PERIODIC THYMIDINE KINASE PRODUCTION IN SYNCHRONOUS PLASMODIA OF PHYSARUM POLYCEPHALUM: INHIBITION BY ACTINOMYCIN AND ACTIDION +)

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Mitosis in multinuclear plasmodia of the myxomycete Physarum polycephalum occurs synchronously every 9 hrs at 26°C. Nuclear DNA replicates during the first 3 hrs following telophase (Nygaard et al.,1960; Sachsenmaier, 1964). Activity of the enzyme thymidine kinase (ATP : thymidine 5'-phosphotransferase, EC 2.7.1.21) increases periodically at the end of interphase and reaches a maximum shortly after mitosis (Sachsenmaier and Ives, 1965). Periodicity of thymidine kinase has been observed in a variety of systems (Bollum and Potter,1959; Hotta and Stern, 1963; Stubblefield and Mueller, 1965; Brent et al.,1965; Littlefield, 1966). This communication concerns with the mechanism controlling the periodic fluctuation of thymidine kinase activity in Physarum polycephalum. Actinomycin C and actidion were employed to inhibit synthesis of RNA and protein at different stages prior to and during the period of enzyme increase. The effects of the antibiotics on enzyme activity and on synchronous mitosis were studied. The results suggest that the increase of kinase activity reflects synthesis of new enzyme protein which in turn depends on the prior formation of messenger-RNA (m-RNA).

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METHODS. Disk shaped synchronous macroplasmodia of Physarum polycephalum were cultivated axenically as described earlier (Daniel and Rusch, 1961; Sachsenmaier and Rusch, 1964). Cultures were cut into three equal segments at various stages between the second and third mitosis. One segment was immediately frozen and stored at -30° C; the second was transferred onto medium containing 250 µg/ml actinomycin C (Bayer, Leverkusen) or 50 µg/ml actidion (supplied by the Cancer Chemotherapy National Service Center, Bethesda). The third piece (control) and the treated segment were frozen at the end of control mitosis. Samples were sonicated with a Branson sonifier (3 x 1 second, stage 3) at 0° C following the addition of 1.0 ml 0.15 M KCl and centrifuged 20 min. at 20.000 g. Protein (Lowry et al., 1951) and thymidine kinase activity (Sachsenmaier and Ives, 1965) were assayed in the supernatant. Synthesis of RNA and protein was measured by pulse labelling of plasmodial segments with ^{3}H-uridine (2 µc/ml,0.25 c/mM) and ^{3}H-DL-leucine (2 to 4 µc/ml, 5 c/mM). Labelled samples were processed as described earlier (Sachsenmaier and Becker, 1964).

RESULTS. Fig 1 demonstrates the effects of actinomycin C on RNA and protein synthesis during late interphase of a synchronous plasmodium. Incorporation of ^{3}H-uridine decreases rapidly below 20% of the controls within 20 min following the addition of the inhibitor. Incorporation of ^{3}H-leucine on the other hand drops slowly over a period of several hours suggesting an average half life time of about 3 hours for the bulk of m-RNA molecules.

Treatment with actidion (Table I) at concentrations above 5 µg/ml reduces protein synthesis below 25% during the first 20 minutes and stops further synthesis completely in cultures

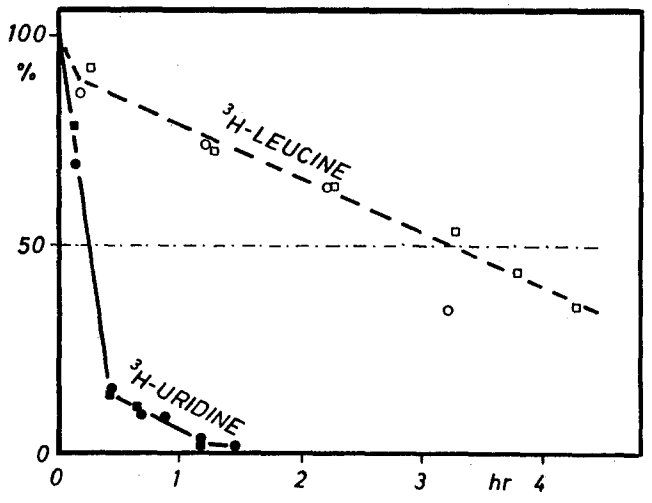

Fig. 1: Effects of actinomycin C on RNA and protein synthesis. 250 µg/ml actinomycin C were added to the medium of two plasmodia 1.6 hrs prior to telophase. At the same time and after various intervals small segments (2 to 4 cm^2) were pulse labelled on medium containing 2 µc/ml ^{3}H-uridine (15 min) or 2 to 4 µc/ml ^{3}H-leucine (20 to 30 min) plus inhibitor. Control plasmodia were labelled simultaneously in the absence of inhibitor. Experiment I (o,•); experiment II (□,■). Abscissa: time following the addition of actinomycin C; ordinata: % incorporation of radioactivity relative to controls.

TABLE I: Effects of actidion on protein and RNA synthesis.

Actidion µg/ml	Specific radioactivity of protein and RNA after 20 and 40 min incorporation of ^{3}H-leucine: dpm/mg prot. (% incorp.)		^{3}H-uridine: dpm/µg RNA (% incorp.)	
	20 min	40 min	20 min	40 min
0	3820 (100)	8320 (100)	2750 (100)	7150 (100)
2	1540 (40)	1990 (24)	2230 (81)	3940 (55)
5	920 (24)	1950 (23)	1690 (61)	3760 (53)
10	685 (18)	1500 (18)	1735 (63)	3570 (50)
20	920 (24)	920 (11)	1765 (64)	2580 (36)
50	900 (23)	680 (8)	1610 (58)	2530 (35)

Plasmodial segments were pulse labelled (20 and 40 min) with ^{3}H-leucine or ^{3}H-uridine in G2-phase (2.5 hrs prior to telophase) immediately following the addition of the inhibitor.

treated with 20 and 50 µg/ml. Actidion, at all concentrations, also affects RNA synthesis to some extent.

Thymidine kinase activity (fig. 2) starts to increase approximately 50 min prior to prophase and reaches its maximum at the end of mitosis, as observed earlier (Sachsenmaier and Ives, 1965). Addition of 50 µg/ml actidion at any time prior to or during the period of activity increase prevents or immediately interrupts this increase. This suggests that enzyme activity does increase due to synthesis of enzyme protein. Actinomycin C on the other hand only prevents the increase of kinase activity when added at least one hour prior to the expected onset of this increase. Enzyme induction is no longer inhibited by actinomycin C during the last hour of the

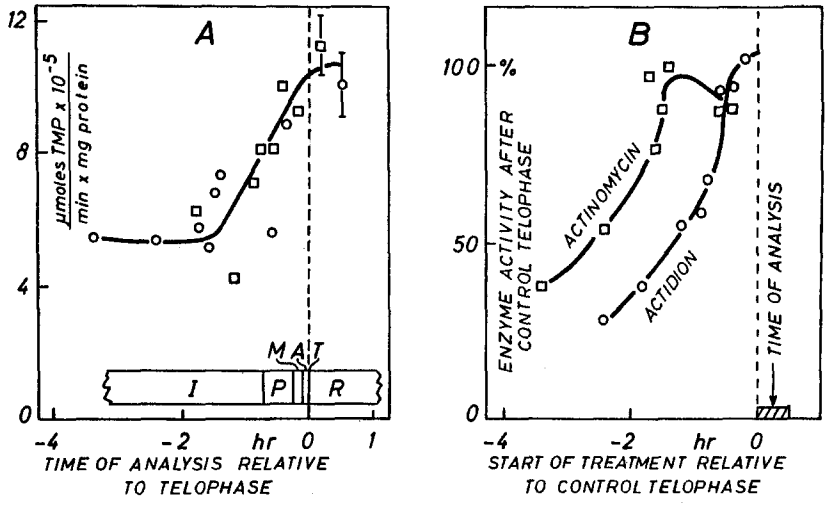

Fig. 2: Effects of actinomycin C and actidion on thymidine kinase induction. A: Points indicate enzyme activities of control plasmodia at the time shown on the abscissa. Cytological stage of nuclei: I (Interphase); P (Prophase); M (Metaphase); A (Anaphase); R (Reconstruction of the nucleolus). B: The abscissa indicates the time when 250 µg/ml actinomycin C or 50 µg/ml actidion were added. All cultures were analyzed within a period of 30 min after control telophase. Activity is expressed as % of the mean of control values.

"actidion sensitive" period. This suggests that m-RNA for thymidine kinase is formed about one hour prior to enzyme synthesis.

The effects of actidion on thymidine kinase induction closely parallel the effects of this agent on mitosis. Addition of the inhibitor prior to late prophase prevents the onset or delays further progress of mitosis (Table II). Similar observations have been reported by Cummins et al. (1965). This indicates that at least some proteins required for the onset and for progress of mitosis are formed during the period of thymidine kinase induction. Actinomycin C which no longer inhibits enzyme induction when added later than 90 min prior

TABLE II. Effects of actinomycin C and actidion on mitosis.

Start of treatment (hrs relative to telophase)		Cytological stage of nuclei: End of treatment (0.5 hrs after control telophase)		
		Control	Actinomycin	Actidion
-2.4	I	R	I	I
-1.7	I	R	IP+	I^{o}
-1.4	I	R	IP+	I^{o}
-0.6	IP	R	M	IP^{o}
-0.4	IP	R	R	M
-0.3	P	R	R	M
-0.2	M	R	R	R

Plasmodial segments were transferred onto medium containing 250 µg/ml actinomycin C or 50 µg/ml actidion at the time indicated in column (1). The cytological stage of nuclei was observed at the start of treatment (column 2) and 0.5 hrs after control telophase (columns 4 + 5). I: Interphase; IP: Early prophase; P: Prophase; M: Metaphase; R: Reconstruction of nucleolar material (0.2 to 1.5 hrs after telophase). (+,o): abnormally condensed chromatin and small (+) or normal (o) nucleoli.

to telophase, still affects mitosis when added as late as 35 min before telophase. This may indicate that m-RNA molecules required for certain steps of mitosis are formed later, or function shorter, than thymidine kinase specific m-RNA.

Our results suggest that thymidine kinase activity increases periodically at the end of the division cycle as a result of periodic enzyme induction. This process may be controlled by a clock mechanism which triggers one of the following events prior to each synchronous mitosis: a) synthesis of a pulse of m-RNA molecules at the thymidine kinase cistron (Jacob and Monod, 1961); b) stabilization or activation of thymidine kinase m-RNA at the translation level; c) block of enzyme turnover by stabilization of the enzyme molecule.

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