RNA-dependent Release of Androgen- and Other Steroid-Receptor Complexes from DNA

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Shutsung Liao,‡ Stephanie Smythe,‡ John L. Tymoczko.§ Gian Paolo Rossini.¶ Chunshing Chen,‡ and Richard A. Hiipakka‡

From the ‡Ben May Laboratory for Cancer Research and the Department of Biochemistry, University of Chicago, Chicago, Illinois 60637, and the §§Department of Biology, Carleton College, Northfield, Minnesota 55057

Certain poly- and oligonucleotides, at low concentrations, promoted the release of androgen-• and other steroid• receptor complexes that were bound to DNA. DNA-cellulose and gradient centrifugation, were used to demonstrate that release of receptor was selective with respect to the base composition of the polymer. Among the homopolyribonucleotides studied, poly(U), poly(G), poly(X), poly(I), and others having bases with uracil/guanine ratios of 1 to 5 were more active than poly(G), poly(U) or equivalent mixtures of poly(G) and poly(U), indicating that the activity was dependent on the nucleotide sequence. The minimum length of the oligonucleotide needed to show activity appeared to be dependent on the type of nucleotide in the oligomer. Since various polynanions were significantly less active than poly(U,G), the release of receptor by polynucleotides, was not due merely to a nonspecific polyionic interaction. Ethidium bromide, heparin, and rifamycins showed some activity at high concentrations but rifampicin, actinomyacin D, and chloroquine were inactive.

Active polyribonucleotides, such as poly(U,G), also promoted the release of rat uterine estrogen • and progesterone • receptor complexes and rat liver dexamethasone • receptor complex from DNA. These findings may be in line with the suggestion that in target cells of steroid hormones, a steroid• receptor complex may recognize and bind to specific RNA having appropriate nucleotide sequences, and thus play an important role in post-transcriptional control.

In target cells, a steroid hormone can form a complex with a specific receptor protein. The steroid• receptor complex can then interact with chromatin and presumably enhance the synthesis of certain RNA (1-5). It is generally believed that such an interaction involves binding of the receptor complex to DNA.

There are also indications that the steroid• receptor complex can bind to certain RNA or RNP1 particles in the cell nuclei and cytoplasm of target tissues (7-12). We report here that polyribonucleotides with certain types of bases can compete effectively with DNA for binding to a steroid• receptor complex and promote the release of the receptor complex from DNA. These observations may be important since steroid• receptor complexes in target cells may participate in the regulation of the synthesis of certain RNA and by binding to RNA may also be involved in post-transcriptional control.

EXPERIMENTAL PROCEDURES

Materials—5α-Dihydro[1,2,6,7-3H]testosterone (90 Ci/mmole); 17β-[2,4,6,7,16,17-3H]testadiol (140 Ci/mmole); [1,2,4-3H]dexamethasone (23 Ci/mmole); [1,2-3H]progesterone (40 Ci/mmole) were obtained from New England Nuclear. Pure enzymes were obtained from Worthington and Sigma. Poly- and oligonucleotides were obtained from P-L Biochemicals, Miles Laboratories, and Collaborative Research, Inc., or prepared in this laboratory with polynucleotide phosphorylase (13). The base composition and the size of the polymers were determined by a Waters Associate high pressure liquid chromatography system (14) equipped with an absorbance detector and a solvent programmer, ion-exchange chromatography (15), and gradient centrifugation (16). Unless otherwise specified, the polyribonucleotides used have sedimentation coefficients of 5 ± 1 S and, for heteropolynucleotides, have equal amounts of individual bases. Sprague-Dawley rats (250 to 300 g) were purchased from Sasco Co., Omaha, Neb. Soluble RNA was extracted from the cytosol fraction of rat liver and chromatographed on oligo(dT)-cellulose as described by Miller and McCarthy (17). RNA that was not bound to oligo(dT)-cellulose was used as transfer RNA. Analysis of this RNA by sucrose gradient centrifugation (18) revealed only 4 S RNA. For the preparation of other RNA, rat ventral prostate was homogenized with an all glass Potter-Elvehjem homogenizer in 4 volumes of 40 mM Tris/HCl, pH 7.5, and centrifuged at 16,000 × g for 15 min. The supernatant was centrifuged again at 130,000 × g for 1 h and the pellet used to prepare polyribosomal RNA. The pellets were resuspended in 10 mM Tris/HCl, pH 7.5, containing 3 mM MgCl2, 250 mM sucrose, 150 mM NaCl, and 0.5% SDS. RNA was extracted and then fractionated on oligo(dT)-cellulose as previously described (17). RNA retained by oligo(dT)-cellulose was used as poly(A)-RNA. RNA not retained was used as ribosomal RNA. Ribosomal RNA was fractionated into 5 S, 18 S, and 28 S ribosomal RNA by sucrose gradient centrifugation (18). The cytosol 1.5 S RNA was prepared as described elsewhere (19). Radioactive steroid• receptor complexes were prepared by mixing radiolabeled steroid with cytoplasmic soluble fractions from ventral

1 The abbreviations used are: RNP, ribonucleoprotein; 5α-dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one; SDS, sodium dodecyl sulfate; SV-40, simian virus 40. The nomenclature and symbols for nucleotides follow the IUPAC-IUB Commission on Biochemical Nomenclature (6).
prostate (dihydrotestosterone), uterus (estradiol) and progestrone), or liver (dexamethasone) of rats castrated or adrenalectomized. The radioactive steroid-receptor complexes thus formed were precipitated by the addition of ammonium sulfate to 40% saturation and then desalted by passing through a Sephadex G-25 gel column (20). The specific radioactivities of the steroid-receptor complexes used in the experiments were generally within the range of 20,000 to 100,000 cpm/mg of protein. DNA-cellulose was prepared as described by Alberts and Hsveric (21) using calf thymus DNA (29% hyperchromicity at 290 nm) and Whatman CF-11 cellulose powder. The adduct contained about 1 mg of DNA/ml (packed volume) of DNA-cellulose. Oligo(dA), oligo(dT), and oligo(dC)-cellulose were obtained from P-L Biochemicals.

DNA-Cellulose Column Assay—For binding studies, DNA-cellulose was equilibrated with Medium ET (20 mM Tris/HCl, pH 7.5, containing 1.5 mM EDTA) and packed into a glass column. The volume of the packed DNA-cellulose was 0.5 ml (0.5 mg of DNA)/column. H-Labeled steroid-receptor complex, normally 10,000 cpm in 0.1 to 0.2 ml of Medium ET, was applied to the column. The column was washed with seven aliquots (0.5 ml each) of Medium ET to remove free steroid or the steroid-protein complex that did not bind to DNA-cellulose. The washed column was then eluted with seven aliquots of Medium ET (0.5 ml each) containing a polyribonucleotide or other test compounds (Fraction E). Finally, the steroid-receptor complex that remained attached to the DNA-cellulose was eluted from the column with seven aliquots of Medium ET (0.5 ml each) containing 0.6 M KCl (Fractions R). The specific radioactivities of the steroid-receptor complexes used in the experiments were generally within the range of 20,000 to 100,000 cpm/mg of protein. DNA-cellulose was prepared as described by Alberts and Hsveric (21) using calf thymus DNA (29% hyperchromicity at 290 nm) and Whatman CF-11 cellulose powder. The adduct contained about 1 mg of DNA/ml (packed volume) of DNA-cellulose. Oligo(dA), oligo(dT), and oligo(dC)-cellulose were obtained from P-L Biochemicals.

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For the convenience of comparing the abilities of various test compounds to elute the receptor complex, we determined the radioactivity in Fraction E (e) and in Fraction R (r) and calculated the percentage of the receptor complex that could be eluted from DNA-cellulose by the test compound at the specified concentration according to the equation:

\[
\text{eluted} = \frac{e - r}{e} \times 100
\]

The concentration of the individual test compound needed for 50% elution is termed EC50.

DNA-Cellulose Centrifugation Assay—In some experiments, we mixed DNA-cellulose (20 to 100 μg of DNA) and the radioactive complex (1 to 5 μg) in 0.5 ml Medium ET and then added polyribonucleotides to study receptor binding by nucleic acids. The tubes containing all the components were incubated at 30°C for 5 min and then centrifuged at top speed in a clinical centrifuge or a Beckman microfuge. The DNA-cellulose pellet was washed with seven aliquots of Medium ET (0.5 ml each) containing a polyribonucleotide or other test compounds (Fraction E). Finally, the steroid-receptor complex that remained attached to the DNA-cellulose was eluted from the column with seven aliquots of Medium ET (0.5 ml each) containing 0.6 M KCl (Fractions R).

The retained radioactivity could not be washed out from the column with Medium ET (Fig. 1). If the KCl concentration of the medium was brought to 0.4 M or higher, all the radioactivity could be removed from the column. The initial flow-through fraction (Fractions 0 to 7) contained free steroid or other steroid-binding proteins that, unlike the androgen-receptor complex, were not retained by DNA-cellulose or by prostate cell nuclei (24, 25). The major prostate cytosol protein (α protein) that binds sex steroids but not glucocorticoids (26-27) was also found in this flow-through fraction. When the radioactive androgen-receptor complex was inactivated by heating at 50°C for 30 min no radioactivity was retained by DNA-cellulose. The radioactivity was measured in a Packard liquid scintillation spectrometer, with a scintillation fluid containing Triton X-100 and toluene (1:3), 0.4% (w/v) diphenylohexamole, and 0.005% (w/v) 1,4-bis[2-phenoxazo]yl)benzene. The counting efficiency was about 30%.

The amount of polynucleotide was measured spectrophotometrically; the polymer concentration that, at pH 7.5, gave an absorbance of 1.0 at 260 nm (light path, 1 cm) was assumed to be 40.0 μg/ml for natural RNA, 35.4 μg/ml for poly(A), 32.5 μg/ml for poly(U), 58.7 μg/ml for poly(C), 39.2 μg/ml for poly(G), 35.8 μg/ml for Poly(U), and 50.0 μg/ml for DNA. The amount of polymer used in experiments was also expressed in monomer concentrations. DNA was also measured by the diphenylamine test, with calf thymus DNA as the standard (22). Protein was determined by the method of Lowry et al. (23) with bovine serum albumin as standard.

RESULTS

Retention of 5α-Dihydro[3H]testosterone-Receptor Complex by DNA-Cellulose—The quantity of DNA-cellulose used in all the experiments reported here had the capacity for binding at least 10 times the radioactive steroid-receptor complex employed. Cellulose, free of DNA, did not retain the radioactive receptor complex to any significant extent. When the radioactive androgen-receptor complex was prepared in the manner described under "Experimental Procedures" and applied to the DNA-cellulose column under our assay conditions, about 50 to 70% of the radioactivity was retained and could not be washed out from the column by Medium ET (Fig. 1). If the KCl concentration of the medium was brought to 0.4 M or higher, all the radioactivity could be removed from the column. The initial flow-through fraction (Fractions 0 to 7) contained free steroid or other steroid-binding proteins that, unlike the androgen-receptor complex, were not retained by DNA-cellulose or by prostate cell nuclei (24, 25). The major prostate cytosol protein (α protein) that binds sex steroids but not glucocorticoids (26-27) was also found in this flow-through fraction. When the radioactive androgen-receptor complex was inactivated by heating at 50°C for 30 min no radioactivity was retained by DNA-cellulose. The radioactive steroid-receptor complex was inactivated by heating at 50°C for 30 min no radioactivity was retained by DNA-cellulose.

Gradient Centrifugation Assay—We also used gradient centrifugation to compare the relative abilities of various polynucleotides to compete with DNA for binding to the steroid-receptor complex. For this purpose, the radioactive steroid-receptor preparations were treated briefly with a small quantity of dextran-coated charcoal to minimize the amount of free steroid present. The receptor preparation (5,000 cpm) was mixed with 1 to 5 μg of DNA in 0.15 ml of Medium ET. The test polymer was then added to the tube and the mixture was incubated at 0°C for 10 min.

Gradient centrifugation was performed in a Spinco ultracentrifuge with an SW 60 rotor. The sucrose gradient (10 to 30% sucrose) contained 1.5 mM EDTA and 20 mM Tris/HCl at pH 7.5. The incubated sample was layered on top of the sucrose gradient and centrifuged with the length of time specified in the individual experiments. After centrifugation, fractions (0.2 ml each) were collected by an Isco fractionator and numbered from the top of the centrifuge tubes.

Under the conditions of our assay, the radioactive steroid-receptor complex bound to DNA (>20 S) sedimented at the bottom of the tube whereas the receptor complex, free or bound to polyribonucleic acid, stayed in the upper portion of the sucrose gradient. The gradient centrifugation assay, although more tedious, is useful when only limited quantities (1 to 5 μg) of DNA or the test polymers are available.

Other Methods—Radioactivity was measured as the amount of poly(U) on the release of 5α-dihydro[3H]testosterone-receptor complex from DNA-cellulose. DNA-cellulose column assay was carried out as described under "Experimental Procedures." The androgen-receptor complex prepared from the rat ventral prostate was applied to the individual columns. Each column was washed with seven aliquots (0.5 ml each) of Medium ET to remove unbound radioactivity. The washed columns were then eluted with seven aliquots of Medium ET (0.5 ml each) (●), or Medium ET containing poly(U) (150 μM monomer concentration) (○), UMP (150 μM), or sodium phosphate (10 mM) (△). The retained radioactivity was removed from the column by the addition of 0.6 M KCl.

Fig. 1. Effect of poly(U) on the release of 5α-dihydro[3H]testosterone-receptor complex from DNA-cellulose. DNA-cellulose column assay was carried out as described under "Experimental Procedures." The androgen-receptor complex prepared from the rat ventral prostate was applied to the individual columns. Each column was washed with seven aliquots (0.5 ml each) of Medium ET to remove unbound radioactivity. The washed columns were then eluted with seven aliquots of Medium ET (0.5 ml each) (●), or Medium ET containing poly(U) (150 μM monomer concentration) (○), UMP (150 μM), or sodium phosphate (10 mM) (△). The retained radioactivity was removed from the column by the addition of 0.6 M KCl.
active complex that was retained by DNA-cellulose and that was eluted from the column by 0.6 M KCl (Fractions 16 to 20) sedimented as a 3 to 4 S entity after sucrose gradient centrifugation, supporting our contention that the retained radioactivity was associated with the 5α-dihydrotestosterone-receptor complex (25).

Effect of Homopolynucleotides on the Release of Androgen-Receptor Complex from DNA-Cellulose—The capability of poly(U) to promote the release of the radioactive 5α-dihydrotestosterone-receptor complex from DNA-cellulose is shown in Fig. 1. In this experiment the radioactive receptor complex was loaded onto DNA-cellulose columns and, after the initial washing, the columns were eluted with Medium ET alone (control) or with the Medium ET containing poly(U). The radioactivity that was not eluted was then removed from the column by Medium ET containing 0.6 M KCl.

Elution of the radioactive receptor complex after the addition of poly(U) proceeded rapidly. Most of the receptor complex that could be eluted at a set concentration of the polymer emerged from the column within five fractions, taking only about 5 min. The difference in the amounts of the receptor complex that could be eluted from duplicate columns at a set concentration of the polymer was generally within 10%. The effectiveness of poly(U) was not mimicked by high concentrations (1 to 5 mM) of inorganic phosphate, inorganic pyrophosphate, UMP, UDP, UTP, or other mononucleotides tested (see below).

When the radioactive complex eluted by poly(U) (Fractions 10 to 16 in Fig. 2A) was treated with pancreatic RNase to destroy poly(U) and then reapplied to a DNA-cellulose column, practically all the radioactivity was retained on the column. The retained radioactivity could again be eluted by poly(U) (Fig. 2B). The radioactive complex eluted by poly(U) and treated with RNase also sedimented as a 3 to 4 S entity in sucrose gradients containing 0.6 M KCl (Fig. 2C). These observations indicated that poly(U) eluted the receptor complex from DNA-cellulose without significantly altering the steroid- and DNA-binding activities and the sedimentation property of the receptor complex.

When the abilities of various synthetic polynucleotides to promote the release of the receptor complex from DNA-cellulose were compared, we found a striking base specificity. As shown in Table I and Fig. 3, poly(G) and poly(U) were active, whereas poly(A) and poly(C) were essentially inactive at monomer concentrations up to 150 µM (about 50 µg/ml). Since the activity of poly(G) could be suppressed by the addition of poly(C) but not poly(A), whereas the activity of poly(U) could be reduced by poly(A) but not poly(C) (Table I), the activity appeared to be dependent on an unpaired base structure.

Besides poly(G) and poly(U), other homopolymers, such as poly(X), poly(I), poly(4-thio-U), and poly(7-methyl-G), were very active, whereas poly(dU), poly(dT), and poly(dG) were much less active than the corresponding ribopolymers. Poly(dC) was inactive. The radioactive androgen-receptor complex could also be retained by columns packed with various oligodeoxyribonucleotide-celluloses. The relative effectiveness of the four major homopolyribonucleotides in promoting the release of the receptor complex from these columns (Table II) was similar to those observed in the experiments using calf thymus DNA-cellulose.

Effect of Heteropolynucleotides on the Release of Androgen-Receptor Complex from DNA-Cellulose—Since poly(G) and poly(U) were effective in promoting the release of the androgen-receptor complex from DNA-cellulose, we also studied poly(U1,G1). For comparison, we fractionated the polymers by gradient centrifugation into groups with different sedimentation coefficients (2 to 4 S, 4 to 6 S, 6 to 8 S). We originally eluted by poly(U) could again bind to DNA-cellulose (O) and be eluted by poly(U) (Δ). Another portion of the radioactive complex eluted by poly(U) and treated with RNase (C) was analyzed by gradient centrifugation (C) as described under “Experimental Procedures.” Centrifugation was performed at 60,000 rpm for 18 h. For comparison, the radioactivity that did not bind to DNA-cellulose (Fractions 1 to 3 in A) (E) and the original 5α-dihydro[3H]testosterone-receptor preparation (R) were also subjected to gradient centrifugation.

**TABLE I**

| Polymer | Per cent eluted |
|---------|----------------|
| Poly(G) | 77             |
| Poly(U) | 54             |
| Poly(C) | 6              |
| Poly(A) | 6              |
| Poly(G) + poly(C) | 46         |
| Poly(G) + poly(A) | 73          |
| Poly(U) + poly(A) | 54           |
| Poly(U) + poly(U) | 38           |

**FIG. 2** Identification of the radioactivity released by poly(U) from DNA-cellulose. The experiment was carried out as in Fig. 1 except that the radioactive androgen-receptor complex (51,000 cpm) was applied to the column and was eluted (Fractions 10 to 16 in A) with Medium ET containing poly(U) (0.9 ml monomer concentration). The eluted complex was treated with pancreatic RNase A (10 µg) at 0°C for 10 min to destroy poly(U). A portion of the complex eluted by poly(U) was applied to another DNA-cellulose column (B) and was eluted in the same manner to show that the complex...
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TABLE II

| Polynucleotide | Oligo(dA)-cellulose | Oligo(dT)-cellulose | Oligo(dC)-cellulose |
|---------------|---------------------|---------------------|---------------------|
| None          | 6                   | 4                   | 7                   |
| Poly(A)       | 11                  | 10                  | 9                   |
| Poly(C)       | 8                   | 6                   | 6                   |
| Poly(G)       | 47                  | 51                  | 33                  |
| Poly(U)       | 22                  | 14                  | 16                  |

Effect of Oligonucleotides on the Release of Androgen-Receptor Complex from DNA Cellulose—In an attempt to study the minimum length of polynucleotides needed to promote the release of the receptor complex from DNA-cellulose, we tested various oligoribonucleotides listed in Table V. ApUpU, ApUpG, and other oligomers that contained uracil and had a nucleotide chain length of six or less were inactive.

TABLE III

| Polymer               | Monomer concentration (μM) | % eluted |
|-----------------------|-----------------------------|---------|
|                       | 7.5                         | 15.0    | 30.0    | 150.0   |
| Poly(U1,G1)           | 53                          | 71      | 85      | 94      |
| Poly(U2,G1)           | 36                          | 62      | 77      | 89      |
| Poly(U3,G1)           | 42                          | 57      | 80      | 92      |
| Poly(U4,G1)           | 54                          | 69      | 73      |         |
| Poly(A1,G1)           | 32                          | 46      | 75      |         |
| Poly(A1,U1)           | 22                          | 33      | 63      |         |
| Poly(A1,C1)           | 8                           | 13      | 34      |         |
| Poly(C1,U1)           | 35                          | 48      | 80      |         |
| Poly(A1,U1,G1)        | 55                          | 69      |         |         |
| Poly(A1,C1,U1)        | 11                          | 18      | 44      |         |
| Poly(A1,U1,G1,C1)     | 25                          | 40      |         |         |

Among other synthetic heteropolymers tested, poly(I1,G1), poly(A1,U1,G1), and poly(A1,U1,G1,C1) were fairly active at 15 μM, whereas poly(C1,U1) was active at high concentrations (~150 μM), and poly(A1,C1) and calf thymus DNA were inactive. Calf thymus DNA, sonicated and heat-denatured, exhibited an activity comparable to that of poly(dT) (Table I). Various RNA fractions isolated from rat ventral prostate were not as active as poly(U1,G1) but were moderately active at 30 μM (Table IV).

Fig. 3. Effect of various synthetic polynucleotides on the release of the 5a-dihydro[3H]testosterone-receptor complex from DNA-cellulose. The experiment was carried out by the DNA-cellulose column assay as in Fig. 1 except that the receptor complex was eluted by the polymers at the concentrations shown on the abscissa. The extent of elution (% eluted) was calculated (see “Experimental Procedures”) and is shown on the ordinate. The polymers tested were poly(U1,G1) (■), poly(G) (□), equal amounts of poly(G) and poly(U) (■), poly(U) (△), poly(A) (●), or poly(C) (X).

Fig. 4. Effect of various uracil- and guanine-containing polynucleotides on the release of the 5a-dihydro[3H]testosterone-receptor complex from DNA-cellulose. The experiment was carried out by the DNA-cellulose column assay as in Fig. 1 except that the polynucleotides used for the elution of the receptor complex had the uracil/guanine ratios shown on the abscissa (■). The concentration of the polymer was 15 μM (monomer concentration). Some of the polynucleotides were treated with T1-RNase (0.15 ng) at 37°C for 10 min (○) before the polymers were used in the assay. The percentage of the receptor complex eluted from the column is shown on the ordinate.
The experiment was performed by the DNA-cellulose column assay using prostate RNA at the concentrations shown.

| RNA         | % eluted |
|-------------|----------|
| Total rRNA  | 12       |
| 5S rRNA     | 15       |
| 18S rRNA    | 22       |
| 28S rRNA    | 14       |
| 4S rRNA     | 14       |
| 1.5S rRNA   | 15       |
| Poly(A)-RNA | 15       |

Table IV

| Synthetic polynucleotides | % eluted at 150 μM |
|---------------------------|--------------------|
| Poly(U,G)                 | 8                  |
| Poly(U,G)                 | 10                 |
| Poly(U,G)                 | 11                 |
| Poly(U,G)                 | 14                 |
| Poly(U,G)                 | 14                 |
| Poly(I)                   | 28                 |
| Poly(U,G)                 | 28                 |
| Poly(U,G)                 | 32                 |
| Poly(G)                   | 36                 |
| Poly(A,G)                 | 40                 |
| Poly(A,G)                 | 60                 |
| Poly(4-thio-U)            | 65                 |
| Poly(7-methyl-G)          | 130                |

Table V

Effect of various compounds on the elution of 5α-dihydro[3H]testosterone-receptor complex from DNA-cellulose

The experiments were performed by the DNA-cellulose column assay. The activities of oligonucleotides were also determined by DNA-cellulose centrifugation assay.

A. Active group (EC50 lower than 150 μM)

| Synthetic polynucleotides | EC50 | Prostate RNA | EC50 |
|---------------------------|------|--------------|------|
| Poly(U,G)                 | 8    | 5S rRNA      | 37   |
| Poly(U,G)                 | 10   | 18S rRNA     | 36   |
| Poly(U,G)                 | 11   | 28S rRNA     | 39   |
| Poly(U,G)                 | 14   | 1.5S rRNA    | 50   |
| Poly(A,G)                 | 14   | 1.5S rRNA    | 25   |
| Poly(I)                   | 28   | Poly(A)-mRNA | 60   |

B. Weakly active group (EC50 higher than 150 μM)

| Synthetic polynucleotides | % eluted at 150 μM | Other compounds | % eluted at 150 μM |
|---------------------------|-------------------|-----------------|-------------------|
| Poly(U)                   | 47                | Ethidium bromide| 26                |
| Poly(T)                   | 46                | Polyvinylsulfate| 25                |
| Poly(G)                   | 30                | Poly(D-glutamic acid) | 19  |
| Poly(C)                   | 34                | Poly(t-glutamic acid) | 15  |
| Poly(A,G)                 | 44                |                 |                  |

C. Inactive group (no activity at 150 μM)

ATP, GTP, UMP, UDP, UTP, GMP, guanosine 5'-diphosphate 3'-diphosphate, guanosine 5'-triphosphate 3'-diphosphate, guanosine 5'-diphosphate 3-phosphate, guanosine 5'-pentaphosphate, oligo(U)20, oligo(A)20, oligo(G)20, oligo(U), ApUpU, ApUpG, poly(A), poly(C), poly(A,C,G), poly(dA), poly(dC), actinomycin D, rifampicin, alloxaan, riboflavin, menadione, MgCl₂, ZnCl₂, L- aspartic acid, L-glutamic acid, poly(L-leucine) (M₅ = 5,100), poly(L-lysine) (M₅ = 50,000), poly(L-proline) (M₅ = 30,000), L-alamyl-L-aspartic acid, L-arginyl-L-aspartic acid, L-glutamyl-L-glutamic acid, α-L-glutamyl-L-glutamic acid, α-L-glutamyl-L-phenylalanine, α-L-glutamyl-L-lysine, α-L-glutamyl-L-valine, L-tryptophanyl-L-glutamic acid, cyloheximide, calf thymus DNA.

at 150 μM nucleotide concentrations. Surprisingly, oligo(I)10-20 was moderately active at 30 μM, but homo-oligomers with either adenosine, cytosine, or uracil and with nucleotide chain lengths of 10 to 20 were inactive at this concentration. The effectiveness of the oligo(I)10-20 was also confirmed by the DNA-cellulose centrifugation assay. By the centrifugation assay, oligo(A)10-20, oligo(G)10-20, and oligo(U)10-20 were not only inactive in promoting the release of the receptor but also slightly increased the amount of the receptor complex that could bind to DNA-cellulose.

As described above (Fig. 4), uracil- and guanine-containing polymers with high U/G ratios were less active than those with low U/G ratios. When various poly(U,G) with different U/G ratios were treated with T₁-RNase, which could cleave the nucleotide chains at the site next to guanine, we found that the activities of the polymers were essentially abolished if the U/G ratio was below 10. Nuclear treatment, however, did not affect the activity of the polymers with U/G ratios of 20 or above. These results suggested that the effective minimum chain length needed for Up(Up),G to exhibit activity was about 15 to 20 nucleotides.

Use of Gradient Centrifugation to Demonstrate Release of Androgen-Receptor Complex from DNA by Polyribonucleotides—Since the receptor complex is not bound to cellulose in the absence of DNA, the phenomena described above were apparently due to binding of the receptor complex by the DNA moiety of the DNA-cellulose adduct. To show that cellulose was not a necessary participant in the polynucleotide-dependent release of the receptor complex from DNA, we used the gradient centrifugation assay method. As shown in Fig. 5, the radioactive androgen receptor complex stayed near the top of the tube after gradient centrifugation if no nucleic acid was present. If φ DNA was added to the tube, a large quantity of the radioactive bond was found to associate with DNA that sedimented at the bottom of the tube. When
poly(U1,G1) (S S) was added to the receptor complex and 3H DNA before centrifugation, the radioactivity was not found with DNA in the bottom of the tube, but was found associated with poly(U1,G1). Poly(A,C) was able to bind to the receptor complex if no DNA was present; however, it could not release the receptor complex from phage DNA. A similar result was obtained when SV-40 DNA was employed.

### Effect of Nonnucleotide Compounds on the Release of Androgen-Receptor Complex from DNA-Cellulose—Table V summarizes the effect of various compounds on the release of 5α-dihydro[3H]testosterone-receptor complex from DNA-cellulose columns. Group A includes compounds that can promote elution of 50% of the DNA-bound receptor complex at monomer concentrations lower than 150 μM (i.e. EC50 < 150 μM). In addition to various nucleotides described above, aurintricarboxylic acid which can dissociate nucleic acid-protein complexes (28) was as active as poly(U1,G1). Poly(L-aspartic acids) with molecular weights of 5,400 and 27,000 were active but required much higher concentrations. In contrast, poly(D-glutamic) required much higher concentrations. In contrast, poly(D-glutamic) was active but required much higher concentrations. In contrast, poly(D-glutamic) was active but required much higher concentrations. In contrast, poly(D-glutamic) was active but required much higher concentrations. In contrast, poly(D-glutamic) was active but required much higher concentrations.

### Table VI

| Polymer | DHT-R Rat prostate | Est-R Rat uterus | Calf uterus | Rat tumor | Prog-R Rat tumor | Des-R Rat liver |
|---------|-------------------|----------------|-------------|-----------|-----------------|----------------|
| Poly(A) | 7.5 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 2 | 2 | 1 | 1 | 1 |
| | 30 | 2 | 2 | 2 | 0 | 1 |
| | 150 | 2 | 6 | 1 | 1 | 0 |
| Poly(C) | 7.5 | 1 | 1 | 1 | 1 | 1 |
| | 15 | 2 | 3 | 1 | 1 | 6 |
| | 30 | 2 | 3 | 1 | 2 | 6 |
| | 150 | 2 | 6 | 3 | 2 | 8 |
| Poly(G) | 7.5 | 14 | 14 | 45 | 45 | 45 |
| | 15 | 27 | 45 | 92 | 82 | 10 |
| | 30 | 27 | 50 | 90 | 90 | 90 |
| | 150 | 27 | 90 | 90 | 90 | 90 |
| Poly(U) | 7.5 | 10 | 10 | 19 | 9 | 9 |
| | 15 | 20 | 26 | 15 | 15 | 9 |
| | 30 | 30 | 30 | 30 | 30 | 30 |
| | 150 | 30 | 43 | 43 | 43 | 43 |
| Poly(U1,G1) | 7.5 | 38 | 38 | 38 | 38 | 38 |
| | 15 | 67 | 92 | 76 | 76 | 76 |
| | 30 | 80 | 96 | 96 | 96 | 96 |
| | 150 | 80 | 97 | 97 | 97 | 97 |
| Poly(X) | 7.5 | 89 | 89 | 89 | 89 | 89 |
| | 15 | 89 | 89 | 89 | 89 | 89 |
| Poly(A,C,G) | 15 | 73 | 85 | 85 | 85 | 85 |

### Discussion

The differences in the effectiveness of various polynucleotides to release steroid-receptor complexes from DNA may reflect the differences in the relative receptor-binding affinities for these polymers in comparison with the receptor-binding affinity for DNA. Thus, the receptor-binding affinities for single-stranded ribo- or deoxyribonucleotide polymers containing uracil and guanine may be higher than that for double-stranded calf thymus or viral DNA, whereas the affinities of receptor for poly(A) and poly(C) may be lower than that for these DNA. The inability of poly(A) and poly(C) to release the androgen-receptor complex from DNA-cellulose was not due to their degradation or binding to DNA during the assay, since we could recover quantitative all poly(A) or poly(C) that was used during the assay and we could show that the sedimentation properties of these polymers were not different before and after assay. For a polynucleotide to be active, the polymer appears to need non-hydrogen-bonded bases with an oxygen or a sulfur atom at C-6 of purines or C-4 of pyrimidines (Fig. 6). Since binding of the steroid-receptor complexes to DNA or nuclear chromatin is inhibited by pyridoxal phosphate and appears to involve amino groups on the receptor protein, the release of the receptor complex from DNA-cellulose by polynucleotides may involve an interaction of the carbonyl groups on the nucleotide bases and the amino groups on the receptor protein. Since poly(U1,G1) was more active than the equivalent mixture of poly(U) and poly(G) (Fig. 3), the nucleotide sequence may be an important factor. The experiment with T-RNase treated poly(U,G) with different U/G ratios (Fig. 4) suggested that for the polynucleotide to bind the receptor complex tightly, the polymer needed a chain length of at least 15 to 20 nucleotides, although this...
appeared to be dependent on the type of base present (Table V).

It is not very clear why only a certain proportion of the receptor complex that is bound to DNA could be eluted at a set nucleotide concentration. This could be due to involvement of other DNA-binding proteins in the receptor interaction with DNA (5, 12). In fact, we have found that poly(U,G) can release from DNA-cellulose a large number of prostate proteins that can not be released by poly(A). Differences in the local DNA sequence and structure may also contribute to the creation of multiple receptor binding sites. In addition, a change in the local DNA bihelical structure, including partial chain separation may occur during the binding and release of the receptor from DNA, creating binding sites with different affinities. It is conceivable that certain RNA having appropriate nucleotide sequences may be more effective than poly(U,G) and may show high specificities toward different DNA-binding proteins and the steroid-receptor complexes.

The present study suggests that various steroid-receptor complexes may have higher affinities for certain types of RNA than for DNA. Since the concentration of RNA needed (1 to 5 µg/ml) to show this may be well within the range expected in the intact cell nuclei (35), preferential RNA binding of the steroid-receptor complexes in the nuclei is not inconceivable. Such a process may be important in the recycling of the receptor protein from nuclei to cytoplasm (8, 9, 36). The removal of RNA from DNA may also make the genetic template available for further transcription while receptor binding of RNA may be involved in the post-transcriptional control as we hypothesized before (7-9). In this scheme, different RNA molecules may contain, for example, identical or similar nucleotide sequences so that more than one RNA species can be selected, although with some preference, by the same steroid-receptor complex. These diversified specificities together with other cellular factors may provide the selectivity and multiplicity observed in the induction of different proteins by steroid hormones (7).

The interaction of the steroid-receptor complexes with RNA should be studied further since there are indications that steroid hormones may be involved in the stabilization of mRNA for proteins being induced by the hormones (37, 38). It is also plausible to speculate that the specific splicing of certain mRNA and removal of introns (39-42) may be controlled by a mechanism involving RNA binding by a steroid-receptor complex. Although we have not studied binding of the steroid-receptor complex to polydeoxynucleotide in detail, the receptor complex appears to have higher binding affinity toward the single-stranded deoxypolynucleotides than to the double-stranded DNA. Whether such a preferential interaction may play a role in the local unwinding of DNA during the replication or transcription of DNA is worthy of further exploration.

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