SYNCHRONIZATION OF MITOSIS BY THE FUSION OF THE PLASMODIA OF *PHYSARUM POLYCEPHALUM*

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INTRODUCTION

Nuclear division in the plasmodium of *Physarum polycephalum* is naturally synchronous (1). Such synchrony could result either from some regulatory "biological clock" within each nucleus or from a common cytoplasmic factor. Previous work in our laboratory has shown that plasmodia obtained from various periods of the mitotic cycle will fuse with one another, and the influence of one plasmodium on the mitotic timing of another can thus be tested (2–5). Furthermore, shaken cultures of the growing organism consist of tiny plasmodia (microplasmodia), each of which exhibits within itself synchronous nuclear divisions, but there is no synchrony among the various microplasmodia in the culture. The fusion of these microplasmodia forms one large plasmodium in which the very first mitosis following fusion is synchronous and occurs after a period of approximately one-half the time required for one complete mitotic cycle. It would appear from these observations that substances leading to mitosis increase in amount in the plasmodia, starting at the completion of mitosis and attaining a level at the end of interphase which induces mitosis. To obtain further information on this problem, plasmodia of various sizes in various stages in the mitotic cycle were placed in contact with each other, allowed to coalesce, and the timing of the next mitosis was observed.

METHODS AND RESULTS

All plasmodia were obtained from shaken cultures. To obtain these, 0.3 ml of a previously shaken culture was placed in 20 ml of growth medium contained in a 500-ml Erlenmeyer flask which was shaken in a reciprocating shaker (6). The shaker was in an incubator maintained at 24 ± 0.5°C. At the appropriate time the microplasmodia were collected by light centrifugation and then suspended in an equal volume of distilled water; 0.3 ml of the suspension was pipetted onto a Millipore membrane supported by a layer of glass beads contained in the bottom of a Petri dish. Fusion of the microplasmodia into one large surface plasmodium was completed in approximately 1 hr, at which time about 13 ml of growth medium was added, an amount just sufficient to cover the beads and wet the Millipore membrane. The cultures were then incubated in the dark at 26 ± 0.5°C (7). The time of mitosis was determined by removing, with a spatula, pieces about 2 mm^2 from the plasmodium and examining smears of the pieces under the phase contrast microscope as previously described (1). It was possible to determine the time of onset of metaphase to an accuracy of a few minutes. Under the conditions of this experiment the first mitosis (MI) occurred about 5 hr after the addition of the growth medium, and the second mitosis (MII) occurred about 10 hr after the first. Thus, it was possible to obtain, at any one time, plasmodia in known stages of the mitotic cycle. Since the first mitosis following fusion contains a small percentage of abnormal nuclei, presumably caused by premature stimulation of mitosis in such nuclei, plasmodia were not used until the first mitosis (MI) had been completed.

Three types of fusion were performed. In one, a plasmodium was placed directly on top of another plasmodium of approximately the same size; in another experiment, small plasmodia were fused with larger ones; and in the third, the end of one plasmodium was placed in contact with the end of another plasmodium of approximately equal size. In all cases, the plasmodia which were thus fused were from different stages of the mitotic cycle.

FUSION OF PLASMODIA OF EQUAL SIZE BUT FROM DIFFERENT PERIODS OF THE MITOTIC CYCLE: Two sets of plasmodia A and B were prepared at such times that the first mitosis (MI) was about 2 hr earlier in A than in B. The following day at approximately 2 hr prior to mitosis III (MIII) in plasmodium A, one piece, approximately one-fourth the size of the whole plasmodium, was cut from each plasmodium (pieces

TABLE I

The Effect of Fusing Two Plasmodia of the Same Size but from Different Periods of the Mitotic Cycle on the Timing of Mitosis

Plasmodia	After MIII in A
	hr
Exp. 1	
A (control)	—
B "	2:05
A over B (fused)	1:05
B over A "	1:00
Exp. 2	
A (control)	—
B "	2:55
A over B (fused)	1:15
B over A "	1:10

A and B). Piece A was removed from the Millipore membrane by submerging the membrane in the salt solution (6) and then carefully slipping a thin spatula between the plasmodium and the membrane; the plasmodium floated to the surface of the salt solution. Then piece B, which was also on a Millipore membrane, was placed under the floating plasmodium (piece A), and the two were slowly lifted out of the solution with plasmodium A directly overlying plasmodium B. The procedure was repeated in the same manner, except that piece B overlay piece A. The membranes were then placed on glass beads in a Petri dish without nutrient medium and allowed to fuse for 90 min, after which nutrient medium was added as described above. The time of mitosis in the various plasmodia was determined, and the results are shown in Table I. A second, essentially similar experiment differed from the one described in that approximately 3 hr separated the time of MIII in the two sets. The results are also shown in Table I. It will be noted that the time of mitosis in the fused pieces occurred approximately midway between the times of mitosis in the control pieces in one experiment and slightly prior to the midway point in the other experiment. This was true whether piece A was placed over piece B, or B over A.

FUSION OF PLASMODIA OF UNEQUAL SIZE AND FROM DIFFERENT PERIODS OF THE MITOTIC CYCLE: Two sets of plasmodia A and B were prepared with a time difference in mitosis of 4.5 hr between the two sets. One hour after mitosis II (MII) appeared in the cultures prepared first (plasmodium A), rectangular pieces 1.5 cm^2 and 3 cm^2 were cut from both sets (plasmodia A and B), and these were placed on filter paper supported by glass beads in a Petri dish without medium. Piece A was from early interphase between MII and MIII, whereas piece B was from late in the cycle between MI and MII. One piece of each parent plasmodium served as a control. Fused plasmodia were formed as described previously, and pieces of varying sizes were taken in order to make fused plasmodia with size ratios of A:B of 2:1, 1:1, and 1:2. Ninety minutes were allowed for the fusion of A and B, and then the nutrient medium was added to all the cultures. The time of mitosis was checked at three different points around the edges as well as in the center of all cultures.

TABLE II

The Effect of Fusing Two Plasmodia of Different Sizes and from Different Periods of the Mitotic Cycle on the Timing of Mitosis

Exp.	Ratio of pieces A:B	Acceleration of MIII in nuclei derived from A	Retardation of MII in nuclei derived from B	Total interval between MII in B and MIII in A
		hr	*hr*	*hr*
1	2:1	0.8	5.0	5.8
2	2:1	0.0	3.6	3.6
3	2:1	0.0	3.6	3.6
4	2:1	1.0	4.3	5.3
2	1:1	1.1	2.5	3.6
4	1:1	2.1	3.2	5.3
1	1:2	2.1	3.7	5.8
2	1:2	2.0	1.6	3.6
3	1:2	2.1	1.5	3.6
4	1:2	3.3	2.0	5.3

The timing of the plasmodium composed of fused A and B was compared with that of the control pieces. When A and B were fused, there was an acceleration of the appearance of MIII in the nuclei which were derived from piece A and a retardation of MII in the nuclei derived from piece B. The acceleration of MIII in the nuclei from piece A was greatest when piece B was larger than piece A, and the retardation of MII in the nuclei from piece B was greatest when piece A was larger than piece B (Table II).

OVERLAPPING THE ENDS OF PLASMODIA OF EQUAL SIZE BUT FROM DIFFERENT PERIODS

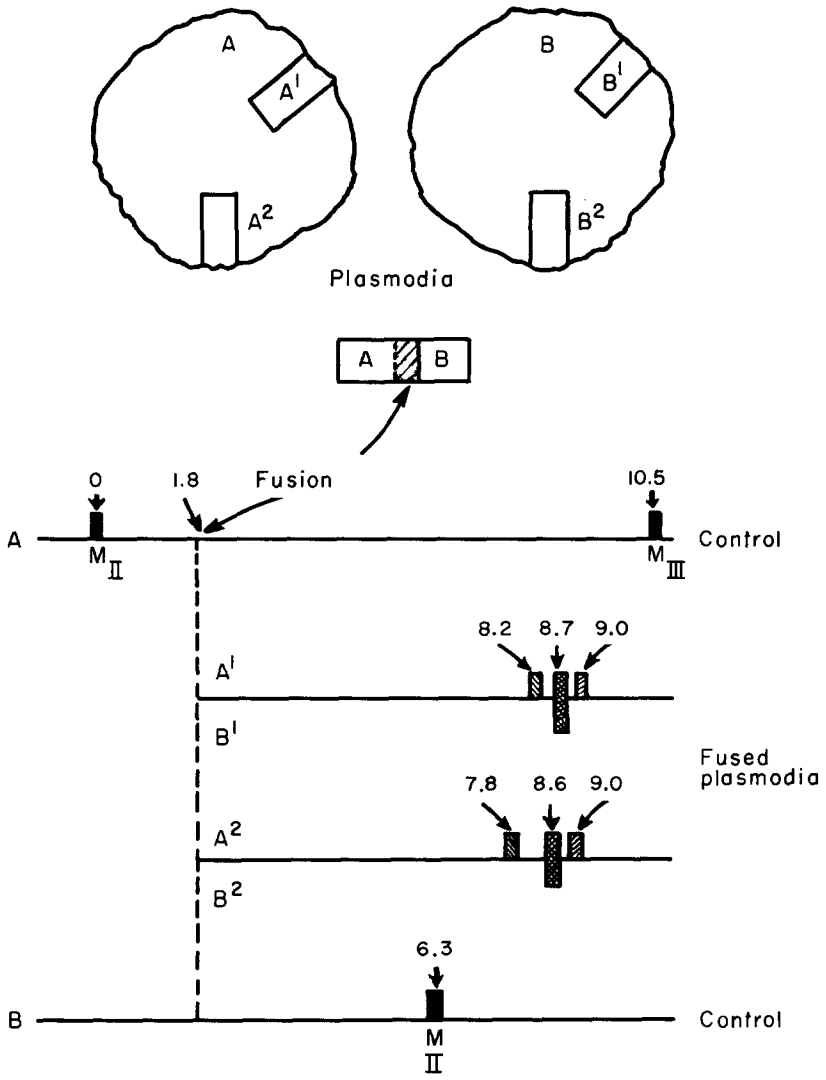

FIGURE 1 The effect of overlapping the ends of two plasmodia from different periods of the growth cycle on the timing of mitosis. The top of the figure depicts two plasmodia and shows the relative positions from which pieces were removed. The time of mitosis in control piece A is indicated as 0 time, and the interval in hours between this and other mitoses is shown by the numbers. The time of mitosis III (MIII) near the A^1 and A^2 ends of the fused plasmodia is depicted by the bars ▨; the time of mitosis II (MII) near the B^1 and B^2 ends of the fused plasmodia is depicted by the bars ▧; and the time of the synchronous mitosis in the center, where the two pieces A^1 to B^1 and A^2 to B^2 have fused, is shown by bars ▩.

OF THE MITOTIC CYCLE: Experiment 1: Two surface cultures were prepared on Millipore membranes at such times that MII in culture A occurred 6.3 hr prior to MII in culture B. One and one-half hours after MII in A, several rectangular pieces (1 × 2 cm) were cut from both plasmodia A and B and removed from the Millipore membrane with a thin spatula. Fused plasmodia were formed by overlapping a small portion of the end of piece A^1 with an equal sized portion at the end of piece B^1 and overlapping A^2 and B^2 in the same manner (Fig. 1). These pieces, together with the controls, were placed on filter paper supported by glass beads in Petri dishes. After fusion, nu-

TABLE III

The Effect of Overlapping Two Plasmodia from Different Periods of the Mitotic Cycle on the Timing of Mitosis

Plasmodia*	Time difference of mitosis relative to controls	
	Before MIII in control A	After MII in control B
	hr	*hr*
End		
A^1	1.5	
A^2	1.5	
Fused center		
A^1-B^1	1.8	2.4
A^2-B^2	1.9	2.3
End‡		
B^1	—	1.9
B^2	—	1.5

* Time difference between MII in B and MIII in A of the controls was 4.2 hr.

‡ Not completely synchronous; a few nuclei remained in interphase at a time when most were in mitosis.

trient medium was added and the time of mitosis was checked at the free ends of the fused pieces and within the fused region.

Mitosis at the opposite ends of the fused plasmodia was accelerated (pieces A^1 and A^2) or retarded (pieces B^1 and B^2) relative to the controls, and mitosis in the fused region occurred approximately midway between that at the opposite ends and was completely synchronous (Fig. 1; Table III).

Experiment 2: This experiment was similar to the one just described, except that whole plasmodia were used for fusion instead of cut pieces, and in addition mitosis was checked at five points in the fused plasmodia as indicated by the black dots in Fig. 2. In this experiment the plasmodia were prepared at such times that MIII occurred approximately 3 hr earlier in plasmodium A than in plasmodium B. Two separate experiments were performed, and the time of mitosis in the different portions of these fused plasmodia indicated that a gradient effect was present (Fig. 2).

TIME OF MITOSIS IN PLASMODIAL HOMOGENATES OR ISOLATED PARTICULATES: Approximately 2 hr prior to the expected time of MIII, a plasmodium was homogenized in 15 ml of nutrient medium for 55 sec in a Potter-Elvehjem homogenizer. This homogenate was layered

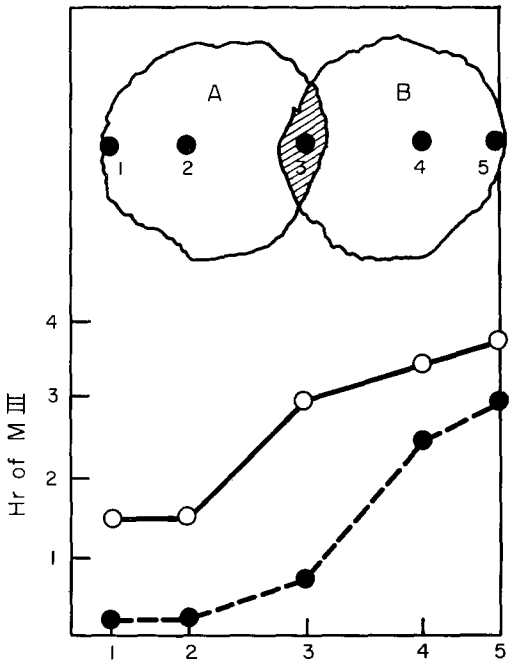

FIGURE 2 The effect of overlapping parts of two whole plasmodia from different times of the mitotic cycle on the timing of mitosis. The abscissa depicts the relative distances between the places on the plasmodia from which pieces were removed and examined for mitoses. The place from which the pieces were removed is shown by the black dots on the diagram. The relative distances between the black dots on the plasmodia and the numbers along the abscissa are identical. The curves show the results of two separate experiments.

on a filter paper supported by glass beads in a Petri dish, and one-half of a surface plasmodium in which MIII was expected in 1 hr was placed on top of the homogenate. The other half of the plasmodium was placed on fresh nutrient medium as a control. There was no difference in the time of MIII between the plasmodium in contact with the homogenate and the control.

Homogenates were also prepared by homogenizing plasmodia in 0.15 N NaCl, and these homogenates likewise had no influence on the time of mitosis in a plasmodium layered on top of the homogenate. In addition, plasmodial ribosomes and nuclei were prepared by centrifugation according to the usual procedures. These particulates, prepared from plasmodia taken at various times of the mitotic cycle, were without effect on mitosis when placed in contact with whole plasmodia.

DISCUSSION

Several alternatives may be considered as explaining the results of the present experiment. First, the stimulus for mitosis is formed in the nucleus, is irrevocably determined, and triggers mitosis at a set time. The present observations are sufficient to discard this hypothesis, since the time of mitosis can be altered.

Second, the stimulus for mitosis is formed in the nucleus and may pass from one nucleus to another. Goldstein showed that some nuclear proteins were transferred between the nuclei of an artificially binucleated amoeba, and the transfer occurred in as short a time as 4 hr (8). This suggestion cannot be discarded as an explanation of our results, but it is not the most probable explanation, since it appears unlikely that the stimulating factor would equilibrate among all the nuclei with sufficient rapidity to produce the observed degree of synchrony. A further complication is that the formation of the stimulating factor would probably start sooner in some nuclei than in others, thus reducing the chance of uniform equilibration among all the nuclei.

A third possibility is that the stimulus for mitosis is formed in the cytoplasm and is then transferred to the nuclei shortly before the occurrence of mitosis. This appears to be the best explanation for the synchronous mitosis throughout the plasmodium. The formation of the stimulus in the cytoplasm is also substantiated by the classical observations of Hartmann (9), who found that the repeated removal of cytoplasm from amebae prevented mitosis. A summary of this work and related experiments is discussed in a review by Swann (10). The present results also indicate that the mitotic stimulants increase from a low to a high level during the mitotic cycle, reaching a peak just prior to mitosis. This suggestion is based on the observation that mitosis occurs at an intermediate time when plasmodia from early in the mitotic cycle are fused with plasmodia from a stage just prior to mitosis. In addition, larger pieces from the later period are more effective in such stimulation than smaller pieces. Furthermore, there is gradient spreading of the substance as shown by the experiment in which the ends of two plasmodia were overlapped. Protein is an essential part of the mitotic initiator since actidione, an inhibitor of protein synthesis, blocked mitosis when added to the growth medium at any time during interphase up to 20 min before the expected metaphase. Actidione did not inhibit mitosis when added 10 min prior to metaphase (11).

The lack of effect of homogenates and of ribosomes and nuclei on the timing of mitosis has little meaning, since there was no evidence as to whether any of these substances gained entrance to the plasmodium. Work in this direction is continuing.

SUMMARY

The plasmodium of *Physarum polycephalum* is multinucleated and exhibits synchronous mitosis. To obtain information on whether the timing of mitosis is regulated by cytoplasmic or nuclear substances, plasmodia from one period of the mitotic cycle were permitted to fuse with plasmodia from other periods of the mitotic cycle. The sizes of the plasmodia to be fused were also varied to combine various proportions of material from periods early or late in the mitotic cycle into the fused plasmodium. The results suggest that substances responsible for mitosis are formed in the cytoplasm and increase in amount during the mitotic cycle, reaching a maximum just prior to mitosis.

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