Studies of the Decline of Deoxyribonucleic Acid Polymerase Activity during Embryonic Muscle Cell Fusion *in Vitro**

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ABSTRACT: DNA polymerase activity associated with single chicken myogenic cells in culture drops off rapidly during periods of active cell fusion. A study is made of an unpurified DNA polymerase system derived from these cells to determine the mechanism for this abrupt decline in polymerase activity. The characteristics of the crude enzyme system are determined by a study of reaction kinetics as a function of pH, primer dependence, magnesium ion dependence, and substrate dependence.

The muscle cell system displays extremely low levels of DNase activity throughout the cell fusion period and there is no evidence for an increase, during fusion, in enzyme activity which would cause degradation of substrates for DNA synthesis. Mixing experiments made on cell extracts before and during cell fusion fail to disclose the presence of any soluble inhibitor of DNA polymerase. Finally, when protein synthesis is inhibited by cycloheximide the apparent rate of decay of DNA polymerase activity is not sufficient to account for the rapid decay in activity observed during periods of cell fusion. These studies offer a biochemical approach to further work on the rapid inhibition of DNA polymerase activity during cell fusion *in vitro*.

One of the early characteristics of muscle cell differentiation is the fusion of single myogenic cells and the resulting formation of multinucleated myotubes (Firket, 1958; Holtzer *et al.*, 1958). Cell fusion is clearly associated with large scale changes in metabolic patterns of the participating cells. For example, glycogen synthetase (DeLa Haba *et al.*, 1968), creating phosphokinase, and myosin (Coleman and Coleman, 1968), and phosphorylase (Love *et al.*, 1969) begin to be synthesized around the time of fusion and it is very probable that abrupt changes in the synthesis of other enzymes and structural proteins will be found to occur at this time (Love *et al.*, 1969). In addition, there appears to be either a drastic change in the precursor pool or a decrease in the rate of total RNA synthesis in fused as compared with unfused cells (Yaffe and Fuchs, 1967; Marchok, 1966).

As an important part of this shift in metabolism there is an abrupt and irreversible inhibition of DNA synthesis in fused cells (Stockdale and Holtzer, 1961). We have reported previously on the rapid decline in DNA polymerase activity associated with cell fusion in muscle cultures (O'Neill and Strohman, 1969). In this paper we report on the characteristics of the crude polymerase enzyme system from cultured muscle cells together with some preliminary studies which attempt to identify the mechanism by which DNA polymerase activity is so rapidly reduced at the time of cell fusion. A description of the crude enzyme system is an important initial phase of this study because one obtains information on the presence or absence in the cellular extract of DNase activity, inhibitors, or enzymes which function to degrade substrates of the DNA polymerase reaction. It is possible that any of these agents may be changing during the time of cell interaction leading to cell fusion.

Methods and Materials

Cultures. Cultures were prepared using cells from 11-day chick embryo thigh muscle (White Leghorn stock, obtained from Kimber Farms, Niles, Calif.) as described by Konigsberg (1960). The cells were plated as described previously (O'Neill and Strohman, 1969).

Prior to harvest, the cultures were rinsed free of medium with BSS;[1] the cells were then either scraped from the plate in BSS or removed with 0.05% pronase (Calbiochem, Grade B)–0.01 M EDTA in BSS, 45 sec, 22°. When cells were removed with pronase, they were washed immediately with BSS containing 20% horse serum, and then twice more with BSS. The cells were then homogenized at 4° with 100 strokes of a Dounce homogenizer with B pestle. The homogenate was centrifuged at 540*g* for 5 min, and the supernatant was used as the enzyme preparation. Assays were performed immediately, without storage of the enzyme.

For the experiments in Figure 1, the medium was changed at an increasing rate in the attempt to avoid kinetic changes specifically due to the depletion and/or conditioning of the medium. The cells for these experiments were obtained from cultures which were 2–5-days old.

DNA Polymerase Assay. [^{3}H]Thymidine triphosphate was purchased from New England Nuclear Corp. Unlabeled deoxynucleotides were purchased from P-L Biochemicals and from California Foundation for Biochemical Research. Calf thymus DNA and crystalline pancreatic DNase were purchased from Worthington Biochemical Corp. "Activated" calf thymus DNA primer was made by limited digestion of the DNA with pancreatic DNase (Aposhian and Kornberg, 1962). The activated primer showed a hyperchromicity of 22% at 265 mμ after heating and rapid cooling. DNA was heat dena-

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[1] BSS is a balanced salt solution containing, in milligrams per milliliter, NaCl, 6.8; KCl, 0.4; NaH_2PO_4, 0.14; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.2; and glucose, 1.0.

tured by heating a solution of DNA (1.75 mg/ml in 0.01 M KCl) at 100° for 10 min and then rapidly cooling in ice.

The standard polymerase reaction mixture, subject to the exceptions of the particular experiments, contained the following in a total volume of 0.2 or 0.225 ml; 25 μmoles of Tris-maleate buffer, pH 7.4; 5 μmoles of $MgCl_2$; 0.5 μmole of KCl; 100 mμmoles each of d-ATP, d-GTP, and d-CTP; 125 mμmoles of [^{3}H]TTP (0.83×10^4 dpm/mμmole, 1.04×10^6 dpm total); 290 mμmoles of "activated" calf thymus DNA-P; and either 0.025 or 0.050 ml of the enzyme preparation, as indicated.

The assay measures the incorporation of [^{3}H]thymidine triphosphate into an acid-insoluble product. After a period of incubation at 37°, the reaction was stopped with 1 ml of 0.5 N perchloric acid containing 0.01 M sodium pyrophosphate (Loeb *et al.*, 1967). After 30 min at 4°, the acid-insoluble material was collected on Whatman GF/C glass fiber filters and washed successively with 15 ml each of the cold perchloric acid solution above, cold double-distilled water, and cold 95% ethanol. The filters were counted in a Nuclear-Chicago scintillation counter. A zero time acid control count was subtracted from all assays.

DNA Measurements. The DNA measurements were made on the hot trichloroacetic acid extracts (5% trichloroacetic acid, 30 min at 100°) following the method of Schneider (1945) or Schmidt and Thannhauser (1945). The Keck (1956) modification of the Cerriotti reaction was used for all DNA measurements. The total volume of the assay was 0.8 ml. (This procedure is extremely sensitive to the quality of the reagents used. Perchloric acid has proved more reliable than trichloroacetic acid. Bad spectra have also been traced to impurities in the ether.) Duplicate determinations on the same sample or on duplicate plates had a maximum range of 20%.

Isolation of Mononucleate Cells from Partially Fused Cultures. Mononucleate cells and myotubes exhibit a differential sensitivity to EDTA. In the presence of EDTA, the myotubes begin to detach from the plate and can be swirled free leaving the majority of the mononucleate cells in place. The cultures were exposed to 0.01 M EDTA in BSS, pH 7.0, for 25 min at room temperature and then swirled to "peel" off the myotube fraction. The myotube fraction was then allowed to sediment for 10 min at 1*g* after which the supernatant was discarded. The material remaining on the plates after EDTA treatment was removed with pronase as described above, washed, and passed through silk screens of 160 mesh, 250 mesh, and then through five folds of lens paper. The filtered preparation contains approximately 90% mononucleate cells, the contaminant being small myotubes each containing a few nuclei. Observations, coupled with thymidine-pulse experiments, indicate that the 1*g* sediment contains most of the myotubes plus 10–50% of the single cells.

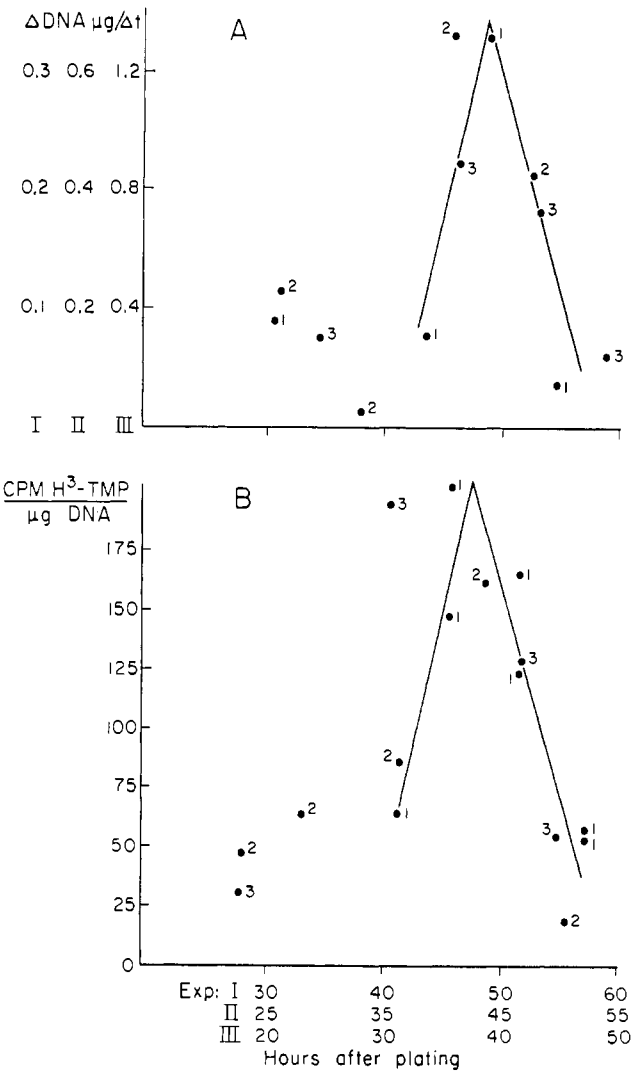

FIGURE 1: Kinetics of DNA synthesis (A) and DNA polymerase activity (B). Numbered data points refer to three separate experiments differing in initial cell concentrations; expt 3, 2×10^6 cells per plate plotted in actual time, expt 2, 0.8×10^6 cells per plate plotted with forward time shift of 5 hr, expt 1, 0.3×10^6 cells per dish plotted with forward time shift of 10 hr. As indicated in the text, the time-base shift plot brings the data points of all three experiments into phase. The ordinate scale factors in the plot of DNA synthesis have been chosen to normalize out the differences in initial DNA concentration in the three experiments. The average interval represented by Δt was 7 hr. The incubation time in the polymerase reactions was 75 min.

Results and Discussion

(*1*) *Polymerase Activity, DNA Synthesis, and Cell Fusion.* In Figure 1, the polymerase activity and the rate of synthesis of DNA are plotted as a function of time after plating of the cells in primary cultures. Represented are the results of three separate experiments in which the major variable was the initial plating concentration of cells. There is a lag period between the plating of the cells and the initial growth phase and this lag is inversely related to but not inversely proportional in the strict sense to the initial cell concentration. The time-base shift used in Figure 1 serves to bring the growth characteristics of the three experiments into phase. At any single-plating density of cells the kinetics of DNA synthesis, polymerase activity, and cell fusion are highly reproducible. We have discussed the shape of these curves in detail elsewhere (O'Neill and Strohman, 1969), and we need only point out here that the DNA polymerase activity is proportional to the rate of DNA synthesis and that the abrupt fall in both rate of DNA synthesis and polymerase activity occurs at the time of maximum cell fusion. It is, as yet, uncertain whether the decline

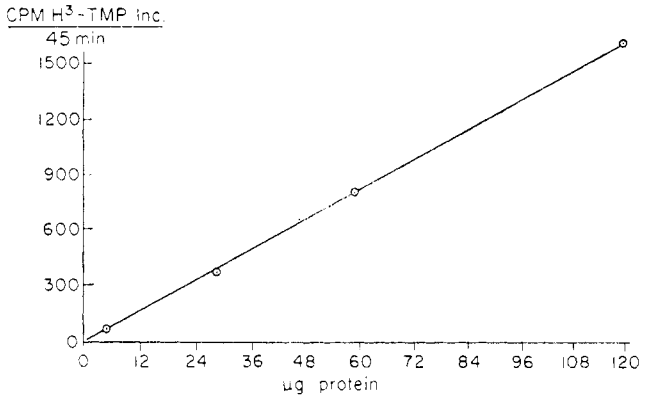

FIGURE 2: Enzyme dependence of the *in vitro* reaction. The protein content in the enzyme preparation was determined by the method of Lowry. Aliquots of incresing size were taken from a single enzyme preparation and incubated with the standard 0.2 ml of reaction mix; the incorporation figure was corrected for differences in the total reaction volume.

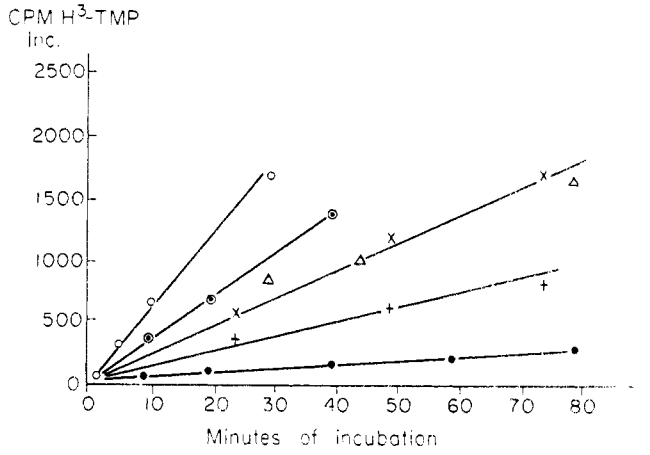

FIGURE 3: Kinetics of the *in vitro* reaction. Six different enzyme preparations, representing a wide range of activities, were used to produce these curves.

in polymerase activity slightly precedes the decline in DNA synthesis.

(*2*) *Requirements of the Reaction.* Table I shows that the enzyme in this system is similar in its requirements to other crude DNA-polymerase preparations (Kornberg, 1961; Keir, 1965). In the absence of two of the four triphosphates, the incorporation of [^{3}H]TTP is reduced to 28% of that with the complete reaction mixture. This, less than absolute dependence on the addition of all four triphosphates, is typical of crude enzyme preparations. When an enzyme preparation was dialyzed (0.5 ml of enzyme against 1 l. of Tris-maleate buffer, pH 7.4, 18 hr at 4°), and then assayed with only [^{3}H]TTP, the incorporation was reduced to less than 2% of that of the complete mixture. The addition of primer and Mg^{2+} ion is required. The product is sensitive to degradation by DNase and is not hydrolyzed in base. Figure 2 shows the reaction is linear with respect to enzyme concentration. The rate here is comparatively high for a crude enzyme preparation (Keir, 1965). The presence of an ATP generating system had no effect.

Kinetics. Figure 3 shows that the reaction is linear for at least 75 min at several different enzyme concentrations. Preincubation of the enzyme for 75 min at 37° in Tris-maleate

TABLE I: Requirements for the DNA Polymerase Reaction.

Reaction Mixture	cpm of [^{3}H]TMP Incorpd	Incubn Period (min)
Complete	1000	75
Minus enzyme	0-5	75
Complete	1500	90
Minus dCTP, dGTP	412	90
Complete dialyzed enzyme	512	45
Minus dATP, dCTP, dGTP	7	49
Complete	547	75
Minus activated primer	0-5	75
Complete, activated DNA primer	992	20
Complete, heat denatured	283	20
Complete, heat denatured	124	75
Complete, native primer (not activated)	8	75
Complete	250	25
Minus $MgCl_2$	10	25
Complete	1500	90
Plus DNase (30 min, 37°) 40 µg/ml	2	90
Complete	397	25
Acid precipitable after 1 N NaOH hydrolysis (20 min, 100°)	357	25

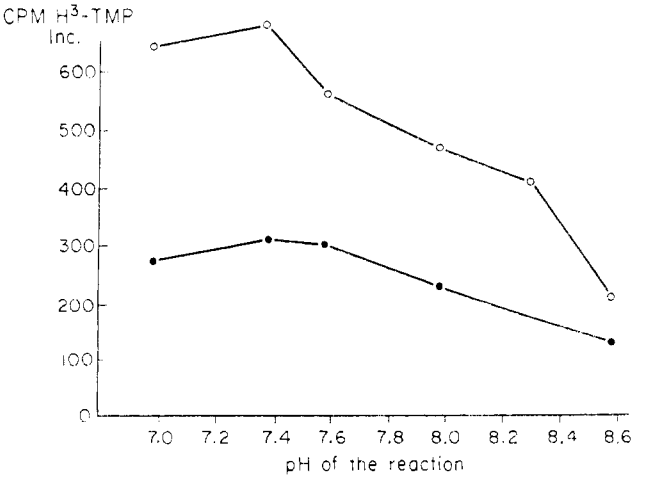

FIGURE 4: pH dependence of the *in vitro* reaction. A single enzyme preparation was used for all points. Solid circles represent a 25-min incubation period; open circles represent a 50-min incubation period.

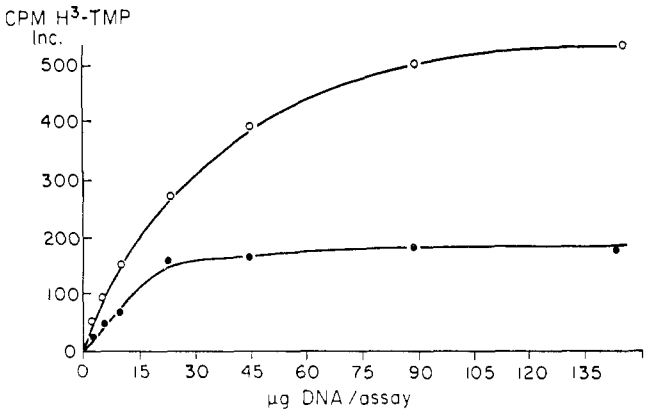

FIGURE 5: Primer dependence of the *in vitro* reaction. Activated DNA was used as primer. A single enzyme preparation was used for all points. Solid circles represent 25-min incubation periods; open circles represent 75-min incubation periods. The assay volume was 0.225 ml.

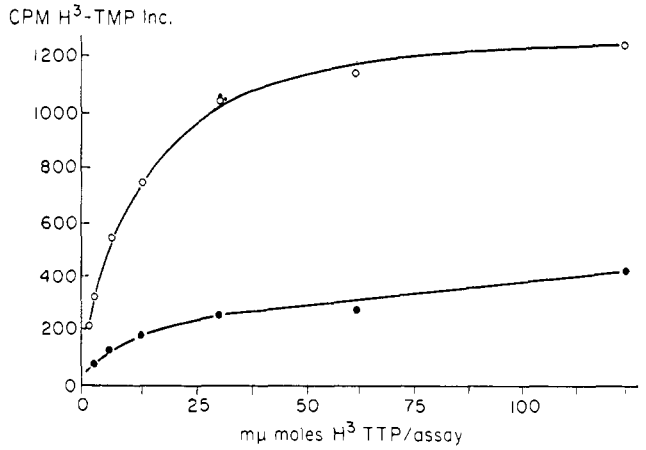

FIGURE 7: Substrate dependence of the *in vitro* reaction. The concentration of the three unlabeled substrates was varied simultaneously as indicated. The assay volume was 0.225 ml. Solid circles represent 25-min incubation periods; open circles represent 75-min incubation periods.

buffer results in a loss of 55% of the activity relative to a non-preincubated control when both are incubated in a standard assay for 25 min. If the preincubation medium is Tris-maleate buffer, 1.45 mM in DNA-P, the loss of activity is reduced to less than 10%. Preincubation for 130 min, even in the presence of DNA, shows a loss of 25% relative to the control.

Long-term incubations of 5 and 10 hr showed the same amount of radioactive product present in each. (This was approximately three times the amount of incorporation that occurred in the first 60 min.) Even through the crude enzyme preparation should contain all soluble proteins, there is no apparent product degradation. This suggests very low DNase levels.

(*3*) *Optimal Conditions*. The pH dependence of the reaction is shown in Figure 4. In Tris-maleate buffer, the observed maximum is pH 7.4. The dependence on added primer is shown in Figure 5; the region of strong dependence on primer concentration ends at approximately 50 µg or 166 mµmoles of DNA-P, well below the 290 mµmoles of DNA-P in the standard mixture. Despite the fact that the supernatant fraction which is used as the enzyme preparation contains as much as 25% of the total DNA that was in the homogenate, no endogenous priming has been observed. Figure 6 shows the the DNA polymerase activity as a function of $MgCl_2$ concentration. The optimum is seen to be at 4 to 5 µmoles (17 to 22 mM). Figure 7 shows the effect of varying the concentration of the three unlabeled triphosphates in the assay reaction. Figure 8 shows the effect of varying the concentration of the labeled triphosphate. In each case, the highest concentration is that

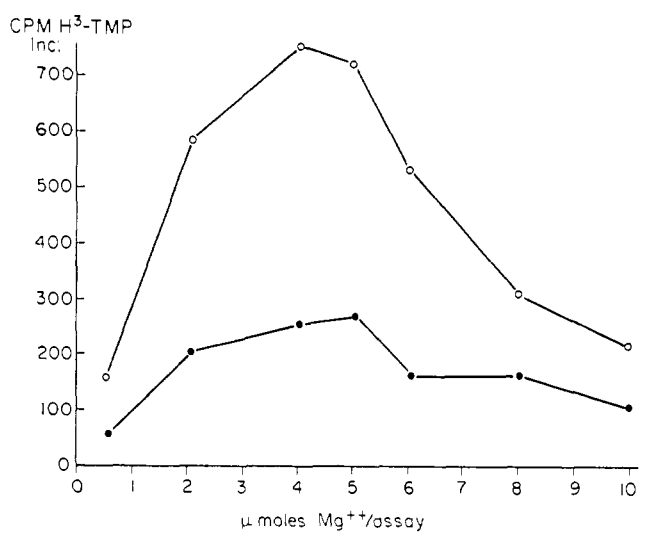

FIGURE 6: Magnesium ion dependence of the *in vitro* reaction. The Mg^{2+} ion concentration was varied using the specified amounts of $MgCl_2$ in an assay of 0.225-ml total volume. A single enzyme preparation was used for all points. Solid circles represent 25-min incubation periods; open circles represent 75-min incubation periods.

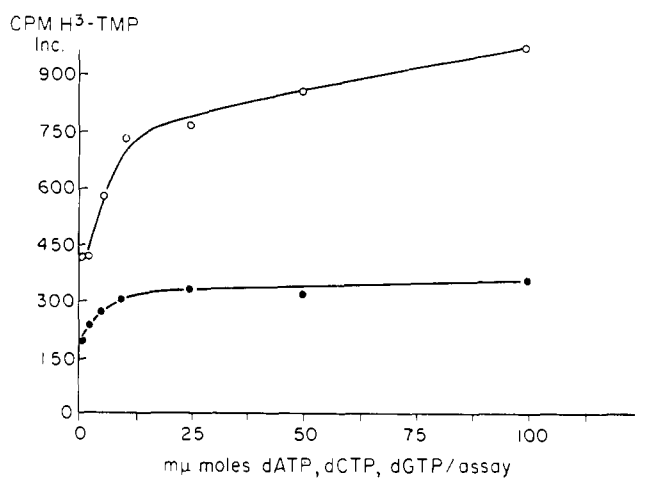

FIGURE 8: [^{3}H]TTP dependence of the *in vitro* reaction. The concentration of the labeled substrate was varied by diluting the most concentrated stock solution. Thus, the specific activity of this substrate remains constant; whereas the total radioactivity added to the assay decreases in proportion to the dilution. The assay volume was 0.225 ml. Solid circles represent 25-min incubation periods; open circles represent 75-min incubation periods.

TABLE II: Primer Preference in the Polymerase Reaction.

Type of Primer	cpm of [^{3}H]TMP Incorporated, 60 min	% of Maximum Incorporation
Activated	729	100
Activated, heat denatured	245	34
High molecular weight, heat denatured	109	15
High molecular weight, native	48	7

employed in the standard reaction mixture, and it is seen that this represents approximately a fivefold excess over the minimum saturating concentration. Comparisons between the 25-min and 75-min curves in Figures 5, 6, 7, 8, suggest that under nonoptimum conditions the reaction is often not linear to 75 min.

(*4*) *Nature of Primer.* Table II also shows the order of preference for primers which have been pretreated in various ways. In descending order of priming ability these are: activated double-stranded primer, activated heat-denatured primer, heat-denatured primer, and native double-stranded primer. Activated primer is about 2.5 to 3 times as effective as the heat-denatured primers; native double-stranded DNA does not serve as primer in this system to a detectable degree with any but the most active preparations. This bias suggests that it may be the single-stranded regions of the activated DNA primer which serve as the actual substrate of the enzyme, with the effectiveness of these regions for priming being somehow enhanced by the presence of adjacent double-stranded regions (Okazaki *et al.*, 1968; Yudelvich *et al.*, 1968).

(*5*) *Localization.* Table III compares the fraction of the total DNA polymerase activity found in the 540*g* "nuclear" pellet and in the supernatant from cultures of several different ages. Generally less than 10% of the total activity was found in the pellet, even though the pellets were unwashed. It is not claimed that less than 10% of the polymerase was originally in the nuclei, but merely that almost all the polymerase activity is in the supernatant under the preparation conditions employed, which include a relatively mild homogenization. There is also the untested possibility that the pellet contains degradative enzymes not present in the supernatant. Nonetheless, complete nuclear localization of the DNA polymerase activity has not been a general finding (Keir *et al.*, 1962; Bach, 1962; Keir and Gold, 1963; Littlefield *et al.*, 1963; Adams and Linsay, 1969). The sea urchin system is a possible exception (Loeb *et al.*, 1969).

(*6*) *Mechanism of Inactivation.* The loss of polymerase activity follows the depletion of mononucleate cells so closely that the mechanism involved must work its effect within a few hours (Figure 1). These preliminary attempts to determine the mechanism focus on two possibilities: (1) that there is a fusion-associated polymerase inhibitor; or (2) polymerase

TABLE III: Relative Amounts of Polymerase Activity in the Low-Speed Pellet and in the Supernatant.

Time Since Plating (hr)	Fraction	Total Activity in Fraction cpm of [^{3}H]-TMP Incorporated/75 min	% of Total Activity in "Nuclear" Pellet
17	Pellet	39	19
17	Supernatant	166	
17	Pellet	65	19
17	Supernatant	282	
30	Pellet	111	7.5
30	Supernatant	1376	
30	Pellet	48	5.7
30	Supernatant	918	
46	Pellet	413	5.7
46	Supernatant	3526	
46	Pellet	411	5.7
46	Supernatant	3344	
65	Pellet	84	13
65	Supernatant	541	

synthesis is stopped by the fusion process with subsequent rapid turnover of the enzyme.

Two types of mixing experiments were performed in the effort to demonstrate a soluble inhibitor: (1) enzyme preparations were made from prefusion cultures and from fused cultures; these were assayed individually and in mixtures; (2) mononucleate cells and myotubes from partially fused cultures were separated as described in Methods; enzyme preparations from these fractions were assayed individually and in mixtures.

The results of these experiments are given in Table IV. In all cases, the mixtures were additive, suggesting the absence of a soluble inhibitor. The fused cultures employed were 12 to 24 hr past their peak fusion. It occurred to us that the inhibitor might be present only during the early hours of fusion, but additional experiments of this type with actively fusing cultures yielded the same result. We then attempted to examine the alternative possibility, that the normal turnover rate of the enzyme is rapid enough to account for the loss of activity at fusion. There have been reports in other systems that protein synthesis is necessary for continued DNA synthesis (Taylor, 1965; Young, 1966; Weiss, 1969). If this were due, in this case, to the rapid turnover of polymerase, no other special mechanism would be necessary beyond stopping synthesis of the enzyme.

Lacking the purified enzyme, we could not measure enzyme turnover directly. We, therefore, measured the loss of activity subsequent to blocking protein synthesis with cycloheximide. Cycloheximide, at 1 μg/ml, reduced [^{3}H]leucine incorporation

TABLE IV: Mixing Experiments Employing Enzyme Preparations from Fused Cells and from Unfused Cells.

Fraction	No. of Assays Averaged	Cpm [^{3}H]TMP incorpd/50 min 50 μl of enzyme	Expected Value If Additive[a]
1 Single cells	1	703	
Myotubes	2	111	
Mixture			
50 μl + 50 μl	2	641 (100 μl)	666
2 Single cells	6	1260	
Myotubes	4	217	
Mixture			
25 μl + 25 μl	3	855	738
3 Unfused 2nd day cultures	4	295	
50% Fused 4th day cultures	4	896	
Mixture			
25 μl + 25 μl	2	513	596

[a] Values corrected for differences in the final total volume of the reaction mixture.

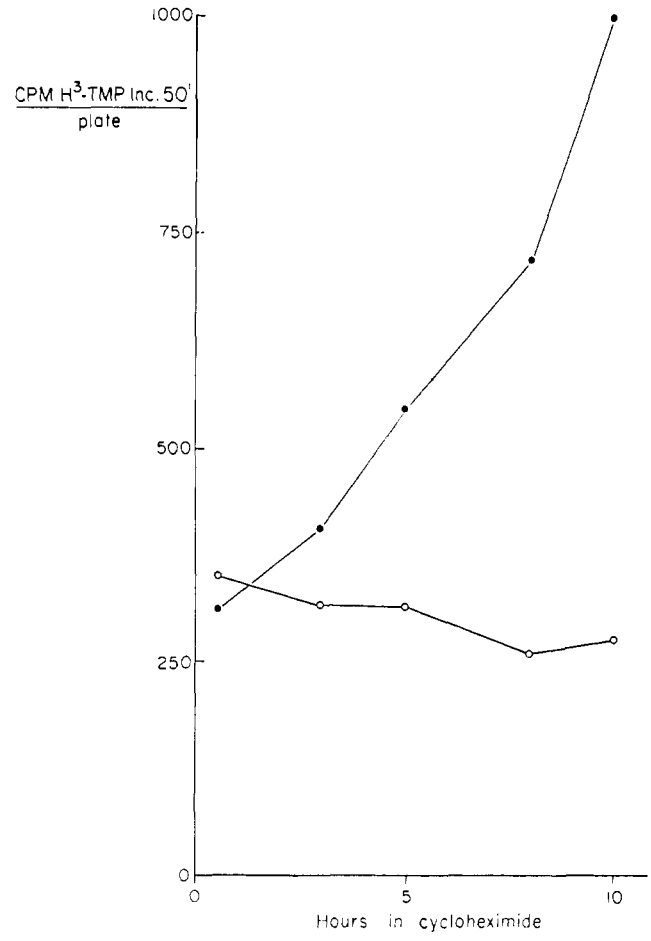

FIGURE 9: Effects of cycloheximide on DNA-polymerase activity. Cycloheximide (1 μg/ml) was added at 22 hr after plating. Treated (open circles) and control (solid circles) cultures were assayed for changes in DNA polymerase activity over the succeeding 10-hr period. The cells were plated at a density of 4 $\times$ 10^6 cells per 100 mm dish. The medium was unchanged from the time of plating. These were prefusion cultures, fusion occurring under these conditions at about 40 hr.

by 93–95% within 40 min. Figure 9 shows the effect of this treatment on DNA polymerase activity in prefusion cultures. The increase in activity which is occurring during this period in the untreated cultures is prevented in the treated cultures; however, the level of activity in the treated cultures does not drop more than 20% from the zero-time level in 10 hr. Taken at face value, this says that turnover does not occur rapidly enough to explain completely the rapid decline of DNA polymerase during cell fusion. There are some obvious objections to this interpretation of the experiment. First, the inhibitor does not completely block amino acid incorporation, leaving the possibility of some continued synthesis of polymerase. However, the large disparity in activity which develops between treated and untreated cultures, even on a per cell basis, suggests that polymerase synthesis is effectively blocked by the inhibitor. A second, more serious objection arises from the practice of measuring the turnover of enzyme activity rather than the turnover of the enzyme. For instance, if this system included a polymerase inhibitor which turned over faster than the enzyme, blocking protein synthesis might result in an increase in activity despite actual turnover of the enzyme. This has been reported to occur in another enzyme system (Kerney, 1967; Grossman and Manrides, 1967; Tomkins *et al.*, 1969). Cultures for this experiment were taken well in advance of fusion to minimize this possibility, but it cannot be ruled out.

The possibility was considered that turnover might be accelerated during fusion in the fusing cells. This could not be tested using inhibitors because these stop the fusion process and cause differential detachment of mononucleate cells and myotubes. However, if the turnover rate is substantially different in fusing cells it might involve a degradative enzyme, which should be demonstrable in the proper mixing experiment. Note this is not the same as looking for a soluble inhibitor—the difference being the expected time course of the loss of activity. Here, turnover would have to have a half-time rate of 4 hr or less as opposed to a shorter time course of action that might be expected for an inhibitor. Therefore, a mixing experiment in which an enzyme preparation from unfused cells is preincubated with an enzyme preparation from recently fused cells could, under this assumption, be expected to show an increased loss of activity greater than that occurring in the separate preincubations. This was attempted both with actively fusing cultures and with postfusion cultures. The results are given in Table V. In both cases, mixtures were additive. In the preincubation experiments mentioned above in the discussion of the reaction kinetics, substrate was present throughout the preincubation. In the experiment presented here, a complete substrate complement was added back after the preincubation, yielding little or no loss of activity. (The former preincubation experiment described under Kinetics must be considered an upper limit on the amount of inactivation occurring during preincubation; it allows for degradation

TABLE V: Mixing Experiments: Long-Term Preincubation of Enzyme Preparations from Fused Cells and from Unfused Cells.

Fraction	No. of Assays Averaged	Preincubation Time (min)	Incubation (min)	cpm of [^{3}H]TMP Incorpd Vol	cpm of [^{3}H]TMP 50 μl of Enzyme	% Loss in Preincubn	Expected Value If Additive[a]
1 Single cells	2	0	50	0.225	432		
Myotubes	2	0	50	0.225	590		
Single cells	2	150	25	0.225	143	34	
Myotubes	2	150	25	0.225	206	27	
Mixture 25 μl + 25 μl	4	150	25	0.225	167		174
2 Single cells	4	0	50	0.225	620		
Myotubes	3	0	50	0.225	697		
Mixture 25 μl + 25 μl	2	0	25	0.225	356		329
3 Single cells	1	150	25	0.325	143	33	
Myotubes	1	150	25	0.325	255	0	
Mixture 25 μl + 25 μl	3	150	25	0.325	251	0	199

[a] Values corrected for differences in the final total volume of the reaction mixture.

of the substrate, possibly to the extent that the subsequent reaction is not zero order.)

In summary, the decline in polymerase activity associated with myoblast fusion is not the effect of degradative enzymes on the substrates in the *in vitro* assay. There is no detectable DNase in the crude enzyme preparation; and, although there is phosphatase activity, the substrate levels remain saturating at all times. The recent report of a sharp increase in phosphatase activity associated with muscle formation *in vivo* (Stockdale, 1970), prompted us to repeat the substrate dependence curves using cultures harvested specifically during the period of most rapid fusion. These curves showed essentially the same saturation characteristic as those in Figure 7. The decline in activity is not the result of a cytoplasmic soluble inhibitor. Nor, from the somewhat circumstantial evidence presented, does it seem likely that the enzyme is turned over rapidly enough to account for the observed rate of decline. Work on the turnover time is continuing. We are also initiating study to see if the enzyme becomes inactivated in the process of becoming membrane bound and/or moving into the nucleus (Gurdon and Speight, 1969; Fansler and Loeb, 1969; Adams and Linsay, 1969).

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Characterization of a Yeast Endonuclease*

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ABSTRACT: A deoxyribonuclease from *Saccharomyces cerevisiae* has been purified approximately 300-fold. The enzyme has a requirement for Mg^{2+} or Mn^{2+}, which cannot be replaced by Ca^{2+}.

Its mode of action is primarily endonucleolytic, yielding oligonucleotides ending in 5′-phosphates, and it exhibits a 750-fold preference for single-stranded DNA over native DNA.

Recent evidence (Lehman, 1967; Takagi *et al.*, 1968; Grossman *et al.*, 1968) that various kinds of deoxyribonucleases are involved in the replication, repair, and recombination of genetic material make a study of the number and kind of DNase specificities in an organism such as yeast of great interest. Yeast has several characteristics which make it well suited to the molecular investigation of genetic recombination: (1) it is unicellular and has a stable diplophase in which meiosis can be induced easily and with high efficiency. Meiotic recombination thus occurs only during a restricted period during the life cycle; (2) a relatively low rate of recombination also occurs in mitotic cells and this rate can be increased greatly by ultraviolet irradiation, X-rays, or chemical mutagens (Roman and Jacob, 1958; Manney and Mortimer, 1964; Zimmerman and Schwaier, 1967). Furthermore, mitotic recombination is most readily induced at a certain stage in the division cycle, *i.e.*, just prior to the S period (Esposito, 1967); (3) the mating-type alleles a and α, influence the frequency of recombination, in that aα diploids undergo recombination, but aa or $\alpha\alpha$ diploids do not or do so rarely (Friis and Roman, 1968); (4) the genetics of yeast has been extensively studied in the past few years, and data have accumulated on various aspects of recombination (Mortimer and Hawthorne, 1969). Such data may be particularly useful in studies which attempt to relate enzyme activity with recombination. In contrast to studies with *Escherichia coli*, where extensive descriptions of purified DNases have been reported, no distinct DNases have been described in yeast. The purpose of this communication is to describe the partial purification and characterization of a DNase (endonuclease A) from *Saccharomyces cerevisiae* which exhibits a decided preference for single-stranded DNA.

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Materials and Methods

Yeast Strains. The haploid strain C252 (2d) from the yeast stocks of Dr. H. Roman, and the diploid 9-D1 were used in the work reported here. 9-D1 was the result of a cross between C252 (2d) and X173 obtained from Dr. R. K. Mortimer.

Media. Three different types of media were used for growing the two strains, and were tested with regard to the yield of DNase. Supplemented YEP medium (YEP + Ad) contained as follows (grams per liter): yeast extract, 10; peptone, 20; glucose, 20; and adenine, 0.1. Wickerham's synthetic complete medium (SC) contained: yeast nitrogen base (without amino acids), 6.7; glucose, 20; adenine, 0.01; uracil, 0.01; arginine, 0.01; and tryptophan, 0.01. Supplemented minimal medium (MA) contained: yeast nitrogen base, 6.7; glucose, 20; and adenine, 0.01.

Chemicals. Alkaline phosphatase from *E. coli* was purchased from Miles Research Laboratories. Venom phosphodiesterase and bovine spleen phosphodiesterase were purchased from Worthington Biochemical Corp. Sodium deoxycholate was obtained from Mann Research Laboratories. Carboxymethylcellulose (CM-52) was a Whatman product. Highly polymerized calf thymus DNA and phenylmethylsulfonyl fluoride were purchased from Sigma Chemical Co., and 2-mercaptoethylamine-HCl were obtained from Calbiochem. The detergent Brij-58 was a gift from McKesson Chemical Co. Glusulase, a trade name for snail digestive juice, was purchased from Endo Laboratories. Yeast extract, peptone, and yeast nitrogen base were purchased from Difco Laboratories.

^{3}H-Labeled T5 DNA. ^{3}H-Labeled T5 DNA was prepared with the cooperation of Dr. M. Feiss by the method outlined by Thomas and Abelson (1966). *E. coli* B was grown in minimal medium supplemented with 0.2% Casamino Acids and 0.25% glucose. $CaCl_2$ was added to 2×10^{-3} M at the time of phage infection. Phage T5 was added when the bacterial titer was 1×10^8/ml at a multiplicity of infection