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THE CONTROL OF MITOSIS IN *PHYSARUM POLYCEPHALUM*: THE EFFECT OF DELAYING MITOSIS AND EVIDENCE FOR THE OPERATION OF THE CONTROL MECHANISM IN THE ABSENCE OF GROWTH

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SUMMARY

Possible mechanisms coordinating the control of mitosis and DNA synthesis with growth were experimentally tested in *Physarum polycephalum* by the response of plasmodia to 2 different kinds of perturbation of the DNA:mass ratio. Mitosis and DNA synthesis were delayed without stopping growth either by the use of fluorodeoxyuridine (FUdR) or puromycin, in both cases the delayed mitosis was followed by a single shortened intermitotic period, as predicted by all the mechanisms considered and substantiating a basic assumption made that the time of mitosis is homeostatically controlled to maintain a constant DNA:mass ratio. When more than 50% of the DNA was destroyed by ultraviolet irradiation 2 consecutive mitoses could occur even when growth was completely stopped by starvation. This result can only be accounted for by 2 of the 5 models considered, a finding which agrees with the results of previous experiments which were also consistent with only these 2 mechanisms.

INTRODUCTION

In two previous papers some hypothetical mechanisms which might coordinate the onset of mitosis with growth were considered (Fantes, Grant, Pritchard, Sudbery & Wheals, 1975; Sudbery & Grant, 1975). It was deduced how each would react to perturbations of the DNA:mass ratio by various methods, specifically with regard to lengths of intermitotic periods subsequent to the perturbation. The predicted responses of the various models following, for example, destruction of a proportion of the DNA were sufficiently different to provide a means of experimentally testing each possible mechanism, allowing the rejection of those where the experimentally observed response was not consistent with that predicted. The results of experiments using *Physarum polycephalum* as a test system in which to destroy a proportion of the DNA have already been presented (Sudbery & Grant, 1975). The effect of a further way of perturbing the DNA:mass ratio was also previously considered; this was to delay mitosis and DNA synthesis without stopping growth. With the exception of a special case of one of the models, the so called Linear-Exponential Model (Fantes *et al.* 1975), all the models should theoretically respond in the same way to this

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perturbation. A single shortened intermitotic period should follow the experimentally extended period, the shortened period being shortened by exactly the same amount as the previous period was extended. Although a distinction between the mechanisms using this perturbation as an analytical tool is not possible, this kind of experiment does provide an additional test of the basic assumption made (Fantes *et al.* 1975) that cells attempt to maintain a constant DNA:mass ratio, an assumption that is not implicit in other theoretical treatments of the control mechanism (Smith & Martin, 1973; Kauffman, 1974). Accordingly this paper presents results of experiments in which the DNA:mass ratio was lowered by delaying mitosis without stopping growth.

A further important prediction can be deduced from the fact that in 3 of the mechanisms mass increase is an integral part of the system but is not in the remaining 2 mechanisms. This allows the former to be distinguished from the latter experimentally by testing to see whether control of the DNA:mass ratio still occurs in the complete absence of growth. This can be done in *P. polycephalum* by removing more than 50% of the DNA by ultraviolet irradiation. After the mitosis subsequent to this treatment the DNA:mass ratio will still be below the steady state value at which mitosis is normally triggered. If the control mechanism is independent of growth then another mitosis should occur in conditions of starvation which prevent growth. The results of such an experiment are presented in this paper.

P. polycephalum was used throughout these investigations as the naturally synchronous mitosis in the multinucleate plasmodial stage allows easy and accurate measurement of the intermitotic period. In addition the DNA:mass ratio can readily be reduced either by ultraviolet irradiation which destroys a proportion of the nuclei (Sachsenmaier, Donges, Rupff & Czihak, 1970; Devi, Guttes & Guttes, 1968) or by delaying mitosis without stopping growth by the inhibitors fluorodeoxyuridine (FUdR) (Sachsenmaier & Rusch, 1964) or puromycin (Sachsenmaier, 1967). As DNA synthesis immediately follows mitosis it seems likely that these 2 events are coordinately controlled. This simplifies the study of the relationship between cell growth and the timing of these events which may be separately controlled in other cell types (Nurse, 1975).

MATERIALS AND METHODS

Physarum polycephalum, strain CL, plasmodia were maintained in shaken liquid culture, from which synchronous surface plasmodia were prepared as described previously (Daniel & Baldwin, 1964) on Schleicher and Schull 576 filter paper. The citrate-buffered nutrient medium described by Daniel & Baldwin was used throughout, it was changed following the second mitosis (MII) after fusion of microplasmodia and after every subsequent mitosis (MIII, MIV, MV, etc.). The time of mitosis was determined by examination of ethanol-fixed smears under a phase-contrast microscope (Guttes, Guttes & Rusch, 1961).

Growth as determined by measurements of protein synthesis was followed as described previously (Sudberg & Grant, 1975). FUdR was a gift from Roche products Ltd, Puromycin, uridine, and thymidine were obtained from Sigma.

Plasmodia were irradiated with an Englehardt Hanovia germicidal lamp (emission maximum = 265 nm) at a dose rate of 5 J m^{-2} s^{-1} as measured by a Latarjet ultraviolet dosimeter (maximum sensitivity at 253·7 nm).

RESULTS

The effect of delaying mitosis without stopping growth

Two methods were employed to delay mitosis without stopping growth.

A 3-h pulse of FUdR in S-phase was used to delay the subsequent mitosis by an equivalent amount (Sachsenmaier & Rusch, 1964). Ten replicate plasmodia were prepared. Fifteen minutes after MII, 5 were placed on medium containing FUdR (2×10^{-5} M), and uridine (4×10^{-4} M) which prevents RNA metabolism being inhibited by FUdR, the remainder serving as controls. After 3 h the treated plasmodia

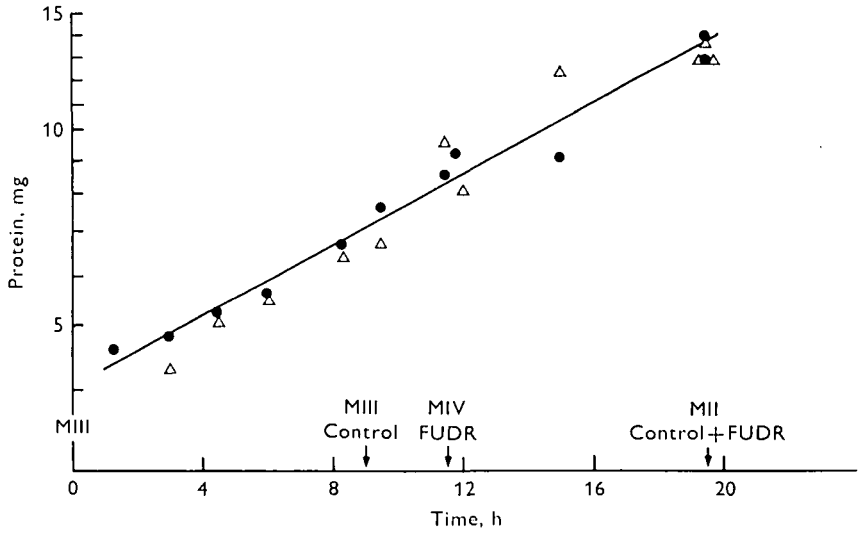

Fig. 1. The effect of a pulse of FUdR and uridine on growth. Replicate 2-cm-square plasmodia were prepared from a large plasmodium grown in rocker culture as described previously (Sudbery & Grant, 1975). Thirty minutes after MII, half were subjected to a 3-h pulse of FUdR and uridine. At intervals plasmodia were harvested and the protein content determined. The arrows indicate the mean times of mitoses. ●, control; △, treated.

were placed on medium containing thymidine (4×10^{-4} M) which reversed the block on DNA synthesis imposed by FUdR and uridine. Then 90 min after MIII in the control plasmodia both the treated and the control plasmodia were placed on fresh medium with no additions. The times of MIII, MIV and MV were recorded in both the treated and the control plasmodia, the medium being changed after every mitosis. The result of such an experiment is shown in Table 1. As observed by Sachsenmaier & Rusch (1964) treatment with FUdR delayed the subsequent mitosis. The next period was shortened, however, such that the total length of MII–MIV was the same in both the treated and control plasmodia. The period MIV–MV was of the same length in both sets of plasmodia; thus the oscillations, predicted by one form of the Linear-Exponential model, did not occur. Rates of growth as measured by protein increase were compared in treated and control plasmodia. The result is

shown in Fig. 1. It can be seen that the rate of protein increase was the same in both sets of plasmodia.

A high concentration of puromycin (600 μg/ml) *was added to the medium after the completion of S-phase.* This delays the onset of the next mitosis (Sachsenmaier, 1967) without interfering with growth, provided the drug is removed from the medium at the time of the next mitosis (J. Mohberg, personal communication). Replicate

Table 1. *The effect of delaying mitosis with FUdR and uridine on the length of subsequent intermitotic periods*

Treatment	MII–MIII	MIII–MIV	MIV–MV	MII–MIV
Uridine + FUdR, then TdR	9·6 ± 0·1	6·5 ± 0·3	10·1 ± 0·6	16·1 ± 0·3
Control	7·6 ± 0·5	8·4 ± 0·2	10·0 ± 0·6	16·0 ± 0·7

Values given are mean ± S.E.M. in h.

Table 2. *The effect of delaying mitosis with puromycin on the length of the subsequent intermitotic period*

	MII–MIII	MIII–MIV	MII–MIV
Control	10·1 ± 0·2	10·4 ± 0·2	20·4 ± 0·1
Puromycin	11·8 ± 0·2	8·7 ± 0·1	20·5 ± 0·2

Values given are mean ± S.E.M. in h.

plasmodia were prepared and 3 h after MII half were placed on medium containing puromycin (600 μg/ml). This delayed the next mitosis by 2 h compared to the control plasmodia. At MIII treated plasmodia were placed on fresh media with no additions. Under these conditions no net inhibition of protein synthesis takes place (J. Mohberg, personal communication). The times of MII and MIV in the treated and control plasmodia are shown in Table 2. As observed with FUdR and uridine treatment, the next intermitotic period following the delayed mitosis was shortened by the amount the previous period was extended.

The effect of a large reduction in the DNA:mass ratio in the absence of growth

To stop growth, plasmodia were starved by culturing them on a balanced salts solution buffered at pH 4·6. The time taken for growth to be inhibited and the extent of the inhibition is shown in Fig. 2. It can be seen that on the non-nutrient medium protein increase drops to zero after 2 h and thereafter the overall protein content starts to fall. Mitosis in the starved and control plasmodia occurred at the same time, presumably because mitosis had been initiated in the experimental plasmodia before growth stopped.

The effect of starvation on the shortened period following irradiation was then determined. Twenty-two replicate plasmodia were prepared and 3 h after MII 14 were irradiated with a total dose of 10^{-3} J. From data published previously (Sudbery & Grant, 1975) this dose ensured that more than 50% of the DNA was destroyed.

Two hours before the expected time of the delayed mitosis 7 of the irradiated plasmodia were transferred to the non-nutrient medium and 7 to fresh nutrient medium as unstarved controls. Of the remaining 8 unirradiated plasmodia, 4 were transferred to non-nutrient medium 2 h before the expected MIII and 4 were transferred to fresh nutrient medium. In both the irradiated and non-irradiated plasmodia the time of MIII was the same in the starved and corresponding non-starved plasmodia. The lengths of the period MIII–MIV under the various conditions are shown in Table 3. It can be seen that there is no significant difference in the lengths of shortened intermitotic period between the irradiated starved and non-starved plasmodia, whereas non-irradiated, starved plasmodia had not gone through mitosis after 24 h, when the observations were discontinued.

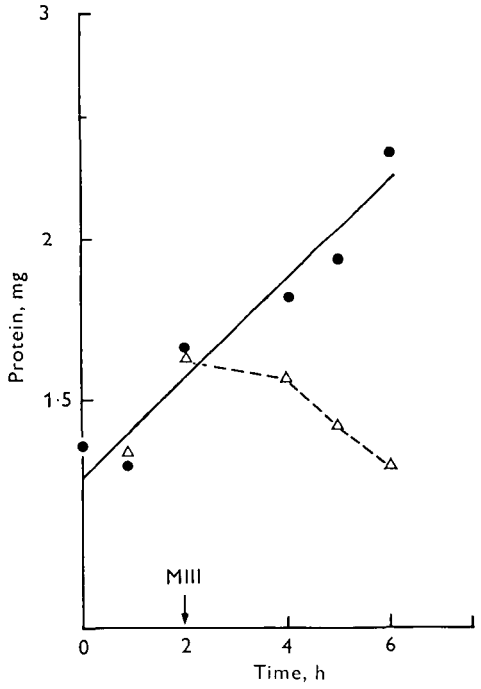

Fig. 2. Growth of plasmodia on non-nutrient medium. Plasmodia 1·5 cm square were used to follow the increase of protein in plasmodia growing on nutrient medium (●) or balanced salts solution (△) as described in the text.

Table 3. *Duration of the period MIII–MIV under various conditions*

Treatment	MIII–MIV
Irradiated, then into nutrient medium	7·3 ± 0·1
Irradiated, then into non-nutrient medium	6·9 ± 0·1
Non-irradiated, then into nutrient medium	8·9 ± 0·1
Non-irradiated, then into non-nutrient medium	>24

Values given are mean ± S.E.M. in h.

DISCUSSION

Brewer & Rusch (1968) reported that in *P. polycephalum* following a mitosis delayed by heat shock, the next intermitotic period was shortened by the amount the previous period was extended such that the subsequent mitosis occurred at the same time as an untreated control. We report here the confirmation of the result noted by Brewer & Rusch together with data to show that growth is not affected by the methods employed to delay mitosis. This result is that predicted by all the mechanisms considered previously (Fantes *et al.* 1975; Sudbery & Grant, 1975) and substantiates the basic postulate that the time of mitosis is controlled to maintain a constant DNA:mass ratio. In *Tetrahymena* it has been shown that delay of cell division results in not one but several of the subsequent cell cycles being shortened (Zeuthen & Scherbaum, 1954). This is not necessarily inconsistent with our prediction that only one shortened cycle should occur. Previously we have shown that in *P. polycephalum* there is a minimum period of some 6 h which must elapse between successive mitoses (Sudbery & Grant, 1975). Ultraviolet-induced changes in the DNA:mass ratio were made such that regulation of the DNA:mass ratio within one period required a period much shorter than 6 h. Since this did not happen, under these conditions a period of the minimum length ensued, necessarily followed by one or more shortened periods (or minimum periods). It is possible that the constraints of a minimum cell cycle length prevents recovery from a delayed cell division within one cycle in *Tetrahymena*.

Enucleation experiments carried out by Frazier (1973) using *Stentor coeruleus* discussed by Sudbery & Grant (1975) suggested a further aspect of the control mechanism, namely that control occurred in the absence of growth. An observation by Devi & Guttes (1972) suggested that the control system in *P. polycephalum* may have a similar property. They showed that the period between the first and control post-irradiation mitoses was only slightly extended by transfer to a non-nutrient medium. In their experiments, however, the irradiation procedure did not lead to the period between the first and second post-irradiation mitoses being shortened in the irradiated non-starved plasmodia compared to the non-irradiated, non-starved controls, indicating doubt as to whether a substantial fraction of the DNA was destroyed. Furthermore, estimations of mass increase were not made. This experiment can be used to distinguish between the models only if it is known that more than 50% of the DNA was destroyed and that no mass increase occurred between the first and second post-irradiation mitoses. We report here the result of an experiment where the irradiation procedure is known from previous experiments to destroy slightly more than 50% of the DNA (Sudbery & Grant, 1975) and in which it was shown that no mass increase occurs between the first and second post-irradiation mitoses. The result clearly shows that the absence of growth does not affect the length of this period, showing that control of the DNA:mass ratio takes place in the absence of growth, an observation which must have a significant bearing on consideration of possible control mechanism. We suggest that this property is consistent only with the Unstable Inhibitor Model (Fantes *et al.* 1975) and the Class II Structured Model (Sudbery & Grant, 1975) and not with the other models considered. Significantly, the results of

previous experiments designed to test different predictions were consistent only with these same 2 models (Sudbery & Grant, 1975). While the Class II structural model will satisfactorily explain the results from *Physarum* some difficulties in applying the model to other cell types have already been pointed out (Sudbery & Grant, 1975). Nevertheless it does not seem possible at present to distinguish definitively between these 2 models from the experimentally observed response to perturbations of the DNA:mass ratio.

It is hoped that new ways will become available to study this problem, in particular the use of conditional mutants defective in specific mitotic cycle functions should provide a promising approach.

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