Delta-type DNA polymerase characterized from *Drosophila melanogaster* embryos

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ABSTRACT

Genetic and biochemical evidence suggests there are at least three DNA polymerases required for replication in eukaryotic cells. However, *Drosophila* embryonic cells have a very short duration S phase which is regulated differently. To address the question of whether embryos utilize different DNA polymerases, we employed Mono Q anion exchange chromatography to resolve the DNA polymerase activities. Two types of DNA polymerase, DNA polymerase δ and DNA polymerase α , were distinguished by: 1. copurification of DNA primase or 3'–5' exonuclease activities; 2. immunoblot analysis with α -specific polyclonal antisera; 3. sensitivity to aphidicolin and BuPdGTP; and 4. processivity measurements with and without Proliferating Cell Nuclear Antigen. These observations suggest that *Drosophila* embryos, similar to nonembryonic cells, have both α - and δ -type DNA polymerases.

INTRODUCTION

The requirement for three distinct replicative DNA polymerases in eukaryotic cells has introduced a fundamental new concept to the field of eukaryotic replication (1–3). Evidence to support the generality of three essential replicative DNA polymerases is derived primarily from two sources. Simian Virus-40 DNA replication has been reconstituted by adding the viral origin-containing DNA and the origin recognition protein, large T antigen, to mammalian cell extracts (for review see 4–6). Two DNA polymerases, (pol α), DNA polymerase δ (pol δ), and at least six other proteins have been identified which are required for viral replication (7,8). Another DNA polymerase, DNA polymerase ϵ (pol ϵ), has been purified from calf thymus, human cells and from the yeast *Saccharomyces cerevisiae* (9–11). Further evidence of three replicative DNA polymerases is illustrated by *S. cerevisiae* genetics. It has been shown that the pol ϵ gene, in addition to the pol α and pol δ genes, is essential for cell viability; Temperature sensitive mutants in any of the three genes arrest with a dumbbell morphology typical of arrest in S phase (12–14).

The DNA polymerases discussed above have been characterized in mammalian and yeast cells.; however, the regulation of replication and the chromatin structure of *Drosophila* embryos differs dramatically from nonembryonic cells. Specifically, the embryos are different in: 1) the rapid rate of replication during the syncytial blastoderm stage [S phase = 3.4 min]; 2) the number of active origins [20,000 per haploid genome]; 3) the synchronous initiation of all replicons; (15) 4) the maternally supplied transcripts and proteins; 5) the lack of zygotic transcription; 6) the lack of cell membranes; 7) the lack of G1 and G2 phases; (reviewed in 16) and 8) the apparent lack of DNA polymerase δ and DNA polymerase ϵ (reviewed in 17). These differences suggest that the organization of the replication fork might be different in rapidly replicating embryos, i.e. embryos may utilize a simpler, single DNA polymerase mechanism for replicating the chromatin.

To address the question of whether early stage *Drosophila* embryos do or do not contain pol δ and/or pol ϵ , we utilized a purification scheme which simultaneously resolved all three replicative DNA polymerases from human and yeast cells (11, 18). The results of our purification procedure and the implications for embryonic replication are presented in this report. We have characterized at least two types of DNA polymerase from *Drosophila* embryos; a pol α /primase, with a low processivity, and a pol δ activity with PCNA-inducible high processivity.

EXPERIMENTAL PROCEDURES

DNA polymerase purification

The 0–2.5 hour *Drosophila* embryos were collected and processed as described (19) through the high speed supernatant fraction (S100). The extract was stored at -80°C in the presence of 50 μM EDTA, 1 mM phenyl methylsulfonyl fluoride, 10 mM sodium bisulfite, and 2 $\mu\text{g/ml}$ leupeptin until further purification. All steps were performed at 4°C . The S-100 extract was diluted to 50% ammonium sulfate by the slow addition of a 75% saturated solution. It was gently stirred for 45 minutes, then centrifuged at $12,000 \times g$ for 30 minutes. The resulting pellet was resuspended and diluted with Buffer H (20 mM Tris base, pH 8.6, 10% glycerol, 10 mM 2-mercaptoethanol, and the protease

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inhibitors listed above, except that EDTA was 500 μ M and EGTA was added to 50 μ M). The resuspension was diluted to the conductivity of Buffer H containing 0.2 M NaCl. This fraction, designated the ammonium sulfate fraction, was applied to a pre-equilibrated 18 $\times$ 1.5 cm heparin sepharose CL-4B column (Pharmacia). The column was washed with Buffer H containing 0.2 M NaCl, step-eluted with 0.45 M NaCl, and the eluted protein diluted to the conductivity of Buffer H containing 0.1 M NaCl. This fraction, designated the heparin fraction, was applied to a pre-equilibrated fast protein liquid chromatography (FPLC) Mono Q HR 5/5 column (Pharmacia) at a flow rate of 0.5 ml/min. The proteins were eluted with a 60 ml linear gradient from 0.1 M to 0.4 M NaCl and Buffer H. One ml fractions were immediately assayed for DNA polymerase activity. The active fractions, designated Q pool I, II, and III, were diluted to a final concentration of 50% glycerol and stored above liquid nitrogen. Pool II and III fractions were relatively stable without repeated freeze-thawing. Pool I fractions stored longer than four months showed a dramatic loss of activity, and were highly sensitive to freeze-thawing.

Enzyme Assays

DNA Polymerase assays for quantitation, inhibitor sensitivity, and processivity measurements. Two different assays were used to detect DNA polymerase activity. When activated DNA was the template, the assay was performed as described (19) except that 17.5 μ g of activated salmon testes DNA (Worthington Biochemicals, Catalog # LS03554) was the template, and the reaction volume was 60 μ l. The reaction was incubated for 30 min at 30°C. When poly(dA)-oligo(dT) was the template, poly(dA) (Midland Certified Reagents, Midland, TX) was annealed to 14-mer oligo(dT) (Pharmacia) at a ratio of 232 μ g of poly(dA) to 7.65 μ g of oligo(dT)₁₄ to yield a 400-nucleotide interprimer distance. The assay was performed as described (9) except that 10 mM MgCl₂ and 1 mM dTTP (200 cpm [³H]dTTP/pmol) were added and ATP was deleted. One unit of DNA polymerase activity was defined as the amount that catalyzes the incorporation of 1 nmol of dNMP into acid-insoluble material in 60 min at 30°C. To determine inhibitor sensitivity, DNA polymerase assays were performed using the activated DNA assay except that aphidicolin sensitivity was determined with 10 μ M dCTP, and dideoxythymidine triphosphate sensitivity was determined with 10 μ M dTTP. To determine processivity, the poly(dA)-oligo(dT) assay was performed with 20 μ M template, and [α -³²P]dTTP was substituted for [³H]dTTP. Enzyme activity was adjusted to yield approximately equal incorporation, with less than 1 pmol dNMP incorporated per pmol of 3' termini. Products of the reaction were separated by 20% polyacrylamide, 8 M urea gel electrophoresis and visualized by autoradiography. PCNA (63 μ g protein/ml), purified from *Drosophila* embryos as described in a 300 mM NaCl buffer (20), was approximately 50% pure as estimated by Coomassie-stained gel (personal communication, Angela Smallwood, Stanford University, CA).

Gel Assay for simultaneously detecting DNA polymerase and 3'-5' exonuclease activities. A highly sensitive gel assay for simultaneously detecting both DNA polymerase and 3'-5' exonuclease activities was developed by modifying a procedure previously described (21). Oligo(dT) was 5' end-labeled with [γ -³²P]ATP (Amersham) by polynucleotide kinase (Biolabs), then annealed to poly(dA) as described for the poly(dA)-oligo(dT) assay.

Assays were performed in 10 μ l volumes, as described for the poly(dA)-oligo(dT) assay except that poly(dA)-[³²P-oligo(dT)] DNA (3 $\times$ 10⁴ cpm/0.06–6.0 pmol) was substituted for unlabeled poly(dA)(dT) and [³H]dTTP. Incubation was for 15 min at 30°C. The products were visualized as described for the processivity assay. To quantitate exonuclease activity, dTTP was omitted from the assay and the degradation products were quantified by a Bio-Rad Model 620 Video Densitometer.

Primase assays. Primase assays were carried out as described (19) except that [α -³²P] dATP was substituted for [³H]dATP.

Protein quantitation, gels, and immunoblotting

Protein concentrations were determined by the BCA microassay (Pierce, Rockville, IL). SDS-PAGE and gel transfer were performed by standard procedures (22,23). The anti-DNA polymerase α polyclonal antiserum (a kind gift from Dr. I.R. Lehman, Department of Biochemistry, Stanford School of Medicine, Stanford, CA) was raised by injection of rabbits with purified DNA polymerase α /primase (specific activity = 5 $\times$ 10⁴ units/mg protein). Rabbits were bled by ear, and specific IgG

Figure 1. FPLC Mono Q column chromatography resolves three pools of DNA polymerase activity, one of which copurifies with a 3'-5' exonuclease. FPLC Mono Q column fractions, eluted with a 0.1 M–0.4 M, 60 ml NaCl gradient, were assayed for DNA polymerase activity with activated DNA or poly(dA)-oligo(dT) (Fig. 1A). The same fractions were assayed for 3'-5' exonuclease activity (Fig. 1B). The three pools of DNA polymerase, denoted as I, II and III, are discussed in the text.

production assayed by ELISA, and purified by Protein A sepharose affinity chromatography. Antiserum was diluted 1:100. [125 I]-conjugated goat anti-rabbit antibody (1:1000) was used to detect the antigen-antibody complex.

RESULTS

A DNA polymerase purification scheme was designed which resolved three pools of DNA polymerase activity (Fig. 1). Fig. 1A illustrates the DNA polymerase activities eluted from a FPLC Mono Q anion exchange column at 175 mM NaCl (pool I), 265 mM NaCl (pool II), and 320 mM NaCl (pool III).

Figure 2. Simultaneous detection of DNA polymerase activity and 3'-5' exonuclease activity in pool I. Pools I-III are assayed by the gel method for both DNA polymerase and 3'-5' exonuclease activities as described in 'Experimental Procedures'. Nucleotide size standards, indicated at the left, were made by 5'-end labeling oligo(dT)₁₄, and partially ligating it. TOP=top of gel, NE=reaction with no enzyme added.

The pool I DNA polymerase activity copurified with a 3'-5' exonuclease activity as shown in Fig. 1B and Fig. 2. Pool I had no significant primase activity. Both DNA polymerase and 3'-5' exonuclease could be detected simultaneously with the gel assay in which [32 P]-labeled reaction products were detected by autoradiography (Fig. 2). Pools II and III both copurified with DNA primase activity (19.4 and 45.0 units/mg protein, respectively), but did not have detectable exonuclease activity (Fig. 1,2). Other fractions containing DNA polymerase activity were occasionally detected eluting at 120 mM, 225 mM and 360 mM NaCl, but were unstable, and could not be characterized further.

As shown in Table 1, pool I DNA polymerase preferred a poly(dA)-oligo(dT) homopolymeric template to activated DNA with short single strand gaps. Most of the DNA polymerase activity, found in pools II and III, strongly preferred the activated DNA template as expected for an α -type DNA polymerase. The very low activity in assays with poly(dA)-oligo(dT) during the earliest stages of the purification (the S-100 and ammonium sulfate fractions) might be due to competition of poly(ADP-ribose) polymerase for the template as reported by others (24).

Immunoblot analysis of pools I, II, and III with polyclonal antisera raised against purified pol α is shown in Fig. 3. The polyclonal antisera specifically detected a 183 kDa peptide in pool III (lane 3) which was not observed using identical conditions without the anti-DNA polymerase antisera (lane 6). Pool II contained immunoreactive peptides of 160-140 kDa (lanes 2 versus 5) which correspond to the proteolytic products of the 183 kDa peptide previously reported (19). The 74 kDa peptide in pool III corresponds to the tightly associated 74 kDa subunit of pol α . The dark band of approximately 48 kDa in pools II and III at the bottom of the blot could represent a primase subunit. Also, bands of 45-60 kDa apparent molecular weight were detected in pool I. Bands of approximately 45-60 kDa were detected in all fractions eluting from the Mono Q column fractions (unpublished data, V.M.P., A.E.C, and E.W.G.). Thus, these bands might correspond to the highly immunogenic embryonic antigens reported by Sauer and Lehman (25). No high molecular weight peptides were detected in pool I, consistent with the presence of a non α -type DNA polymerase.

All three pools of DNA polymerase were sensitive to aphidicolin (Table 2) as expected of α -, δ -, and ϵ - type DNA polymerases (26,27). The DNA polymerases were relatively resistant to dideoxythymidine triphosphate and thus are not β - or γ -type DNA polymerases which are repair and mitochondrial

Table 1 Purification of DNA polymerases from two-hour *Drosophila melanogaster* embryos.

Step	Protein (mgs)	Activity ¹		Specific Act ¹		Ratio Act. DNA/ p(dA)-(dT)
		Act. DNA ²	p(dA)-(dT) ²	Act. DNA	p(dA)-(dT)	
S-100	106	8493	1064	80.1	10.0	8.0
(NH ₄) ₂ SO ₄	67.4	8000	1040	119	15.4	7.7
Heparin-sepharose	10.6	7224	1617	681	153	4.5
MONO Q						
Pool I	.21	44	56	214	272	0.8
Pool II	.40	690	210	1725	525	3.3
Pool III	.40	1380	635	3538	1628	2.2

¹Units of DNA polymerase activity are defined in 'Experimental Procedures'. Specific Activity is defined as units of DNA polymerase activity per mg protein. ²Act DNA = activated DNA, p(dA)-(dT) = poly(dA)-oligo(dT)

Figure 3. Immunoblot analysis of DNA polymerases from pools I-III. Mono Q pools I, II, and III were separated by SDS-PAGE, and the gel was immunoblotted as described in 'Experimental Procedures' with polyclonal antiserum raised against pol α . The total protein and units of DNA polymerase activity measured with the preferred template are 4 μ g, 0.7 units (lanes 1 and 4), 1 μ g, 1.0 unit (lanes 2 and 4), and 1.8 μ g, 5.3 units (lanes 3 and 6). Protein molecular weight standards in kDa are indicated at the left, and the pools of DNA polymerase are indicated at the top of the figure. The presence or absence of the primary antiserum during the blotting procedure is indicated (+/-).

Table 2. Effect of inhibitors on DNA polymerase activities.

Inhibitor	Concentration	Pool I	% Relative Activity Pool II	Pool III
Control		100	100	100
Aphidicolin (μ g/ml)	4	15	ND ¹	<1
	10	<1	ND	<1
	100	<1	2	<1
ddTTP ² (μ M)	10	89	ND	78
	50	48	ND	56
BuPdGTP (μ M)	10	85	9	<1
	100	80	<1	<1
KCl	80	4	ND	36
	200	<1	ND	<1

¹Not Determined

²Dideoxythymidine triphosphate

DNA polymerases, respectively (28,29). In addition, all DNA polymerase pools were inhibited by 80 mM KCl. This confirms that they are not pol γ , since pol γ is optimally active in the presence of 200 mM KCl (29). To distinguish between α -, δ -, and ϵ -type enzymes, BuPdGTP sensitivity was determined. Pools II and III were sensitive to even 10 μ M BuPdGTP, but pool I retained 80% of its activity with 100 μ M BuPdGTP. These data are consistent with pools II and III containing α -type enzymes, and pool I containing a δ - or ϵ -type DNA polymerase (26,27).

Another characteristic of pol δ is the ability of PCNA to increase the processivity of the enzyme. Fig. 4A demonstrates the relative processivity of pools I and III. Both pools elongated the 14-mer oligonucleotide by 9-19 nucleotides which would be expected for pol α using poly(dA)-oligo(dT) template, or pol δ separated from its processivity factor, PCNA (30). Fig. 4B demonstrates the PCNA-dependent nature of pool I DNA

Figure 4. Processivity of DNA polymerases from pool I and pool III. Processivity measurements were performed using poly(dA)-oligo(dT) as described under 'Experimental Procedures'. Size standards are indicated in nucleotides, and were prepared as described in Fig. 2 (panel A—lane ST, and panel B—lane 1), or by 5'-end labeling TaqI-digested Φ X174 (panel B—lane 2). A, Processivity was measured with equal units of pools I and III. I=Pool I, III=Pool III, ST=Size Standards B, Processivity of pool I was measured in the absence (lanes 3-5) or the presence (lanes 6-8) of 11 μ g/ml PCNA, with the activity adjusted to yield equivalent incorporation at the 15 min time point. Aliquots were removed from the reaction and processed after 5 min (lanes 3,6), 10 min (lanes 4,7) or 15 min (lanes 5,8).

polymerase. Upon addition of PCNA, products were detected which ranged from 30 to greater than 400 nucleotides (lanes 6-8). The constant size of the products detected over a 15 min time course illustrates that the enzyme was not reassociating with an already elongated template molecule; thus, the assay accurately reflects the processivity of the pool I DNA polymerase.

DISCUSSION

Hamatake *et al.* (11) utilized the FPLC Mono Q column to simultaneously resolve five pools of yeast DNA polymerase activity: pool A was pol δ , pool B was a mixture of pol δ and degraded pol α , pool C was intact pol α , and pools D and E were two forms of pol ϵ . With the purification scheme utilized for *Drosophila* embryo DNA polymerases, the order of elution of pool I, II, and III from the Mono Q column, and the designation of these DNA polymerases as δ , degraded α , and intact α , is consistent with the results of Hamatake *et al.* (11). It may be of interest to note that a highly unstable DNA polymerase activity was occasionally detected in conjunction with a 3'-5' exonuclease activity which eluted just after the pool III pol α .

Of the three replicative DNA polymerases in nonembryonic cells, both pol δ and pol ϵ , but not pol α , express a readily detectable 3'-5' proofreading exonuclease. In *Drosophila* embryos, however, it has been reported (21) that pol α expressed a cryptic 3'-5' proofreading exonuclease upon separation from

the 74 kDa subunit. This feature was not observed in calf thymus pol α preparations (31). The apparent lack of *Drosophila* homologs of pol δ and pol ϵ suggested that *Drosophila* embryos might utilize a single DNA polymerase (pol α) and a single cryptic proofreading exonuclease as compared with the more complex nonembryonic systems with multiple DNA polymerases, and multiple proofreading enzymes. However, our results (Fig. 1) suggest that *Drosophila* embryos employ an editing strategy utilizing more than one proofreading DNA polymerase, similar to mammalian and yeast systems.

In the presence of PCNA, pol δ can synthesize products greater than 1000 nucleotides in one cycle of polymerization. Pol ϵ also has a very high processivity, but it is PCNA independent under standard assay conditions (30). Thus pool I probably does not contain an ϵ -type DNA polymerase (Fig. 4A). The decrease in activity detected with the poly(dA)-oligo(dT) template comparing the heparin sepharose fraction with the Mono Q pools is consistent with the separation of PCNA from the pol δ , as was the case during the purification of yeast pol δ and PCNA (11). The lack of PCNA could be responsible for the low specific activity of pool I compared to the pol α in pools II and III (Table 1). Indeed, the addition of PCNA to pool I stimulated the DNA polymerase activity 11-fold. Fig. 4B illustrates that PCNA also induces pool I DNA polymerase processivity; products were detected ranging from 30 to greater than 400 nucleotides in size. This measurement may be an under-estimate of the true processivity stimulation due to the destabilizing effect of the NaCl in the PCNA fraction, as reported by others (32). The ability of PCNA to stimulate both DNA polymerase activity and processivity is a distinctive characteristic of a δ -type DNA polymerase (30).

The low apparent yield of pol δ activity relative to pol α activity could be partially due to the extreme PCNA dependence of pol δ , as demonstrated in Fig. 4B. Proteolysis, a common problem encountered during purification of DNA polymerases, was reported by Bauer *et al.* (33) to cause a low recovery of pol δ activity. They found that even in the presence of protease inhibitors, there was a low recovery. When they utilized a PEP4 protease deficient strain of yeast, they recovered 10% more pol δ activity. Our purification utilized protease inhibitors, and pol α was recovered at least partially as the full length 183 kD form. However, we cannot assess the state of the pol δ peptides, since we have been unsuccessful in determining the pol δ -specific peptides in pool I by immunologic reactivity with human pol δ -specific antibodies. We also tried to further purify pol δ , but were hindered by loss of activity. This may reflect an *in vitro* inefficiency of pol δ due to missing accessory proteins and/or inappropriate templates. Alternatively, the low yield of pol δ may be a reflection of the *in vivo* roles of this DNA polymerase at the replication fork. Pol δ is less abundant than pol α and pol ϵ in human and yeast systems (26,33). Whatever the explanation, the low activity of pol δ is probably responsible for the previous lack of detection of pol δ in *Drosophila* embryos.

As noted in the introduction, there are dramatic differences between embryonic and nonembryonic cell DNA replication; a short duration S phase, ten-fold higher number of active origins, maternally supplied replicative proteins, and the apparent lack of pol δ and pol ϵ in *Drosophila* embryos. These differences may reflect a mechanism of replication which differs from the dual DNA polymerase, bidirectional replication fork model proposed for nonembryonic cells (4–6). An alternative, single DNA polymerase model of replication has been suggested for amphibian embryos (34). The amount of single stranded DNA observed in *Xenopus* embryos was inversely proportional to the

length of the S phase. It was suggested that single stranded parental DNA might be replicated by discontinuous synthesis carried out by pol α /primase. A similar model was proposed for the repeated rDNA genes in the pea plant (35). Two-dimensional agarose gel electrophoresis indicated that the rDNA was replicated by a single stranded displacement loop mechanism which, again, could be accomplished by a single DNA polymerase mechanism. A second possibility is that embryos have multiple DNA polymerases organized differently to allow initiation, elongation, and proofreading of single stranded displacement loop configuration during replication. Finally, *Drosophila* embryos might utilize a mechanism similar to the nonembryonic cell model; pol δ and/or pol ϵ DNA polymerases may also be present in the embryos, and the replication fork may be organized as proposed for nonembryonic cell replication. If this is the case, the rapid rate of embryonic replication would not be determined by the replication fork organization, but might be determined by the number of origins utilized as suggested (15). We characterized at least two distinct DNA polymerases in *Drosophila* embryos, an observation most consistent with the latter two models. Further, we have found that 9-hour embryos, which utilize fewer origins of replication and have a 6-fold longer duration S phase relative to 2-hour embryos, also contain both types of DNA polymerase with enzymatic properties indistinguishable from 2-hour preparations (unpublished data). It is possible that activation of the DNA polymerases is developmentally controlled by post-translational mechanisms which also control origin usage.

In summary, we have characterized two types of DNA polymerase from early stage *Drosophila* embryos. One DNA polymerase activity corresponds to the pol α previously purified from later stage *Drosophila* embryos (19). The other DNA polymerase appears to be distinct from pol α , and resembles a δ -type enzyme based on the following properties: 1. associated 3'–5' exonuclease and lack of primase; 2. PCNA-dependent, high processivity; 3. lack of recognition by pol α -specific antisera; and 4. sensitivity to aphidicolin and resistance to BuPdGTP. These results are consistent with the suggestion that multiple DNA polymerases function in both embryonic and nonembryonic cells for the replication of DNA.

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ABBREVIATIONS

pol α , DNA polymerase α /primase; pol δ , DNA polymerase δ ; pol ϵ , DNA polymerase ϵ ; PCNA, proliferating cell nuclear antigen; S-100, supernatant from the 100,000 $\times$ g centrifugation; BuPdGTP, N₂-(p-butylphenyl) deoxyguanosine 5'-triphosphate; and FPLC, fast protein liquid chromatography.

REFERENCES

1. Downey, K.M., Tan, C.-K., and So, A.G. (1990) *Bioessays* 12, 231–235.
2. Syvaoja, J.E. (1990) *Bioessays* 12, 533–536.
3. Bambara, R.A., and Jesse, B.C. (1991) *Biochim Biophys Acta* 1088, 11–24.
4. Hurwitz, J., Dean, F.B., Kwong, A.D., and Lee, S.-H. (1990) *J. Biol. Chem.* 265, 18043–18046.
5. Stillman, B. (1989) *Annu. Rev. Cell Biol.* 5, 197–245.
6. Challberg, M.D. and Kelly, T.J. (1989) *Annu. Rev. Biochem.* 58, 671–717.
7. Melendy, T. and Stillman, B. (1991) *J. Biol. Chem.* 266, 1942–1949.
8. Weinberg, D.H. and Kelly, T.J. (1989) *Proc. Natl. Acad. Sci. USA* 86, 9742–9746.
9. Crute, J.J., Wahl, A.F., and Bambara, R.A. (1986) *Biochemistry* 25, 26–36.
10. Nishida, C., Reinhard, P., and Linn, S. (1988) *J. Biol. Chem.* 263, 501–510.
11. Hamatake, R.K., Hasegawa, H., Clark, A.B., Bebenek, K., Kunkel, T.A., and Sugino, A. (1990) *J. Biol. Chem.* 265, 4072–4083.
12. Morrison, A., Araki, H., Clark, A.B., Hamatake, R.K. and Sugino, A. (1990) *Cell* 62, 1143–1151.
13. Johnson, L.M., Snyder, M., Chang, L.M.S., Davis, R.W. and Campbell, J.L. (1985) *Cell* 43:369–377.
14. Sitney, K.C., Budd, M.E. and Campbell, J.L. (1989) *Cell* 56:599–605.
15. Blumenthal, A.B., Kriegstein, H.J., and Hogness, D.S. (1973) *C.S.H.S.Q.B.* 38, 205–223.
16. Glover, D.M. (1991) *Trends in Genet.* 7, 125–132.
17. Linn, S. (1991) *Cell* 66, 185–187.
18. Lee, M.Y.W.T., Jiang, Y., Zhang, S.J., and Toomey, N.L. (1991) *J. Biol. Chem.* 266, 2423–2429.
19. Kaguni, L.S., Rossignol, J.-M., Conway, R.C. and Lehman, I.R. (1983) *Proc. Natl. Acad. Sci. USA* 80, 2221–2225.
20. Ng, L., Prelich, G., Anderson, C.W., Stillman, B., and Fisher, P.A. (1990) *J. Biol. Chem.* 265, 11948–11954.
21. Cotterill, S.M., Reyland, M.E., Loeb, L.A., and Lehman, I.R. (1987) *Proc. Natl. Acad. Sci. USA* 84, 5635–5639.
22. Laemmli, U.K. (1970) *Nature* 227, 680–685.
23. Towbin, H., Staehelin, T., Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* 76, 4350–4354.
24. Lee, S.-H., Kwong, A.D., Ishimi, Y., and Hurwitz, J. (1989) *Proc. Natl. Acad. Sci. USA* 86, 4877–4881.
25. Sauer, B. and Lehman, I.R. (1982) *J. Biol. Chem.* 257, 12394–12398.
26. Syvaoja, J., Suomensaaari, S., Nishida, C., Goldsmith, J.S., Chui, G.S.J., Jain, S., and Linn, S. (1990) *Proc. Natl. Acad. Sci. USA* 87, 6664–6668.
27. Burgers, P.M.J., and Bauer, G.A. (1988) *J. Biol. Chem.* 263, 925–930.
28. Sakaguchi, K., and Boyd, J.B. (1985) *J. Biol. Chem.* 260, 10406–10411.
29. Wernette, C.M., and Kaguni, L.S. (1986) *J. Biol. Chem.* 261, 14764–14770.
30. Bauer, G.A., and Burgers, P.M.J. (1988) *Proc. Natl. Acad. Sci. USA* 85, 7506–7510.
31. Perrino, F. and Loeb, L.A. (1990) *Biochemistry* 29, 5226–5231.
32. Bauer, G.A., and Burgers, P.M.J. (1988) *Biochim. Biophys. Acta* 951, 274–279.
33. Bauer, G.A., Heller, H.M., and Burgers, P.M.J. (1988) *J. Biol. Chem.* 263, 917–924.
34. Gaudette, M.F., and Benbow, R.M. (1986) *Proc. Natl. Acad. Sci. USA* 83, 5953–5957.
35. Van't Hof, J., and Lamm, S.S. (1991) *EMBO J.* 10, 1949–1953.