REGULATION OF DNA REPLICATION IN THE NUCLEI OF THE SLIME MOLD *PHYSARUM POLYCEPHALUM*

Transplantation of Nuclei by Plasmodial Coalescence

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ABSTRACT

Nuclei in G_2 phase of the slime mold *Physarum polycephalum*, when transplanted, by plasmodial coalescence, into an S-phase plasmodium, failed to start another round of DNA synthesis. In the reciprocal combination, S-phase nuclei in a G_2-phase host continued DNA synthesis for several hours without appreciable decrease in rate. It is suggested that the beginning of DNA replication is determined by an event, either during or shortly after mitosis, which renders the chromosomes structurally competent for DNA replication.

INTRODUCTION

The replication of nuclear DNA is a discontinuous process: replication begins at a defined stage of the mitotic cycle[1] and, once the initial amount of DNA has been duplicated, another "round of replication" (25) is not initiated until after the next mitosis. The factors which control the onset and, after doubling, the termination of the replication process are unknown. A priori, any of the known prerequisites for DNA replication, e.g. availability of primer, precursors, kinases, DNA polymerase, might be limiting and, by being provided at a specific stage in the mitotic cycle, might "control" the onset of DNA replication (1, 24).

Prescott and Goldstein (29) have recently reported that DNA replication can be initiated in G_2-phase nuclei of *Amoeba proteus* by transplanting them into S-phase host cells.[2] Similarly, DNA replication was initiated in G_1-phase macronuclei of *Stentor coeruleus* (6) by implanting them into S-phase recipient cells. Graham et al. (8) observed that nuclei from various tissues of *Xenopus laevis*, when injected into unfertilized eggs of the same species, commenced DNA synthesis. Employing plasmodial coalescence as a method of transplantation (15, 17), we have performed similar experiments in the multinucleated, coenocytic slime mold, *Physarum polycephalum*. In this organism, DNA replication begins immediately after mitosis (26) and is completed in most nuclei within approximately 3–4 hr (2). After this period, the number of nuclei that are able to incorporate thymidine-^{3}H decreases rapidly and reaches almost zero a few hours before mitosis. The results reported below show that G_2-phase nuclei of *Physarum polycephalum*, when transplanted by coalescence into an S-phase host plasmodium, do not start another round (25) of DNA replication until after their next mitosis which occurs in synchrony with the mitosis of the host nuclei. In the converse combination, S-phase nuclei continue to synthesize DNA in a G_2-phase environment. A preliminary

[1] The term "mitotic cycle" will be used to denote the period from one stage of synchronous mitosis in a plasmodium to the same stage of the next mitosis.

[2] For brevity, the following terms (19) will be used to denote parts of the intermitotic period before, during, or after DNA replication: G_1 phase, period preceding DNA replication; S phase, period of DNA replication; G_2 phase, period following DNA replication.

account of these experiments has been given previously (11, 13).

MATERIALS AND METHODS

Preparation of Plasmodia

The organism was grown in the form of microplasmodia (5) in agitated culture. Mitotically synchronized surface plasmodia (12, 14, 20) were obtained by coalescence of microplasmodia as described previously (15).

Transplantation

We were interested in the following two combinations: (*a*) transplantation of postmitotic (S phase) nuclei into late interphase host plasmodia (mostly G_2-phase nuclei) and (*b*) transplantation of late interphase (G_2 phase) nuclei into postmitotic (S phase) host plasmodia. These two stages of the mitotic cycle are readily distinguishable from one another by morphological criteria (12) in ethanol-fixed smear preparations under phase contrast: shortly after telophase the daughter nuclei contain a number of small nucleolar bodies which, during the next 60 min, gradually fuse with one another to form one large central nucleolus. This period was approximately of the same length as the time required for coalescence plus subsequent incubation with thymidine-^{3}H. Hence, in an experiment concerned with the behavior of postmitotic nuclei in a late interphase host, or vice versa, correct timing of the experiment was essential. Successful were those combinations in which the exchange of constituents between the plasmodia was just beginning at the time when mitosis was underway in one of the plasmodia.

So that nuclei at a predetermined stage of the mitotic cycle could be transplanted into host plasmodia of another, also predetermined, stage, plasmodia in different stages of the mitotic cycle had to be available simultaneously. For this purpose several sets of surface plasmodia were prepared, at intervals of 2–3 hr, from aliquots of the same microplasmodial culture (15). These groups of plasmodia went through the first and second postfusion mitoses (for determination of mitotic stages see Cytological Techniques) with phase differences approximately equal to the time intervals at which the various plasmodia were prepared by fusion (15).

For transplantation of nuclei from one plasmodium (donor) into another one (host), we made use of the known tendency of the plasmodia to coalesce spontaneously when brought into contact with one another (15, 17). When two plasmodia are allowed to coalesce (Fig. 1), an exchange of cytoplasmic constituents (Fig. 2), including nuclei and mitochondria, commences. If the plasmodia are separated within approximately 15 min after the beginning of this exchange, each plasmodium contains a few constituents from the other. Thus, the result of coalescence is tantamount to transplantation of plasmodial constituents (nuclei, mitochondria, etc.) from one plasmodium into another.

Locomotion and high mobility were essential prerequisites for prompt coalescence. As the plasmodia exhibit virtually no locomotion on growth medium, the two plasmodia which were to serve, respectively, as donor and host, were placed (Fig. 1), at a distance of approximately 1.0 cm, along with the supporting filter paper, on nonnutrient agar (1% Bacto-Agar from Difco Laboratories, Detroit, Mich.) approximately 2 hr before they reached the stages which we wanted to combine. This transfer was followed by rapid expansion of the plasmodial periphery over the agar surface. The delay of mitosis caused by lack of nutrients did not interfere with the experiment since it was similar for all plasmodia which were transferred to agar at late interphase.

Soon after the expanding plasmodia (Fig. 1 *a*) touched each other, they coalesced (Fig. 1 *b*) and, within the next 5–10 min, thin plasmodial strands began to appear reaching from one plasmodium into the other (Fig. 1 *c*). The plasmodia were separated, by a cut along the line where they had coalesced, shortly after they had reached the stage of coalescence shown in Fig. 1 *b*, and before the formation of plasmodial strands was as pronounced as seen in Fig. 1 *c*. For determination of the best time for separation of the plasmodia, the appearance of nuclei from the prospective donor in the prospective host was conveniently monitored by inspection under phase contrast of smear preparations made from small explants removed from the host piece at a distance of approximately 5 mm from the line of contact. As soon as nuclei from the prospective donor were found among the nuclei of the host plasmodium, both "donor" and "host" were separated as described above. A piece of approximately 0.5 cm^2 adjacent to the region of previous coalescence was removed, along with the underlying agar, from the plasmodium that was to serve as host, and placed on another plate of nonnutrient agar to allow the constituents that were received before separation from the other plasmodium to become more evenly distributed. Pieces were excised from the distant sites of both donor and host, at a distance of approximately 2.5 cm from the line of coalescence, and likewise placed on nonnutrient agar. They served as controls (donor control and host control) since they contained no nuclei from the other plasmodium at the time of excision. Unless either the donor or the host nuclei were prelabelled with thymidine-^{3}H (see under Results), the host pieces containing the implanted nuclei were immersed, along with the host control

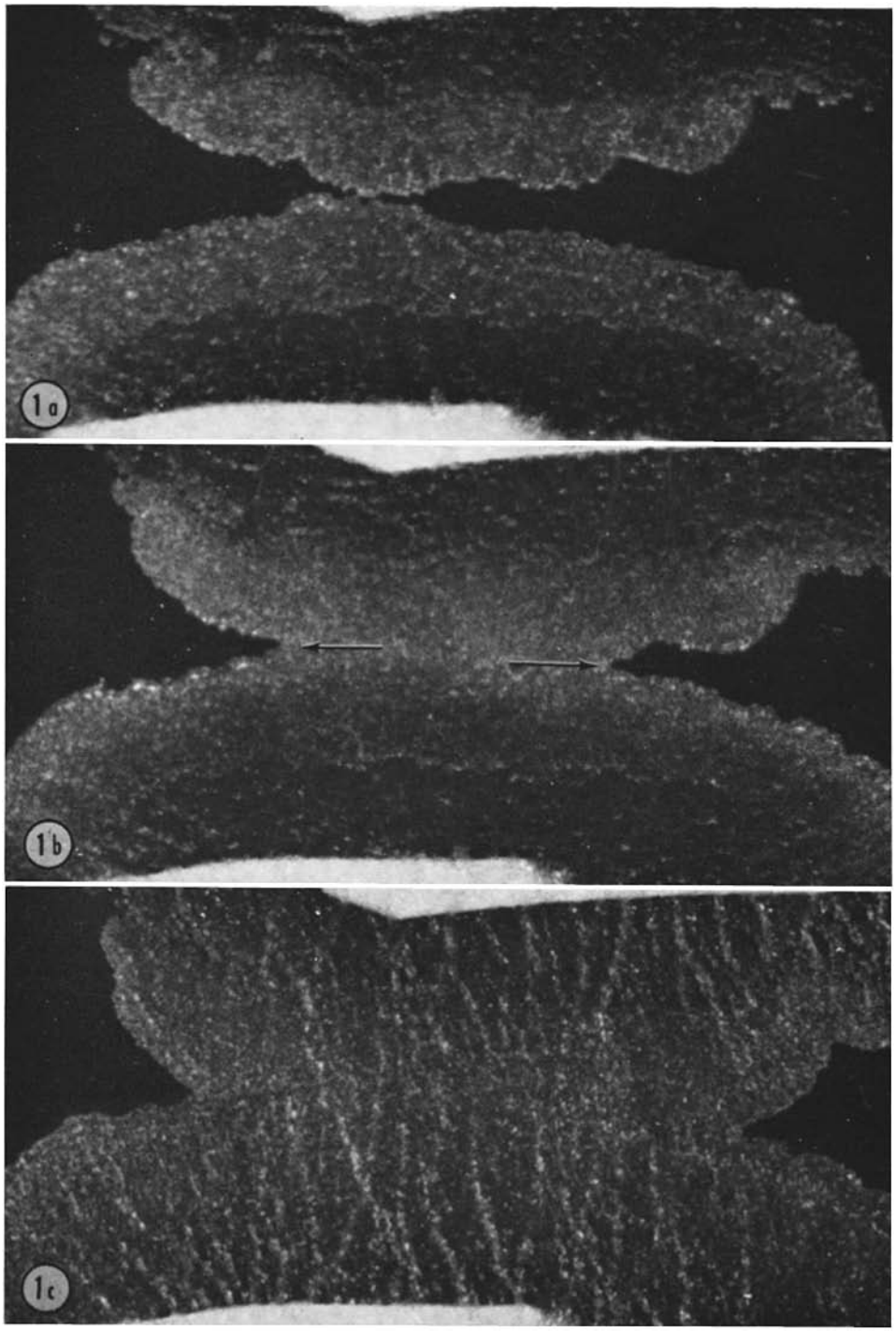

FIGURE 1 Coalescence of two plasmodia on nonnutrient agar. *a*, 95 min after being placed on agar. *b*, 10 min later. Coalescence is underway. For transplantation of plasmodial constituents the plasmodia are separated, shortly after reaching this stage of coalescence, along a line between the two arrows. *c*, 10 min later. Numerous plasmodial strands extending from one plasmodium into the other. × 5.

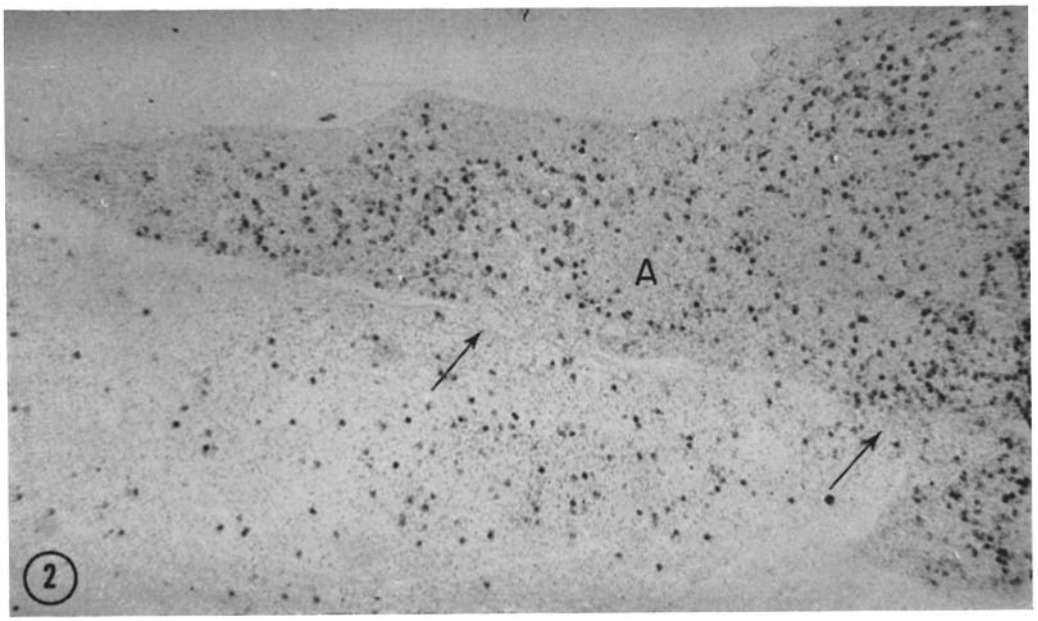

FIGURE 2 Section through two plasmodia shortly after coalescence. Radioautograph. Fixation, ethanol–acetic acid; unstained. The nuclei of one of the plasmodia (A) were labeled, prior to coalescence, with thymidine-^{3}H. Incubation for 3 hr. Concentration, 5 μc/cc of growth medium. The labeled nuclei appear as black dots. Arrows point to areas of coalescence. × 150.

and donor control pieces, in agitated growth medium containing thymidine-^{3}H (Schwarz Bio Research, Inc., Orangeburg, N. Y., specific activity, 0.36 c/mmole). The time of incubation and the concentrations used are indicated under Results.

Cytological Techniques

The nuclei within one plasmodium divide in synchrony (12, 14). For establishment of the position of the experimental and control plasmodia in the mitotic cycle during the experiment, the time of mitosis of these plasmodia before and after coalescence and incubation with thymidine-^{3}H was determined by examination under phase contrast of ethanol-fixed, unstained smear preparations of small explants from the plasmodial periphery. After incubation with thymidine-^{3}H a number of pieces were fixed as smear preparations, on cover slips, in 95% ethanol. Some of the pieces were embedded in glycerol jelly, and the number of transplanted nuclei (per cent) was determined with phase-contrast microscopy. The preparations were processed for radioautography (Kodak AR-10 stripping film), stored in the dark at 4°C, and developed with Kodak D-19 developer (10 min at 20°C). Preparations from postmitotic host plasmodia that were incubated with thymidine-^{3}H shortly after receiving G_2-phase nuclei were stored in the dark for 2 months, under nitrogen atmosphere, prior to development. All other preparations were stored for 1 wk. Only very thin areas in the radioautographs were evaluated.

For precise correlation of isotope uptake with the stage in the mitotic cycle of individual nuclei, stained sections of the experimental pieces were made after incubation with thymidine-^{3}H as follows. The experimental and the control plasmodia were fixed in Champy's fluid for 6 hr, followed by incubation in 3% potassium dichromate for 3 days in the dark. After having been embedded in paraffin (melting point 61°C), sections (thickness, 2 μ) were prepared and stained with acid-fuchsin (Altmann's procedure). As the nuclei of a single plasmodium at a given stage of the mitotic cycle are of uniform morphology (12), the transplanted nuclei, representing a stage of the mitotic cycle different from that of the host nuclei, were readily identified. The sections were evaluated as follows: areas containing one or two transplanted nuclei surrounded by nuclei of the host were photographed, and the location of the section on the slide and of the area in the section was recorded. The stain was then extracted with 95% ethanol overnight and the slides were processed for radioautography. After exposure and development, the previously recorded areas were photographed again. The nuclei were identified by comparison with the pre-

vious pictures and the number of unlabeled and labeled donor and host nuclei was determined. Although only a limited number of nuclei was examined, this method had the advantage that label, or failure to be labeled, was readily correlated with previously identified nuclei.

TABLE I

Failure of G_2-Phase Nuclei to Incorporate Thymidine-^3H after Transplantation into Early S-Phase Host Plasmodia (Radioautographs of Smear Preparations)

	Stage of host plasmodium at time of fixation*	
	I	II
	+35 min	*+50 min*
Time of fixation before (−) metaphase of donor control on agar, *min*	−65	−45
Nuclei unlabeled in host control, %	<0.5	<0.5
Nuclei unlabeled in donor control, %	85.4	>99
Transplanted nuclei in host at time of fixation, %	1.5	4.2
Nuclei unlabeled in host, %	1.1	4.4

Both donor and host were placed on agar approximately 60–80 min before mitosis of the latter. For heavy label, the preparations were incubated for 2 months in the dark before development.

* After (+) metaphase.

RESULTS

In the first group of experiments (Tables I and II, Fig. 3) the ability of G_2-phase nuclei to incorporate thymidine-^3H shortly after transplantation into S-phase host plasmodia had been determined. At the time of coalescence the nuclei of the host were about to enter metaphase, and the exchange of plasmodial constituents was allowed to continue until the host nuclei were in late telophase. At various times after separation, donor control, host control, and pieces of host plasmodia containing the transplanted nuclei were incubated with tritiated thymidine (20 μc/cc) for a period of 15 min. As seen in Table I, all nuclei in the host control piece, with the exception of less than 0.5%, were labeled and, except for the combination shown in the first column of Table I, almost all nuclei in the donor-control piece remained unlabeled. The nuclei that were found unlabeled in radioautographs were clearly identifiable as G_2-phase nuclei received from the G_2-phase donor plasmodium, and their number was close to the number of nuclei that were identified with phase-contrast microscope as G_2-phase nuclei in ethanol-fixed, parallel, smear preparations. There was no indication that an appreciable number of the

TABLE II

Failure of G_2-Phase Nuclei to Incorporate Thymidine-^3H after Transplantation into S-Phase Plasmodia

	Stage of host plasmodium at time of fixation*			
	I	II	III	IV
	+30 min	*+45 min*	*+50 min*	*+30 min*
Time of fixation before (−) metaphase of donor-control, min	−120	−50	−30	−30
No. late interphase (donor) nuclei recorded	9	5	6	8
No. labeled nuclei among these	1	0	0	0
No. postmitotic (host) nuclei recorded in same areas of section as the above donor nuclei	17	18	19	19
No. labeled nuclei among these	17	18	19	19

Similar experiment as above. After incubation of the host with thymidine-^3H, pieces were fixed with Champy's liquid and processed (see under Methods) so that label in radioautographs could be correlated with nuclei previously identified in stained sections.

* After (+) metaphase.

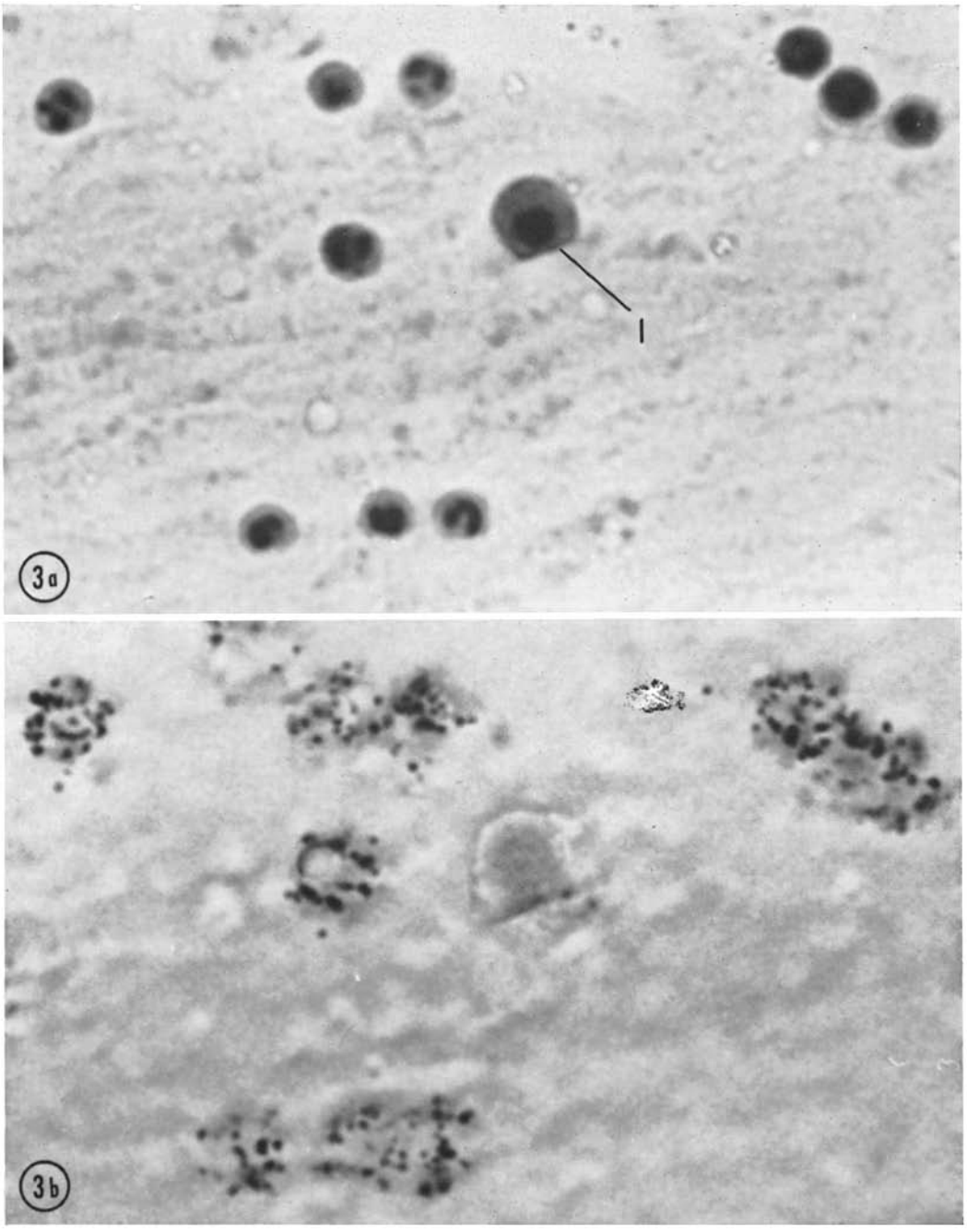

FIGURE 3 Section through postmitotic (45 min after metaphase) host plasmodium containing transplanted G_2-phase nuclei. Fixation shortly after incubation with thymidine-^{3}H. *a*, stained with acid fuchsin. *b*, radioautograph made after destaining. *I*, implanted G_2-phase nucleus containing one central nucleolus. The other nuclei are postmitotic host nuclei. × 2500..

transplanted G_2-phase nuclei had become labeled in the S-phase environment during the 15 min period of incubation.

In another, similar, experiment (Table II, Fig. 3), donor controls and host controls were fixed as smear preparations, as in Table I. The host pieces containing the transplanted nuclei were fixed, after incubation, with Champy's liquid, and a number of host nuclei and transplanted nuclei were identified in stained preparations and examined for label as described above (see under Methods).

In Table II, column 1, only one of the nuclei identified as having been transplanted became labeled; in the other combinations (columns 2–4), all transplanted nuclei remained unlabeled.

The following experiment (Table III) was done to determine whether G_2-phase nuclei would incorporate thymidine-^{3}H after prolonged exposure to an S-phase environment in the host. In this experiment coalescence of a late interphase donor with the host plasmodium was just underway at the time when the nuclei of the latter had arrived at late prophase, and both plasmodia were separated when the nuclei of the host were at late telophase. After separation, smear preparations were made so that the number of transplanted nuclei could be determined. The host piece (Table III) was then removed from the agar and placed, along with donor and host control pieces, on growth medium containing thymidine-^{3}H (5 μc/cc). 3 hr later the plasmodia were fixed and processed for radioautography. At this time, transplanted nuclei and host nuclei were not morphologically distinguishable. However, the number of unlabeled nuclei in the host was close to that of the nuclei that were identified in the host as donor nuclei shortly after transplantation. As all of the nuclei in thin areas of smear preparations of the host controls were labeled and most of the nuclei in the donor controls were unlabeled, we believe that the unlabeled nuclei which were found 3 hr after transplantation in the S-phase host were the transplanted G_2-phase nuclei and that these were unable to incorporate thymidine-^{3}H even after prolonged exposure to an S-phase environment.

In order to determine whether the failure of G_2-phase nuclei to incorporate thymidine-^{3}H after transplantation into S-phase hosts was due to a loss of viability or lack of functional integration with the host plasmodia, we devised the following two experiments. In one experiment the prospective donor plasmodium was incubated for 30 min with thymidine-^{3}H (5 μc/cc) during early interphase and allowed to go through another mitosis and S phase. The growth medium was changed twice during this time. Nuclei from this plasmodium were then transplanted, at late G_2 phase, into another, unlabeled, plasmodium approximately 5 hr before mitosis of the latter, and the percentage of labeled nuclei in the host was subsequently determined at various times until after the next mitosis. The result is shown in Table IV. The percentage of labeled nuclei found in the host plasmodium varied somewhat during the period between transplantation and prophase of the host nuclei, but it was not very different after mitosis from what it was shortly before mitosis. The number of nuclei which did not participate in mitosis (interphase nuclei found in smear preparations at late prophase) was negligible (less than 0.5%). For two preparations, which were fixed 30

TABLE III

Failure of G_2-Phase Nuclei to Incorporate Thymidine-^{3}H During an Extended Period of Time Following Transplantation into a Postmitotic Host Plasmodium (Radioautographs of Smear Preparations)

	Stage of the donor plasmodium at the time of coalescence*		
	−60 min	*−55 min*	*−45 min*
Transplanted nuclei found in host at 15 min after estimated beginning of coalescence, %	5.5	6.2	4.8
Unlabeled nuclei in donor control, %	>99	>99	>99
Unlabeled nuclei in host control, %	<0.5	<0.5	<0.5
Unlabeled nuclei in host	4.8	6.5	5.1

* Before (−) metaphase.

TABLE IV

Participation of Transplanted G_2-Phase Nuclei in the Next Mitosis of the Host (Radioautographs of Smear Preparations)

Time of fixation*	Labeled nuclei in host
hr	%
−4.2	5.4
−3.4	4.6
−0.5	5.1
+0.5	6.3
+1.0	6.1

G_2-phase nuclei prelabeled with tritiated thymidine were transplanted into an unlabeled host plasmodium approximately 5 hr before mitosis of the latter.

* Before (−) and after (+) metaphase of mitosis in host plasmodium.

TABLE V

Incorporation of Thymidine-^3H into Nuclei Transplanted During G_2-Phase after Having Undergone Mitosis Along with the Host Nuclei (Radioautographs of Smear Preparations)

Time of fixation*	Unlabeled nuclei
hr	%
−8.3	4.2
−6.2	3.7
−3.9	3.9
−0.5	3.5
+0.5	0
+1.0	0

G_2-phase nuclei were transplanted into an S-phase host plasmodium approximately 2 hr after mitosis of the latter. The host plasmodium, after having received the transplanted nuclei, was returned to growth medium containing thymidine-^3H.

* Before (−) and after (+) metaphase of host plasmodium.

min before and 1 hr after metaphase, respectively, we determined the number of grains per labeled nucleus. The number was 9.2 prior to mitosis and 4.2 after mitosis. This decrease of label and the finding that the percentage of labeled nuclei was approximately the same before and after mitosis, suggest that the transplanted nuclei had divided along with the host nuclei.

In another experiment we determined the ability of transplanted G_2-phase nuclei to incorporate thymidine-^3H after undergoing mitosis along with the host nuclei. In this case, G_2-phase nuclei were transplanted into another plasmodium (Table V) approximately 2 hr after mitosis of the latter. The host plasmodium, after receiving the transplanted nuclei, was returned to growth medium containing thymidine-^3H (5 μc/cc). Between 3.5 and 4.2% unlabeled nuclei were found in smear preparations taken at various times during the remaining part of the intermitotic period and during prophase, whereas in smear preparations from host controls all nuclei were labeled. Approximately 30 min after metaphase the number of unlabeled nuclei was negligible (below 0.5%). As the previous experiment had shown that the transplanted G_2-phase nuclei divided along with those of the host plasmodia, it follows that the implanted G_2-phase nuclei became labeled after mitosis. Both experiments indicate that the G_2-phase nuclei after transplantation were viable and fully integrated with the host environment.

In the reciprocal combination (Tables VI and VII, Fig. 4) S-phase nuclei were transplanted into late G_2-phase host plasmodia. In one group of experiments, incubation with thymidine-^3H (concentration, 5 μc/cc; duration, 10 min) began between 10 and 30 min after termination of coalescence. As seen in Table VI, all implanted donor nuclei were labeled (Fig. 4) at approximately the same rate as the nuclei in the donor controls whereas almost all of the host-control nuclei were unlabeled. In one experiment we determined whether the rate of incorporation of thymidine-^3H into transplanted S-phase nuclei was different, several hours after their transfer into G_2-phase hosts, from that in donor controls at the same time (Table VII). For this purpose, S-phase nuclei were transplanted, immediately after mitosis, into host plasmodia approximately 2½ hr before metaphase of the latter. The host pieces were incubated, along with the donor control and host-control pieces, for 10 min with tritiated thymidine (10 μc/cc) at a time when the nuclei of the host nuclei were beginning to prepare for mitosis, as indicated by a gradual movement of the nucleolus toward the nuclear membrane. As seen in Table VII, the number of nuclei which were labeled in the host at that time was not appreciably different from the number of implanted nuclei found shortly after transplantation in smear preparations. The grain count over these donor nuclei was only slightly

TABLE VI

Rate of Incorporation of Thymidine-^3H into S-Phase Nuclei after Transplantation into G_2-Phase Host Plasmodia (Radioautographs of Smear Preparations)

	Stage of host plasmodium at time of fixation*		
	−15 min	*−40 min*	*−20 min*
Time of fixation after (+) metaphase of donor control on agar, min	+30	+35	+40
Nuclei labeled in donor control, %	>99	>99	>99
No. grains per nucleus in donor control	12.4	10.2	11.4
Nuclei labeled in host control, %	<0.5	<0.5	<0.5
No. grains per labeled nucleus in host control	6.8	5.9	7.9
Transplanted nuclei found in host at time of fixation, %	3.4	6.3	5.9
Nuclei labeled in host, %	4.1	5.8	6.5
No. grains per labeled nucleus in host	14.2	9.8	12.5

The prospective host plasmodia were placed on agar at various times during the latter part of the intermitotic period. The prospective donor plasmodia were placed on agar approximately 60 min before mitosis. Coalescence between these plasmodia began shortly after the nuclei of the donor had finished mitosis. The values given for number of grains/nucleus were not corrected for background (= 1.1 grains per 100 μ^2).

* Before (−) metaphase.

lower than that over nuclei of the donor-control pieces.

DISCUSSION

The results show that G_2-phase nuclei of *Physarum polycephalum*, when transplanted into an S-phase host plasmodium, did not incorporate thymidine-^3H before they had completed the next mitosis. On the other hand, nuclei in S phase, when transplanted into a G_2-phase host, continued to incorporate thymidine-^3H at a rate which was not appreciably different from that of the donor controls during the period of observation. The failure of G_2-phase nuclei to incorporate DNA precursors from an S-phase host was apparently not due to loss of viability or lack of functional integration of the G_2-phase nuclei with the host plasmodia. This result is in agreement with previous results on mitotic synchronization of large populations of microplasmodia (10): when a large number of nuclei from different microplasmodia are forced, by coalescence, to share a common cytoplasmic environment, the first synchronous postfusion mitosis begins after an interval which is approximately one-half of the average generation time of the microplasmodial culture, without any delay which would be indicative of significant nuclear damage.

Recent studies (4) suggest that DNA replication in *Physarum polycephalum* occurs as a succession of rounds of replication, and that the initiation of each of these rounds requires formation of a protein which is newly synthesized after a previous round has been completed. The results of these experiments and experiments by Braun et al. (2) suggest that these rounds of replication concern different replicons (23) of the *Physarum* genome whose replication follows an ordered pattern as in other organisms (21, 22, 28, 32–34). From the present experiments it appears that, for each replicon, after DNA replication is completed, the occurrence of mitosis is an additional prerequisite for the beginning of the next round of replication.

Recent studies in different organisms have shown that DNA synthesis was initiated, apparently without being preceded by mitosis, in nuclei which were introduced, either by transplantation (6–9, 29) or by cell fusion (18), into a DNA-synthesizing (S phase) environment. Conversely, DNA synthesis was depressed in S-phase nuclei of *Stentor coeruleus* (6) and *Amoeba proteus* (29) by transplanting them into either G_1-phase (6) or G_2-phase (29) host cells. The results of the work with *Stentor* (6) and *Amoeba proteus* (29) suggest that DNA replication is initiated by a cytoplasmic factor which is present in S-phase cells but absent during G_1 phase and

TABLE VII

Incorporation of Thymidine-^3H and Rate of Incorporation into S-Phase Nuclei after Prolonged Exposure to G_2-Phase Environment (Radioautographs of Smear Preparations)

	Stage of host plasmodium at time of estimated coalescence*
	$-2\frac{1}{2}$ hr
Nuclei labeled in donor control at time of fixation, %	>99
No. grains per nucleus in donor control at time of fixation	8.4
Nuclei labeled in host control at time of fixation, %	<0.5
No. grains per labeled nucleus in host control at time of fixation	6.1
Transplanted nuclei found in host at 15 min after estimated beginning of coalescence, %	7.2
Nuclei labeled in host, %	6.8
No. grains per labeled nucleus in host	7.8

Postmitotic nuclei were transplanted, immediately after mitosis, into host plasmodium approximately $2\frac{1}{2}$ hr before metaphase of the latter. The pieces were then returned, along with donor controls and host controls, to growth medium and incubated with thymidine-^3H shortly before the nuclei of the host had begun to prepare for mitosis.

* Before (−) metaphase.

G_2 phase. The difference between these results and those obtained with a similar experimental approach in *Physarum polycephalum* could be due to the different organization of the organisms employed. *P. polycephalum* is a multinucleated organism and as many as 10^8 nuclei may share the same cytoplasmic environment in a given plasmodium (15). The nuclei have no measurable G_1 phase, and incubation of plasmodia with thymidine-^3H for short periods of time immediately after mitosis or during the first 3 hr after mitosis results in heavy labeling of all nuclei (2, 16). During the transition period between very early and very late interphase the number of nuclei incorporating thymidine-^3H declines gradually (2), and heavily labeled and unlabeled nuclei may be found side by side. This would suggest that, possibly because of regional differences within a plasmodium, DNA replication is more advanced in some nuclei than in others and that some nuclei enter G_2 phase at a time when earlier rounds of replication in other nuclei have not been completed. A mechanism for initiation of DNA replication such as that found in *Amoeba proteus* (29) and *Stentor coeruleus* (6) could lead, by diffusion of initiating factors from more advanced areas to less advanced regions of the plasmodium, to replication of the same part of the genome more than once within one intermitotic period. This would be prevented if replication of all parts of the genome, including those which are replicated at a later time of the intermitotic period, were rigidly controlled by a common signal related to mitosis.

The finding that G_2-phase nuclei, when implanted into S-phase host plasmodia, were not labeled with thymidine-^3H even after prolonged exposure to the S-phase environment, and that incorporation of thymidine-^3H in these nuclei began immediately after division, would suggest that the beginning of DNA replication, even of those replicons which might start replication at a later time during the intermitotic period (2, 4), is controlled by an event which occurs either during or immediately after mitosis. It is possible that the chromosomes are rendered competent for DNA replication by a structural alteration which they undergo as part of the mitotic process. Another, less likely, possibility cannot be excluded at present, namely, that a diffusible, cytoplasmic factor initiating DNA synthesis, of the type discovered in *A. proteus* (29) and in *S. coeruleus* (6), is present in *P. polycephalum* for a few minutes during mitosis. Such a factor could not be demonstrated by our experiments. For transplantation of G_2-phase nuclei into an S-phase environment we used only those combinations of plasmodia in which the exchange of plasmodial constituents was beginning at the time of prophase, or a little later, of the prospective host. For demonstration of the presence or absence, during this short period, of factors which could initiate DNA synthesis in nuclei that have not undergone mitosis, it would have been necessary to use combinations of plasmodia in which the G_2-phase nuclei entered the host plasmodia just prior to mitosis of the latter, without subsequently undergoing mitosis along with the nuclei of the host. Unfortunately, such a combination results in advanced mitosis of the G_2-phase nuclei in synchrony with a somewhat delayed mitosis of the host nuclei (14, 30).

The finding that S-phase nuclei continued to incorporate DNA precursors from a G_2-phase host

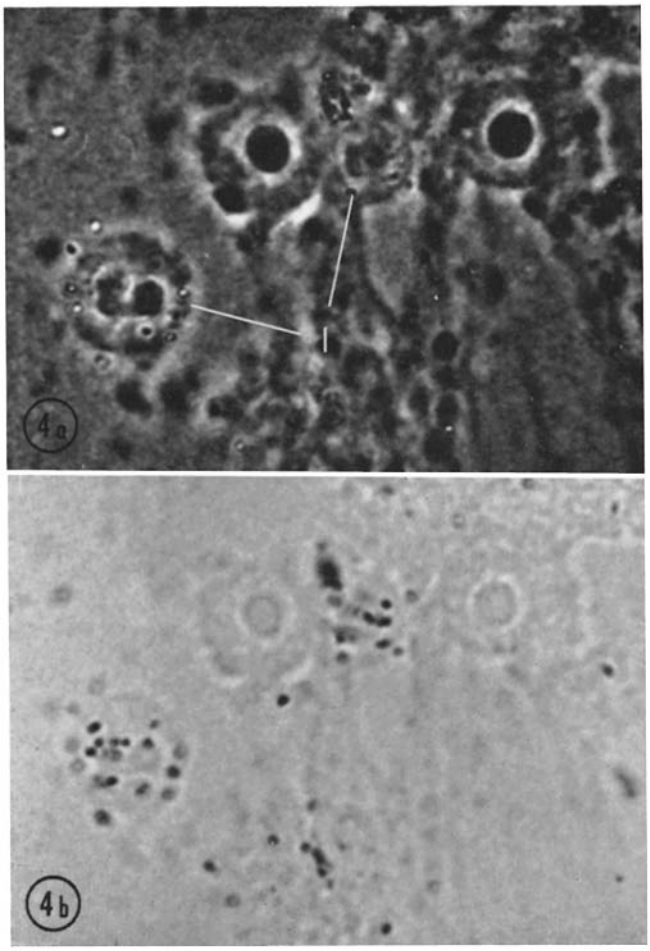

FIGURE 4 Smear preparation (fixation, ethanol) from late interphase (40 min before metaphase) host plasmodium which had received postmitotic nuclei. Fixed shortly after incubation with thymidine-^{3}H. Radioautograph. *I*, implanted S-phase nuclei. × 2500. *a*, with phase contrast. *b*, without phase contrast.

plasmodium at a rate not vastly different from that in S-phase plasmodia is not surprising since it is known that DNA polymerase (27) and thymidine kinase (31) are present in *Physarum* throughout the intermitotic period and that DNA polymerase is present in isolated nuclei of this organism (3). The high rate of incorporation of thymidine-^{3}H does not indicate, however, that G_2-phase plasmodia would be able to support a complete round of replication of a large number of nuclei. The number of implanted S-phase nuclei was always small compared to the total number of nuclei which served as acceptors for DNA precursors during S-phase.

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REFERENCES

1. BOLLUM, F. J. 1963. *J. Cellular Comp. Physiol.* **62** (Suppl. 1):61.
2. BRAUN, R., C. MITTERMAYER, and H. P. RUSCH. 1965. *Proc. Natl. Acad. Sci. U.S.* **53**:294.
3. BREWER, E. N., and H. P. RUSCH. 1965. *Biochem. Biophys. Res. Commun.* **21**:235.
4. CUMMINS, J. E., and H. P. RUSCH. 1966. *J. Cell Biol.* **31**:577.
5. DANIEL, J. W., and H. H. BALDWIN. 1964. *In* Methods in Cell Physiology. D. M. Prescott, editor. Academic Press Inc., New York. **1**:9.

6. DeTerra, N. 1965. *Proc. Natl Acad. Sci. U.S.* **57**:607.
7. Graham, C. F. 1966. *J. Cell Sci.* **1**:363.
8. Graham, C. F., K. Arms, and J. B. Gurdon. 1966. *Develop. Biol.* **14**:349.
9. Gurdon, J. B. 1967. *Proc. Natl Acad. Sci. U.S.* **58**:545.
10. Guttes, E., S. Guttes, and H. P. Rusch. 1959. *Federation Proc.* **18**:479.
11. Guttes, E., and S. Guttes. 1961. Abstracts of the First Annual Meeting of the American Society of Cell Biology 79.
12. Guttes, E., S. Guttes, and H. P. Rusch. 1961. *Develop. Biol.* **3**:588.
13. Guttes, E., and S. Guttes. 1962. *Federation Proc.* **21**:381.
14, Guttes, E., and S. Guttes. 1963. *Experientia.* **19**:13.
15. Guttes, E., and S. Guttes. 1964. *In* Methods in Cell Physiology. D. M. Prescott, editor. Academic Press Inc., New York. **1**:43.
16. Guttes, E., P. C. Hanawalt, and S. Guttes. 1967. *Biochim. Biophys. Acta.* **142**:181.
17. Guttes, E., and S. Guttes. 1967. *Experientia.* **23**:713.
18. Harris, H., J. F. Watkins, C. E. Ford, and G. I. Schoefl. 1966. *J. Cell Sci.* **1**:1.
19. Howard, A., and S. R. Pelc. 1953. *Heredity.* **6** (Suppl.):261.
20. Howard, F. L. 1932. *Ann. Botany.* **46**:461.
21. Hsu, T. C. 1964. *J. Cell Biol.* **23**:53.
22. Hsu, T. C., W. Schmid, and E. Stubblefield. 1964. *In* The Role of Chromosomes in Development. M. Locke, editor. Academic Press Inc., New York. 83.
23. Jacob, F., S. Brenner, and F. Cuzin. 1963. *Cold Spring Harbor Symp. Quant. Biol.* **28**:329.
24. Lark, K. G. 1963. *In* Molecular Genetics. J. H. Taylor, editor. Academic Press Inc., New York. **1**:153.
25. Maaloe, O. 1963. *J. Cellular Comp. Physiol.* **62** (Suppl. 1):31.
26. Nygaard, O. F., S. Guttes, and H. P. Rusch. 1960. *Biochim. Biophys. Acta.* **38**:298.
27. Nygaard, O. F. 1961. *In* The Effects of Ionizing Radiations on Immune Processes. C. A. Leone, editor. Gordon & Breach Science Publishers, Inc., New York. 47.
28. Plaut, W., D. Nash, and T. Fanning. 1966. *J. Mol. Biol.* **16**:85.
29. Prescott, D. M., and L. Goldstein. 1967. *Science.* **155**:469.
30. Rusch, H. P., W. Sachsenmaier, K. Behrens, and V. Gruter. 1966. *J. Cell Biol.* **31**:204.
31. Sachsenmaier, W., D. v. Fournier, and K. F. Gürtler. 1967. *Biochem. Biophys. Res Commun.* **27**:655.
32. Stubblefield, E., and G. C. Mueller. 1962. *Cancer Res.* **22**:1091.
33. Taylor, J. H. 1960. *J. Biophys. Biochem. Cytol.* **7**:455.
34. Wimber, D. E. 1961. *Exptl. Cell. Res.* **23**:402.