DNA SYNTHESIS BY ISOLATED NUCLEI OF PHYSARUM POLYCEPHALUM

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Nuclei isolated from various sources are capable of protein and RNA synthesis (Allfrey et al., 1964; Birnstiel et al., 1962; Rendi, 1960; Malec et al., 1964; Sirlin and Schor, 1962; Allfrey and Mirsky, 1962; Rho and Chipchase, 1962; Mittermayer et al., in press; Rozijn et al., 1964; Rees and Rowland, 1961). Incorporation of precursors into DNA in isolated nuclei has also been reported (Friedkin and Wood, 1956; Mazia and Hinegardner, 1963; Behki and Schneider, 1963), but little incorporation was observed without added primer. Furthermore, no evidence was obtained to indicate that any of these nuclear preparations were autonomous with regard to control of DNA synthesis.

This communication reports the synthesis of DNA, without added primer, by isolated nuclei of Physarum polycephalum, in which synchronous mitosis occurs naturally. Synthesis occurred only in nuclei isolated during the DNA-synthesizing period of the mitotic cycle; this indicates that these nuclei retain, after isolation, the same mechanism for control of DNA synthesis as is exerted in vivo. The implications of these findings for DNA replication are discussed.

METHODS AND MATERIALS

Methods for culturing the organism and preparation of synchronously dividing cultures have been described (Daniel and Rusch, 1961; Mittermayer et al., 1965).

Nuclei were isolated by the method of Mohberg and Rusch (1964). For all experiments except those shown in Fig. 4, nuclei from 40 cultures were isolated between 15 and 60 min after mitosis, pooled, divided into 20 equal portions, and suspended in 2.0 ml of incubation medium. Incubation was for 30 min at 25 C; reactions were stopped by adding an equal volume of cold 0.5 M PCA. Pellets obtained by centrifugation at 30,000 g for 10 min were dissolved in 0.4 N NaOH, reprecipitated with 0.25 M PCA, and the process repeated. This suspension was filtered on Type E glass fiber filters (Gelman Instrument Co., Ann Arbor), and radioactivity was determined with a Packard Tri-Carb liquid scintillation counter. Protein was determined by the method of Lowry et al. (1951).

The incubation medium consisted of 0.2 M sucrose; 0.05 M Tris-HCl buffer, pH 7.2-7.4; 0.4 mM each dGTP, dCTP, and TTP; 7.0 - 10.0 μM ^{3}H-dATP; and 0.045 M $Mg(OAc)_2$.

Randomly labeled ^{3}H-dATP (0.67 c/mmole) was from Schwarz BioResearch; dGTP, dCTP, and TTP from P-L Biochemicals, Milwaukee. Other chemicals were reagent grade.

RESULTS

The effect of Mg^{++} on the incorporation of ^{3}H-dATP into acid-insoluble material is shown in Fig. 1. The extent of incorporation was linear with increasing Mg^{++} concentration up to about 0.035 M and remained maximal to at least 0.08 M; this represents a much higher requirement for Mg^{++} than that reported for in vitro DNA synthesis (Bessman et al., 1958; Davidson et al., 1958; Bollum and Potter, 1958). The effect is not one of osmolarity since the effect is independent of sucrose molarity (triangles, Fig. 1).

Incorporation of ^{3}H-dATP increased with time of incubation up to about 40 min (Fig. 2), with pH optimum at about 7.4 (Fig. 3).

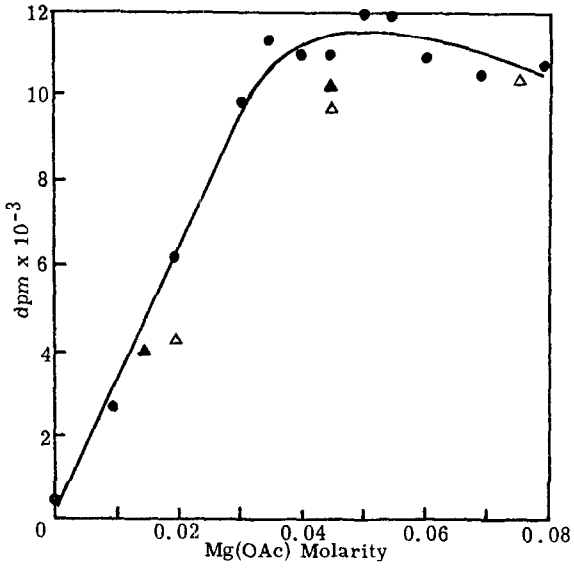

Fig. 1. Effect of Mg^{++} concentration on incorporation of ^{3}H-dATP (7.5 μM) by isolated nuclei. ●, 0.2 M sucrose; ▲, 0.1 M sucrose; △, 0.35 M sucrose. Other conditions described under METHODS.

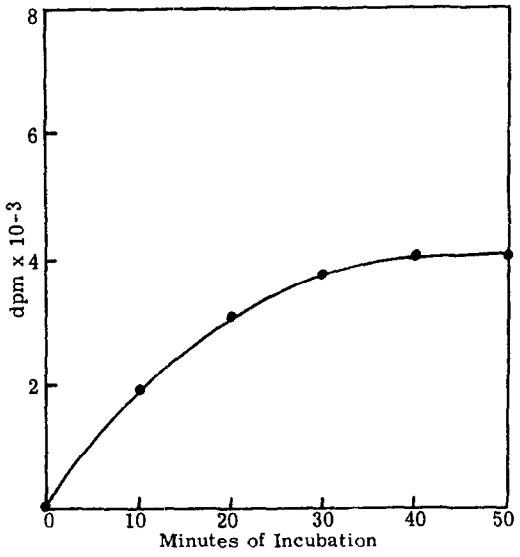

Fig. 2. Time course of incorporation of ^{3}H-dATP (10.0 μM) by isolated nuclei. Conditions described under METHODS.

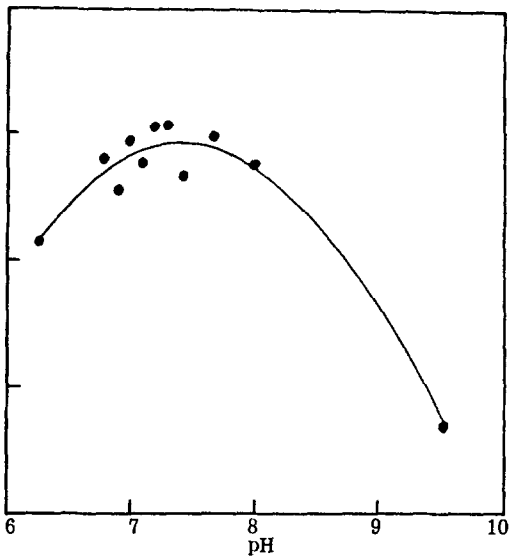

Fig. 3. pH curve for incorporation of ^{3}H-dATP (10.0 μM). Conditions described under METHODS.

The requirement for all four deoxynucleoside triphosphates is shown by the data in Table 1. Addition of all four nucleoside triphosphates, amino acid mixture, or glucose had little effect on the incorporation, except for ATP which was stimulatory. Na^{+} had no effect in contrast to the inhibitory effect of Ca^{++}, which is known to inhibit DNA polymerase (Smellie et al., 1959; Mantsavinos and Canellakis, 1959). The inhibitory effect of dithiothreitol is surprising, since mercaptoethanol has been used frequently in vitro.

Incubation at 37 C essentially doubled incorporation as compared with that at 25 C (optimal growth temperature for the intact organism); little incorporation was observed at 0 C.

The incorporation of ^{3}H-dATP into acid-insoluble material by nuclei isolated at various times of the mitotic cycle is shown in Fig. 4. Although the shape of the curve is somewhat different from that reported

TABLE 1

Effect of Additions and Deletions on Incorporation of ^{3}H-dATP

System (See METHODS)	cpm ^{3}H-dATP Incorporated
complete (7.5 μM ^{3}H-dATP)	6,740
-dGTP	2,090
-dCTP	2,000
-TTP	2,150
-dGTP,dCTP	440
-dCTP,TTP	700
-dGTP,TTP	480
-dGTP,dCTP,TTP	90
1/2X dGTP,dCTP,TTP	6,540
2X dGTP,dCTP,TTP	7,130
+2.5 mM ATP	9,600
+four nucleoside triphosphates, 0.4 mM each	8,690
+amino acid mixture, 0.25 mM each	5,780
+nucleoside triphosphates + amino acids	6,680
+0.07 M glucose	7,300
+6.5 mM dithiothreitol	3,470
+0.4 mM $CaCl_2$	2,280
+0.02 M NaCl	6,260
0 C	820
37 C	12,750

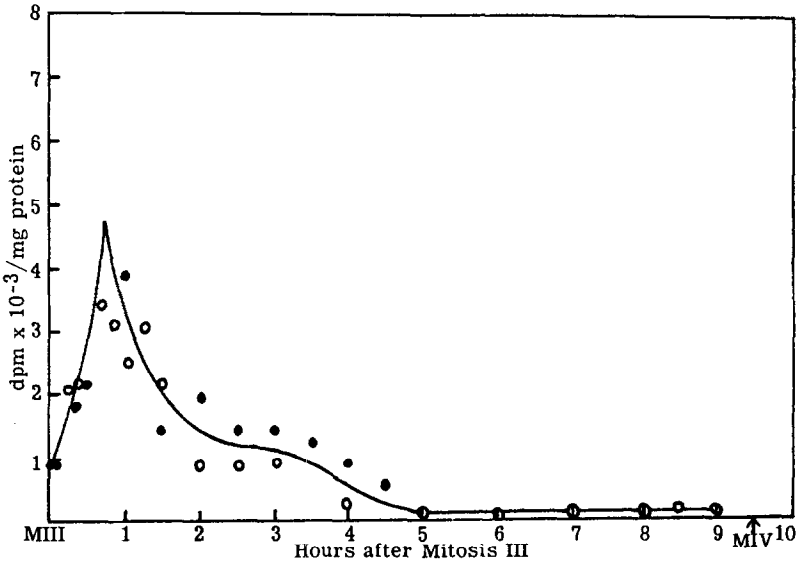

Fig. 4. Incorporation of ^{3}H-dATP by nuclei isolated at various times in the mitotic cycle. Nuclei from two stationary cultures were isolated at the indicated times relative to Mitosis III (third synchronous division after fusion of microplasmodia), and incubated as described under METHODS in 7.0 μM ^{3}H-dATP. The curve represents the combined results of two separate experiments, shown by ● and O.

for incorporation *in vivo* (Braun *et al.*, 1965), the same conclusions can be drawn: maximal synthesis occurs shortly after mitosis and a lower level of synthesis continues until 4-4 1/2 hours after nuclear division, after which little or no synthesis occurs until the next mitosis.

DISCUSSION

Isolated nuclei of *Physarum polycephalum* incorporate precursors into RNA to an extent depending on the time of the mitotic cycle at which the nuclei are isolated (Mittermayer *et al.*, in press). The pattern closely resembles RNA synthesis *in vivo* at corresponding times of the division cycle in this organism.

Evidence has now been obtained that the pattern of DNA synthesis by nuclei isolated at various times of the mitotic cycle is also similar to that observed *in vivo*. This is the first report of nuclei which retain physiological control of DNA replication after isolation.

The requirements for incorporation of labeled precursor into DNA include a relatively high Mg^{++} concentration (0.035 M) and the presence of all four deoxynucleoside triphosphates. The reaction is stimulated by addition of ATP, but not by mixtures of nucleoside triphosphates (the observed stimulation is presumably due to ATP) or amino acids.

The data support the suggestion of Braun *et al.* (1965) that control of DNA replication in this organism is probably owing to template structure or availability rather than to supply of substrates. Synthesis does not occur in nuclei isolated during the G_2 period of the division cycle even if all necessary substrates are present in the incubation mixture. On the other hand, DNA synthesis *in vivo* begins at a maximal rate immediately after mitosis (Braun *et al.*, 1965), whereas nuclei isolated at that time have a low rate of incorporation (Fig. 4). This finding indicates that isolated nuclei may be unable to *initiate* DNA replication but can carry on active synthesis after initiation.

Work is in progress to clarify this point, as well as to determine the nature of the acid-insoluble product.

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