Exonuclease activity has been measured in preparations of both enzymes. The activity associated with DNA polymerase a requires denatured DNA, Mg2+ and is ATP-dependent. The polymerase b-associated nuclease requires denatured DNA and Mg2+ or Ca2+.

Studies of the distribution of these enzymes during the cell cycle have shown that extracts of cells harvested at the time of nuclear DNA synthesis (Chiang & Sueoka, 1967) have DNA polymerase b as the predominant DNA-synthesizing activity, whereas DNA polymerase a is the major activity in cells harvested at a resting phase of growth. The two major DNA polymerases of Chlamydomonas reinhardii therefore seem to differ in physical and enzyme properties and in the time of appearance during the vegetative cell cycle.

We thank the Medical Research Council for the award of a studentship to C. A. R.


Role of Deoxyribonucleic Acid Polymerase β in Nuclear Deoxyribonucleic Acid Synthesis

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Homogenization of mouse L929 cells in hypo-osmotic buffer releases most of the DNA polymerase a into the supernatant fractions. However, nuclei isolated under these conditions still contain both DNA polymerase a and DNA polymerase b (Adams et al., 1973). DNA polymerase a shows an increase in activity as cells leave a resting phase and enter into S phase (Lindsay et al., 1970; Chang et al., 1973), but the activity of DNA polymerase b is independent of the state of growth of the cells. Although this points to a possible role for DNA polymerase b in repair of DNA, it does not exclude some role in DNA replication. It has been reported (Adams & Wood, 1973) that removal of up to 50% of the total nuclear DNA polymerase has little effect on the ability of isolated nuclei to synthesize DNA. Here we present some evidence that DNA polymerase b alone is sufficient to catalyse this nuclear DNA synthesis in vitro.

L929 cells were grown as described by Lindsay et al. (1970), and nuclei were prepared by homogenizing the cells in 0.25M-sucrose containing 20mM-Tris/HCl, pH 7.5, and 5mM-2-mercaptoethanol. DNA synthesis was assayed in nuclei, washed twice with the same buffer, by incubation in the reaction mixture of Hershey et al. (1973). This contains 10mM-MgCl2, 40mM-Tris/HCl, pH 7.8, 100mM-NaCl, 0.5mM-EDTA, 4mM-2-mercaptoethanol, 5mM-ATP, 0.1mM each of dATP, dCTP, dGTP and [3H]dTTP (specific radioactivity 50μCi/μmol and 2mCi/μmol).

To extract DNA polymerase α differentially, nuclei from S-phase cells were suspended gently in Tris/sucrose buffer containing 0.2M-KCl, left for 15 min and sedimented at 800g.

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The above procedure was repeated once more, which gives nuclei completely free of DNA polymerase $\alpha$. Evidence of the absence of DNA polymerase $\alpha$ was obtained by exhaustively extracting nuclei with Tris/sucrose buffer containing 0.4M-KCl. The extract was layered on to 5–20% linear sucrose gradients containing 0.4M-KCl. The centrifugation was performed at 4°C in SW56 rotor at 50000rev./min for 12–16h in a Beckman L2-65B ultracentrifuge. Then 15 fractions were collected by using an MSE gradient harvester, and the fractions were assayed for DNA polymerase activity as described previously (Adams et al., 1973), but with 'activated' DNA as template. Markers of haemoglobin (4.1S), immunoglobulin G (7S) and catalase (11.3S) were used.

Characterization of DNA synthesized in vitro was performed on alkaline sucrose gradients (Krokan et al., 1975), with Simian virus 40 DNA as marker.

Nuclei isolated from S-phase cells are capable of synthesizing DNA when incubated with the standard test mixture, and this synthesis continues for about 30min. The maximum amount of dTTP incorporated into DNA by such nuclei was 300pmol/mg of DNA. These results are quite comparable with results obtained by Hershey et al. (1973), who showed that incorporation represented the continuation of DNA synthesis initiated in vivo. Calculations show that an average of 160 nucleotides could be added to each growing chain.
Fig. 2. Alkaline-sucrose-density-gradient centrifugation of DNA synthesized in isolated nuclei

Nuclei were prepared from cells pre-labelled with 5 μCi of [14C]thymidine (○). They were incubated for 5 min in standard test mixture as described in the text, except that the final concentration of [3H]dTTP was 12.5 μM (specific radioactivity 2 mCi/μmol) (●). Samples (50 μg of DNA) were prepared for centrifugation as described by Krokan et al. (1975), except that centrifugation was performed at 4°C in a SW56 Spinco rotor for 4 h at 50,000 rev./min.

As predicted, nuclei isolated from stationary-phase cells fail to synthesize any DNA, demonstrating that the observed DNA synthesis reflects the state of the initial cells.

There are two DNA polymerases present in nuclei isolated from S-phase cells. They are DNA polymerase β (4S) and DNA polymerase α (8S), which are easily separated on sucrose gradients (Fig. 1a). Extraction of these nuclei with 0.4 M-KCl removes all DNA polymerase activity and abolishes their DNA-synthesizing capability in vitro. It is possible to remove all the assayable DNA polymerase α from these nuclei by gentle washing with 0.2 M-KCl (Fig. 1b). Surprisingly, this treatment has little effect on the DNA-synthesizing capability of these nuclei, thus excluding the role of polymerase α in the reaction studied in these nuclei. Characterization on alkaline sucrose gradients of DNA synthesized in vitro by nuclei containing both DNA polymerases α and β shows that only small ‘Okazaki’-type pieces are synthesized, and the same is true for nuclei containing only DNA polymerase β (Fig. 2). These results show the involvement of DNA polymerase β in the synthesis of DNA observed in these nuclei.

Thanks are due to Professor R. M. S. Smellie and Professor A. R. Williamson for their interest and for providing necessary facilities. T. R. B. is supported by a grant from M.R.C., who also provided funds.

Vol. 4
The Nature of 'Activated' Deoxyribonucleic Acid used in Deoxyribonucleic Acid Polymerase Studies

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DNA polymerases catalyse the template-directed transfer of nucleotide residues to the 3'-hydroxyl group of a poly- or oligo-nucleotide chain hydrogen-bonded to that template. The preferences of various polymerases towards different forms of DNA template have been the subject of much study, and in particular, DNA that has been 'activated' by the action of certain DNAases* has found wide application. 'Activated' DNA falls into two groups:

(i) lightly 'nicked' DNA, or duplex DNA into which a low density of single-strand breaks has been introduced by the action of nanogram amounts of pancreatic DNAase (Aposhian & Kornberg, 1962);

(ii) extensively degraded DNA, which has been acted on by microgram amounts of pancreatic DNAase until an appreciable amount (usually 5–20%) has been rendered acid-soluble or else by lower amounts of DNAase followed by limited digestion by a 3':5'-exonuclease acting non-processively on duplex DNA (e.g. exonuclease III from Escherichia coli). This latter is sometimes termed 'gapped' DNA (Kornberg & Gefter, 1972).

Use of type (i) DNA has been largely restricted to work with prokaryotic polymerases of the poll class, as these are the only enzymes so far described which are capable of the strand displacement and/or 5':3' hydrolysis necessary for extensive synthesis on this template (Kornberg, 1974). Type (ii) DNA has been widely used in studies on polymerases from eukaryotic and bacteriophage-infected sources, as well as the prokaryotic class II and III enzymes. It should be noted that almost all methods for the preparation of activated DNA involve a heating step intended to inactivate the DNAase used. The importance of this heating in the activation process does not appear to have been evaluated to date.

In the course of studies on the DNA polymerase and DNA exonuclease induced by herpes simplex virus, DNA of both types has been extensively used. Several useful properties of the purified, virus-induced enzymes have been exploited in order to gain a better understanding of the nature of 'activated' DNA.

In order to decrease the number of experimental variables, the DNA was rendered small and relatively homogeneous by ultrasonic vibration before DNAase treatment. This had the added advantage of allowing comparability with the very useful physical studies of Hays & Zimm (1970). On the basis of hydrodynamic studies, these authors concluded that the main effect of single-strand breakage on duplex DNA occurred when two neighbouring breaks were sufficiently close to permit either (i) release of short oligo-nucleotides, when the breaks were on the same strand, or (ii) breakage of the duplex into two shorter lengths with single-stranded tails, when the breaks were on opposite strands. Both of these structures are, of course, ideal substrates for polymerase activity.

Herpes-virus-induced DNA polymerase has proved a useful reagent for these studies because of its low apparent $K_m$ for both deoxyribonucleoside triphosphates and DNA.

* Abbreviation: DNAase, deoxyribonuclease.