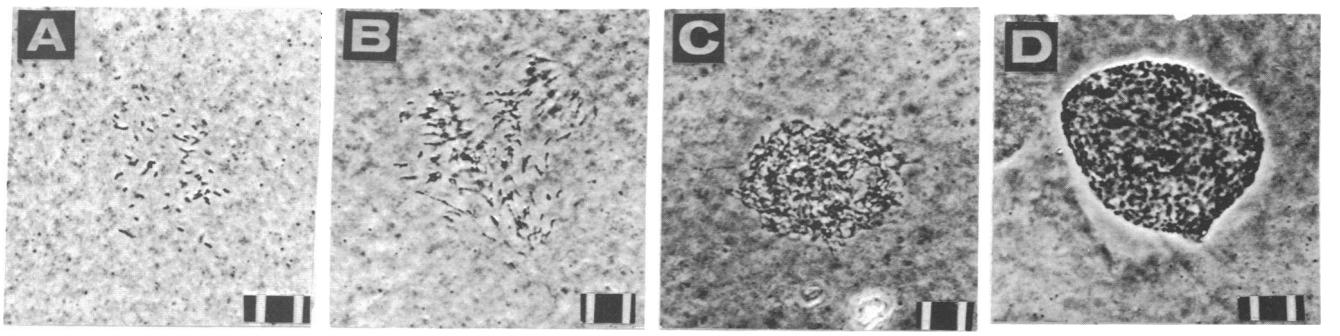

FIG. 2. Continuation of chromosome cycles in unfertilized eggs of *Lytechinus pictus* which were exposed to NH_4OH–sea water for 1 hr, then transferred to sea water. Scale division = 10 μm. (*A*) Chromosomes at 200 min; diploid chromosome number; not all chromosomes in view. (*B*) Chromosomes at 6 hr, showing indications of centrifugal movement. (*C*) Chromosomes at 18 hr. (*D*) Nucleus at 18 hr; there is still a single nucleus and the egg has not divided.

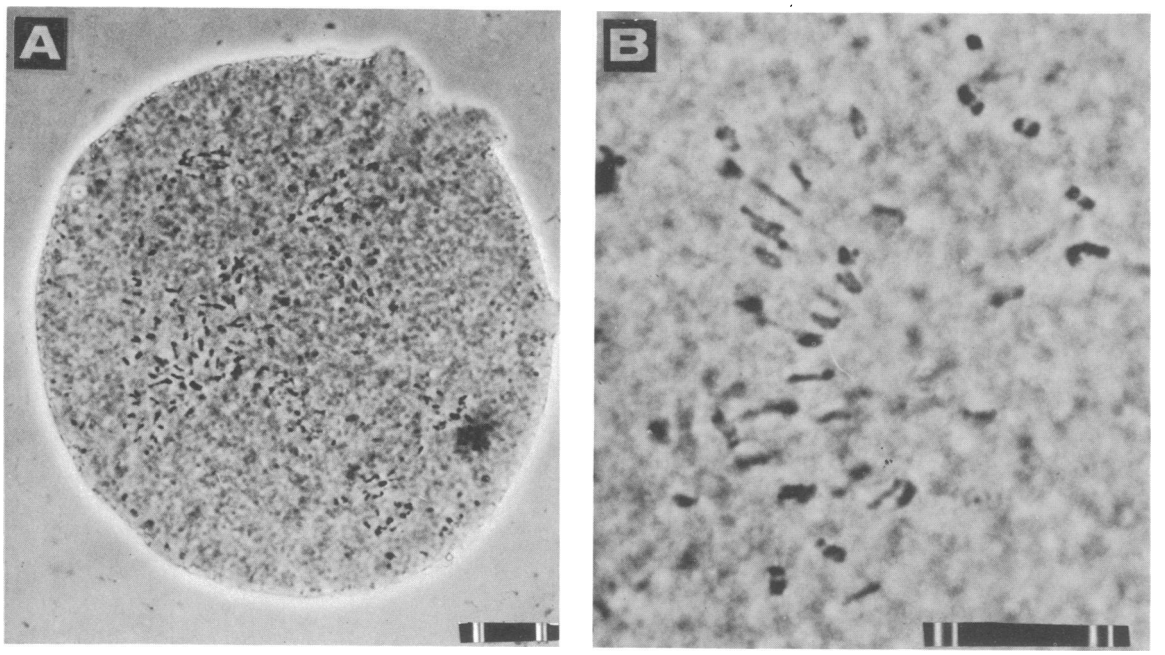

FIG. 3. Premature condensation of male chromosomes in eggs of *Strongylocentrotus purpuratus*. Unfertilized eggs were kept in NH_4OH–sea water for 60 min, transferred to sea water, inseminated heavily, and fixed at 45 min after fertilization. Scale division = 10 μm. (*A*) Squash of whole egg. Many sperm had entered and all the male chromosomes have condensed. (*B*) Greater magnification of part of an egg. Thicker chromosomes (*upper right*) interpreted as maternal, thin chromosomes as paternal. Alignments and stretching of chromosomes are signs of spindle activity.

the mitotic apparatus can be seen quite clearly in living fertilized eggs in normal mitosis. No asters or spindles are seen, and the egg does not divide.

Later Cycles. Some time after the interphase nucleus has been reconstituted, the chromosomes are again seen to be in prophase. They continue into the chromosome cycle, and at the second prophase-equivalent or metaphase-equivalent it is evident that the chromosome number is twice that in the first cycle (Fig. 2*B*). The progressive and regressive changes are seen and the interphase nucleus reforms. The cycles repeat themselves and at later cycles very large numbers of chromosomes are seen (Figs. 2*C* and *D*). It is in the later cycles where the evidence of centripetal movement of the chromosomes is most conspicuous. In some cells, the chromosomes may seem to move outward radially, as though they are disposed around the surface of an expanding sphere. In other cells, the chromosomes give the impression of moving in groups and in an arrangement that is roughly reminiscent of a multipolar mitotic figure (Fig. 2*D*). However, no poles are detected and the groups of chromosomes are not separated; they come together in the regressive phase and form a single nucleus.

Ultimately one observes a single egg with a huge nucleus, often with long-lobed extensions (Fig. 2*E*).

Fertilization of NH_4OH-Treated Eggs. As has been mentioned, the eggs that have been provoked into the chromosome cycle by treatment with NH_4OH are regarded as unfertilized eggs because they can later be fertilized. If these eggs are fertilized at any time after the beginning of the exposure to NH_4OH, the male nuclei will be started on their way to chromosome condensation that much later than the maternal nuclei. The experiment to be described in detail is one in which the eggs were kept in NH_4OH–sea water for 1 hr, then transferred to sea water and fertilized. They were fixed 45 min later. At that time, ordinary fertilized eggs would be in mitosis, with condensed chromosomes. Unfertilized eggs treated with NH_4OH would also have condensed chromosomes, the haploid maternal set. The sperm nuclei in eggs fertilized 1 hr after the beginning of the treatment with NH_4OH would be only 45 min into their cycle; in a normal fertilized egg they would be decondensed in interphase at that time.

In the experiment described, the basic observation is that the paternal chromosomes are observed as condensed chromosomes; the condensation is premature by about an hour. The NH_4OH treatment makes the eggs somewhat susceptible to polyspermy, so that one generally sees several groups of condensed paternal chromosomes, along with one group of condensed maternal chromosomes (Fig. 3*A*).

The condensed paternal chromosomes appear to be thinner than the maternal chromosomes (Fig. 3*B*). One possible explanation is that they have not replicated, either because they have not had sufficient time or because the egg had shut down its machinery for initiating DNA synthesis after the maternal chromosomes had completed (or nearly) their replication at the time of fertilization. An alternative explanation is that the apparent thinness of the paternal chromosomes is a structural abnormality, since they have not had the normal time to go from their supercompact state in the sperm head to their decondensed interphase condition before being forced into mitotic condensation.

These results confirm the illuminating discovery of Johnson and Rao (6), who fused cells of different kinds in different phases of the cell cycle, that the presence of condensed chromosomes in a cell will force the premature condensation of all other chromosomes in that cell.

The Mitotic Apparatus. In the NH_4OH-treated eggs, no mitotic apparatus is formed. In normal fertilization, the mitotic apparatus forms in close coordination with the chromosome cycle, a coordination that is one of the general problems in understanding mitosis. In the experiments just discussed, in which eggs are fertilized after the maternal chromosomes have advanced in their cycle, a mitotic apparatus forms. Asters are seen; chromosomes seem to be connected to poles by fibers. In these preliminary studies, the connection of the male chromosomes to a nearby aster is especially easy to observe though hard to photograph; whether the maternal chromosomes are engaged is not entirely clear, but they are sometimes seen to be aligned, as though on metaphase plates. Pending further work, the important point is not that a mitotic apparatus forms, which it should do in a fertilized egg, but that it forms precociously in real time. Relative to the time of fertilization, the mitotic apparatus is forming an hour prematurely. Relative to the stage of the chromosomes, it is forming at the right time. It is as though the same state of the cell that forces the premature condensation of chromosomes also imposes a premature formation of a mitotic apparatus.

The absence of a mitotic apparatus in the eggs treated with NH_4OH and its appearance after these eggs have been fertilized is easy to explain in classical terms. The older theories of fertilization (8) stated that the spermatozoon introduced the centriole, which is the focus for the formation of astral rays and spindle fibers. Artificial parthenogenetic activation of eggs is successful when it provokes the appearance of asters in which centrioles develop. In the experiments with NH_4OH, we do not see the recognized signs of parthenogenetic activation, neither the initial changes in membrane potential (9) nor the appearance of asters. When the eggs are fertilized later, the admission of the sperm, presumably the centrioles, sets in motion the formation of the mitotic apparatus.

DISCUSSION

To the demonstration that the treatment of unfertilized sea urchin eggs with NH_4OH turns on DNA synthesis (4), the present work adds these findings: (*i*) the DNA synthesis leads to chromosome condensation and (*ii*) the DNA synthesis is a replication, since the chromosomes are not only seen as doubled but go through successive cycles yielding ever-increasing numbers of chromosomes. The effect of the NH_4OH is that of a triggering or releasing process, since the chromosome cycles described proceed after the eggs have been returned to the normal sea water environment. In fact, the progress through the first cycle is impeded if the eggs are left in NH_4OH, although they begin their condensation on time. The interpretation of what the NH_4OH as such does will not be discussed here; some possibilities are considered in other publications (1, 4). If chromosome replication or condensation require protein synthesis, that is provided for, since the NH_4OH treatment turns on polyadenylation of RNA (3) and protein synthesis (2).

The cycle of the chromosomes themselves is fairly normal up to the time when sister chromosomes split apart. The very clear splitting is additional evidence for the generalization (10) that the first step in chromosome separation at mitosis does not depend on the action of the mitotic apparatus but is a distinct event. There is no further separation; the split chromosomes remain more or less side by side in the regressive phase leading to the reconstitution of the interphase nucleus. The decondensation of the chromosomes in the regressive phase looks different from the normal anaphase–telophase transition. In the normal mitosis in the sea urchin egg, the condensed anaphase chromosomes swell abruptly to form chromosome vesicles. In the eggs treated with NH_4OH, the chromosomes elongate after splitting, but here too they form vesicles that fuse to form the interphase nucleus.

The apparent centrifugal movement of the still-unsplit chromosomes in the progressive phase, and the apparent inward movement after they have split, is a very tentative interpretation of phase-microscopic observations of fixed material. What is interesting about it, and will be investigated by electron microscopy, is what might be learned about chromosome movement in a system where there is no evidence of poles or a spindle.

When the eggs are fertilized some time after the beginning of exposure to NH_4OH, the paternal chromosomes condense prematurely at the time the maternal chromosomes condense. The experiments confirm the discovery (6, 7) of a chromosome-condensing condition or factor that prevades the whole cell and condenses all the chromosomes in that cell. With this confirmation, eggs may be considered as experimental material for analytical studies of the chromosome-condensing factor, since they would provide large quantities of cells entering mitosis synchronously.

A new observation is that the mitotic apparatus forms prematurely when the male chromosomes are made to condense prematurely. The close coordination of the condensation of chromosomes and the formation of the mitotic apparatus is a fundamental problem of normal mitosis. One can now propose the simplest explanation: that the factor that induces chromosome condensation and the factor that induces the formation of the mitotic apparatus are the same.

This work was supported by Program-Project Grant GM-13882 from the National Institutes of Health.

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