Radman in whose laboratory this work was initiated.

References

Abbott, P. J., & Saffhil, R. (1977) *Nucleic Acids Res. 4*, 761–769.
Cone, R., Bonura, T., & Friedberg, E. C. (1980) *J. Biol. Chem. 255*, 10354–10358.
Drake, J. W., & Baltz, R. H. (1976) *Annu. Rev. Biochem. 45*, 11–37.
Duncan, B. K., & Miller, J. H. (1980) *Nature (London) 287*, 569–571.
Herschfield, M. S., & Nossal, N. G. (1972) *J. Biol. Chem. 247*, 3393–3404.
Inman, R. B., & Baldwin, , R. L. (1964) *J. Mol. Biol. 8*, 452–469.
Kato, T., & Shinoura, Y. (1977) *Mol. Gen. Genet. 156*, 121–131.
Kornberg, A. (1974) in *DNA Synthesis*, pp 5369–5372, Freeman, San Francisco, CA.
Kornberg, T., & Gefter, M. L. (1972) *J. Biol. Chem. 247*, 5369–5372.
Lackey, D., Wald-Krauss, S., & Linn, S. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 330–334.
Laval, J., & Laval, F. (1980) *IARC Sci. Publ. 27*, 55–72.
Leblanc, J. P., Martin, B., Cadet, J., & Laval, J. (1982) *J. Biol. Chem. 257*, 3477–3483.
Lecomte, P., Boiteux, S., & Doubleday, O. P. (1981) *Nucleic Acids Res. 9*, 3491–3501.
Lindahl, T. (1982) *Annu. Rev. Biochem. 51*, 61–87.
Lindahl, T., & Anderson, T. (1972) *Biochemistry 11*, 3618–3623.
Lindahl, T., & Nyberg, B. (1974) *Biochemistry 13*, 3405–3410.
Livingston, D. M., Hinkle, D., & Richardson, C. C. (1975) *J. Biol. Chem. 250*, 461–469.
Pierre, J., & Laval, J. (1980a) *Biochemistry 19*, 5018–5024.
Pierre, J., & Laval, J. (1980b) *Biochemistry 19*, 5027–5031.
Pierre, J., & Laval, J. (1981) *J. Biol. Chem. 256*, 10217–10220.
Radman, M., Villani, G., Boiteux, S., Kinsella, A. R., Glickman, B. W., & Spadari, S. (1979) *Cold Spring Harbor Symp. Quant. Biol. 43*, 937–945.
Richardson, C. C., Schildkraut, C. L., Aposhian, H. V., & Kornberg, A. (1964) *J. Biol. Chem. 239*, 222–232.
Schaaper, R. M., & Loeb, L. A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1773–1777.
Setlow, P., Brutlag, D., & Kornberg, A. (1972) *J. Biol. Chem. 247*, 224–231.
Shearman, C. W., & Loeb, L. A. (1979) *J. Mol. Biol. 128*, 197–218.
Singer, B. (1982) *Annu. Rev. Biochem. 51*, 655–693.
Sirover, M. A., & Loeb, L. A. (1976) *Science (Washington D.C.) 194*, 1434–1437.
Strauss, B., Rabkin, S., Sagher, D., & Moore, P. (1982) *Biochimie 64*, 829–833.
Ullman, J. S., & McCarthy, B. J. (1973) *Biochim. Biophys. Acta. 294*, 396–404.
Villani, G., Boiteux, S., & Radman, M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3037–3041.
Warner, H. R., & Duncan, B. K. (1978) *Nature (London) 272*, 32–34.

Nuclear Matrix-Bound Deoxyribonucleic Acid Synthesis: An in Vitro System†

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ABSTRACT: A nuclear matrix-bound in vitro replicational system that utilizes α-polymerase and matrix-attached DNA has been isolated from regenerating rat liver and characterized for in vitro requirements. β-Polymerase is a minor component of the matrix in vitro system. Evidence is presented for the native association of α-polymerase with the nuclear matrix and for the parallel induction of matrix-bound α-polymerase and in vivo DNA synthesis during the proliferative response following partial hepatectomy. Our data suggest that the attachment of α-polymerase to the matrix may be an important step in the assembly of functional replisomes. We also demonstrate prereplicative and late-replicative rises in nuclear and matrix-bound β-polymerase activity and discuss these results in terms of possible genome screening.

Many cellular functions involve the regulation and integration of macromolecular assemblies, for example, the pyruvate dehydrogenase complex of *Escherichia coli*, the fatty acid synthetase of yeast, mitochondrial oxidative phosphorylation, and protein synthesis (Stryer, 1981). Significant advances have been made to define the components of the macromolecular assemblies or replisomes responsible for procaryotic DNA replication (Kornberg, 1980). It is intriguing to consider whether eucaryotic DNA replication involves mechanisms analogous to those in procaryotes. For example, Jacob et al. (1963) proposed that procaryotic DNA is synthesized on membrane-bound replisomes while Berezney & Coffey (1975, 1976) suggested that eucaryotic replication occurs in association with the matrix of the cell nucleus.

Although the precise spatial localization of functional replisomes remains to be clarified in both procaryotic and eucaryotic cells, a number of recent findings support the concept that DNA replication is a matrix-bound process in eucaryotes.

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It is known that chromatin is organized into characteristic repeating domains or loops within the cell nucleus (Cook & Brazell, 1975; Benyajati & Worcel, 1976; Georgiev et al., 1978; Igō-Kemenes & Zachau, 1978). These supercoiled loops or domains are attached to the nuclear matrix of interphase nuclei (Wanka et al., 1977; Volgelstein et al., 1980; Berezney & Buchholtz, 1981a) and its metaphase counterpart the chromosomal scaffold of mitotic cells (Paulson & Laemmli, 1977). In vivo incorporation studies suggest that DNA replication occurs at or close to the matrix attachment sites for the DNA loops (Berezney & Coffey, 1975, 1976; Dijkwel et al., 1979; McCready et al., 1980; Pardoll et al., 1980; Berezney & Buchholtz, 1981b). This conclusion has received additional support from light microscopic autoradiographic analysis of newly replicated DNA with the DNA halo technique (McCready et al., 1980; Volgelstein et al., 1980). During the replicative process, it is proposed that DNA is either reeled through fixed replicational complexes bound to the matrix or that the entire matrix-bound replisome is translocated along the DNA loops (Dijkwel et al., 1979; McCready et al., 1980; Pardoll et al., 1980; Berezney & Buchholtz, 1981b).

The above-mentioned models assume that functional replicational complexes are actually bound to the matrix. Our laboratory is attempting to test this hypothesis directly. As a first step, we demonstrated that isolated nuclear matrices contain significant levels of the eucaryotic replicative enzyme, DNA polymerase α (Smith & Berezney, 1980). We now report that the nuclear matrix is an effective in vitro system that synthesizes DNA on the matrix-attached DNA fragments. Single-stranded lengths of 100–200 nucleotides are produced that may represent the characteristic primary replicational intermediates or Okazaki fragments (Edenberg & Huberman, 1975; Hand, 1978). Matrix-bound DNA polymerase α (the presumptive replication enzyme) is the principle DNA polymerase activity driving this in vitro DNA synthesis system and represents a cell cycle specific, inducible component. We also demonstrate the similarity of matrix-bound DNA synthesis to total nuclear DNA synthesis and provide evidence which suggests that dynamic domains of polymerase activity are present within the nucleus. Finally, we report prereplicative and late-replicative rises in nuclear and matrix-bound DNA polymerase β (the presumptive repair enzyme) and discuss its possible significance in terms of genome screening prior to and possibly after replication.

Experimental Procedures

Nuclear and Matrix Preparations. Nuclei were prepared from normal and two-thirds partially hepatectomized rats (Sprague-Dawley 230–280 g, King Animal Labs) as described previously (Berezney & Coffey, 1975, 1976) with slight modifications. The Tris[1] concentration was lowered from 50 to 5 mM and the pH from 7.4 to 7.0 (unless indicated otherwise) in all nuclear isolation buffers in order to minimize leaching of nuclear polymerase activities (Lynch et al., 1975).

Following dense sucrose purification, nuclei were resuspended to 2.5 mg of DNA/mL in 0.25 M sucrose, 5 mM $MgCl_2$, and 5 mM Tris-HCl, pH 7.4 (23 °C), and nuclei equivalent to 12–15 μg of DNA were used for each in vitro DNA synthesis assay. Nuclear activities were corrected to 100% nuclear recovery on the basis of DNA measurements. Actual recovery of purified nuclei varied from 55% to 85%. Total cellular α- and β-polymerase activities were calculated as the sum of the corrected nuclear activities and activities measured in nuclei-free cytosol.

Endogenously digested nuclei (37 °C for 45 min) were extracted 4 times with LS buffer [0.2 M $MgCl_2$, 10 mM Tris-HCl, pH 7.4 (23 °C), 1 mM PMSF (phenylmethanesulfonyl fluoride)] to yield low-salt soluble bulk chromatin and the insoluble low-salt matrix. The bulk chromatin contained 70–80% of the total DNA while the remaining 20–30% of the chromatin was associated with the low-salt (LS) matrix (Berezney & Coffey, 1976; Berezney, 1979). LS matrix was then extracted 3 times with HS buffer [2 M NaCl, 0.2 M $MgCl_2$, 10 mM Tris-HCl, pH 7.4 (23 °C), 1 mM PMSF], once with 0.4% (w/v) Triton X-100 in LS buffer, and twice with LS buffer. Centrifugations were at 1000g for 15 min during the initial LS buffer extractions and at 6000g for 15 min for all subsequent extractions. Final nuclear matrices corresponding to matrix III structures (Berezney, 1979) and containing 1–3% of the total nuclear DNA were resuspended in LS buffer ($^1/_3$–$^1/_2$ the original volume of nuclei), and 15 μL was used per in vitro assay.

In Vivo DNA Synthesis. [^{3}H]Thymidine (50–60 Ci/mmol, New England Nuclear) was injected directly into the hepatic portal vein (100 μCi/250 g of body weight) as previously described (Berezney & Buchholtz, 1981b). After the appropriate pulse time, the livers were excised, minced in ice-cold buffer, and processed as described above. For long-term in vivo labeling, rats were injected intraperitoneally with 200 μCi of [^{3}H]thymidine at 18, 20, and 22 h of liver regeneration. Livers were harvested at 24 h as described above. Specific labeling of DNA was determined as reported previously (Berezney & Buchholtz, 1981b).

In Vitro DNA Synthesis. α-Polymerase activity was assayed in a 50-μL reaction mix (α-polymerase buffer) containing 50 mM Tris-HCl, pH 7.2 (23 °C), 600 μg/mL DNase free bovine serum albumin, 15% glycerol, 2 mM dithiothreitol, 2 mM EGTA, 8 mM $MgCl_2$, 15 mM KCl, 0.1 mM phenylmethanesulfonyl fluoride, 2 mM ATP, 80 μM dATP, dCTP, and dGTP, and 40 μM [^{3}H]-TTP (0.48 Ci/mmol, New England Nuclear). The α-polymerase assays were corrected for NEM (*N*-ethylmaleimide)-resistant incorporation measured in a parallel reaction after preincubation of sample with 10 mM NEM at 0 °C for 30 min.

β-Polymerase activity was assayed in a 50-μL reaction mix (β-polymerase buffer), containing 50 mM Tris-HCl, pH 8.8 (23 °C), 600 μg/mL DNase free bovine serum albumin, 15% glycerol, 2 mM EGTA, 8 mM $MgCl_2$, 100 mM NaCl, 0.1 mM phenylmethanesulfonyl fluoride, 2 mM ATP, 80 μM dATP, dCTP, and dGTP, and 40 μM [^{3}H]-TTP (0.48 Ci/mmol, New England Nuclear). All samples were preincubated with 10 mM NEM at 0 °C for 30 min.

For alkaline sucrose gradient analysis, [^{32}P]-dCTP (400 Ci/mmol, New England Nuclear) was used instead of [^{3}H]-TTP in the in vitro assays after diluting it to the same specific activity as the [^{3}H]-TTP.

All assays were run in triplicate at 37 °C for 10 min. Reactions were terminated by the addition of trichloroacetic acid to 5% on ice. Acid-precipitable counts were trapped on GF/A Whatman filters and counted in 4a20 liquid scintillant (Research Products International Corp.) with a Delta 300 liquid scintillation system (Tracor Analytic). The counting efficiency was 37%. Incorporation on exogenous template [391 μg/mL activated calf thymus DNA (Baril et al., 1977)] was corrected for incorporation in its absence (endogenous template activity).

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

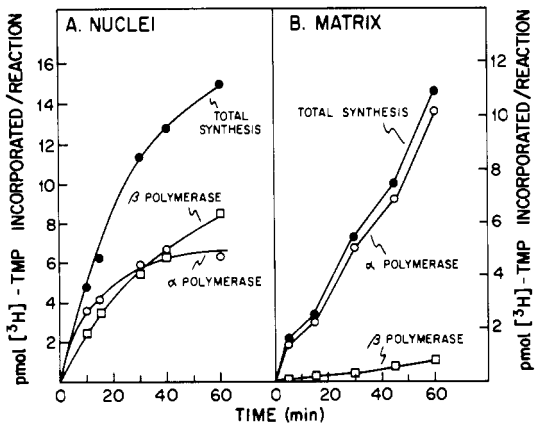

FIGURE 1: Kinetics of endogenous in vitro DNA synthesis. Purified nuclei (A) and matrices (B) were prepared from regenerating liver 22 h after partial hepatectomy (see Experimental Procedures) and assayed in α-polymerase buffer with [β-polymerase (□)] or without [total synthesis (●)] *N*-ethylmaleimide (NEM). α-Polymerase (○) represents the difference in incorporation with and without NEM.

Alkaline Sucrose Gradient Analysis. In these experiments, DNA was labeled in vivo with [^{3}H]thymidine and in vitro with [^{32}P]-dCTP. The DNA was enzymatically and chemically purified from each sample as described previously (Basler et al., 1981). Samples were denatured at 37 °C for 30 min in 0.2 N NaOH–10 mM EDTA and layered onto 5–20% sucrose gradients containing 0.2 N NaOH, 0.9 M NaCl, and 1 mM EDTA (Berezney & Buchholtz, 1981a,b). Eleven-milliliter gradients were centrifuged in an SW-41 rotor (Beckman Instrument Inc.) at 34000 rpm for 16 h at 20 °C. Aliquots (0.2 mL) were collected from the top of each gradient and precipitated with 5% trichloroacetic acid. Acid-precipitable counts were determined by liquid scintillation counting as described above. Parallel gradients were calibrated with ^{3}H-labeled φX-174 DNA (16.4 S). Under these conditions mononucleosomal core DNA prepared after micrococcal nuclease (Worthington) digestion of isolated rat liver nuclei sedimented at approximately 4 S (Berezney & Buchholtz, 1981b).

Results

Kinetics of in Vitro DNA Synthesis. High salt resistant nuclear matrices (nuclear matrix III; Berezney, 1979) containing 8–12% of the total nuclear protein and 1–3% of the total nuclear DNA were isolated from normal or regenerating rat liver (see Experimental Procedures). Figure 1 shows the kinetics of in vitro DNA synthesis in nuclei and matrices isolated from regenerating liver when in vivo replication is maximal. Matrix-bound DNA synthesis continued at a linear rate for at least 1 h, whereas the rate of total nuclear DNA synthesis decreased after 30 min. Although the in vitro assays were performed under conditions optimal for α-polymerase, considerable amounts of β-polymerase activity were also detected in the nuclear reactions. In the matrix, α-polymerase predominated, and only trace amounts of β-polymerase were measured (<10% of the total matrix polymerase activity). The kinetics for nuclear and matrix-bound β-polymerase assayed under conditions optimal for β-polymerase were essentially the same as shown in Figure 1. It is important to emphasize that the rate of α-polymerase reaction was linear for over 1 h in the matrix but rapidly declined after 15 min in total nuclei. To obtain meaningful comparison data for nuclei and matrices we have chosen 10 min as a standard assay time.

Inhibitors of Nuclear and Matrix-Bound DNA Synthesis. Our in vitro system has operationally defined α- and β-polymerase activities on the basis of their well-characterized sulfhydryl requirements and NEM (*N*-ethylmaleimide) sensitivities (Falaschi & Spadari, 1978). In Table I we show our results with other specific inhibitors of α- and β-polymerases. Nuclear and matrix-bound α-polymerases were almost completely inhibited by aphidicolin, a specific inhibitor of α-polymerase (Ikegami et al., 1978), whereas β-polymerase was resistant to inhibition. Dideoxy-TTP, an inhibitor of β-polymerase (Wagar et al., 1978), did not inhibit nuclear or matrix α-polymerase but did inhibit β-polymerase. Finally, α-polymerases from nuclei and matrices required a full complement of deoxynucleoside triphosphates, while β-polymerases continued to synthesize DNA at an appreciable rate when three of the deoxynucleoside triphosphates were left out of the in vitro reaction. These results verify our definition of α- and β-polymerase activities on the basis of NEM sensitivity, and we therefore use this criterion to discriminate between these two activities in all subsequent studies.

Table I: Inhibition of Nuclear and Matrix-Bound in Vitro DNA Synthesis[a]

	inhibition (%)			
	α-polymerase		β-polymerase	
condition	nuclear	matrix	nuclear	matrix
aphidicolin (10 μg/mL)	92	99	9.2	16
dideoxy-TTP (5:1, ddTTP:TTP)	0	0	66	83
minus dATP, dCTP, dGTP	90	78	47	15

[a] Nuclei and matrices were prepared from actively replicating regenerating rat liver and assayed for endogenous template activity as described under Experimental Procedures. Samples assayed in the presence of aphidicolin were preincubated with the inhibitor (10 μg/mL) for 10 min on ice and assayed in the presence of the inhibitor at the indicated concentration. The effect of dideoxy-TTP was measured by adding the inhibitor directly to the assay at the indicated molar ratio to TTP. Values are expressed as the percent of the activity in the absence of inhibitor (control). Control incorporations were 1778, 533, 220 and 12 pmol/g of tissue for nuclear α-, nuclear β-, matrix α-, and matrix β-polymerase activities, respectively.

General Properties of Nuclear and Matrix-Bound DNA Synthesis. In vitro DNA synthesis increased linearly with increasing sample concentration (10–250 μg of protein per reaction) for both α- and β-polymerase in nuclei and matrix (data not shown). The optimal buffer conditions for in vitro DNA synthesis (see Experimental Procedures) in nuclei and matrices were similar to those described in other systems (Falaschi & Spadari, 1978). α- and β-polymerase had markedly different monovalent salt and pH requirements. Nuclear and matrix-bound α-polymerase functioned maximally at pH 6.8–7.2 while β-polymerase required pH 8.4–8.8 for optimal DNA synthesis. Similarly, nuclear and matrix-bound α-polymerases were strongly inhibited by monovalent salt concentrations above 100 mM while β-polymerases functioned maximally at salt concentrations of 100–200 mM. Moreover, the conditions we used fell on sharp optima for the nuclear enzymes but represented the midpoint in much broader optima seen with matrix-bound enzymes (data not shown).

Nuclear and matrix-bound α-polymerases required ATP for optimal in vitro DNA synthesis (Figure 2). Both sources of α-polymerase were maximally stimulated by 1 mM ATP and strongly inhibited by ATP concentrations greater than 3 mM. Nuclear β-polymerase required ATP for optimal DNA synthesis but was relatively resistant to inhibition by high ATP concentrations. Matrix-bound β-polymerase required 1–2 mM ATP for maximal activity and, like α-polymerase, was in-

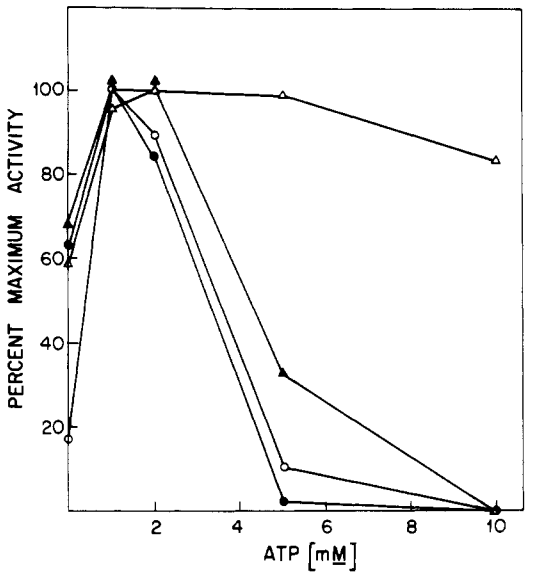

FIGURE 2: Effect of ATP on nuclear and matrix-bound in vitro DNA synthesis. Nuclei and matrices from 22-h regenerating liver were assayed for α- and β-polymerase activities with the indicated ATP concentrations: nuclear α-polymerase (O); matrix α-polymerase (●); nuclear β-polymerase (Δ); matrix β-polymerase (▲). Incorporations in the absence of ATP were 193, 212, 568, and 56 pmol/g of tissue for nuclear α-, matrix α-, nuclear β-, and matrix β-polymerase activity, respectively.

Table II: Stimulation of DNA Synthesis by Exogenous Template[a]

	x-fold stimulation	
	α-polymerase	β-polymerase
nuclei	7.1 ± 1.5	20.2 ± 4.4
matrix	5.6 ± 0.5	2.2 ± 0.6

[a] Nuclei and matrices were prepared from 22-h regenerating liver. The x-fold stimulation is calculated as the ratio of the polymerase activity measured in the presence of activated calf thymus template (exogenous DNA synthesis) and the activity in the absence of exogenous template (endogenous DNA synthesis). Values represent the average of three different nuclear and matrix preparations ± SEM. In these calculations, the endogenous activity was not subtracted from the activity in the presence of calf thymus DNA. Incorporations in the absence of exogenous template were 1778, 533, 220, and 12 pmol/g of tissue for nuclear α-, nuclear β-, matrix α-, and matrix β-polymerase activities, respectively.

hibited by higher ATP concentrations.

The endogenous template activity is subtracted as background from the activity in assays with the exogenous template. The limited information available suggests that treating the endogenous and exogenous template activities as distinct entities is a valid approach, as the two template activities may not compete for template-engaged enzyme but may only compete for free enzyme (Gold & Helleiner, 1964; Phillipe et al., 1976; Seki & Mueller, 1976; Chan et al., 1977; Fry et al., 1980; Yagura & Seno, 1980). We will therefore refer to these two activities as endogenous and exogenous template activities.

Table II shows the effect of adding activated calf thymus DNA to the in vitro reaction. The nuclear and matrix-bound α-polymerases were stimulated 5–7-fold by exogenous DNA template. In contrast, the matrix-bound β-polymerase was stimulated only 2-fold compared to the 20-fold stimulation of nuclear β-polymerase.

Size of the in Vitro Product. The in vitro product of α- and β-polymerases was DNase I sensitive and RNase A, proteinase

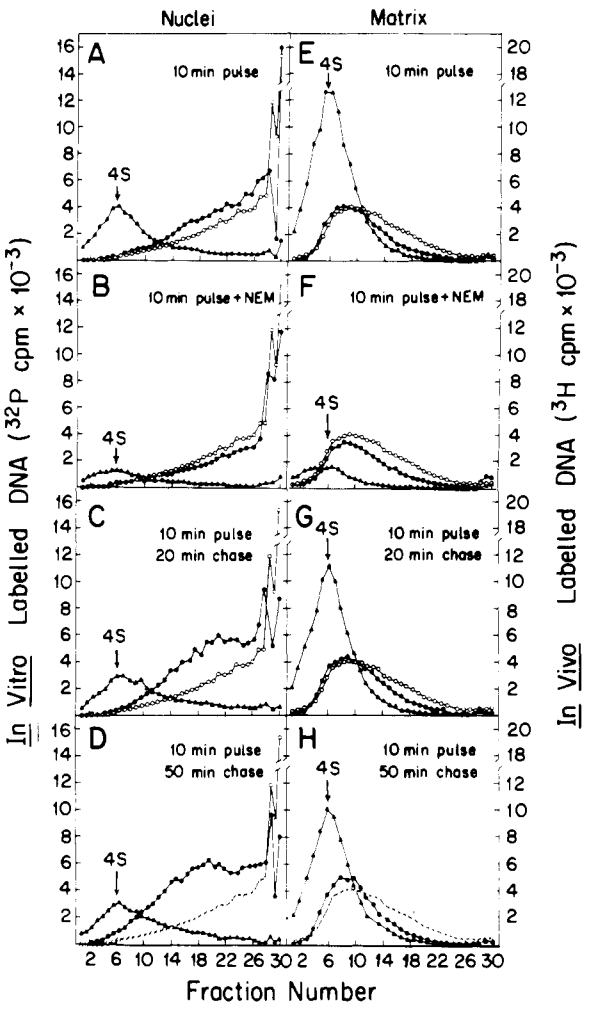

FIGURE 3: Alkaline sucrose gradients of in vitro synthesized DNA in nuclei and matrices. Nuclei and matrices were prepared from actively replicating regenerating liver that had been long-term-labeled in vivo with [^{3}H]thymidine (see Experimental Procedures). Nuclei and matrices were incubated in vitro in α-polymerase buffer with or without NEM in the presence of [^{32}P]-dCTP. DNA isolation and alkaline sucrose gradient analysis of all samples are described under Experimental Procedures. In vivo labeled DNA before incubation (O) and after incubation (●); in vitro [^{32}P]-dCTP labeled DNA (▲).

K, and alkaline resistant (data not shown). We purified nuclear and matrix DNA before and after in vitro DNA synthesis and analyzed the size distribution of total in vivo labeled [^{3}H]-DNA and in vitro labeled [^{32}P]-DNA on alkaline sucrose gradients (Figure 3). Our assay buffers contained 2 mM EGTA to reduce the nicking of DNA by the Ca^{2+}/Mg^{2+} endogenous nuclease of rat liver (Burgoyne & Hewish, 1978). As shown in Figure 3A,B, EGTA prevented extensive nuclease activity during our standard 10-min incubation of isolated nuclei. However, EGTA could only slow down the rate of nuclear DNA nicking under conditions of prolonged incubations (Figure 3C,D). There was no indication of nuclease activity in our matrix fractions (Figure 3G,H). Moreover, the absence of EGTA in the assay buffers had no effect on the single-stranded size of matrix DNA for incubations up to 60 min. In contrast, the DNA of isolated nuclei was extensively nicked under these conditions (data not shown).

The in vitro DNA product of nuclear and matrix-bound α-polymerase sedimented as a discrete 4–5S peak on alkaline sucrose gradients (Figure 3A,E). This corresponds to a single-stranded fragment length of 100–200 nucleotides, similar to that demonstrated by other investigators in vivo and in vitro (Edenberg & Huberman, 1975; Tseng & Goulian, 1975;

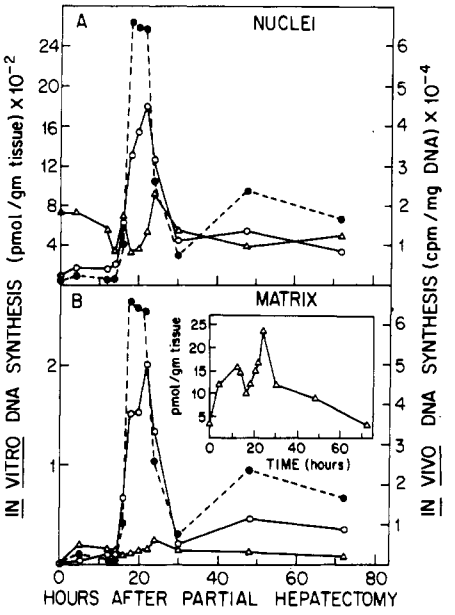

FIGURE 4: Temporal relationship of nuclear and matrix in vitro DNA synthesis to in vivo replication. In vivo DNA replication and in vitro DNA synthesis on the endogenous template were determined as described under Experimental Procedures for nuclei (A) and matrices (B): 30-min in vivo pulse-labeled DNA (●), α-polymerase (○), and β-polymerase (△). Insert shows β-polymerase on an expanded scale.

Fraser & Huberman, 1977; Knopf & Weissbach, 1977; Seale, 1977; Brun & Weissbach, 1978; Funderud et al., 1978; Hand, 1978).

Since the matrix-bound in vitro DNA synthesis was driven predominantly by α-polymerase, it was of interest to determine the size of the DNA synthesized by the trace amount of β-polymerase. As shown in Figure 3F, the discrete 4S peak was abolished, and the labeled DNA migrated as a heterodisperse population of fragments from the top of the gradient to approximately 6 S. Virtually identical results were found for total nuclear β-polymerase driven DNA synthesis (Figure 3B).

Chasing the in vitro labeled matrix DNA for up to 50 min had no effect on the alkaline sucrose gradient profiles (Figure 3G,H). This suggests the absence of functional ligation activities in these isolated matrix preparations. It is possible that ligase activity is present but not expressed due to the relatively short double-stranded length of the nicked, matrix-attached DNA fragments (average of 3000–4000 base pairs on nondenaturing agarose gels, data not shown). The absence of significant ligation in total nuclear DNA synthesis (Figure 3C,D) suggests that these factors were extracted or inactivated during nuclear purification. This appears to be characteristic of highly purified nuclear in vitro systems that require the addition of cytosolic factors to achieve effective ligation (Hershey et al., 1973; Tseng & Goulian, 1975; Muller et al., 1981). We cannot rule out the possibility, however, that nuclear ligation activity is present but masked by the trace amount of nuclease activity still present in the nuclear reactions (Figure 3C,D).

Temporal Relationship of Matrix-Bound in Vitro DNA Synthesis Activity to in Vivo Replication. Since nuclear matrices from quiescent normal liver are deficient in α-polymerase activity (Smith & Berezney, 1980), we determined the temporal relationship between matrix-bound in vitro DNA synthesis and in vivo replication. The first wave of DNA replication in regenerating liver began 16 h after partial hepatectomy and was maximal between 18–22 h before rapidly declining at 24 h (Figure 4). The temporal induction of in vitro DNA synthesis driven by the matrix-bound α-polymerase showed a remarkable resemblance to that of the in vivo replicational rate. Matrix-bound α-polymerase activity began to increase at 16 h after partial hepatectomy and reached a maximum at 22 h before rapidly declining at 24 h in synchrony with in vivo replication (Figure 4B). A similar response was observed for nuclear DNA synthesis driven by α-polymerase (Figure 4A). As anticipated from previously reported studies (Spadari & Weissbach, 1974; Chiu & Baril, 1975; Craig et al., 1975), in vitro DNA synthesis driven by β-polymerase did not show a direct temporal correlation to in vivo replication in either matrix or total nuclei (Figure 4). However, we did observe reproducible prereplicative (12–16 h after partial hepatectomy) and late-replicative (24 h) rises in both total nuclear and matrix-bound β-polymerase activity (Figure 4A and insert of Figure 4B). Similar prereplicative and late-replicative rises in total cellular β-polymerase activity were also measured (data not shown).

Table III: x-Fold Induction of α-Polymerase Activity[a]

	endogenous template act.	exogenous template act.
total cellular		2.9
total nuclei	20	13
bulk chromatin	1.9	3
low-salt matrix	23	9
high-salt matrix	100	40

[a] x-Fold induction was calculated as the in vitro DNA polymerase activity in actively replicating regenerating liver divided by that in normal liver. The in vivo rate of replication was stimulated approximately 50-fold as determined by in vivo [^{3}H]thymidine pulse (see Figure 4). Fractionation and in vitro assays were conducted as described under Experimental Procedures. Values represent the average of three to six separate determinations from two separate experiments. Normal liver values for endogenous template activity in total nuclei, bulk chromatin, low-salt matrix, and high-salt matrix were 77, 45, 3.5, and 1.3 pmol/g of tissue, respectively. Normal liver values for exogenous template activity in total nuclei, bulk chromatin, low-salt matrix, and high-salt matrix were 1218, 1078, 215, and 15.8 pmol/g of tissue, respectively. The recovery of total nuclear DNA in the various fractions was as follows: bulk chromatin, 76%; low-salt matrix, 24%; high-salt matrix, 2%.

Table III summarizes the fold induction of α-polymerase activity in total cellular enzyme and in several nuclear fractions coincident with the peak of in vivo replication. Increases in α-polymerase activity over normal liver values were observed in all fractions. The matrix-bound α-polymerase activities showed the highest level of induction coincident with in vivo replication (100-fold and 40-fold for endogenous and exogenous template activities, respectively). This is to be compared with an approximately 50-fold increase measured for in vivo replication (Figure 4). Increases in total nuclear α-polymerase activities also coincided with in vivo replication but were not as pronounced as those of the matrix-bound enzymes (20-fold and 13-fold for endogenous and exogenous template activities, respectively). The total cellular α-polymerase activity was increased only 2.9-fold. Interestingly, the bulk chromatin showed a similar low level of enzyme induction (1.9-fold and 3-fold for endogenous and exogenous template activities, respectively). These results demonstrate that the high salt resistant matrix is the intranuclear domain of highest α-polymerase activity induction and that the process of fractionation enriches for the site(s) of maximum induction.

Table IV shows the total and specific activities (pmol/mg DNA) of endogenous template engaged α- and β-polymerases. Though β-polymerase activities in all nuclear fractions varied slightly during regeneration, striking changes occurred for α-polymerase. In addition to a marked induction of total

Table IV: Total and Specific Activities of Endogenous Template-Engaged DNA Polymerases[a]

fraction	DNA polymerase α		DNA polymerase β	
	total act. (pmol/g of tissue)	sp act. (pmol/mg of DNA)	total act. (pmol/g of tissue)	sp act. (pmol/mg of DNA)
normal liver				
total nuclear	60	30	1031	568
bulk chromatin	35	23	4816	3784
low-salt matrix	5.3	7.0	2237	3268
high-salt matrix	0.6	0.4	54	826
regenerating liver				
total nuclear	1074	563	980	516
bulk chromatin	493	62	2064	2752
low-salt matrix	440	845	1256	2580
high-salt matrix	342	4560	83	1049

[a] Normal and actively replicating regenerating liver was fractionated and assayed in vitro for endogenous template activities as described under Experimental Procedures. Values represent the average from 10 separate nuclear preparations. The recovery of total nuclear DNA was as follows: bulk chromatin, 76%; low-salt matrix, 24%; high salt resistant matrix-attached DNA, 2%.

Table V: Effect of Endogenous Digestion on in Vitro DNA Synthesis[a]

	activities		% recovery of total nuclear activity	
	pmol/g of tissue	pmol/mg of protein	before digestion	after digestion
α-polymerase				
nuclei	1074	146		
digested nuclei	828	118		
matrix	342	399	31.8	41.3
β-polymerase				
nuclei	980	134		
digested nuclei	6963	997		
matrix	83	94	8.5	1.2

[a] Actively replicating regenerating rat liver was fractionated and assayed for in vitro endogenous template activities as described under Experimental Procedures. Values represent the average of nine separate nuclear preparations.

activity, the specific activity of the template-engaged α-polymerase on the high salt resistant matrix increased during regeneration to levels higher than other nuclear fractions (Table IV). The specific activity of α-polymerase in bulk chromatin (70–80% of the total nuclear DNA) was nearly 2 orders of magnitude lower than that of the high salt resistant matrix (1–3% of total DNA). Taken together, the above results suggest that the bulk chromatin is depleted in active replicational forks while the high salt resistant matrix-bound DNA is highly enriched in these sites.

Further analysis revealed that the relative distribution of α-polymerase in the bulk chromatin and low-salt matrix changed strikingly during liver regeneration (Figure 5). Nearly 50% of the total α-polymerase-driven DNA synthesis shifted to the low-salt matrix (20–30% of the total nuclear DNA) by 24 h after partial hepatectomy compared with less than 5% in quiescent normal liver (Figure 5). In contrast, DNA synthesis driven by β-polymerase did not show this shift (Figure 5). The β-polymerase activity distributed in a random fashion with respect to the bulk chromatin and low-salt matrix, independent of the replicative state of the cell. It is this shift in distribution from the bulk chromatin to the low-salt matrix in cells undergoing DNA replication that is the basis for the relatively high α-polymerase total and specific activities on the high salt resistant matrix (Figure 4 and Tables IV and V).

Distribution of in Vitro DNA Synthetic Activity during Nuclear Matrix Isolation. Table V shows a comparison of the in vitro DNA synthetic activity in 22-h regenerating liver nuclei before and after nuclease digestion (see Experimental Procedures for details of nuclear matrix isolations). Although

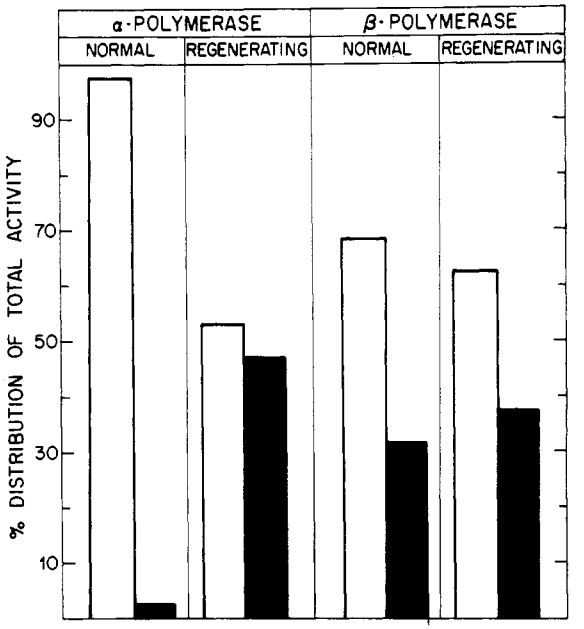

FIGURE 5: Distribution of in vitro DNA synthesis in bulk chromatin and low-salt matrix. Endogenously digested nuclei from normal and regenerating liver were fractionated into bulk chromatin and low-salt matrix (see Experimental Procedures). The relative percent distribution of total endogenous template α- and β-polymerase activities was determined for bulk chromatin (open bars) and low-salt matrix (closed bars). Total activities were as follows: normal liver bulk chromatin and low salt matrix α-polymerase, 35.0 and 5.3 pmol/g of tissue, respectively; normal liver bulk chromatin and low salt matrix β-polymerase, 4.82 and 2.24 nmol/g of tissue respectively; regenerating liver bulk chromatin and low salt matrix α-polymerase, 493 and 440 pmol/g of tissue, respectively; regenerating liver bulk chromatin and low salt matrix β-polymerase, 2.06 and 1.26 nmol/g of tissue, respectively.

nuclear DNA was cleaved to relatively small fragments by the endogenous nuclease digestion, 98% of the total nuclear DNA was still recovered in the nuclear pellet. A previous report demonstrated that the endogenous nuclease of rat liver activates total nuclear DNA synthesis (Burgoyne et al., 1970). There was a differential effect of endogenous digestion on α- and β-polymerase-driven DNA synthesis (Figure 6). In normal and regenerating liver, β-polymerase was stimulated 5–7-fold while α-polymerase was actually depressed.

Small amounts of nuclear α- and β-polymerase endogenous and exogenous template activities were released into the supernatant during endogenous nuclease digestion. Enzyme activities in the postdigestion supernatant were too low to account for the changes seen in postdigestion nuclei (Figure

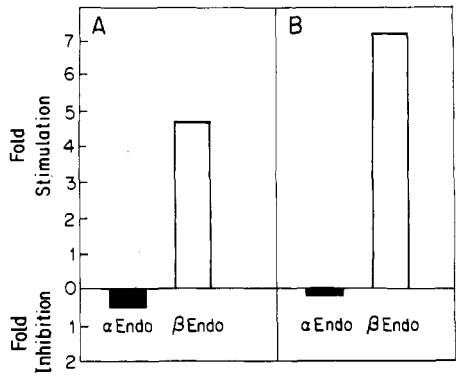

FIGURE 6: x-Fold stimulation or inhibition of nuclear endogenous template-engaged polymerase activity following endogenous digestion. Normal and actively replicating regenerating liver nuclei were prepared, endogenously digested, and assayed in vitro on the endogenous template as described under Experimental Procedures. Levels of template-engaged in vitro DNA polymerase activity after endogenous digestion were compared to those in nuclei before endogenous digestion in calculations of fold stimulation or inhibition: (A) normal liver; (B) 22-h regenerating liver; (closed bars) α-polymerase; (open bars) β-polymerase. Nuclear activities before digestion are as follows: (normal α) 60 pmol/g of tissue; (normal β) 1.03 nmol/g of tissue; (regenerating α) 1.1 nmol/g of tissue; (regenerating β) 980 pmol/g of tissue.

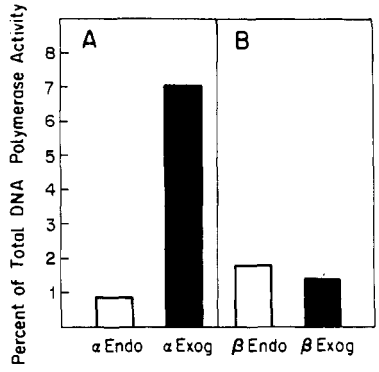

FIGURE 7: Percent of total nuclear DNA polymerase activity released into postdigestion supernatant. Actively replicating regenerating rat liver nuclei (22 h) were prepared and endogenously digested as described under Experimental Procedures. Nuclei were pelleted (1000g, 15 min), and the postdigestion supernatant was removed and assayed for DNA polymerase activities as described under Experimental Procedures. In vitro DNA polymerase activities in nuclei before digestion were used to calculate the percent of total nuclear activities released into the postdigestion supernatant: (A) α-polymerase; (B) β-polymerase; (closed bars) enzyme activities measured on exogenous template; (open bars) enzyme activities measured on the endogenous template. Incorporations in the postdigestion supernatant were 8.3, 846, 15, and 235 pmol/g of tissue for α endogenous and exogenous template activities and β endogenous and exogenous template activities, respectively.

7). The results demonstrate that β-polymerase was very active on the nicked nuclear template but α-polymerase apparently was not. The nuclease-induced inhibition of α-polymerase, the lack of significant solubilization of enzyme activity, and the absence of activation during 1-h kinetics (Figure 1) all suggest that α-polymerase does not effectively use nicks introduced by the endogenous nuclease.

It is important to note that the matrix-attached DNA studied in these experiments had an average single-stranded size on both alkaline sucrose gradients and denaturing acrylamide gels similar to the total population of endogenously digested DNA (800–1200 nucleotides). We, therefore, compared the DNA synthetic activities measured on the matrix with those of postdigestion nuclei. Approximately 40% of the total α-polymerase activity measured after the endogenous

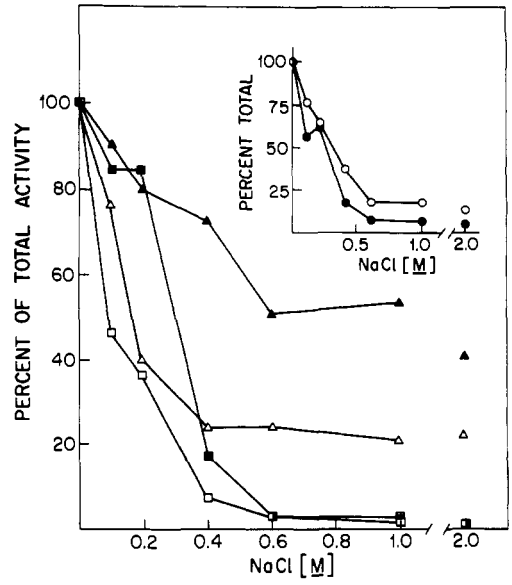

FIGURE 8: Effect of increasing ionic strength on extraction of matrix-bound DNA polymerase activity. Low-salt matrix prepared according to Experimental Procedures from 24-h regenerating liver was extracted with the indicated NaCl concentrations, and the final matrix was assayed under α- and β-polymerase conditions for in vitro DNA synthesis. All results are expressed as relative percent recovery of low-salt matrix prior to high-salt extraction. Low salt matrix total polymerase activities: α endogenous template activity, 440 pmol/g of tissue; α exogenous template activity, 2.46 nmol/g of tissue; β endogenous template activity, 1.26 nmol/g of tissue; β exogenous template activity, 3.79 nmol/g of tissue. α-Polymerase endogenous template activity (△), β-polymerase endogenous template activity (□), α-polymerase exogenous template activity (▲), and β-polymerase exogenous template activity (■). Inset shows the relative percent recovery of low salt matrix protein (○) and DNA (●).

digestion was recovered on the matrix (Table V). In contrast, only 1% of the total β-polymerase was matrix bound. Expressed on a specific activity basis, the matrix-bound α-polymerase activity was enriched over 3-fold and 10-fold per milligram of protein and DNA, respectively, when compared with postdigested nuclei. The matrix β-polymerase activity was depleted over 10-fold and 3-fold per milligram of protein and DNA, respectively.

Effect of Salt Extraction on Matrix-Bound DNA Polymerases. It could be argued that the α-polymerase tightly bound to the matrix is an artifact created by aggregation of the enzyme on the matrix following high-salt extraction. This seems unlikely since high-salt solutions are used to prevent aggregation of the enzyme during purification (Matsukage et al., 1975; Fichot et al., 1979). To explore this possibility further, we extracted the low-salt matrix with increasing concentrations of NaCl. Between 0.1 and 0.4 M NaCl, we observed a progressive increase in the extraction of α-polymerase activity (Figure 8). No additional activity was removed after 0.4–0.6 M NaCl for the α-polymerase endogenous and exogenous template activities, respectively, even though proteins and DNA were released in significant amounts up to 0.6 M NaCl. Moreover, β-polymerase activity was released progressively until only trace amounts remained matrix bound at 2.0 M NaCl. The recovery of α- and β-polymerases with the final matrix was not affected by the order in which the low- and high-salt extractions were performed. These results demonstrate that a discrete proportion of the total α-polymerase-driven DNA synthesis is high-salt resistant and bound to the matrix.

Relationship between Isolation Conditions and Matrix-Bound in Vitro DNA Synthesis. If there is a discrete popu-

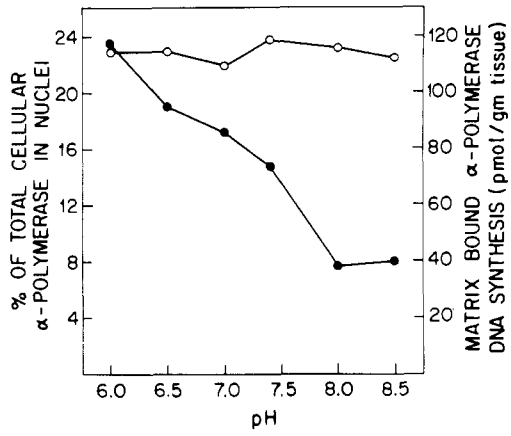

FIGURE 9: Effect of pH of nuclear isolation on matrix in vitro DNA synthesis. Nuclei were isolated from 24-h regenerating livers at the indicated pHs. Total cellular α-polymerase activity was calculated as the sum of activity measured with exogenous calf thymus DNA in cytosol and nuclei (corrected to 100% nuclear recovery). Over the pH scale investigated (6.0–8.5), the total cellular α-polymerase activity varied by approximately 10%. Total cellular α-polymerase activities at pH 6.0 and 8.5 were 22.2 and 19.7 nmol/g of tissue, respectively. Percent of total cellular α-polymerase recovered in the nuclei (●); matrix-bound endogenous template-engaged α-polymerase activity (○).

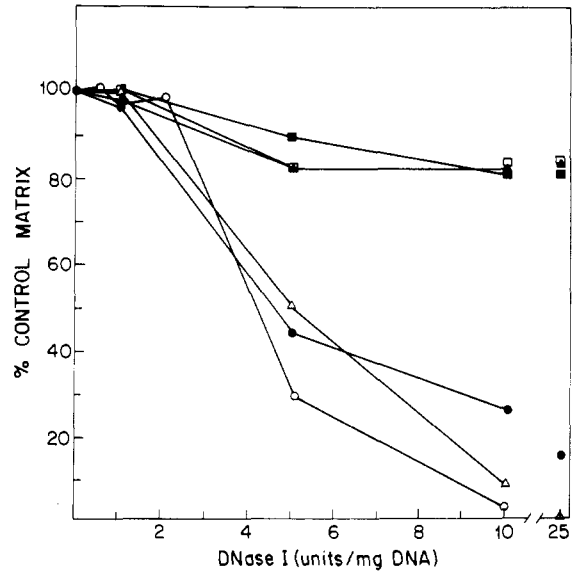

FIGURE 10: Nuclease sensitivity of matrix-bound polymerase activity. Prior to matrix preparation, endogenously digested nuclei were resuspended in 0.25 M sucrose, 10 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, and 1 mM PMSF and incubated with the indicated levels of DNase I (Sigma) at 0 °C for 15 min. Control matrix polymerase activities were as follows: α endogenous template activity, 342 pmol/g of tissue; α exogenous template activity, 1.74 nmol/g of tissue; β endogenous template activity, 83 pmol/g of tissue; β exogenous template activity, 222 pmol/g of tissue. 1-min in vivo pulse-labeled DNA (○); total DNA (●); α-polymerase endogenous template activity (△); α-polymerase exogenous template activity (▲); β-polymerase endogenous template activity (□); β-polymerase exogenous template activity (■).

lation of α-polymerase tightly bound to the matrix at the replicational sites, then the activity of these presumptive matrix-bound replisomes should remain relatively constant under conditions where more loosely bound nuclear α-polymerase is extracted. Lynch et al. (1975) reported that increasing the pH during homogenization results in a considerable decrease in total nuclear-bound α-polymerase activity. We examined matrix-bound DNA synthesis following isolation of nuclei in buffers ranging from pH 6.0 to 8.5. It is important to stress that only the nuclei were isolated at different pHs. Following isolation, all the nuclear preparations were treated identically (see Experimental Procedures). There was no significant change in total cellular α-polymerase activity over the examined pH scale. As shown in Figure 9, there was a progressive decrease in nuclear-bound α-polymerase measured with exogenous template from 24% of total cellular activity at pH 6.0 to 8% at pH 8.0. In contrast there was no appreciable change in matrix-bound α-polymerase-driven DNA synthesis over the entire pH range. This result further supports the possibility that there are a fixed number of functional replicational complexes bound to the matrix.

Sensitivity of Matrix-Bound DNA Polymerase α to DNase I. We have previously reported that in vivo newly replicated DNA bound to the matrix is preferentially released by digestion of isolated nuclei with DNase I or micrococcal nuclease (Berezney & Buchholtz, 1981b). We therefore examined the effect of DNase I digestion of nuclei on subsequent in vitro DNA synthesis in regenerating liver matrices. As shown in Figure 10, the matrix-bound α-polymerase activity decreased markedly with increasing DNase I concentration similar to the 1-min in vivo pulsed replicating DNA. In contrast, the β-polymerase-driven DNA synthesis was only slightly decreased by high levels of DNase I. Assay of exogenous template polymerase activity revealed that over 80% of the α-polymerase activity was still bound to the matrix even though the endogenous, template-engaged α-polymerase activity was not detected (Figure 10). β-Polymerase exogenous template activity was also resistant to DNase I treatment. In these experiments the recovery of total nuclear DNA on the matrix was reduced from 3.1% to <0.1% at the highest concentration of DNase I (25 units/mL). This corresponded to a reduction in the single-stranded length of the matrix-attached DNA from approximately 1200 nucleotides to <50 nucleotides (data not shown).

Discussion

Nuclear matrices prepared from regenerating rat liver engaged in in vivo DNA replication carry out in vitro DNA synthesis on the fragments of matrix-attached DNA. We have shown with specific DNA polymerase inhibitors (Table I) and buffer requirements that matrix-bound in vitro DNA synthesis is driven predominantly by α-polymerase, the replicative enzyme (Falaschi & Spadari, 1978). Moreover, the association of α-polymerase with the matrix depends on the proliferative state of the cell and shows a remarkable temporal parallel with the induction of in vivo replication during liver regeneration (Figure 4). Interestingly, the size of the DNA synthesized in vitro by the matrix (Figure 3) is similar in single-stranded length to the primary replicational intermediates or Okazaki fragments detected during in vivo replication and also reported in other eucaryotic in vitro systems (Edenberg & Huberman, 1975; Tseng & Goulian, 1975; Fraser & Huberman, 1977; Knopf & Weissbach, 1977; Seale, 1977; Brun & Weissbach, 1978; Funderud et al., 1978; Hand, 1978).

The discovery of matrix-bound in vitro DNA synthesis is extremely significant in light of recent evidence demonstrating that in vivo replicating DNA is enriched on the matrix in a wide variety of organisms (Berezney & Coffey, 1975; Dijkwel et al., 1979; Shaper et al., 1979; Pardoll et al., 1980; Berezney & Buchholtz, 1981b; Hunt & Volgelstein, 1981). The kinetics of in vivo labeling of newly replicated DNA further suggest that DNA is synthesized at or near its matrix attachment site (Berezney & Coffey, 1976). These findings have led to the proposal that functional replication complexes or replisomes are bound to the matrix (Dijkwel et al., 1979; McCready et al., 1980; Pardoll et al., 1980; Berezney & Buchholtz, 1981a,b). Our study, therefore, represents the first direct

evidence consistent with this proposal.

The matrix-bound α-polymerase may be a highly processive enzyme as it synthesizes continuous stretches of DNA (100–200 nucleotides) on the short matrix-attached DNA fragments (800–1200 nucleotides, Figure 3). This is clearly different from purified α-polymerase, which is not very processive (Das & Fujimara, 1979; Fisher et al., 1979; Detera et al., 1981; Villani et al., 1981), and may be related to the preservation of the specific orientation of enzyme and template found on the matrix. Moreover, the matrix-bound α-polymerase effectively utilizes exogenous DNA template (Table II) and is stimulated by low concentrations of ATP (Figure 2). Since purified α-polymerase is not stimulated by ATP (Fansler, 1974), it is intriguing to consider whether the ATP requirement, which is characteristic of in vitro DNA synthetic systems (Fraser & Huberman, 1977; Knopf & Weissbach, 1977; Brun & Weissbach, 1978), is related to the processivity of chain growth. Matrix-bound in vitro DNA synthesis driven by α-polymerase also may have a high degree of fidelity. Removal of three deoxynucleoside triphosphates causes marked inhibition (Table II). Consistent with previous reports (Fry et al., 1980; Kunkel & Loeb, 1981), nuclear and matrix-bound β-polymerases appear to lack in vitro fidelity.

Several investigators have shown that the cellular distribution of DNA polymerase activity is dynamically controlled during the cell cycle (Littlefield et al., 1963; Gold & Helleiner, 1964; Fansler & Loeb, 1969,1972; Spadari & Weissbach, 1974; Chiu & Baril, 1975; Craig et al., 1975; Ono et al., 1979; Chang, 1980) and have suggested that the nuclear localization of α-polymerase and, hence, DNA synthesis are regulated by the absolute levels of polymerase in the cell. We have also observed an increased retention of α-polymerase activity during liver regeneration from 8% of the total cellular activity in normal liver to 40% of the total activity in 20–22-h regenerating liver (data not shown).

Concomitant with the increased nuclear retention of α-polymerase, we find a redistribution of α-polymerase activity within nuclear subfractions (Figure 5). The marked temporal induction in α-polymerase activity bound to the high salt resistant matrix (Figure 4), together with its redistribution from bulk chromatin to the low-salt matrix (Figure 5), suggests that the matrix becomes a high-affinity site for α-polymerase attachment during in vivo DNA replication.

Our results also suggest that the process of fractionation from whole cell to high salt resistant matrix enriches for the site(s) of maximum enzyme induction (Tables III and IV). Put in other terms, the matrix appears to represent the nuclear site or domain where the greatest changes in α-polymerase activity are occurring with the onset and duration of in vivo DNA replication. The sites of DNA synthesis or replication forks associated with the matrix are also more active than in any other nuclear subfraction, particularly the nonmatrix-associated bulk chromatin (Table IV). This is readily apparent in the increased recovery of total nuclear α-polymerase activity on the high salt resistant matrix during replication (Table V).

Though in vitro DNA synthesis has been previously studied in chromatin extracts (Ballal et al., 1970; Lindsay et al., 1970; Loeb, 1970; Wallace et al., 1971; Chiu & Sung, 1972; Klose & Flickinger, 1972; Lynch & Lieberman, 1973; Phillippe et al., 1976; Chan et al., 1977; Nishioka et al., 1977; Seale, 1977; Brewer & Busacca, 1979; Matsukage et al., 1979; Tanuma et al., 1980), to our knowledge this is the first report of an intranuclear redistribution of α-polymerase activity within discrete subfractions or "domains" of the nucleus during active in vivo replication. The nature of this "matrix activation" is unknown but might be related to a specific binding of the polymerase to the matrix in addition to the DNA template. This possibility is supported by experiments in which the matrix-attached DNA is removed by extensive digestion of nuclei with DNase I. While there is no measurable endogenous template engaged α-polymerase activity on the very small fragments of remaining matrix-attached DNA (<50 nucleotides), the matrix-bound α-polymerase activity driven by exogenous DNA template is only slightly reduced (Figure 10).

We have approached the question of artifactual matrix-associated polymerase activity at several levels. Matrix-bound DNA synthesis clearly represents a subset of the total nuclear enzymatic activity on the basis of its buffer requirements, ATP sensitivity (Figure 2), inhibitor sensitivity (Table II), and in vitro product (Figure 3). Importantly, the level of loosely bound nuclear polymerase activity present prior to matrix isolation has no effect on the subsequent recovery of matrix-bound polymerase activity (Figure 9). Artifactual association of α-polymerase activity with the matrix due to nuclease activation of the matrix endogenous template did not occur (Figures 6 and 7, Table V). Moreover, our preliminary results indicate that polymerase association with the matrix may be mediated by matrix components other than DNA (Figure 10). Finally, our results suggest that matrix-bound α-polymerase is not an artifact of high salt induced aggregation (Figure 8) and in fact represents a high salt resistant subset of the total nuclear activity.

In all cellular fractions examined, β-polymerase activity, measured with either exogenous or endogenous template, is elevated prior to (4–16 h) and immediately after (24 h) the peak of α-polymerase activity and in vivo DNA replication [Figure 4 and H. C. Smith and R. Berezney (unpublished experiments)]. Thus while the β-polymerase activity is rapidly rising in regenerating liver between 22–24 h, the α-polymerase and in vivo replicational rates are rapidly decreasing. Following the second increase at 24 h, the β-polymerase activity is rapidly depressed to normal liver levels at 30 h. No additional fluctuations are detected during regeneration, but this is to be expected since later time points lack the degree of synchrony of the first 30 h. Although previous findings suggested that the level of β-polymerase activity remains relatively constant during differentiation and throughout the cell cycle (Chang & Bollum, 1972; Fansler, 1974; Spadari & Weissbach, 1974; Chiu & Baril, 1975; Craig et al., 1975; Hübscher et al., 1977; Giri et al., 1981), small variations were often observed that may have been significant.

The β-polymerase response that we have observed is consistent with cell cycle fluctuations of DNA repair detected in normal WI-38 fibroblasts (Gupta & Sirover, 1980) and supports the theory that the genome is screened (or both prereplicatively and postreplicatively screened in rat liver) prior to its replication (Gupta & Sirover, 1980). Moreover, if β-polymerase functions only when α-polymerase is inactive, one could readily explain the enhanced susceptibility of cells to transforming agents (Bates et al., 1968; Barranco & Humphrey, 1971; Baserga, 1977) and UV-induced lesions affecting sister chromatid exchange (MacRae et al., 1979) during the S phase and the G_1/S transition. We cannot rule out at this point, however, a function for β-polymerase in early- or late-replicative processes (Butt et al., 1978; Hübscher et al., 1979; Geuskens et al., 1981).

The studies reported in this paper provide evidence consistent with, though they do not directly demonstrate that, functional replicational complexes or replisomes being dynamically associated with the nuclear matrix during active replication. In

this regard, it is interesting to note that Reddy & Pardee (1980) demonstrated that both DNA polymerase and several enzymes involved in DNA precursor metabolism were redistributed prereplicatively from the cytoplasm to the nucleus and were associated in a rapidly sedimenting complex. Recent immunocytochemical analysis employing monoclonal antibodies to α-polymerase suggested that the α-polymerase is localized predominantly in the in situ matrix regions of intact cells (Bensch et al., 1982). Further studies employing the matrix-bound in vitro DNA synthesis system may provide a suitable starting point for examining the molecular events of DNA replication in relation to nuclear structure.

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References

Ballal, N. R., Collins, M. S., Halpern, R. M., & Smith, R. A. (1970) *Biochem. Biophys. Res. Commun. 40*, 1201–1208.

Baril, E. F., Mitchener, J., Lee, L., & Baril, B. (1977) *Nucleic Acids Res. 4*, 2641–2653.

Barranco, S. C., & Humphrey, R. M. (1971) *Mutat. Res. 11*, 421–429.

Baserga, R. L. (1977) in *Neoplastic Transformation: Mechanisms and Consequences* (Koprowski, H., Ed.) pp 125–133, Abakon Verlagsgesellschaft, Berlin.

Basler, J., Hastie, N. D., Pietras, D., Matsui, S.-I., Sandberg, A. A., & Berezney, R. (1981) *Biochemistry 20*, 6921–6929.

Bates, R. B., Wortham, J. S., Counts, W. B., Dingman, C. W., & Gelboin, H. V. (1968) *Cancer Res. 28*, 27–34.

Bensch, K. G., Tanaka, S., Hu, S.-Z., Wang, S.-F., & Korn, D. (1982) *J. Biol. Chem. 257*, 8391–8396.

Benyajati, C., & Worcel, A. (1976) *Cell (Cambridge, Mass.) 9*, 393–407.

Berezney, R. (1979) *Cell Nucl. 7*, 413–456.

Berezney, R., & Coffey, D. S. (1975) *Science (Washington, D.C.) 189*, 291–293.

Berezney, R., & Coffey, D. S. (1976) *Adv. Enzyme Regul. 14*, 63–100.

Berezney, R., & Buchholtz, L. A. (1981a) *Biochemistry 20*, 4995–5002.

Berezney, R., & Buchholtz, L. A. (1981b) *Exp. Cell Res. 132*, 1–13.

Brewer, E. N., & Busacca, P. A. (1979) *Biochem. Biophys. Res. Commun. 91*, 1352–1357.

Brun, G., & Weissbach, A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5931–5935.

Burgoyne, L. A., & Hewish, D. R. (1978) *Cell Nucl. 4*, 48–74.

Burgoyne, L. A., Wagar, M. A., & Atkinson, M. R. (1970) *Biochem. Biophys. Res. Commun. 39*, 254–259.

Butt, T. R., Wood, W. M., McKay, E. L., & Adams, R. L. P. (1978) *Biochem. J. 173*, 309–314.

Chan, J. Y. H., Rodriguez, L. V., & Becker, F. F. (1977) *Nucleic Acids Res. 4*, 2683–2695.

Chang, L. M. S. (1980) in *Mechanistic Studies of DNA Replication and Genetic Recombination* (Alberts, B., & Fox, C. F., Eds.) pp 649–653, Academic Press, New York.

Chang, L. M. S., & Bollum, F. J. (1972) *J. Biol. Chem. 247*, 7948–7950.

Chiu, J. F., & Sung, S. C. (1972) *Biochem. Biophys. Res. Commun. 46*, 1830–1836.

Chiu, R. W., & Baril, E. F. (1975) *J. Biol. Chem. 250*, 7951–7957.

Cook, P. R., & Brazell, I. A. (1975) *J. Cell. Sci. 19*, 261–279.

Craig, R. K., Costello, P. A., & Keir, H. M. (1975) *Biochem. J. 145*, 233–240.

Das, S. K., & Fujimura, R. K. (1979) *J. Biol. Chem. 254*, 1227–1232.

Detera, S. D., Becerra, S. P., Swack, J. A., & Wilson, S. H. (1981) *J. Biol. Chem. 256*, 6933–6943.

Dijkwel, P. A., Mullenders, L. H. F., & Wanka, F. (1979) *Nucleic Acids Res. 6*, 219–230.

Edenberg, H. J., & Huberman, J. A. (1975) *Annu. Rev. Genet. 9*, 245–284.

Falaschi, A., & Spadari, S. (1978) in *DNA Synthesis Present and Future* (Molineux, I., & Kohiyama, M., Eds.) pp 487–515, Plenum Press, New York.

Fansler, B. S. (1974) *Int. Rev. Cytol. Suppl. 4*, 363–415.

Fansler, B. S., & Loeb, L. A. (1969) *Exp. Cell Res. 57*, 305–310.

Fansler, B. S., & Loeb, L. A. (1972) *Exp. Cell Res. 75*, 433–441.

Fichot, O., Pascal, M., Méchali, M., & De Recondo, A.-M. (1979) *Biochim. Biophys. Acta 561*, 29–41.

Fisher, P. A., Wang, T. S.-F., & Korn, D. (1979) *J. Biol. Chem. 254*, 6128–6137.

Fraser, J. M. K., & Huberman, J. A. (1977) *J. Mol. Biol. 117*, 249–272.

Fry, M., Shearman, C. W., Martin, G. M., & Loeb, L. A. (1980) *Biochemistry 19*, 5939–5946.

Funderud, S., Andreassen, R., & Haugli, F. (1978) *Cell (Cambridge, Mass.) 15*, 1519–1526.

Georgiev, G. P., Nedospasov, S. A., & Bakayev, V. V. (1978) *Cell Nucl. 6*, 3–34.

Geuskens, M., Hardt, N., Pedrali-Noy, G., & Spadari, S. (1981) *Nucleic Acids Res. 9*, 1599–1613.

Giri, J. G., Reuben, R. C., Rifkind, R. A., & Marks, P. A. (1981) *Exp. Cell Res. 132*, 137–146.

Gold, M., & Helleiner, C. W. (1964) *Biochim. Biophys. Acta 80*, 193–203.

Gupta, P. K., & Sirover, M. A. (1980) *Mutat. Res. 72*, 273–284.

Hand, R. (1978) *Cell (Cambridge, Mass.) 15*, 317–325.

Hershey, H. V., Stieber, J. F., & Mueller, G. C. (1973) *Eur. J. Biochem. 34*, 383–394.

Hübscher, U., Kuenzle, C. C., & Spadari, S. (1977) *Nucleic Acids Res. 4*, 2917–2928.

Hübscher, U., Kuenzle, C. C., & Spadari, S. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2316–2320.

Hunt, B. F., & Vogelstein, B. (1981) *Nucleic Acids Res. 9*, 349–363.

Igő-Kemenes, T., & Zachau, H. G. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 109–118.

Ikegami, S., Taguchi, T., Ohashi, M., Oguro, M., Nagano, H., & Mano, Y. (1978) *Nature (London) 275*, 458–460.

Jacob, F., Brenner, S., & Cuzin, F. (1963) *Cold Spring Harbor Symp. Quant. Biol. 28*, 329–348.

Klose, J., & Flickinger, R. A. (1972) *Exp. Cell Res. 73*, 236–239.

Knopf, K.-W., & Weissbach, A. (1977) *Biochemistry 16*, 3142–3149.

Kornberg, A. (1980) *DNA Replication*, W. H. Freeman, San Francisco.

Kunkel, T. A., & Loeb, L. A. (1981) *Science (Washington, D.C.) 213*, 765–767.

Lindsay, J. G., Berryman, S., & Adams, R. L. P. (1970) *Biochem. J. 119*, 839–848.

Littlefield, J. W., McGovern, A. P., & Margeson, K. B. (1963) *Proc. Natl. Acad. Sci. U.S.A. 49*, 102–107.

Loeb, L. A. (1970) *Nature (London) 226*, 448–449.
Lynch, W. E., & Lieberman, I. (1973) *Biochem. Biophys. Res. Commun. 52*, 843–849.
Lynch, W. E., Surrey, S., & Lieberman, I. (1975) *J. Biol. Chem. 250*, 8179–8183.
MacRae, W. D., MacKinnon, E. A., & Stich, H. F. (1979) *Chromosoma 72*, 15–22.
Matsukage, A., Bohn, E. W., & Wilson, S. H. (1975) *Biochemistry 14*, 1006–1020.
Matsukage, A., Nishioka, N., Nishizawa, M., & Takahashi, T. (1979) *Cell Struct. Funct. 4*, 295–306.
McCready, S. J., Godwin, J., Mason, D. W., Brazell, I. A., & Cook, P. R. (1980) *J. Cell. Sci. 46*, 365–386.
Muller, M. T., Kajiwara, K., & Mueller, G. C. (1981) *Biochim. Biophys. Acta 653*, 391–407.
Nishioka, N., Matsukage, A., & Takahashi, T. (1977) *Cell Struct. Funct. 2*, 61–70.
Ono, Y., Enomoto, T., & Yamada, M.-A. (1979) *Gann 70*, 527–532.
Pardoll, D. M., Vogelstein, B., & Coffey, D. S. (1980) *Cell (Cambridge, Mass.) 19*, 527–536.
Paulson, J. R., & Laemmli, U. K. (1977) *Cell (Cambridge, Mass.) 12*, 817–828.
Phillippe, M., de Recondo, A.-M., & Chevaillier, P. (1976) *Exp. Cell Res. 98*, 424–428.
Reddy, G. P. V., & Pardee, A. B. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3312–3316.
Seale, R. L. (1977) *Biochemistry 16*, 2847–2853.
Seki, S., & Mueller, G. C. (1976) *Biochim. Biophys. Acta 435*, 236–250.
Shaper, J. H., Pardoll, D. M., Kaufmann, S. H., Barrack, E. R., Vogelstein, B., & Coffey, D. S. (1979) *Adv. Enzyme Regul. 17*, 213–248.
Smith, H. C., & Berezney, R. (1980) *Biochem. Biophys. Res. Commun. 97*, 1541–1547.
Spadari, S., & Weissbach, A. (1974) *J. Mol. Biol. 86*, 11–20.
Stryer, L. (1981) *Biochemistry*, W. H. Freeman, San Francisco.
Tanuma, S.-L., Enomoto, T., & Yamada, M.-A. (1980) *Cell Struct. Funct. 5*, 27–37.
Tseng, B. Y., & Goulian, M. (1975) *J. Mol. Biol. 99*, 317–337.
Villani, G., Fay, P. J., Bambara, R. A., & Lehman, I. R. (1981) *J. Biol. Chem. 256*, 8202–8207.
Vogelstein, B., Pardoll, D. M., & Coffey, D. S. (1980) *Cell (Cambridge, Mass.) 22*, 79–85.
Wallace, P. G., Hewish, D. R., Venning, M. M., & Burgoyne, L. A. (1971) *Biochem. J. 125*, 47–54.
Wanka, F., Mullenders, L. H. F., Bekers, A. G. M., Pennings, L. J., Aelen, J. M. A., & Eygensteyn, J. (1977) *Biochem. Biophys. Res. Commun. 74*, 739–747.
Waqar, N. A., Evans, M. J., & Huberman, J. A. (1978) *Nucleic Acids Res. 5*, 1933–1946.
Yagura, T., & Seno, T. (1980) *Biochim. Biophys. Acta 608*, 277–286.

Regulation of Tyrosine Aminotransferase Messenger Ribonucleic Acid in Rat Liver. Effect of Cycloheximide on Messenger Ribonucleic Acid Turnover†

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ABSTRACT: Tyrosine aminotransferase messenger ribonucleic acid (mRNA) activity in rat liver was rapidly increased 3–6-fold following in vivo administration of hydrocortisone acetate, dibutyryladenosine cyclic 3′,5′-phosphate, or the protein synthesis inhibitor cycloheximide. Treatment with the steroid hormone or cyclic nucleotide in combination with cycloheximide resulted in levels of tyrosine aminotransferase mRNA 10–20-fold greater than control values. These changes in mRNA activity were not accompanied by changes in albumin mRNA or total liver template activity. The rapid decline in tyrosine aminotransferase mRNA activity following cordycepin inhibition of de novo RNA synthesis was prevented by cycloheximide treatment. This protection was not observed when pactamycin was substituted for cycloheximide, demonstrating that the inhibition of protein synthesis per se was not responsible for the stabilization of tyrosine aminotransferase mRNA. Based upon the effects of cycloheximide and pactamycin on rat liver polysome structure, it is concluded that the cycloheximide-mediated increase in tyrosine aminotransferase mRNA activity is the result of stabilization of the mRNA molecule which renders the message less susceptible to inactivation and degradation in the cytoplasm. The action of cycloheximide is very specific for tyrosine aminotransferase, phosphoenolpyruvate carboxykinase, and probably several other mRNAs that code for minor liver proteins that turn over rapidly in response to hormonal or metabolic stimuli.

A general feature of steroid hormone and adenosine cyclic 3′,5′-phosphate (cyclic AMP)[1] action in target tissues is an increase in the activity of specific enzymes following hormone treatment. The induction of enzyme activity is due principally to an increase in the rate of enzyme synthesis as a result of a rise in the level of functional mRNA coding for the enzyme (Higgins & Gehring, 1980; Rosenfeld & Barrieux, 1979). One of the best characterized examples of this phenomenon is the regulation of tyrosine aminotransferase mRNA by glucocorticoids and cyclic AMP in rat liver (Ernest & Feigelson,

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[1] Abbreviations: Bt_2cAMP, N^6,$O^{2'}$-dibutyryladenosine cyclic 3′,5′-phosphate; $NaDodSO_4$, sodium dodecyl sulfate; cyclic AMP, adenosine cyclic 3′,5′-phosphate; Tris, tris(hydroxymethyl)aminomethane.

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