CONTROL OF DNA REPLICATION: EFFECT OF SPERMINE ON DNA POLYMERASE ACTIVITY IN NUCLEI ISOLATED FROM PHYSARUM POLYCEPHALUM

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The mechanisms for temporal and quantitative control of DNA replication are not known. A close relationship between replication and the appearance of enzymes involved with deoxythymidine triphosphate synthesis has been observed (reviews by Bucher, 1963; Lark, 1963; Schmidt, 1966). These observations have led to proposals that the control of DNA synthesis in vivo is regulated by levels of these enzymes (e.g. Canellakis et al., 1959; Maley and Maley, 1960). However, evidence for more direct means of control has been obtained (Lark, 1960; Billen, 1962; Gold and Helleiner, 1964; Gogol and Rosenberg, 1964; Taylor, 1965; Brewer and Rusch, 1965; Adams et al., 1966). An observed change in the physical state of the template during the replication cycle has been implicated (Rolfe, 1963; Bollum, 1963; Rosenberg and Cavalieri, 1965). The present communication presents further evidence that changes in template structure or availability, but not precursor or DNA polymerase levels, regulate the synthesis of DNA by isolated nuclei of Physarum polycephalum.

In order to study DNA polymerase levels in isolated nuclei of Physarum, as opposed to the synthetic capacity of such nuclei without added primer, exogenous DNA and spermine were included in the incubation medium. Spermine and other polyamines exhibit metabolic effects in a variety of organisms (review by Tabor and Tabor, 1964). These effects include a stimulation of RNA synthesis in vivo (Mills and Dubin, 1966; Raina and Cohen, 1966) and in vitro (Fox and Weiss, 1964; O'Brien et al., 1966), and an inbibition of the

DNA polymerase reaction *in vitro* (O'Brien *et al.*, 1966). The present report describes a stimulatory effect of spermine on DNA polymerase activity in isolated nuclei.

METHODS AND MATERIALS

Nuclei within individual plasmodia (cf. Mittermayer *et al.*, 1965) of *Physarum polycephalum* divide synchronously. Under the conditions employed in this laboratory, there is no G_1 period, and the mitotic cycle lasts for about 8 hours. In this communication, mitosis refers to the third synchronous nuclear division after fusion of microplasmodia. Nuclei isolated by the method of Mohberg and Rusch (1964) were incubated, and incorporation of ^{3}H-dATP into an acid-insoluble product determined, essentially as described previously (Brewer and Rusch, 1965). Nuclei isolated from several plasmodia were pooled and divided into the same number of fractions; each fraction (containing about 0.1 mg DNA and 1 mg protein) was then incubated for 40 min. at 25 C in 1.0 ml of the standard incubation medium (0.2 M sucrose; 0.045 M $Mg(OAc)_2$; 0.4 mM dGTP, dCTP, dTTP; 1.0 μM ^{3}H-dATP (3.8 c/mmole); 0.05 M Tris-HCl, pH 7.5) with or without added DNA and/or spermine, as indicated below. At the end of the incubation period, aliquots were taken for protein determination (Lowry *et al.*, 1951), and then 5 ml of cold 0.25 M perchloric acid was added. Pellets obtained by centrifugation were dissolved in NaOH, reprecipitated with perchloric acid, and collected on Type E glass fiber filter discs (Gelman Instrument Co., Ann Arbor). Radioactivity was determined with a Packard Tri-Carb liquid scintillation counter.

Salmon sperm DNA and spermine·4HCl were obtained from Calbiochem. DNA was used in the native state. Randomly labeled ^{3}H-dATP was purchased from Schwarz BioResearch. Other chemicals were reagent grade.

RESULTS

The effect of spermine on the incorporation of ^{3}H-dATP into acid-insoluble material by isolated nuclei in the presence of exogenous DNA is shown in Fig. 1. The concentration of spermine which stimulated maximal

incorporation was found to be about 5.7×10^{-4} M, regardless of the time in the mitotic cycle at which the nuclei were isolated.

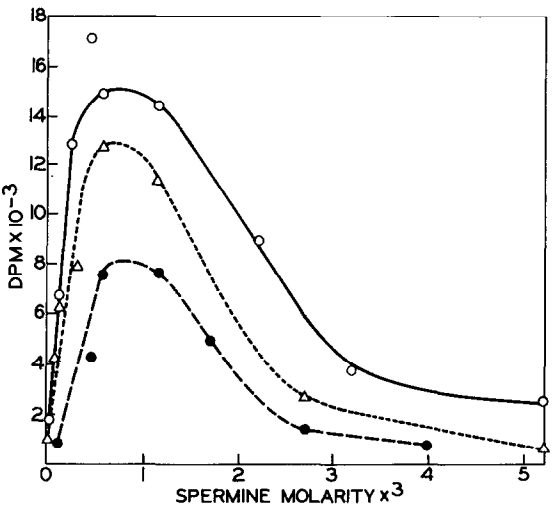

Fig. 1. Effect of spermine on incorporation of ^{3}H-dATP by isolated nuclei. Standard incubation medium + 0.15 mg DNA/ml. Nuclei isolated at 2.5 to 3 h. before mitosis (●), 15 to 60 min. before mitosis (▲), or 2 to 2.5 h. after mitosis (o).

At 5.7×10^{-4} M spermine, the incorporation of the radioactive precursor was found to be proportional to the amount of DNA added to the incubation medium, as shown in Fig. 2. For nuclei isolated at 30 to 60 min. before mitosis, the saturating level of DNA was about 0.1 mg/ml. For nuclei obtained at 1.5 h. after mitosis, saturation was not evident at 0.2 mg of added DNA per ml.

Further data concerning DNA synthesis by nuclei isolated at various times in the mitotic cycle are shown in Fig. 3. For this experiment, nuclei of four stationary cultures at the same point in the division cycle were isolated, pooled, and divided into quarters. Each quarter was then incubated for 40 min. in the standard incubation medium (a) without added DNA or spermine, (b) with spermine, (c) with DNA, or (d) with DNA and spermine, as indicated. The data show that when either DNA or spermine was added to the standard incubation medium, a relatively small but consistant increase

in ^{3}H-dATP incorporation occurred. When both were added, a very large increase was observed, the peak of which coincided with the peak of "physiological" nuclear DNA synthesis (standard incubation medium alone).

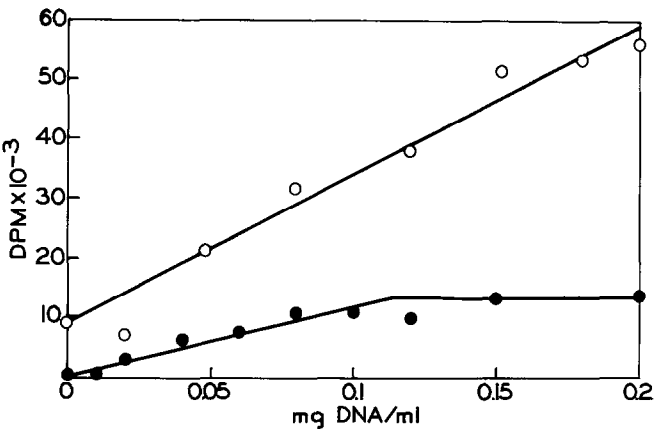

Fig. 2. Incorporation of ^{3}H-dATP by isolated nuclei in the presence of native salmon sperm DNA. Standard incubation medium + 5.7 x 10^{-4} M spermine. Nuclei isolated at 30 to 60 min. before mitosis (•), or at 1.5 h. after mitosis (o).

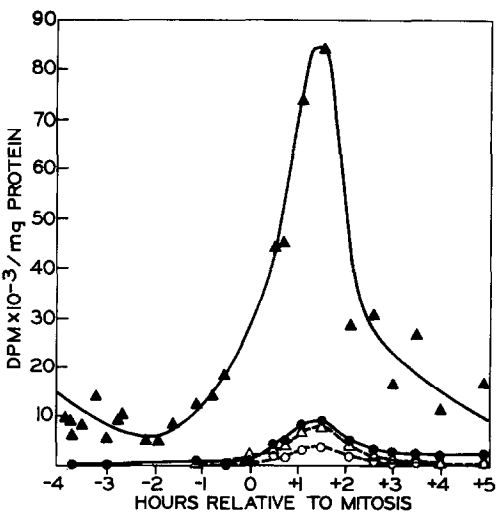

Fig. 3. Incorporation of ^{3}H-dATP by nuclei isolated at various times in the mitotic cycle. Nuclei were incubated in the standard incubation medium with no additions (o), with added spermine, 5.7 x 10^{-4} M (•), with added DNA, 0.15 mg/ml (▲), or with both spermine and DNA (▲).

DISCUSSION

Isolated nuclei of Physarum polycephalum incorporate precursors into DNA to an extent dependent on the time in the mitotic cycle at which the nuclei are isolated (Brewer and Rusch, 1965). The pattern resembles in vivo DNA synthesis at corresponding times of the division cycle in this organism.

The addition of both spermine and DNA to the incubation medium results in a many-fold increase in incorporation of ^{3}H-dATP into acid-insoluble material by isolated nuclei (Fig. 3). When spermine is omitted from the medium, however, only a relatively slight increase in incorporation relative to "physiological" activity is observed. This suggests that the function of spermine is either to increase the penetration of exogenous DNA into isolated nuclei, or to release or otherwise activate the latent DNA polymerase present in such nuclei. The latter suggestion is supported by the observations (Fig. 3) that (1) the apparent level of DNA polymerase is a function of the time in the division cycle at which the nuclei are isolated, (2) the peak polymerase activity coincides with the peak of DNA synthesis by isolated nuclei in the absence of added DNA and spermine, and (3) the polymerase activity corresponds closely to the in vivo levels of thymidine kinase throughout the mitotic cycle in the same organism (Sachsenmaier and Ives, 1965). When exogenous DNA is omitted from the incubation medium, on the other hand, relatively little increase in apparent DNA polymerase activity is seen with added spermine, even though the enzyme is presumably "activated" to the same extent. This indicates that the DNA-synthetic capacity of isolated nuclei is not directly controlled by levels of DNA polymerase. Furthermore, since the other parameters involved in the synthesis of DNA (Mg^{++}, deoxynucleoside triphosphates) are held constant in the incubation medium, direct control of in vivo synthesis by these factors also seems unlikely. Therefore, control of DNA synthesis probably resides with the structure or availability of the template itself. This conclusion is consistent with those of other workers based on data obtained by different means (Billen, 1962; Rosenberg and Cavalieri, 1965; Taylor, 1965).

The coincidence between the periodic rise and fall of DNA polymerase activity and that of thymidine kinase activity (Sachsenmaier and Ives, 1965) during the division cycle of Physarum polycephalum supports the suggestion (Sachsenmaier and Ives, 1965; Shen and Schmidt, 1966) that messengers for the synthesis of enzymes involved with DNA replication may be transcribed from the same operon.

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REFERENCES

Adams, R. L. P.; Abrams, R.; and Lieberman, I., J. Biol. Chem., 241, 903 (1966).
Billen, D., Biochim. Biophys. Acta, 55, 960 (1962).
Bollum, F. J., J. Cell. Comp. Physiol., Suppl. 62, 61 (1963).
Brewer, E. N., and Rusch, H. P., Biochem. Biophys. Res. Commun., 21, 235 (1965).
Bucher, N. L. R., Internat. Rev. Cytol., 15, 245 (1963).
Canellakis, E. S.; Jaffe, J. J.; Mantsavinos, R.; and Krakow, J. S., J. Biol. Chem., 234, 2096 (1959).
Fox, C. F., and Weiss, S. B., J. Biol. Chem., 239, 175 (1964).
Gogol, E. M., and Rosenberg, E., Biochem. Biophys. Res. Commun., 14, 565 (1964).
Gold, M., and Helleiner, C. W., Biochim. Biophys. Acta, 80, 193 (1964).
Lark, K. G., Biochim. Biophys. Acta, 45, 121 (1960).
Lark, K. G., in Molecular Genetics (Taylor, J. H., ed.), Acad. Press, New York, 1963, p. 153.
Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; and Randall, R. J., J. Biol. Chem., 193, 265 (1951).
Maley, F., and Maley, G. F., J. Biol. Chem., 235, 2968 (1960).
Mills, J., and Dubin, D. T., Mol. Pharmacol., 2, 311 (1966).
Mittermayer, C.; Braun, R.; and Rusch, H. P., Exptl. Cell Res., 38, 33 (1965).
Mohberg, E. J., and Rusch, H. P., J. Cell Biol., 23, 61A (1964).
O'Brien, R. L.; Olenick, J. G.; and Hahn, F. E., Proc. Natl. Acad. Sci., 55, 1511 (1966).
Raina, A., and Cohen, S. S., Proc. Natl. Acad. Sci., 55, 1587 (1966).
Rolfe, R., Proc. Natl. Acad. Sci., 49, 386 (1963).
Rosenberg, B. H., and Cavalieri, L. F., Nature, 206, 999 (1965).
Sachsenmaier, W., and Ives, D. H., Biochem. Z., 343, 399 (1965).
Schmidt, R. R., in Cell Synchrony (Cameron, I. L., and Padilla, G. M., eds.), Acad. Press, New York, 1966, p. 189.
Shen, S. R., and Schmidt, R. R. Arch. Biochem. Biophys., 115, 13 (1966).
Tabor, H., and Tabor, C. W., Pharmacol. Rev., 16, 245 (1964).
Taylor, E. W., Exptl. Cell Res., 40, 316 (1965).