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**Cytological Studies of Tumors, XVII. A Phase Microscopy
Study on the Mitotic Process in the MTK-IV Tumor
and the Watanabe Ascites Hepatoma¹⁾**

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(With 2 Plates and 1 Text-figures)

Phase microscopy investigations with several ascites tumors of rats carried out by Makino and his coworkers (Makino and Nakahara, 1953a, b, 1955, Nakahara 1953, Tanaka 1953, Makino and Nakanishi 1955, Nakanishi 1956) have made extensive contributions to the analytical study of various fundamental mitotic phenomena in living tumor cells. The present study was attempted in order to follow mitotic events in two different kinds of transplantable ascites tumors, the MTK-IV tumor and the Watanabe ascites hepatoma which develop in albino rats. The results of the present investigation will supplement those of the former studies as quoted above.

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Material and method

The MTK-IV tumor is a kind of ascites tumor of white rat. It arose in one of the albino rats which had been fed with azo-dye (Tanaka and Kanô, unpublished).

On the other hand, the Watanabe ascites hepatoma was originated from a hepatoma developed in one of several rats by repeated application of hot water (72°C) in their peritoneal cavities. A transplantable ascites tumor was induced by injecting in the peritoneal cavity of the rat crushed pieces of tissue of the hepatoma thus formed (Watanabe and Matsunaga, 1954).

The samples for study were taken from tumor-bearing rats on the 2nd to 4th day after transplantation of the tumor. The tumor ascites at the third day after transplantation proved to be most suitable for observation of the cell divi-

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sion because of the prevalence of mitotic figures.

The hanging-drop method in combination with the application of fluid paraffin as described by Makino and Nakahara (1953b, 1955) was also adopted in this study. The preparations were studied with the aid of phase-contrast optics in conjunction of a bright-medium contrast 90 \times objective with a Periplan 10 \times ocular. The microscope was set in an electric warm chamber; the observations were run at 35°C.

Observations

The MTK-IV tumor cells are roughly classified with regard to their size into small-sized cells and large-sized ones. The small-sized cells are 18 to 20 μ in diameter and large-sized cells are 28 to 30 μ . The Watanabe ascites hepatoma cells are roughly classified into the following three types; small-sized, medium-sized and large-sized cells. The diameters of cells were obtained as 20–21 μ , 23–28 μ and more than 30 μ for the small-, medium- and large-sized cells, respectively.

The resting tumor cell generally contains a number of fine granular cytoplasmic inclusions which are highly different in size, nature and distribution. They show a remarkable Brownian movement in the living cell. The nucleus is oval or kidney-shape, or sometimes of lobated appearance. Especially, the MTK-IV tumor cells are characterized by a high frequency of cells with lobated nuclei (Tomomura 1956, Nakanishi 1956). Conspicuous small bodies two or three in number with well-defined outline, probably the nucleoli, are detected in the nucleus.

I. Regular mitotic division in the small-sized cell of the MTK-IV tumor and in the small- and medium-sized cells of the Watanabe ascites hepatoma

At prophase, the nucleus swells and becomes expanded. Meanwhile the chromosomes become partially visible, and the nucleoli fade from view. The chromosomes become thicker and shorter with time (Figs. 1, 9). Then the nuclear membrane becomes irregularly wrinkled and disappears. The area around the chromosomes remains free of inclusion granules. The spindle body seems to be formed in this area. Shortly after the disappearance of the nuclear membrane, the chromosomes have completed their arrangement on the metaphase plate (Figs. 2, 3, 10, 15). In the Watanabe ascites hepatoma, the spindle body is found in process of formation in one side of the cell body in most cases. The chromosomes remain with no visible change for a considerable length of time. During metaphase, individual chromosomes show a striking oscillation. The spindle body is also movable as a whole. The spindle fibers are usually invisible. In the meantime, a distinct splitting becomes visible in each chromosome. The separation of the chromosomes and their migration to the opposite poles at anaphase take place rather synchronously in all chromosomes (Figs. 4–5, 11, 16–18).

At telophase the nuclear membrane appears around a light area containing the chromosomes at about the time they reach the pole. The chromosomes swell in the newly formed nuclear vesicle, and meanwhile all visible traces of the chromosomes disappear from view (Figs. 8, 13-14, 20). The nucleolar bodies are to be seen in the nucleus by this time. The cleavage furrow begins to appear at about the equator of the cell body at a time earlier or later than the telophase nuclei have been formed. It rapidly cuts the cell into two daughter bodies (Figs. 6-8, 12-14, 19-20).

The duration of each mitotic phase was measured in the successive series of a division process followed through the same cell, beginning with prophase and ending with telophase. The data are presented in Tables 1 and 2.

Table 1. Time relations in the division of the small-sized cells of the MTK-IV tumor at 35°C.

Phase \ Cell size	Cell size					
	18 μ	19 μ	19 μ	19 μ	19 μ	20 μ
Prophase	17 min.	17 min.	15 min.	10 min.	— min.	16 min.
Metaphase	14	23	23	31	13	14
Anaphase	3	6	7	5	3	6
Telophase	22	13	22	11	22	7
Total	56	59	67	57	—	43

Table 2. Time relations in the division of the small- and medium-sized cells of the Watanabe ascites hepatoma at 35°C.

Phase \ Cell size	Small-sized cells			Medium-sized cells		
	21 μ	21 \times 22 μ	22 \times 21.5 μ	24 \times 22 μ	24 μ	24 \times 27 μ
Prophase	15 min.	— min.	— min.	— min.	15 min.	15 min.
Metaphase	45	11+	12+	30	18	26
Anaphase	6	5	8	4	5	5
Telophase	22	30	63	28	30	—
Total	88	—	—	—	68	—

II. Abnormal mitosis in the large-sized cells of the Watanabe ascites hepatoma

1. **Multipolar mitosis:** Multipolar mitoses occur in a rather high percentage as a remarkable mitotic abnormality in tumor cells which are larger than 30 μ in a diameter.

a) *Tripolar division:* Figures 21 to 24 show successively the course of a tripolar division taking place in a large-sized tumor cell of the Watanabe ascites hepatoma. Beginning with metaphase (Fig. 21), the tripolar division of chromosomes at anaphase is shown in Fig. 22. Figures 23 and 24 indicate the formation of the cleavage furrow and the constriction of the cell body into three bodies.

The duration of each mitotic phase measured was 47 minutes for metaphase, 7 minutes for anaphase and 18 minutes for telophase.

b) *Polypolar division*: Figures 25 to 30 show successively the course of a polypolar (hexapolar?) division taking place in a giant tumor cell ($30 \times 29 \mu$ in diameter) of the Watanabe ascites hepatoma. This tumor cell was spherical in late prophase (Fig. 25) while it was transforming itself into an unusual amoeboid-form shape. Figure 26 indicates the change of the cell form, and the arrangement of chromosomes on the equatorial plate at metaphase. The polypolar segregation of chromosomes at anaphase is shown in Figs. 27 and 28, successively. Figure 29 indicates the formation of the cleavage furrow and the constriction of a cell body into six smaller bodies. After the cell body has been constricted, the upper three and the next two of the six daughter cells come together (Fig. 30), resulting in the formation of three and one micronuclei, respectively. This observation made clear the process of the formation of micronuclei. The duration of each mitotic phase measured was 78 minutes for metaphase, 52 minutes for anaphase and 43 minutes for telophase.

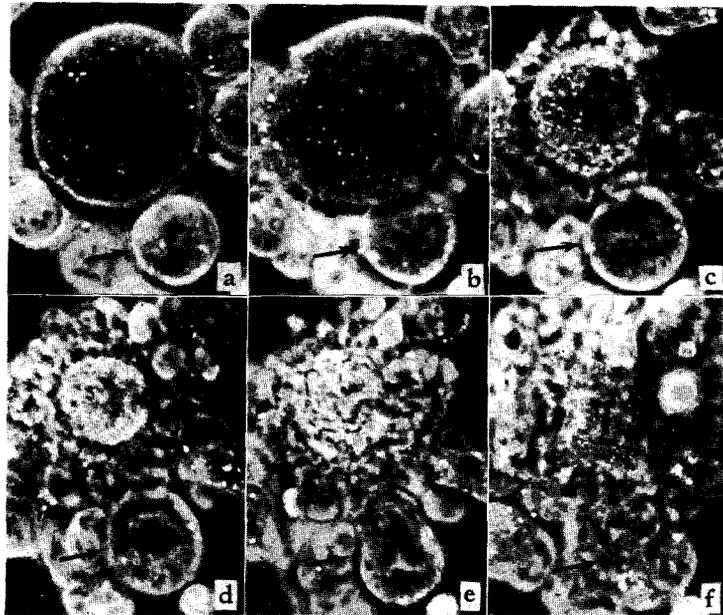
2. **The division of the binucleate cell**: Figures 31 to 36 show a successive process of the division in a large-sized, binucleate tumor cell of the Watanabe ascites hepatoma, 30μ in diameter. At prophase the chromosomes appeared synchronously within two nuclei (Figs. 31, 32). At metaphase two spindles were formed side by side, with two equatorial plates forming the same plane (Fig. 33). At anaphase, the separation of chromosomes took place synchronously into two spindles. The daughter chromosomes coming from two nuclei came together at the poles as seen in Fig. 34. At telophase, the formation of the cleavage furrow was observed to be similar in process to that observed in the regular mitosis (Fig. 35). Then the nuclear membrane was formed; the mother cell thus produced two daughter cells with large-sized nuclei in each (Fig. 36). The duration of each mitotic phase measured was 18 minutes for prophase, 30 minutes for metaphase, 7 minutes for anaphase and 34 minutes for telophase.

3. **Formation of an anuclear cytoplasmic bud**: Figures 37 to 40 show the course of the formation of an anuclear cytoplasmic bud in a large tumor cell of the Watanabe ascites hepatoma, 27μ in a diameter. At metaphase, the spindle was formed on one side of the cell body (Fig. 37). At anaphase, the separation of chromosomes proceeded regularly (Fig. 38). Figure 39 indicates the formation of the cleavage furrow with the constriction of the cell into three bodies. The mother cell produced two daughter cells, one with nuclei, and the other with an anuclear cytoplasmic bud as indicated by the arrow. In the meanwhile the anuclear bud fused with one of the daughter cells resulting in the formation of large-sized cell (Fig. 40). The duration of each mitotic phase measured was 42 minutes for metaphase, 6 minutes for anaphase and 36 minutes for telophase.

III. *The degeneration by blebbing of the large-sized cell and*

the regular mitotic division of the small-sized cell in the MTK-IV tumor under the same condition

Text-fig. 1, a-f. show in succession the course of damage of a large tumor cell, $33\ \mu$ in a diameter, together with the regular mitotic course in a small tumor cell, $20\ \mu$ in diameter. Text-fig. 1, a shows the prophase of the large cell and the interphase of the small one as indicated by the arrow. Text-fig. 1, b indicates the



Text-fig. 1. a-f. Photomicrographs of tumor cells from the MTK-IV tumor, taken with the phase microscope, showing the course of damage of large tumor cell ($33\ \mu$ in diameter), together with the regular mitotic course in a small tumor cell ($20\ \mu$ in diameter) $\times 700$. a, the large cell; prophase, the small cell; interphase, 18 minutes after preparation. b, the large cell; pro-metaphase, the small cell; prophase, 103 min. c, the large cell; showing blebbing in the surface, the small cell; metaphase, 105 min. d, the small cell; anaphase, 135 min. e-f, the large cell; fragmentation of the cytoplasm into irregular masses, with pycnotic aggregation of chromosomes, the small cell; telophase, 139 and 144 min., respectively.

pro-metaphase of the large cell and the prophase of the small one. The first step of the damage in the large cell was observed to be an irregular indentation of the cell surface. Next, the cell began to show blebbing in the surface. The blebbing of the cell surface became more intensive with time. The division was arrested at pro-metaphase: the chromosomes were irregularly condensed (Text-fig. 1, c-e). The final damage was attained by fragmentation of the cytoplasm into many small masses with pycnotic aggregations of chromosomes (Text-fig. 1, f). On the other

hand, the chromosomes of the small tumor cell completed their regular metaphase arrangement (Text-fig. 1, c). The regular division of chromosomes at anaphase is shown in Text-fig. 1, d. Text-fig. 1, e and f indicate the formation of the cleavage furrow and the completion of cell division. The duration of each mitotic phase of this small-sized cell was measured as 16, 14, 6 and 7 minutes for prophase, metaphase, anaphase and telophase, respectively.

Discussion

The existence of the stem-line (or-lines) of tumor cells which are characterized by a high frequency, definite ideograms and regular mitotic behavior has been well accepted at present in many kinds of tumors (Makino and Kanô 1951, 1953, 1955, Makino 1952a, b, 1956). Makino, Kanô and Tonomura (1955), Watanabe and Tonomura (1956) and Makino (1956) have shown that there are stem-cells characterized by subtriploid tumor cells in the MTK-IV tumor, and at least three remarkable populations characterized by subdiploid, subtetraploid and subhexaploid tumor cells in the Watanabe ascites hepatoma. The growth of the tumor is primarily attributed to the regular mitotic proliferation of these tumor cells. The results of the present study have revealed that the stem-cells of the MTK-IV tumor as well as of the Watanabe ascites hepatoma multiply with regular mitotic behavior. Further it has been shown that the course of the multipolar division and the process of degeneration through blebbing in a manner nearly similar to that observed as to podophyllin with large-sized tumor cells. Evidence presented in this study indicates that the small-sized tumor cells observed in the MTK-IV tumor, and both small- and medium-sized tumor cells in the Watanabe ascites hepatoma are just the tumor stem-cells which serve as primary contributors to neoplastic growth. Furthermore, the tumor cells of larger size probably characterized by having aberrant chromosome constitution are no longer able to divide, since they have lost some of their capacity for normal reproduction such as the ability to form functional spindles.

Summary

This paper deals with the mitotic process in tumor cells of the MTK-IV tumor and the Watanabe ascites hepatoma followed by means of phase microscopy, with the use of hanging-drop preparations.

The results from the present observations have revealed that the small-sized tumor cells in the MTK-IV tumor, and both small- and medium-sized tumor cells in the Watanabe ascites hepatoma are the tumor stem-cells which serve as primary contributors to neoplastic growth with regular mitotic behavior. Further it has been shown that the course of multipolar division as well as the process of degeneration does take place in large-sized tumor cells.

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Explanation of Plate XXII

Photomicrographs of the regular mitotic course in the small-sized cells of the MTK-IV tumor and in the small- and medium-sized cells of the Watanabe ascites hepatoma, taken with the phase microscope. $\times 900$.

Figs. 1-8, showing the successive series of a division process in a small tumor cell of the MTK-IV tumor (20μ in diameter) followed in a single cell. 1, late prophase. The chromosomes become partially visible. 62 minutes after preparation. 2, pro-metaphase, 66 min. 3, metaphase, showing the arrangement of chromosomes on the equatorial plate (side view). 71 min. 4-5, anaphase showing the migration of chromosomes to the poles, 75 and 77 min. 6-7, early telophase. The cleavage furrow begins to appear. 77.5 and 82 min. 8, late telophase. The completion of cell division. 100 min.

Figs. 9-14, showing the successive series of a division process in a small tumor cell of the Watanabe ascites hepatoma (21μ in diameter) followed in a single cell. 9, prophase, 12 minutes after preparation. 10, metaphase, 12 min. 11, anaphase, 33 min. 12, telophase, 39 min. 13, the formation of the nuclear membrane, 60 min. 14, completion of cell division, 140 min.

Figs. 15-20, showing the successive series of a division process in a medium tumor cell of the Watanabe ascites hepatoma ($26 \times 24 \mu$ in diameter) followed in a single cell. 15, metaphase, 20 minutes after preparation. 16-18, anaphase, 26, 17 and 29 min. 19, telophase, 31 min. 20, completion of cell division, 38 min.

Explanation of Plate XXIII

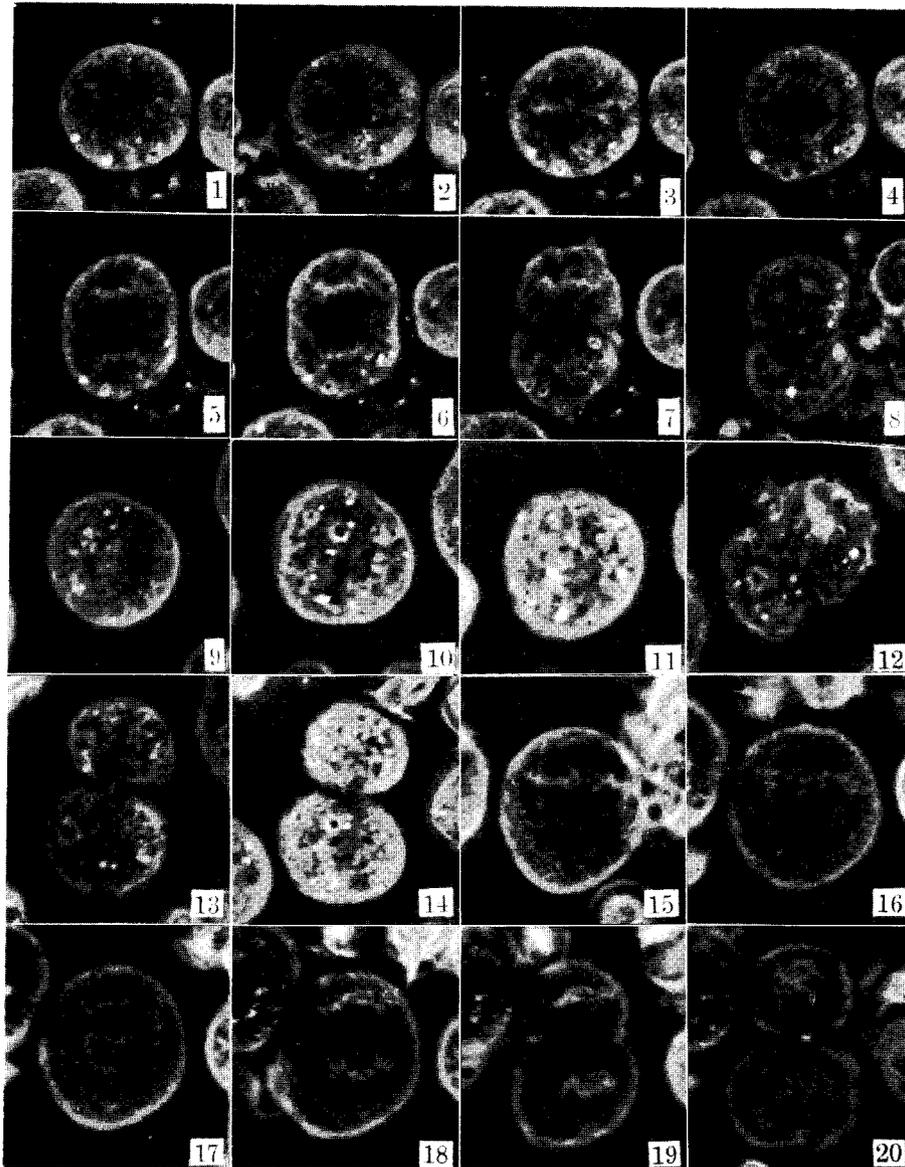
Photomicrographs of the abnormal mitotic course in the large-sized cells of the Watanabe ascites hepatoma, taken with the phase microscope. $\times 900$.

Figs. 21-24, showing the successive series of a tripolar division in a large tumor cell (30μ in diameter) followed in a single cell. 21, metaphase, 16 minutes after preparation. 22, anaphase, 49 min. 23-24, telophase, 54 and 72 min.

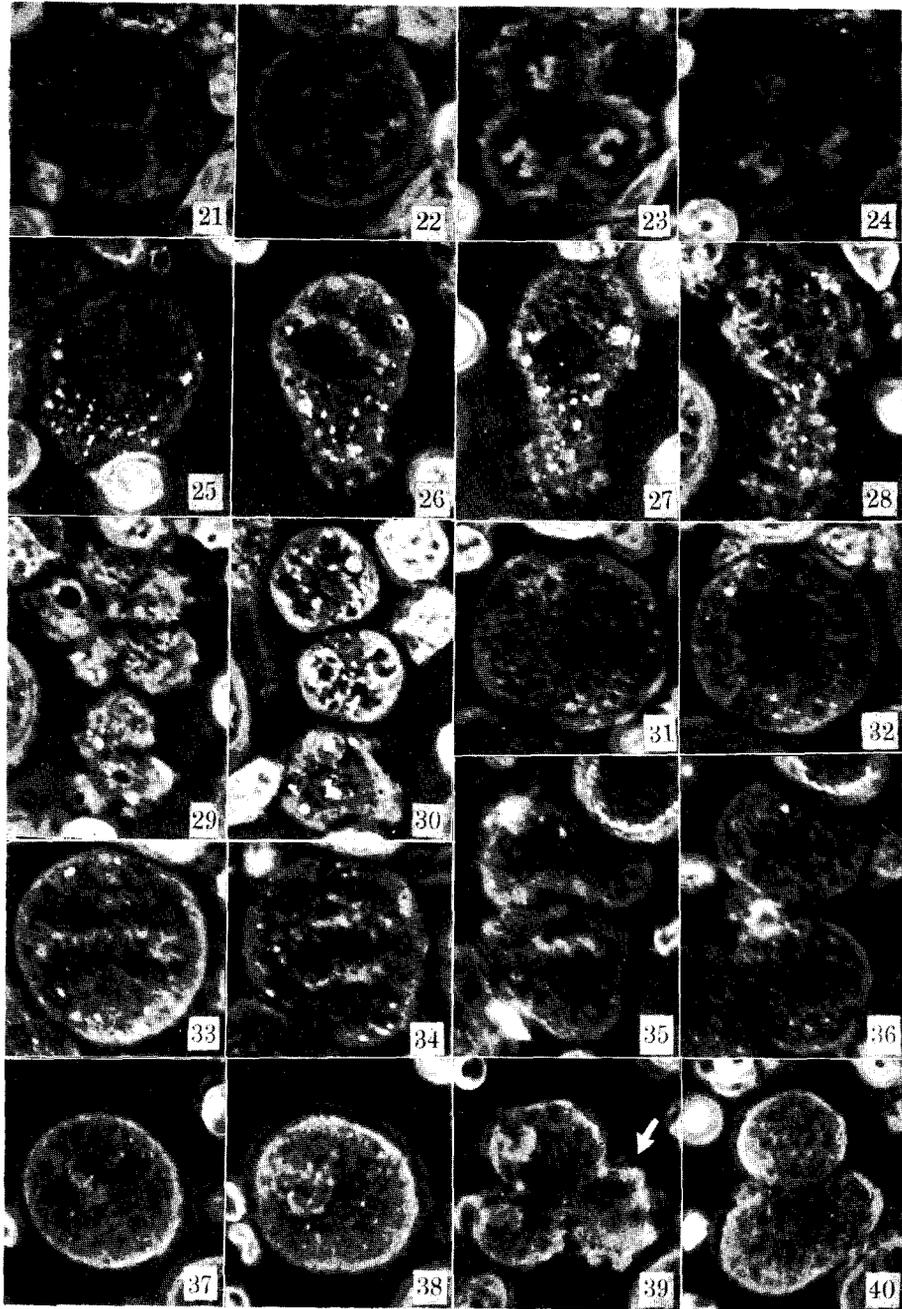
Figs. 25-30, showing the successive series of a polypolar division in a large tumor cell ($23 \times 29 \mu$ in diameter) followed in a single cell. 25, late prophase, 5 minutes after preparation. 26, metaphase. The cell transforms into unusual amoeboid form. 39 min. 27-28, anaphase, 83 and 135 min. 29, telophase, formation of cleavage furrow dividing a cell into six bodies. 153 min. 30, the upper three and next two of the six daughter cells come together, resulting in the formation of three and one micronuclei, respectively, 190 min.

Figs. 31-36, showing the successive series of a division in large tumor cell with two nuclei (30μ in diameter) followed in a single cell. 31-32, prophase, 17 and 23 minutes after preparation. 33, metaphase, 33 min. 34, anaphase, 60 min. 35, telophase, 69 min. 37 completion of cell division, showing the large-sized nucleus reconstructed in each cell. 103 min.

Figs. 37-40, showing the formation of an anuclear cytoplasmic bud in a large tumor cell (27μ in diameter) followed in a single cell. 37, metaphase, 32 minutes after preparation. 38, anaphase, 74 min. 39, telophase. The cleavage furrow cuts the cell body into three bodies. An arrow shows an anuclear cytoplasmic bud. 84 min. 40, the anuclear cytoplasmic bud comes together into the one separated daughter cell. 140 min.



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