SYNTHESIS OF A CYTOPLASMIC DNA DURING THE G_2 INTERPHASE OF PHYSARUM POLYCEPHALUM

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Although the bulk of DNA synthesis in the acellular slime mold Physarum polycephalum occurs during the first 2 to 3 hours of the interphase period ("S" period) (Nygaard et al., 1960), there are indications that a small amount of DNA synthesis occurs during the remainder of interphase ("G_2") (Sachsenmaier and Rusch, 1964; Sachsenmaier, 1964; and Evans, personal observation). Little is known about this synthesis. One possibility is that the nuclear division cycle is not perfectly synchronized. Sachsenmaier (1964) has suggested that the synthesis of DNA during G_2 occurs in the cytoplasm. The autoradiographic study by Guttes and Guttes (1964) strongly supports the latter hypothesis and suggests that the mitochondria are the site of this synthesis. A wide variety of evidence supports the view that mitochondria of many organisms contain DNA (cf. Gibor and Granick, 1964, and Nass et al., 1965). Parsons (1965) has demonstrated autoradiographically that synthesis of DNA in the mitochondria of Tetrahymena can occur during G_1 and G_2. The data presented in this report provide evidence for a cytoplasmic DNA synthesized during G_2 interphase and differing in buoyant density from the DNA of the nucleus.

EXPERIMENTAL

Macroplasmodia of Physarum polycephalum were grown axenically at 27°C. using the methods described by Mittermeyer et al. (1965). The time of the synchronous nuclear division within each plasmodium was determined by microscopic observation of alcohol-fixed smears. The observed interdivision time was about eight hours.

Plasmodia were transferred to a medium containing 3 to 4 μC/ml H^3-thymidine (sp. act. = 6.7 C/mM; New England Nuclear Corp.) four hours after metaphase of the second synchronous division (at a time when the S period is completed) and incubation was continued for two hours (at which time the molds were still in G_2 interphase). For harvesting, the molds were transferred to 0.15 M NaCl + 0.015 M Na citrate (SSC) and heated at 60°C. for 10 minutes. Four to six plasmodia were combined and used as a source of total cellular DNA. Equal numbers of cultures were briefly centrifuged and resuspended in the isolation medium for nuclei described by Mohberg and Rusch (1964); after several homogenizations and centrifugations a low speed (17 g) pellet was obtained which consisted of nuclei without appreciable cytoplasmic contamination. This nuclear pellet served as a second source of DNA.

The isolation and purification of DNA from either the whole plasmodia or the nuclear preparation was carried out according to a method developed previously (T.E. Evans and H. Harrington, unpublished): the sample was suspended in SSC, frozen and thawed, treated for one hour at 60°C. with pronase (1 mg/ml in SSC; Calbiochem), and homogenized with a teflon-glass homogenizer; after centrifugation at 35,000 g for 10 minutes, the supernatant was precipitated with streptomycin sulfate (5 mg/ml); the precipitate was collected by centrifugation and dissolved in 2 M NaCl; following alcohol precipitation and treatment with RNase (100 γ/ml; Worthington Biochemical Corp.) for one hour at 37° C., the preparation was further deproteinized with 0.2% Na

deoxycholate (DOC) and again precipitated with alcohol (the RNase and DOC treatments were repeated as necessary).

The tritium-labelled DNA obtained was 98% acid insoluble and was sensitive to DNase treatment as judged by acid solubility. The DNA preparations were subjected to density gradient centrifugation in CsCl for 68 hours in a Spinco SW-39 rotor at 37,000 rpm. The tubes were punctured and fractions of four drops each were collected. After dilution, the absorbance at 260 mμ was determined and a portion of each fraction was assayed for tritium by scintillation counting. Figure 1 records the results for the "total DNA" preparation. The radioactivity curve shows a peak at a lower density (ca. 1.68) than the majority of the 260 mμ absorbing material (ca. 1.70). Results

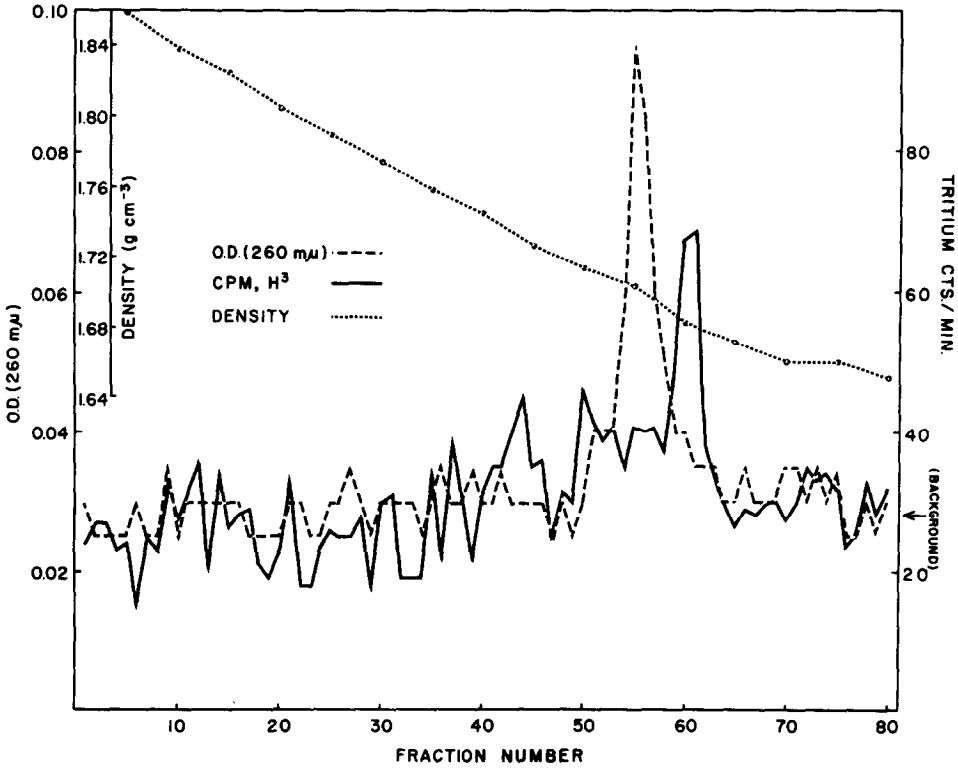

Figure 1. Density distribution of a "total DNA" preparation. The lighter tritium peak is seen to the right of the OD_{260} peak.

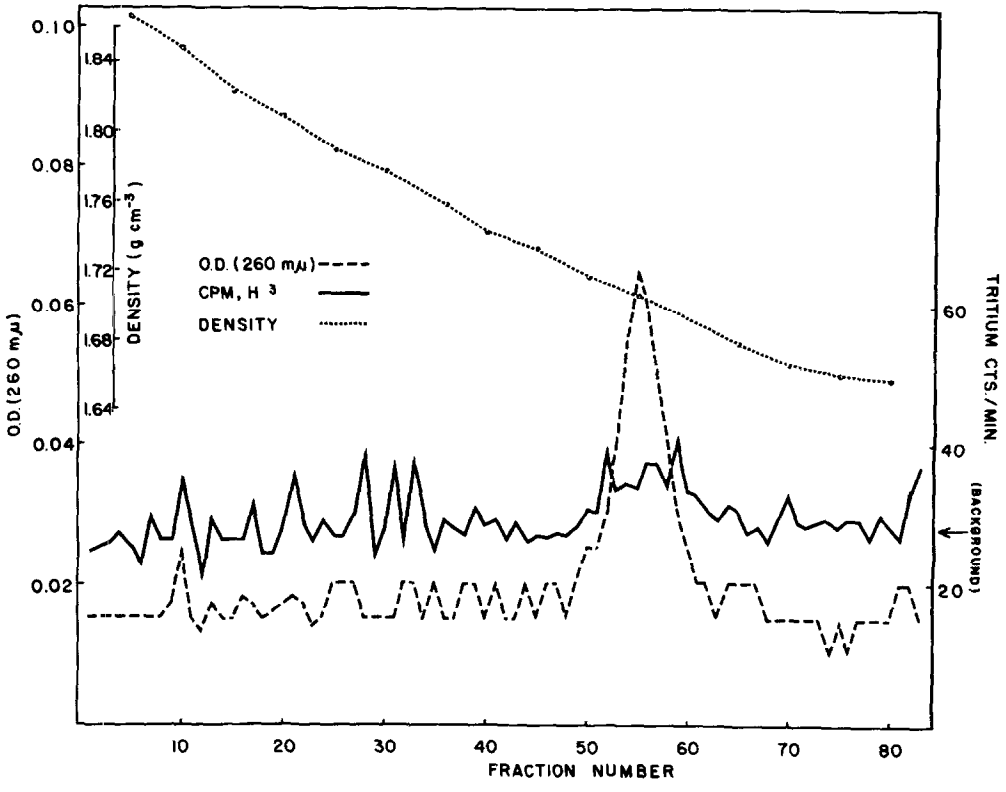

Figure 2. Density distribution of DNA prepared from isolated nuclei.

obtained for the DNA prepared from isolated nuclei are shown in Figure 2; no peak of radioactivity corresponding to that observed with the "total DNA" can be seen. The DNA preparations and CsCl runs have been repeated several times with the same results.

The OD_{260} and tritium peaks were collected from another preparative CsCl run (corresponding approximately to fraction numbers 54-56 and 59-61, respectively, in Figure 1). The tritium peak was concentrated *in vacuo* and both peaks were dialyzed against SSC at 4° C. overnight. The specific activity of the DNA in the tritium peak (210 cpm/γDNA) was about 80-fold greater than that of the OD_{260} peak. Both peaks gave typical DNA absorption spectra (260/230 ratio >1.9); heating at 100° C. for 10 minutes and rapidly cooling in an ice bath resulted in OD_{260} increases indicative of extensive double-strandedness.

Approximately 1 to 2 γ of DNA from each peak and 2 γ of Pseudomonas aeruginosa DNA (ρ = 1.727 g cm^{-3}) were analyzed for buoyant density in CsCl using a Spinco Model E ultracentrifuge (cf. Meselson et al., 1957). A microdensitometer tracing of the UV absorption pattern after 20 hours of centrifugation at 44,770 rpm and 25° C. is shown in Figure 3. Calculation of densities by the method of Sueoka (1961) gave values of 1.700 g cm^{-3} for the OD_{260} peak ("nuclear DNA") and 1.686 g cm^{-3} for the tritium peak ("satellite DNA").*

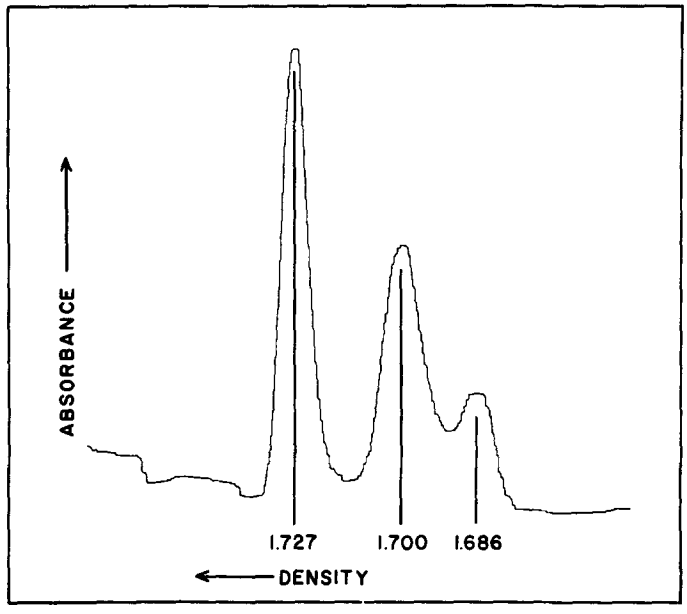

Figure 3. Microdensitometer tracing of the UV absorption pattern of DNA after banding in CsCl in the Spinco Model E analytical ultracentrifuge. The three peaks are (from the left): P. aeruginosa DNA (marker); Physarum nuclear DNA; and the Physarum satellite DNA.

The foregoing results suggest that a non-nuclear DNA is synthesized during G_2 interphase in Physarum polycephalum. On the basis of the OD_{260} patterns obtained with CsCl density gradient centrifugations of "total DNA"

* The corresponding G - C contents, estimated according to Schildkraut et al. (1962), are 41% and 26% for the nuclear and satellite DNAs, respectively.

preparations it is estimated that this fraction represents about 5% of the total cellular DNA. Further biochemical and cytological studies on this satellite DNA are in progress.

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