Desynchronization of cell divisions in the course of egg cleavage and an attempt at experimental shift of its onset

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There is considerable current interest in the study of regularities and modes of regulation of cell cycles in the course of development (Dettlaff, 1964; Graham, Arms & Gurdon, 1966; Chulitskaia, 1967a). The period of cleavage divisions is a very suitable object for such investigations. It consists of two different periods known as periods of synchronous and asynchronous cell divisions (Syngayewskaya, 1931; Balinsky, 1931; Costello, 1955; Sirakami, 1958; Neyfakh & Rott, 1959; Dan, 1960; Pankova, 1963; Agrell, 1964; Engelberg, 1964; Skoblina, 1965; Chulitskaia, 1967b; Rott & Sheveleva, 1968). Peculiarities of these two periods are known rather well but regularities of the transition from synchronous to asynchronous divisions are still imperfectly understood (Rückert, 1899; Hoff-Jørgensen & Zeuthen, 1952; Agrell & Person, 1956; Neyfakh & Rott, 1959; Neyfakh, 1961; Agrell, 1961, 1962; Pankova, 1963; Brown, 1965; Bachvarova, Davidson, Allfrey & Mirsky, 1966; Timofeeva, Kafiani & Neyfakh, 1967; Chulitskaia, 1967a, b; Rott & Sheveleva, 1968).

The present investigation was designed to study the time and character of the transition from synchronous to asynchronous cleavage divisions at different temperatures. The correlation between the time of appearance of different mitotic phases in various cells and the time of increase in interphase duration as expressed by a decrease of the mitotic index has been studied.

Three aspects of cell division have been studied: (a) the stage when nuclei in more than two neighbouring mitotic phases at any one time were first observed in one embryo; (b) the stage when the mitotic index first decreased; and (c) the duration of the period between stage (a) and stage (b).

On the other hand we studied the influence of the cytoplasm on cell cycle in this transitional period. We concluded from data previously obtained (Dettlaff, Nikitina & Stroeva, 1964) that the cytoplasm of immature oocytes inhibits division of somatic nuclei transplanted into it and the cytoplasm of mature

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oocytes stimulates the division of nuclei and exerts a synchronizing effect on nuclei of different origin. Somewhat later these results were confirmed by Graham et al. (1966) and Gurdon (1967). They discovered a new very important phenomenon—the induction of DNA synthesis in somatic nuclei under the influence of the cytoplasm of mature oocytes. In all the experiments mentioned above, the influence of the cytoplasm on nuclei was studied by means of transplantation of somatic cell nuclei into the cytoplasm of oocytes or mature eggs. In the present investigation a converse experiment was carried out—the cytoplasm of mature eggs was injected into developing embryos. We studied the effect of the cytoplasm on desynchronization of cell divisions and on the mitotic index in the period of the transition from synchronous to asynchronous cell divisions.

MATERIALS AND METHODS

Experiments were carried out on the eggs of Acipenser güldenstädti and Rana temporaria, in Spring 1964–66. Sturgeon and frog eggs were inseminated and incubated in a Heppler's ultrathermostat at different temperatures: frog eggs—at 7°, 13°, 18°, 23°C and sturgeon eggs at 17°, 20° and 23°C. Fixations of sturgeon and frog eggs were performed at all the temperatures studied, from the 7th cell division (which is still quite synchronous at all the temperatures studied) onwards during the subsequent eight cell cycles. The interval between fixations was equal to one cell cycle (τ₀); τ₀—means the duration of the shortest cell cycle, i.e. the cell cycle during synchronous cleavage divisions (Dettlaff & Dettlaff, 1961). It corresponds to the time between the appearance of two successive cleavage furrows on the egg surface. Using the previously obtained values of τ₀ at different temperatures (Dettlaff & Dettlaff, 1961; Chulitskaia, 1965) we estimated the time of fixation of embryos. The first fixation was performed after the appearance of the 7th furrow on the egg surface, i.e. after the time necessary for the transition of the cell nucleus from interphase to metaphase (Dettlaff, 1963).

However, as this time is not estimated exactly for various temperatures, sometimes embryos proved to be fixed not at metaphase, but at meta- or prometa- and metaphase. As successive cleavage stages during the period of asynchronous divisions cannot be characterized by the number of nuclear divisions, we designated them by the number of τ₀ from the onset of nuclear divisions, i.e. from metaphase of the first cleavage division.

Sturgeon embryos were fixed with Sanfelice fluid, frog embryos with Bouin’s fluid. Sections of sturgeon embryos 10 μ thick were stained with haematoxylin by the Heidenhain method; sections of frog embryos were stained according to Mann. 5–10 embryos were studied for each temperature and each time of fixation.

In order to study the influence of the cytoplasm on the cell cycle, sturgeon eggs were inseminated and incubated in Heppler's ultrathermostat at 20°. The cyto-
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Plasm to be injected was taken with a glass micropipette from the animal region of a mature unfertilized egg (4–5% of the total volume of cytoplasm) and injected into the animal portion of developing embryos of the same female, in the region between furrows at the stage of the 4th cleavage. At this stage the furrows do not completely divide the cytoplasm and the endoplasm of the blastomeres is not completely disconnected.

In addition, experiments were carried out using an injection of the same volume consisting of the supernatant fluid obtained by homogenization of 250–300 mature eggs in 4 ml Tris-buffer (pH 7.8), followed by centrifugation of the homogenate for 20 min at 5000 g. The supernatant while on ice was evaporated under an air stream to approximately \( \frac{1}{3} \) of the initial volume. As the volume of the sturgeon egg is about 0.014 ml, it follows that the concentration of substances which passed from the cytoplasm into the supernatant increased approximately 2-5 times. After the appearance of the fourth cleavage furrows control embryos were injected with cytoplasm from embryos of the same age. All the injected embryos were cultivated in Holtfreter solution with sulphasin and penicillin. Most of them cleaved normally, those with abnormal cleavage were excluded.

Embryos were fixed from 8 \( \tau_0 \) onwards till 11 \( \tau_0 \) at intervals equal to 1 \( \tau_0 \). Some eggs were fixed at 15 \( \tau_0 \) and 16 \( \tau_0 \) for estimation of the mitotic index (MI), as in normal eggs it decreases sharply at 15 \( \tau_0 \). Finally the time of gastrulation was determined in some eggs from the groups injected with cytoplasm, and some eggs from the control group and untreated group. Embryos were fixed with a mixture containing 7.5 ml 70% alcohol, 2.5 ml 40% formalin and 0.5 ml glacial acetic acid. The use of this method of fixation excluded the passage of the egg contents through the holes in the membranes, produced by the micropipette, which took place after fixation with Sanfelice fluid, Bouin, Zenker and 4% formalin. The mixture used did not change the form of embryos and gave satisfactory results with subsequent treatment. Sections 10 \( \mu \) thick were stained with haematoxylin according to Regaud and Heidenhain. Material fixed at 15–16 \( \tau_0 \) was counterstained with methylgreenpyronin. 15–20 embryos were studied at each cell division in the period of the transition from synchronous to asynchronous divisions.

In all experiments, regularities of the transition from synchronous to asynchronous divisions were studied on blastomeres of the animal region where there were a lot of nuclei by this time. Mitotic phases were determined in all the cell nuclei of the animal region and marginal zone in all the sections of each embryo. In order to follow changes of the mitotic index in the period of transition from synchronous to asynchronous cell divisions, we compared the number of different mitotic phases in nuclei of different cells at successive cleavage stages in the period of asynchronous divisions, with their relative duration in the period of synchronous divisions (Dettlaff, 1963). The number of nuclei at each phase was expressed in % of the total number of nuclei counted. After 15 \( \tau_0 \),
when there were already a lot of nuclei in embryos, the percentage of dividing nuclei (at prometa-, meta-, ana- and telophase) was estimated in 1000 nuclei. As fixation could be performed at the stage of the transition from one mitotic phase to another, the occurrence of two neighbouring phases was still considered characteristic of a synchronous division. Simultaneous occurrence in an embryo of nuclei at three mitotic phases was considered as the onset of desynchronization of cell divisions. A division in which the number of nuclei at the third mitotic phase was more than 5% of the total number of nuclei was regarded as the first desynchronized division.

In addition, in sturgeon embryos the structure of the nucleus was studied at the stages of transition from synchronous to asynchronous cell divisions.

Fig. 1. Occurrence of mitotic phases (in %) in successive cleavage divisions at different temperatures in embryos of *Rana temporaria*. □, 1 = interphase + prophase; ☐, 2 = prometaphase; ☐, 3 = metaphase; ☐, 4 = anaphase; ■, 5 = telophase.
RESULTS

I. The character of the transition from synchronous to asynchronous cleavage divisions in sturgeon and frog embryos at different temperatures

Figs. 1 and 2 give the number of different mitotic phases in the animal region and marginal zone in frog and sturgeon embryos at successive cleavage stages at different temperatures (in % of the total number of nuclei). In both species the transition from the synchronous to asynchronous divisions has some common

Table 1. Number of nuclei at different mitotic phases (in % of the total number of nuclei) in embryos of Rana temporaria in the period between 11 and 14 $\tau_0$ at different temperatures

<table>
<thead>
<tr>
<th>Temperature ($^\circ$C)</th>
<th>Total number of nuclei</th>
<th>Interphase+prophase</th>
<th>Prometaphase</th>
<th>Metaphase</th>
<th>Anaphase</th>
<th>Telophase</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>1668</td>
<td>43-8</td>
<td>2-1</td>
<td>27-0</td>
<td>16-1</td>
<td>11-0</td>
</tr>
<tr>
<td>18</td>
<td>1547</td>
<td>51-5</td>
<td>9-1</td>
<td>28-1</td>
<td>9-5</td>
<td>1-8</td>
</tr>
<tr>
<td>13</td>
<td>3723</td>
<td>47-4</td>
<td>14-8</td>
<td>19-2</td>
<td>12-1</td>
<td>6-5</td>
</tr>
<tr>
<td>7</td>
<td>1753</td>
<td>45-5</td>
<td>11-1</td>
<td>20-8</td>
<td>10-9</td>
<td>11-7</td>
</tr>
<tr>
<td>Average values</td>
<td>8691</td>
<td>47-1</td>
<td>9-3</td>
<td>23-8</td>
<td>12-1</td>
<td>7-7</td>
</tr>
</tbody>
</table>

The same in proportions of the total number of nuclei taken as 1

<table>
<thead>
<tr>
<th>Relative duration of mitotic phases during one nuclear division in the period of synchronous cleavage divisions (in $\tau_0$) after Dettlaff (1964)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.47</td>
</tr>
</tbody>
</table>

Features. At the optimum temperatures (14–20 °C for the sturgeon and 13–21 °C for the frog) the first single nuclei at neighbouring mitotic phases appear among synchronously dividing nuclei and at the next division many nuclei are already at different mitotic phases. At the higher temperatures this transition proceeds within one division when many nuclei are simultaneously at different mitotic phases. At the lower temperatures single nuclei at the third mitotic phase appear during two divisions and only at the third division are nuclei at different mitotic phases (note the distance between lines $b$ and $c$ in Fig. 3). After that, synchronous divisions are still predominant. Later, after complete desynchronization of nuclear divisions for some period the nuclei occur at all mitotic phases but in the same ratio as that of the duration of each mitotic phase during synchronous
cleavage divisions. The end of this period is characterized by a decrease of mitotic index and occurs at all the temperatures synchronously, at the same division.

These regularities are most clearly revealed in frog embryos. At $8 \tau_0$ only synchronously dividing nuclei are found in these embryos: in the animal region all the nuclei still divide with the same rhythm and are at the same, or at two neighbouring mitotic phases. Desynchronized nuclei appear at $23 ^\circ C$ at $9 \tau_0$, at $18 ^\circ C$—at $10 \tau_0$, at $13 ^\circ C$ and $7 ^\circ C$—at $11 \tau_0$ from metaphase of the first cleavage division (see Fig. 1 and Table 2). After complete desynchronization within the interval between 11 and 14 $\tau_0$ (see Table 1) at all the temperatures

### Table 2. Time of the onset of desynchronization of nuclear divisions and of the decrease in mitotic index in different nuclei in blastomeres of the animal region and marginal zone at different temperatures in frog embryos

<table>
<thead>
<tr>
<th>Temperature ($^\circ C$)</th>
<th>Number of embryos studied</th>
<th>Time in $\tau_0$ of the first appearance of desynchronized nuclei in different cells of the same embryo</th>
<th>Occurrence in % (prometa-, meta-, ana- and telophase) in the period between 11–14 $\tau_0$ from the beginning of cell divisions</th>
<th>MI at the 15 $\tau_0$ stage from metaphase of the first cleavage division</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>37</td>
<td>9</td>
<td>56</td>
<td>23-9</td>
</tr>
<tr>
<td>18</td>
<td>40</td>
<td>10</td>
<td>48</td>
<td>17-4</td>
</tr>
<tr>
<td>13</td>
<td>37</td>
<td>11 (10)*</td>
<td>52</td>
<td>15-9</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>11 (9)</td>
<td>54</td>
<td>20-2</td>
</tr>
</tbody>
</table>

(mean value 52) (mean value 19)

* In brackets—time (in $\tau_0$) of desynchronization of cell divisions in single nuclei (meaning that the number of nuclei in a third mitotic phase does not exceed 5%).

about a half of the nuclei (47%) are at interphase + prophase, 9% at prometaphase, 24% at metaphase, 12% at anaphase and 8% at telophase. Relative duration of mitotic phases during one nuclear division in the period of synchronous cleavage divisions equals 0-50, 0-10, 0-20, 0-14 and 0-06 correspondingly. Although in the period of 11–14 $\tau_0$, the number of interphase and prophase nuclei amounts to about 50% of the total number of nuclei, the mitotic index is still close to the average mitotic index during synchronous division. The average mitotic index can be considered equal to 50 during the period of synchronous cleavage divisions as half of this time all the nuclei are in active mitotic phases (MI = 100) and the other half of the time they are at interphase and prophase (MI = 0).

A sharp decrease in MI occurring within the one cell division is observed only in the embryos fixed at 15 $\tau_0$ from metaphase of the first cleavage division. The percentage of nuclei in the active mitotic phases decreases sharply (Table 2:
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from 52% in the period of 11–14 \( \tau_0 \) to 19% at the 15 \( \tau_0 \) stage). Accordingly, the percentage of cells at interphase and prophase increases.

Similar but less precise data were obtained for sturgeon embryos (Figs. 2, 3). In sturgeon embryos, the onset of desynchronization also depends on temperature: at 23 °C desynchronization begins at 8 \( \tau_0 \), at 20 °C—at 9 \( \tau_0 \) and at 17 °C—at 10 \( \tau_0 \). At subsequent stages in the interval between 12–14 \( \tau_0 \) from metaphase

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**Fig. 2.** Occurrence of mitotic phases (in %) in successive cleavage divisions at different temperatures in embryos of *Acipenser guliendrai*. Key as in Fig. 1.

**Fig. 3.** Time of the onset of desynchronization of cell divisions and of decrease in mitotic index in sturgeon embryos at different temperatures. \( a = \) Period of synchronous cleavage divisions; \( b = \) first appearance of desynchronized nuclei; \( c = \) onset of desynchronization; \( d = \) period of desynchronization; \( e = \) asynchronous period; \( f = \) onset of gastrulation.
of the first cleavage division in each embryo, nuclei occur simultaneously at all mitotic phases and the average relative number of different mitotic phases approximately corresponds to their relative duration in the period of synchronous cleavage divisions. Only at 23 °C, i.e. at a temperature close to harmful ones, is there a high percentage of interphases in the early stages of cleavage divisions.

The study of the structure of interphase nuclei in sturgeon embryos at different times during desynchronization has shown that at the beginning of this period nuclei have karyomere structure and nucleoli are absent at 20 °C. Nucleoli appear in 4–5 \( \tau_0 \) after the onset of desynchronization although nuclei still have karyomere structure; the latter disappears only after the decrease of the mitotic index (Chulitskaia, 1967a). Thus, the appearance of typical interphase nuclei is not connected with the onset of desynchronization.

The data obtained on frog and sturgeon embryos show that there is a certain transition period between synchronous and asynchronous cleavage divisions which may be called the period of desynchronization. It differs from the period of synchronous cleavage divisions by desynchronization of mitotic phases in nuclei of different blastomeres, and from the period of asynchronous cleavage divisions—by the absence of a decrease of the mitotic index. Desynchronization begins either at earlier or later cleavage stages at different temperatures. The end of the period of desynchronization is characterized by a sharp decrease of the mitotic index and does not depend on temperature. Hence, there is no strict correlation between the onset of desynchronization and the decrease of the mitotic index at different temperatures.

Unlike the period of desynchronization, the asynchronous period always starts at all the temperatures at the same time (expressed in \( \tau_0 \)). The end of this period being at the same time the onset of gastrulation is attributed to a definite \( \tau_0 \) only at the optimum temperatures, but varies markedly at extreme temperatures (Fig. 3).

II. Injection of the cytoplasm into developing sturgeon embryos

In all the experiments, the injection into developing embryos of the cytoplasm or the supernatant of the mature egg homogenate resulted in prolongation of the period of synchronous cleavage divisions. The data obtained are presented in Figs. 4 and 5.

In normally developing embryos nuclei in all the cells of the animal region still divide synchronously at the stages 7 \( \tau_0 \) and 8 \( \tau_0 \) at 20 °C. Desynchronized nuclei first appear at the 9 \( \tau_0 \) stage. At this stage nuclei occur in four mitotic phases. In embryos injected with the cytoplasm of mature eggs, nuclei divide synchronously not only at the 8 \( \tau_0 \) stage, but also at 9 \( \tau_0 \). At the 8 \( \tau_0 \) stage in all the embryos nuclei are in the same or in neighbouring mitotic phases; at the 9 \( \tau_0 \) stage in more than half the embryos nuclei occur in a third mitotic phase, but their number does not exceed 3 %. At the 10 \( \tau_0 \) stage in all the
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Embryos nuclei occur in three or four mitotic phases simultaneously. At the 11 $\tau_0$ stage in all the embryos studied nuclei occur in five mitotic phases.

In embryos injected with the supernatant from homogenized mature eggs, nuclei divide synchronously not only at the 8 $\tau_0$ and 9 $\tau_0$ stages, as was the case with the cytoplasm injection, but also at the 10 $\tau_0$ stage. At the 8 $\tau_0$ and 9 $\tau_0$ stages nuclei in all the embryos are in the same or in two neighbouring mitotic phases. At the 10 $\tau_0$ stage in the majority of embryos nuclei are still in one or two neighbouring mitotic phases and only in some embryos are there single nuclei in a third or fourth mitotic phase: the number of such nuclei does not exceed 4% of the total number of nuclei counted. At the 11 $\tau_0$ stage nuclei occur in four mitotic phases.

In control embryos injected with cytoplasm of the same age, nuclei still divide synchronously at the 8 $\tau_0$ stage; only in some embryos single nuclei occur in a third mitotic phase, but their number does not exceed 3%. At the 9 $\tau_0$ stage in almost all the embryos nuclei occur in three mitotic phases and the number of such nuclei exceeds 5%. At the 10 $\tau_0$ and 11 $\tau_0$ stages four or five mitotic phases are observed simultaneously.

The desynchronization of cell divisions in control embryos, injected with cytoplasm of the same age, does not differ from that in normal intact embryos. As in normal embryos, in control ones stage 8 $\tau_0$ (the 9th cell division) at 20 °C is the last synchronous division and from stage 9 $\tau_0$ (the 10th cell division) desynchronization of mitotic phases in nuclei of different blastomeres is observed. After the injection of cytoplasm from mature unfertilized eggs into the cytoplasm of cleaving embryos desynchronization starts one division later; after injection of the supernatant of homogenized eggs—two divisions later than in intact embryos.
In all the experimental groups, the mitotic index was estimated for 8–10 embryos. At the 15 $\tau_0$ stage in normal embryos and in those injected with cytoplasm of unfertilized eggs and cytoplasm of the same age the mitotic index decreased sharply and equalled 11·8, 10·8, 16·9 and 13·4 respectively. At the 16 $\tau_0$

**Fig. 5.** Desynchronization of cleavage divisions in sturgeon embryos under the influence of cytoplasm. N, K, C, H—as in Fig. 4. Numbers of nuclei < 5 % at a given mitotic phase are designated by the thick line.

stage the values of the mitotic index in the groups of embryos studied were similar and equal to 8·1, 6·5, 6·4 and 7·9 respectively. These data indicate that the injection of 3–5 % (by volume) of the mature egg cytoplasm into cleaving embryos shifts the onset of desynchronization to a later division, but does not influence the timing of a sharp decrease of the mitotic index. It has no effect on the onset of gastrulation either: in all developing embryos studied the duration of the period
between insemination and the onset of gastrulation was practically constant and equal to $20 \tau_0$.

**Discussion**

In the present paper the period of transition from synchronous to asynchronous cell divisions was studied in blastomeres of the animal region in sturgeon and frog embryos. The period under study differs from the synchronous period by desynchronization of cell divisions and from the asynchronous period—by the absence of a decrease in the mitotic index.

The onset of desynchronization is characterized by the appearance, first, of single desynchronized nuclei and at the next division many such nuclei. The onset of desynchronization depends on the temperature: at high temperatures the disturbance of synchrony occurs at earlier stages and occurs within one mitotic cycle in many nuclei simultaneously. At low temperatures it occurs at a later cleavage stage and in a smaller number of nuclei. The onset of desynchronization, when synchronously dividing nuclei are still predominant, is followed by a complete desynchronization of cell divisions when the relative number of different mitotic phases in the cells corresponds on the average with the relative duration of these phases during the synchronous period. The mitotic index by this time is approximately equal to 53%, if telophase is considered as an active mitotic phase, and equals about 46%, if telophase is counted with interphase.

The end of the period of desynchronization and the onset of the asynchronous period (the middle blastula stage) is characterized by a sharp decrease of the mitotic index: within one cell division it decreases from 53% to 19%. This decrease coincides in time, according to Neyfakh (1961), with the onset of morphogenetic nuclear function. Relative durations of the synchronous period and of the period of desynchronization vary at different temperatures owing to the shift of the onset of desynchronization to earlier or later cleavage stages. Thus, according to the data presented in Fig. 3 the period of desynchronization at 23°C equals 35% of the whole cleavage period (from the first cleavage metaphase to the onset of gastrulation), at 20°C—20%, at 17°C—24%, at 12°C—13–15%. The duration of the asynchronous period is rather constant at optimum temperatures; at low temperatures it increases by $2 \tau_0$ owing to the shift of the onset of gastrulation to later stages (Krönig, 1960).

Low temperatures have a synchronizing effect on the cells of sturgeon and frog embryos, i.e. have the same effect as on HeLa cells (Rao & Engelberg, 1966). However, in blastomeres of frog and sturgeon embryos the frequency of metaphase does not increase at low temperatures. Unlike low temperatures, high temperatures accelerate the onset of desynchronization.

Many years ago Rückert (1899) found in the course of the division of different blastomeres of Elasmobranchia embryos a period during which multiple numbers of blastomeres were still produced, even though nuclei were already dividing asynchronously. A similar phenomenon was found in loach embryos (Rott &
Sheveleva, 1968). They showed that a complete desynchronization of cell divisions in loach embryos occurred one cell cycle earlier than a decrease of the mitotic index. This suggests the occurrence of a period of desynchronization in loach embryos also. In loach embryos it occurs within one cell cycle, whereas in frog and sturgeon embryos it occurs over a considerable period of time (up to 6 $\tau_0$).

In the course of maturation the cytoplasm of eggs acquires the ability to synchronize cell divisions (Dettlaff et al. 1964) and to stimulate DNA synthesis (Graham et al. 1966). Injection of such cytoplasm into cleaving embryos shifts the onset of desynchronization of cell divisions but has no effect (in the amounts used in these experiments) on the time of the decrease in mitotic index and the onset of gastrulation. Accordingly, the duration of the asynchronous period in all experimental groups was constant and equalled 30% of the duration of the whole period of cleavage (see Fig. 4). Unlike the asynchronous period, the duration of the synchronous period and that of desynchronization vary owing to the shift of the onset of desynchronization. Thus, the duration of desynchronization at 20 °C in normal untreated embryos equals 30% of the whole period in cleavage; in embryos injected with the mature cytoplasm it equals 25% and in those injected with the supernatant of the homogenate of mature eggs it equals 20%.

The data obtained suggest the relative independence of the processes studied: desynchronization of cell divisions on the one hand, and prolongation of interphase and decrease of mitotic index, on the other hand. In such a way we interpret the fact that different temperature conditions and injection of the mature egg cytoplasm shift the onset of desynchronization but have no effect on the time of the decrease in mitotic index.

It is rather difficult for the time being to answer the question how desynchronization occurs in cells. One can believe that desynchronization arises from a proportional increase of the duration of all mitotic phases which does not occur simultaneously in all cells. If it were proved experimentally, it would explain the appearance of desynchronization independently of a decrease in the mitotic index.

The preservation of the relative durations of different mitotic phases, which has recently been found during the transition from the 1st maturation division in sturgeon oocytes to the 2nd maturation division and cleavage divisions, was accompanied by sharp changes (shortening) both in the duration of the whole cycle and that of separate mitotic phases (Vassetsky, 1969).

The experiments with the injection of the mature egg cytoplasm lead to the conclusion that synchronous divisions are conditioned by the presence in the cytoplasm of mature eggs of some substances disappearing at subsequent cleavage stages. In this connexion of great interest are the data (Crippa, Davidson & Mirsky, 1967) indicating that in Xenopus a sudden disintegration of the long-lived RNA, synthesized during oogenesis, takes place at the blastula...
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Desynchronization of cleavage divisions stage. Of great interest also is the question about the nature of substances responsible for synchronization of cell divisions. The presence of these substances in the supernatant of homogenized mature eggs and their capacity to shift the onset of desynchronization, offer new possibilities for the investigation of their nature.

SUMMARY

1. The time and character of the transition from synchronous to asynchronous cleavage divisions were studied in the animal region and marginal zone in sturgeon and frog embryos at different temperatures. The effect of the mature egg cytoplasm on this process was also studied.

2. The transitional period, termed as the period of desynchronization, was found to occur between synchronous and asynchronous cleavage divisions. It differs from the synchronous period by desynchronization of mitotic phases in nuclei of different blastomeres and from the asynchronous period by the absence of the decrease in the mitotic index. After complete desynchronization, the relative number of nuclei at different mitotic phases corresponds on the average with the duration of these phases during the synchronous period. During the period of desynchronization nuclei still have karyomere structure.

3. Desynchronization usually begins with the appearance of single desynchronized nuclei. At the next division a considerable proportion of nuclei divide asynchronously. The onset of desynchronization depends on temperature: at extreme temperatures the differences in the time of the onset of desynchronization may amount to 2 $\tau_0$. The end of desynchronization is characterized by a sharp decrease in the mitotic index and does not depend on temperature. Both in sturgeons and frogs it occurs at the same stage: at 15 $\tau_0$.

4. The onset of the asynchronous period is characterized by a sharp decrease in the mitotic index within one cell division and accordingly by an increase of the percentage of interphase cells. Interphase nuclei at this time do not have karyomere structure and do contain nucleoli. A sharp decrease in the mitotic index in sturgeon and frog embryos in the course of cleavage coincides in time with the onset of morphogenetic nuclear function. The duration of the asynchronous period is constant at the optimum temperatures but varies at extreme temperatures, owing to a shift of the onset of gastrulation to earlier cleavage stages (at high temperatures) or to later ones (at low temperatures).

5. In embryos injected with cytoplasm (3–5 % of the total egg volume) from mature unfertilized eggs, desynchronization starts one division later as compared with normal untreated embryos; desynchronization starts two divisions later if embryos are injected with the supernatant fluid from homogenized mature unfertilized eggs. However, these injections have no effect on the time of the sharp decrease in the mitotic index and the onset of gastrulation.
E. V. CHULITSKAIA

Резюме

1. На зародышах осетра (Acipenser giildenstädti colchicus V. Marti) и лягушки (Rana temporaria) изучали время и характер перехода от синхронных делений дробления к асинхронным в blastomерах амниальной области и краевой зоны при разных температурах. Исследовали также влияние на этот процесс цитоплазмы зрелого яйца.

2. У зародышей изученных видов между синхронным и асинхронным периодами дробления лежит период десинхронизации. От периода синхронных делений он отличается десинхронизацией фаз митоза в ядрах разных blastомеров, а от асинхронного периода-отсутствием уловимого нашим методом падения митотического индекса (МИ). После наступления полной десинхронизации в делении разных ядер частота, с которой встречаются ядра на разных фазах митоза в клетках одного зародыша в среднем ближе еще к относительной продолжительности этих фаз во время цикла одного деления в период синхронных делений дробления. В период десинхронизации ядра имеют еще карийомерное строение.

3. Переход от синхронных делений дробления к десинхронизации начинается как правило с нарушения синхронности деления в единичных ядрах, а на следующем делении уже значительная часть ядер начинает делиться асинхронно. Начало периода десинхронизации при разных температурах заметно варьирует; при крайних температурах различия в наступлении десинхронизации измеряются 2 τ0. Конец периода десинхронизации определяется резким падением МИ и не зависит от температуры; как у осетра, так и у лягушки он приурочен всегда к одному и тому же делению — стадии 15 τ0.

4. Начало асинхронного периода характеризуется резким падением МИ в течение одного деления и соответствующим возрастанием процента клеток в состоянии интерфазы. Интерфазные ядра в это время не имеют уже карийомерного строения и содержат ядрышки. Резкое падение МИ у зародышей осетра и лягушки на стадии дробления совпадает со стадией начала морфогенетической функции ядер. Продолжительность асинхронного периода при оптимальных температурах постоянна и может изменяться только при крайних температурах за счет смещения начала гастроуляции на немного более ранние стадии дробления (при действии высоких температур) или на более поздние стадии (при действии низких температур).

5. При инъекции в дробящийся зародыш небольшого количества (3–5 % объема яйца) цитоплазмы из зрелого неплодотворенного яйца десинхронизация в делении ядер начинается на одно деление позднее по сравнению с нормальными неоплодотворенными зародышами, а при добавлении надосадочной жидкости гомогенизованных зрелых неоплодотворенных яиц — на два деления позднее. В то же время на стадию резкого падения МИ и начало гастроуляции подобные инъекции не оказывают влияния.

I am grateful to Professor T. A. Dettlaff for helpful discussion at all stages of this investigation.
Desynchronization of cleavage divisions

REFERENCES


*(Manuscript received 17 October 1968, revised 12 August 1969)*