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Correlations between the activities of DNA polymerase α and the glucocorticoid receptor

(rifamycin AF/013/β-lapachone/aphidicolin/anti-DNA polymerase α IgG)

THOMAS J. SCHMIDT*, F. J. BOLLUM†, AND GERALD LITWACK*‡

*Fels Research Institute and the Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140; and †Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014

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ABSTRACT **Specific inhibitors and anti-DNA polymerase α IgG have been utilized to probe for similarities between cytoplasmic rat hepatic glucocorticoid receptors and DNA polymerase α [DNA nucleotidyltransferase (DNA-directed), EC 2.7.7.7]. Rifamycin AF/013, an inhibitor of RNA and DNA polymerase activities, significantly inhibited the binding of activated [6,7-^{3}H]-triamcinolone acetonide (TA) receptor complexes to DNA-cellulose. β-Lapachone, an inhibitor of DNA polymerase α and reverse transcriptase activities, inhibited the specific binding of [6,7-^{3}H]TA when preincubated with unbound receptors. Aphidicolin, another DNA polymerase α inhibitor, failed to inhibit any of the glucocorticoid-receptor functions tested. Two specific anti-DNA polymerase α IgGs interfered with glucocorticoid receptor functions as measured by their ability to inhibit the binding of [6,7-^{3}H]TA to unbound receptors (85% maximal inhibition) and, to a lesser extent, to inhibit the binding of activated [6,7-^{3}H]TA receptor complexes to DNA-cellulose (50% maximal inhibition). The anti-DNA polymerase α IgG and β-lapachone failed to affect the binding of tritiated estradiol, progesterone, or 5α-dihydrotestosterone to their receptors in appropriate rat target tissues or the binding of [1,2-^{3}H]hydrocortisone to serum transcortin. The most obvious interpretation of these data is that cytoplasmic glucocorticoid receptors and DNA polymerase α share antigenic determinants. An alternative interpretation is that the polyclonal anti-DNA polymerase α antibody contains IgG molecules raised against calf thymus cytoplasmic activated glucocorticoid–receptor complexes that copurified with DNA polymerase α used as the antigen. Taken collectively, however, the antibody and inhibitor data suggest a relationship between DNA polymerase α and the glucocorticoid receptor.**

The precise mechanism by which glucocorticoids, and steroid hormones in general, elicit different specific phenotypic responses in target cells is not completely understood. However, it is clear that most, if not all, of these effects are mediated through cytoplasmic receptors that bind glucocorticoids with high affinity and specificity. Once the steroid has been bound, the glucocorticoid–receptor complex must then undergo a two-step process in order to bind to nuclei. The first step, termed "activation" or "transformation," is temperature dependent and involves a conformational change resulting in the exposure of positively charged residues on the surface of the molecule and an increased affinity for polyanions such as DNA. The second step, termed "translocation," is temperature independent and involves the movement of the "activated" complexes to the nucleus, where they bind to acceptor sites within the chromatin. Ultimately these nuclear bound complexes induce changes in chromatin structures, resulting in increased transcription and ultimate translation of specific mRNAs (1, 2).

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Our laboratory has recently demonstrated (unpublished data) that the activated rat hepatic glucocorticoid–receptor complex exhibits a hierarchy of affinities for binding to deoxynucleotide homopolymers which is identical to that exhibited by DNA polymerase α (3). DNA polymerase α [DNA nucleotidyltransferase (DNA-directed), EC 2.7.7.7] activity and glucocorticoid receptor functions (steroid binding, activation of steroid–receptor complexes, binding of activated steroid–receptor complexes to acceptors) are also both sensitive to a number of common relatively nonspecific inhibitors, including *N*-ethylmaleimide (4–7), pyridoxal 5′-phosphate (8, 9), heparin (10, 11), and 1,10-phenanthroline (12–14). These observations prompted us to investigate further the possible relationship between these two proteins that ultimately must interact with DNA. In the present study we have utilized highly specific chemical and immunological inhibitors to probe for similarities between cytoplasmic glucocorticoid receptors and DNA polymerase α, which is the primary eukaryotic replicative enzyme and can be distinguished from DNA polymerase β (repair enzyme) and DNA polymerase γ (mitochondrial enzyme) (15). First we have studied the effects of several more specific inhibitors—namely, rifamycin AF/013, β-lapachone, and aphidicolin—on glucocorticoid receptor functions. Second, we have utilized two anti-DNA polymerase α IgGs, which do not crossreact with DNA polymerase β or γ (16), to probe for possible antigenic similarities between DNA polymerase α and the glucocorticoid receptor.

MATERIALS AND METHODS

Animals and Preparation of Cytosol. Adrenalectomized male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were used 4–8 days after surgery. The 150- to 175-g rats were fed a normal chow diet and maintained on 0.9% NaCl. The animals were killed by decapitation and the livers were perfused *in situ* through the portal vein with ice-cold 0.9% NaCl. The livers were removed and homogenized in an equal volume of TSM buffer (50 mM Tris·HCl/0.25 M sucrose/3 mM $MgCl_2$, pH 8.0 at 0–4°C). The homogenate was centrifuged at 105,000 × *g* for 1 hr at 4°C in a Beckman L5-50 ultracentrifuge. The upper lipid layer was discarded and the cytosol (40 mg of protein per ml) was either further diluted with TSM buffer (as indicated for each experiment) and used immediately or was stored in aliquots under liquid nitrogen until further use. Cytosol could be stored for several months with no detectable loss in the subsequent binding of labeled glucocorticoid.

Abbreviations: TA, triamcinolone acetonide, 9-fluoro-11β,21-dihydroxy-16α-17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-dione; rifamycin AF/013, 3-formylrifamycin SV *O*-*n*-octyloxime; β-lapachone, 3,4-dihydro-2,2-dimethyl-2*H*-naphthol[1,2-*b*]pyran-5,6-dione; aphidicolin, 3α,16,17,18-tetrahydroxyaphidicolane; TSM buffer, Tris/sucrose/$MgCl_2$ buffer.

‡ To whom reprint requests should be addressed.

Specific Cytosolic Binding of [6,7-^{3}H]Triamcinolone Acetonide. Aliquots of cytosol were incubated for 2 hr at 0–4°C with 60 nM [6,7-^{3}H]triamcinolone acetonide (TA) (31.3 Ci/mmol, New England Nuclear; 1 Ci = 3.7×10^{10} becquerels) in the presence or absence of a 1,000-fold excess of nonradioactive steroid. Specific binding was determined by using the dextran-coated charcoal adsorption technique to remove free steroid (17). Specific binding of glucocorticoid to serum transcortin was determined by incubating diluted serum with 120 nM [1,2-^{3}H]hydrocortisone (47.9 Ci/mmol, New England Nuclear) for 2 hr at 0–4°C in the presence or absence of a 1,000-fold excess of nonradioactive steroid followed by dextran-coated charcoal treatment. Duplicate 25-μl aliquots of the charcoal-treated cytosol or serum were then added to 10 ml of Liquiscint scintillation cocktail (National Diagnostics, Somerville, NJ). Radioactivity was then determined in a liquid scintillation spectrometer with an average counting efficiency for tritium of 30%.

DNA-Cellulose Binding Assay. The binding of thermally activated (25°C for 30 min) [^{3}H]TA–receptor complexes to DNA-cellulose was determined by the procedure of Kalimi *et al.* (11). Aliquots (100 μl) of labeled cytosol were incubated for 45 min at 0–4°C with 50 μl of packed DNA-cellulose (P-L Biochemicals). At the end of the incubation the pellets were washed four times with 2 ml of ice-cold TE buffer (10 mM Tris·HCl/1 mM Na_2EDTA, pH 8.0 at 0–4°C). The final pellet was resuspended in 0.8 ml of the same buffer and an aliquot (0.5 ml) was assayed for radioactivity.

Chemical Reagents and Antibodies. Nonradioactive TA, hydrocortisone, rifampicin, and Tris were obtained from Sigma. Na_2EDTA was obtained from Fisher. Aphidicolin was a gift from the Imperial Chemical Industries, Pharmaceutical Division (Macclesfield, Cheshire, England), and rifamycin AF/013 was a gift from David Toft, Department of Molecular Medicine, Mayo Clinic (Rochester, MN). β-Lapachone was a gift from B. S. Joshi, Ciba–Geigy Research Center (Goregaon, India). The preparation and characterization of the specific IgG antibodies to calf thymus DNA polymerase α and calf thymus terminal deoxynucleotidyltransferase used in this study have been described (16).

RESULTS

Effects of Rifamycin AF/013, β-Lapachone, and Aphidicolin on Cytoplasmic Glucocorticoid Receptor Functions. In light of the common sensitivities of DNA polymerase α and glucocorticoid receptors to a number of relatively nonspecific inhibitors, we chose to study the effects of several additional specific DNA polymerase inhibitors on receptor functions. Rifamycin AF/013, an inhibitor of DNA and RNA polymerases (18–20), was found to significantly inhibit the binding of activated [6,7-^{3}H]TA–receptor complexes to DNA-cellulose, whereas rifampicin, which does not inhibit eukaryotic DNA or RNA polymerase (21), failed to inhibit (Fig. 1). Although incubation of activated complexes with rifamycin AF/103 at low temperature (0–4°C) did not alter steroid binding, incubation with this compound at higher temperature (25°C) significantly accelerated the dissociation of [6,7-^{3}H]TA (data not shown).

Next we tested the effects of β-lapachone, which has been reported to inhibit specifically the activities of DNA polymerase α and viral reverse transcriptase (RNA-directed DNA polymerase, EC 2.7.7.49), while having no effect on the activities of either DNA polymerase β or RNA polymerase (22). As seen in Fig. 2, preincubation of unbound cytoplasmic receptors with β-lapachone blocked the subsequent specific binding of [6,7-^{3}H]TA. The concentration of β-lapachone that resulted in 50% inhibition was approximately 5 μg/ml (20 μM). At this concentration β-lapachone had no effect on either activation of

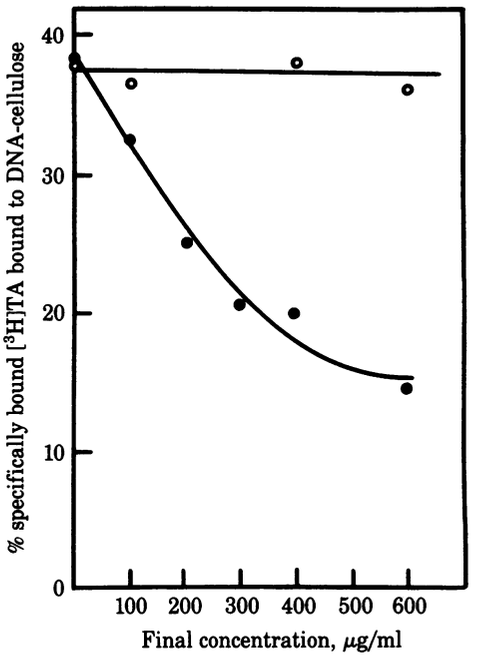

FIG. 1. Effects of rifamycin AF/013 and rifampicin on the binding of activated [6,7-^{3}H]TA–receptor complexes to DNA-cellulose. Undiluted rat liver cytosol was labeled for 2 hr at 0–4°C with 60 nM [6,7-^{3}H]TA. Bound receptor complexes were then activated for 30 min at 25°C and aliquots were subsequently incubated (30 min at 0°C) with increasing concentrations of rifamycin AF/013 (●) or rifampicin (○). Specific cytoplasmic binding of [6,7-^{3}H]TA and DNA-cellulose binding of activated receptor complexes were then determined. For the control sample the specific cytoplasmic binding was 159,000 dpm/100 μl of cytosol and the DNA-cellulose binding was 61,000 dpm/100 μl of cytosol.

[6,7-^{3}H]TA–receptor complexes or the binding of activated complexes to DNA-cellulose. Despite this dramatic effect on cytoplasmic glucocorticoid receptors, β-lapachone failed to in-

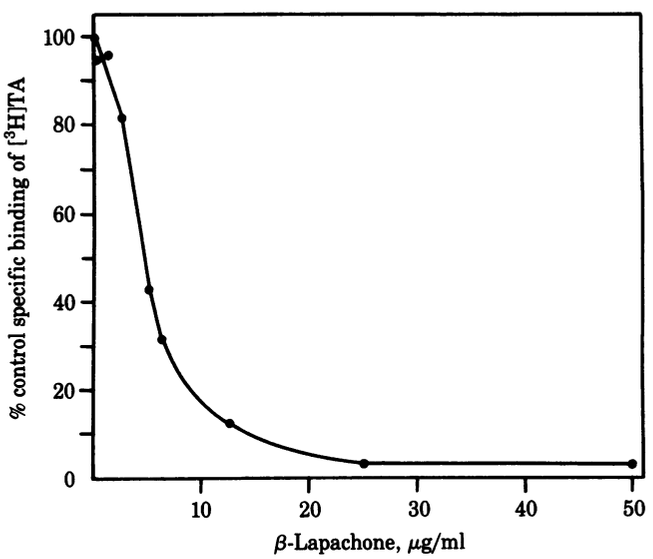

FIG. 2. Effect of preincubation with β-lapachone on subsequent specific binding of [6,7-^{3}H]TA. Rat liver cytosol was diluted 1:1 (vol/vol) with TSM buffer and aliquots were then preincubated for 1 hr at 0°C with increasing concentrations of β-lapachone. The diluted cytosol was then labeled for 2 hr at 0–4°C with 60 nM [6,7-^{3}H]TA and specific binding was then determined. For the control sample the specific cytoplasmic binding was 85,000 dpm/100 μl.

hibit the specific binding of [1,2-^{3}H]dihydrotestosterone to androgen receptors in the rat ventral prostate or the specific binding of [6,7-^{3}H]estradiol or [1,2-^{3}H]progesterone to rat uterine estrogen or progesterone receptors (data not shown).

Finally, the effects of aphidicolin, a specific inhibitor of DNA polymerase α (23), were tested. This steroid-like tetracyclic diterpene tetraol failed to inhibit specific binding of [6,7-^{3}H]TA when added at a 1,000-fold molar excess (data not shown). Likewise the compound had no effect on the activation of [6,7-^{3}H]TA–receptor complexes or the binding of previously activated complexes to DNA-cellulose (data not shown).

Effect of Anti-DNA Polymerase α IgGs on Specific Binding of [6,7-^{3}H]TA to Rat Hepatic Cytoplasmic Glucocorticoid Receptors. Table 1 summarizes the results of two separate experiments in which the effects of preincubation of unbound receptors with anti-DNA polymerase α IgGs on the subsequent specific binding of [6,7-^{3}H]TA were investigated. In both experiments preincubation with either anti-DNA polymerase α IgG (300 μg of IgG per 200 μl of diluted cytosol) significantly inhibited (maximal inhibition >85%) specific binding of [6,7-^{3}H]TA by glucocorticoid receptors, whereas anti-terminal transferase IgG or preimmune IgG had no effect. The potency of the anti-DNA polymerase α IgG in inhibiting specific binding of [6,7-^{3}H]TA was dependent on the final concentration of immunoglobulin, as depicted in Fig. 3. This inhibitory effect was not restricted to rat hepatic glucocorticoid receptors, because anti-DNA polymerase α IgG also blocked the specific binding of [6,7-^{3}H]TA to cytoplasmic receptors of the glucocorticoid-sensitive human lymphoid cell line CEM-C7 (24) (data not shown). However, despite this dramatic inhibitory effect on specific cytoplasmic glucocorticoid binding, anti-DNA polymerase α IgG failed to inhibit specific binding of [1,2-^{3}H]-hydrocortisone to rat serum transcortin (data not shown). Likewise anti-DNA polymerase α IgG failed to inhibit the specific binding of [1,2-^{3}H]dihydrotestosterone to androgen receptors in the rat ventral prostate or the specific binding of [6,7-^{3}H]estradiol or [1,2-^{3}H]progesterone to rat uterine estrogen or progesterone receptors (data not shown).

Effect of Anti-DNA Polymerase α IgG on the Binding of Activated [6,7-^{3}H]TA–Receptor Complexes to DNA-Cellulose. Table 2 summarizes the results of two separate experiments in which the effect of one anti-DNA polymerase α IgG on the binding of thermally activated (25°C for 30 min) [6,7-^{3}H]TA–receptor complexes to DNA-cellulose was investigated. In both experiments incubation (0–4°C for 30 min) of previously activated glucocorticoid–receptor complexes with anti-DNA polymerase α IgG inhibited the subsequent DNA-cellulose binding of these complexes by approximately 50%, whereas incubation

Table 1. Effect of anti-DNA polymerase α IgGs on specific binding of [6,7-^{3}H]TA to rat hepatic glucocorticoid receptors

Preincubation	Specific binding of [6,7-^{3}H]TA, dpm/100 μl	% control
Exp. I		
Control	15,300	100
Anti-terminal transferase IgG	13,800	90
Anti-DNA polymerase α IgG*	3,000	19
Exp. II		
Control	12,400	100
Preimmune IgG	12,200	98
Anti-terminal transferase IgG	11,500	93
Anti-DNA polymerase α IgG*	2,100	17
Anti-DNA polymerase α IgG†	7,900	63

Freshly prepared rat liver cytosol was further diluted 1:4 (vol/vol) with TSM buffer and aliquots were then preincubated at 0–4°C for 30 min with the appropriate IgG (300 μg/200 μl of diluted cytosol). The cytosol was then incubated at 0–4°C for 2 hr with 60 nM [6,7-^{3}H]TA and specific binding was determined.

* Titer of 0.13 (titer defined as μg of IgG required to inhibit 1 unit of DNA polymerase α activity by 50%) (rabbit no. 520).

† Titer of 1.04 (rabbit no. 519).

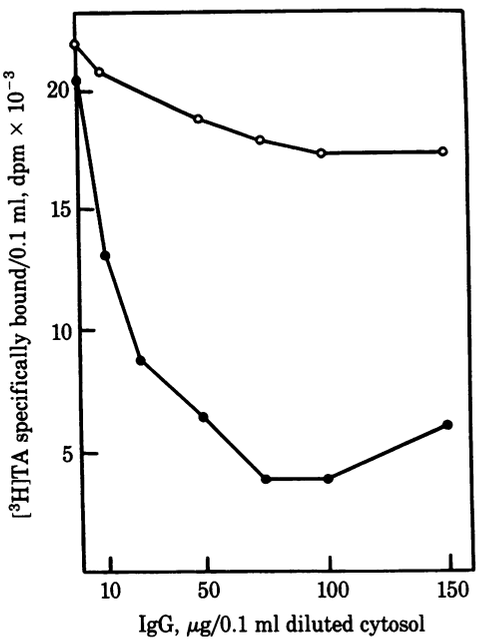

FIG. 3. Effect of preincubation with anti-DNA polymerase α IgG on subsequent specific binding of [6,7-^{3}H]TA. Fresh rat liver cytosol was diluted 1:4 (vol/vol) with TSM buffer and aliquots were then preincubated for 30 min at 0°C with increasing concentrations of anti-DNA polymerase α IgG (titer of 0.13) (●) or preimmune IgG (○). The diluted cytosol was then labeled for 2 hr at 0–4°C with 60 nM [6,7-^{3}H]-TA and specific binding was determined. For the control sample the specific cytoplasmic binding was 20,300 dpm/100 μl.

Table 2. Effect of anti-DNA polymerase α IgG on binding of activated rat hepatic [6,7-^{3}H]TA–receptor complexes to DNA-cellulose

Treatment	[6,7-^{3}H]TA bound to DNA-cellulose dpm	%*	% control
Exp. I			
Control	9,900	45	100
Preimmune IgG	10,400	47	105
Anti-DNA polymerase α IgG†	4,900	22	50
Exp. II			
Control	5,900	54	100
Preimmune IgG	5,600	51	94
Anti-terminal transferase IgG	5,600	51	94
Anti-DNA polymerase α IgG†	2,900	26	48

Freshly prepared rat liver cytosol was further diluted 1:3 (vol/vol) with TSM buffer and then labeled at 0–4°C for 2 hr with 60 nM [6,7-^{3}H]TA. The labeled cytosol was activated (25°C for 30 min) and then incubated at 0–4°C for 30 min with the appropriate IgG (300 μg/200 μl of cytosol). Binding of [6,7-^{3}H]TA–receptor complexes to DNA-cellulose was then determined.

* (DNA-cellulose binding/specific cytoplasmic [6,7-^{3}H]TA binding) × 100. Specific cytoplasmic [6,7-^{3}H]TA binding: Exp. I, 22,000 dpm/100 μl; Exp. II, 11,000 dpm/100 μl.

† Titer of 0.13.

with anti-terminal transferase IgG or preimmune IgG had no effect. Incubation with anti-DNA polymerase α IgG did not accelerate the rate at which [6,7-^{3}H]TA dissociated from the activated receptor complexes.

DISCUSSION

A number of relatively nonspecific inhibitors have been reported to block the activity of DNA polymerase α as well as to interfere with glucocorticoid-receptor functions. In the present report we have extended this list to include several more specific inhibitory compounds. The ability of rifamycin AF/013, an inhibitor of both RNA and DNA polymerase activities (18–20), to inhibit the binding of activated glucocorticoid–receptor complexes to DNA-cellulose (Fig. 1) agrees with the published effects of this rifamycin derivative on the nuclear binding of avian oviduct progesterone–receptor complexes (25). We also have demonstrated that β-lapachone, a specific inhibitor of DNA polymerase α and reverse transcriptase (22), inhibits the specific binding of [6,7-^{3}H]TA when preincubated with unbound glucocorticoid receptors (Fig. 2). Although it is not known exactly how this compound, whose structure is unlike that of a steroid, exerts this dramatic effect, it is clear that its inhibitory effect is not mediated via oxidation of sulfhydryl groups at the steroid-binding site. The inhibitory effect of β-lapachone on the glucocorticoid receptor is unaffected by the presence of dithiothreitol (data not shown). Likewise β-lapachone does not inhibit the binding of androgen, estrogen, or progesterone to their receptors, although all of these receptor proteins require free sulfhydryl groups at their appropriate steroid-binding sites. The failure of aphidicolin, a steroid-like molecule that specifically inhibits DNA polymerase α (23), to inhibit the tested glucocorticoid receptor functions is difficult to interpret in light of the fact that the precise mechanism by which aphidicolin inhibits DNA polymerase α is unknown. The ineffectiveness of aphidicolin in the present study may suggest that the receptor molecule does not contain an aphidicolin-binding site that may be located near the catalytic site of DNA polymerase α or within an accessory subunit (26).

The data presented also demonstrate that two specific anti-DNA polymerase α IgGs interfere with glucocorticoid receptor functions as measured by their ability to inhibit the binding of [6,7-^{3}H]TA to unbound receptors (Table 1, Fig. 3) and, to a lesser extent, to inhibit the binding of activated [6,7-^{3}H]TA–receptor complexes to DNA-cellulose (Table 2). The first and most obvious interpretation of these data is that cytoplasmic glucocorticoid receptors and DNA polymerase α are immunologically related and hence share antigenic determinants. Although the precise nature of this possible relationship cannot be explained presently, several alternatives should be considered. First, DNA polymerase α and the glucocorticoid receptor may be evolutionarily related DNA-binding proteins. Second, the glucocorticoid receptor and DNA polymerase α both may be derived from the same polypeptide precursor. Third, the glucocorticoid receptor may possess DNA polymerase α enzymatic activity. If the last alternative were true then one might predict that, because DNA polymerase α is required for replication, the activated glucocorticoid–receptor complex might stimulate specific gene replication before enhancing transcription. This, however, appears very unlikely in light of the fact that an inhibitor of DNA synthesis, cytosine arabinonucleoside, does not block the ability of glucocorticoids to induce tyrosine aminotransferase in HTC cells (27). DNA polymerase activity (28) and DNA synthesis (29) in the rapidly growing liver are also extremely sensitive to suppression by cortisone administration, despite stimulation of RNA and protein synthesis. This information plus the fact that glucocorticoid–receptor complexes bind to nuclear chromatin acceptor sites closely associated with the synthesis of mRNA, which is required for enzyme induction, might lead one to logically predict that the receptor protein and RNA polymerase may be immunologically related. This hypothesis could be tested as monoclonal antibodies directed against the numerous subunits of eukaryotic RNA polymerase become available (30).

An alternative interpretation of the reported data is that the polyclonal anti-DNA polymerase α IgG preparation tested contains not only IgG molecules specific for DNA polymerase α but also IgG molecules raised against calf thymus cytoplasmic activated glucocorticoid–receptor complexes (presumably present in nonadrenalectomized animals) that copurified with the DNA polymerase α used as the antigen. Careful comparison of the purification scheme for DNA polymerase α with the properties of various forms of the glucocorticoid receptor protein eliminates possible copurification of cytoplasmic unbound or unactivated complexes with the enzyme. The first step in the purification scheme involves the binding of DNA polymerase α to phosphocellulose, whereas neither of the previously mentioned receptor forms (unbound or unactivated) will bind to this material (31). However, none of the purification steps, which include phosphocellulose, DEAE-cellulose, and hydroxylapatite, exclude possible copurification of cytoplasmic activated glucocorticoid–receptor complexes (nuclear activated complexes would not be extracted by homogenization buffer). If in fact copurification occurs, this would reflect the similar biochemical characteristics shared by DNA polymerase α and activated glucocorticoid–receptor complexes. This alternative interpretation cannot be completely eliminated until IgG directed against homogeneous DNA polymerase α or monoclonal antibodies directed against partially purified DNA polymerase α become available.

In addition to their common sensitivities to a number of inhibitors, both chemical and immunological, DNA polymerase α and steroid receptors (especially glucocorticoid receptors), share a number of other biochemical properties. It has been reported that both DNA polymerase α (32) and the partially purified progesterone receptor from avian oviduct (33) catalyze pyrophosphate exchange, and in the latter case this activity can be inhibited by 1,10-phenanthroline and rifamycin AF/013. In light of the fact that aphidicolin inhibits the pyrophosphate exchange reaction catalyzed by DNA polymerase α (34), it would be interesting to test the effect of aphidicolin on the similar activity catalyzed by a steroid receptor protein. Reisher *et al.* (35) reported that phosphorylation of calf thymus DNA polymerase enhances the polymerase reaction, whereas dephosphorylation by a protein phosphatase inhibits the polymerase reaction. Likewise, several studies have suggested that dephosphorylation of the unbound glucocorticoid receptor renders it incapable of binding steroid, and rephosphorylation restores this binding capacity (36, 37). Evidence also suggests that glucocorticoid (38) and progesterone receptors (39), like DNA polymerase α (40), contain nucleotide-binding sites. Also, DNA polymerase α (3) and, as previously mentioned, activated glucocorticoid–receptor complexes (unpublished data) exhibit the same hierarchy of affinities for binding to deoxynucleotide homopolymers [poly(dT) ≥ poly(dG) > poly(dC) ≫ poly(dA)].

The intracellular locations and sizes of steroid receptor proteins and DNA polymerase α also merit discussion. Experiments designed to determine the intracellular location of DNA polymerase α have been difficult to interpret because localization in the cytoplasm may be due to leakage of enzyme from the nucleus and, likewise, localization of enzyme in isolated nuclei is complicated by cytoplasmic contamination. Recently Brown

et al. (41) have utilized anti-DNA polymerase α IgG to examine the intracellular localization of the enzyme in whole cells. They demonstrated that the bulk of DNA polymerase α is located in the perinuclear region of the cell, and this location is consistent with the hypothesis that a small amount of the enzyme may be transported into the nucleus during replication of DNA (42). Similarly, unbound steroid receptors are localized in the cytoplasm and only after the receptor proteins have bound the appropriate steroid and the complexes have been activated does nuclear translocation occur. It has also been reported that DNA replication occurs in close proximity to the nuclear matrix (43) and that estrogens and androgens bind specifically to the nuclear matrix of steroid-responsive tissue (44). Direct comparison of the molecular weights of DNA polymerase α and glucocorticoid receptors suggests that these two proteins differ in size. Although heterogeneity of DNA polymerase has been observed commonly during its purification from a variety of cell systems (45), calf thymus DNA polymerase α is believed to be a single polypeptide of 150,000–160,000 molecular weight which also contains an associated subunit of 50,000–70,000 molecular weight (46). Information concerning the size of the glucocorticoid receptor protein is somewhat conflicting and may reflect species or target tissue differences, differences in experimental conditions, or variable proteolysis. The rat hepatic receptor protein has been reported to be a single polypeptide of approximately 90,000 molecular weight (47, 48).

The data presented suggest that DNA polymerase α activity and glucocorticoid receptor functions are both sensitive to two relatively specific inhibitors. The availability of specific antibodies to eukaryotic DNA polymerase α has enabled us to investigate the possible relationship between these two proteins more directly. Although the precise interpretation of the cross-reactivity of the anti-DNA polymerase α IgG with the glucocorticoid receptor reported here is unclear, the tight coupling between the effects of β-lapachone and the antibody are intriguing. We hope that these provocative results will stimulate additional effort directed at understanding the possible relationship between DNA polymerase α and the glucocorticoid receptor protein.

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