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Nuclear Binding of Steroid Receptors: Comparison in Intact Cells and Cell-Free Systems

(glucocorticoids/estrogens/gene regulation/chromatin)

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ABSTRACT **In regulating gene expression in mammalian tissues, steroid hormones bind to cytoplasmic receptors and the resulting complex associates with the nucleus. In an attempt to study this nuclear association under more precisely controlled conditions, we examined the binding of receptor–[³H]steroid complexes to isolated nuclei. Two systems were used: the glucocorticoid-responsive hepatoma cell and the estrogen-sensitive immature rat uterus. Cell-free nuclear binding resembles that observed in the intact cell; it requires the receptor–steroid complex in the appropriate form ("active" complex); it is of high affinity; and it appears to involve about 4000 nuclear acceptor sites per haploid genome. Furthermore, whether binding occurs in intact cells or in cell-free extracts, complexes dissociate from nuclei at similar rates, and the ability of NaCl to extract either type of nuclear-bound complex and the sedimentation velocities of the extracted complexes are identical. Despite these similarities, acceptor sites detected in isolated nuclei differ from those of intact cells, since in neither hepatoma cells nor in the uterus do complexes bound to the nuclei of intact cells prevent further cell-free binding. We conclude that receptor–steroid complexes bind to acceptor sites in isolated nuclei that are chemically similar to, but topographically different from, those of the intact cell.**

Many aspects of cellular function are influenced by steroid hormones (1–4). As a model, we are studying the induction of tyrosine aminotransferase (EC 2.6.1.5) by glucocorticoids in cultured hepatoma (HTC) cells (5). Initially, the steroid enters the cell and binds specifically to cytoplasmic receptor proteins (6–9). Changes then occur in the receptor–steroid complex such that the complexes associate with the cell nucleus (9, 10). Eventually, there is an increase in the concentration of active mRNA specific for tyrosine aminotransferase (11). Similar steroid-mediated binding of cytoplasmic receptors to cell nuclei occurs in other hormone-responsive tissues, such as the immature rat uterus treated with estradiol (3, 12–15).

We have examined the association of the HTC cell receptor–dexamethasone complex to isolated HTC cell nuclei, and of the uterine receptor–estradiol complex to isolated uterine nuclei (16, 17). In each case, the nuclear binding is specific in that it is of high affinity (K_d at $0° \simeq 2$ to 3×10^{-10} M) and involves a limited number of acceptor sites (16, 17) of the same magnitude as the cellular receptor content (10). Several features suggest that receptor–steroid complexes bind very similarly to nuclei in whole cells and in cell-free preparations (10, 16, 17). (*i*) The steroid must be complexed with the receptors to bind specifically in the nucleus. (*ii*) The affinity of the nuclear association is high (10, 16, 17). (*iii*) The concentration of acceptor sites for receptor–steroid complexes in isolated nuclei is close to the number of complexes found in the nuclear fraction of cells incubated with concentrations of steroid that saturate the receptors (Table 1). (*iv*) Receptor–steroid complexes formed at 0°, at low ionic strength, do not bind to isolated nuclei, but additional changes termed "activation" in the HTC cell system (16, 18) and "transformation" in the uterus (3, 13) and thymus (19) must precede nuclear binding.

We have examined the question of whether the nuclear acceptor sites in both situations are identical by testing the binding capacity of isolated nuclei already containing complexes bound in the intact cell. The results suggest that the acceptor sites involved in the binding by isolated nuclei may be different from those in whole cells. Evidence is presented that the nuclear binding reactions in cell-free and intact-cell systems are nonetheless similar.

METHODS AND MATERIALS

The sources of chemicals, the composition of buffers and media, and the methodology for the binding experiments have been described in detail (9, 10, 16–18, 20) and are briefly outlined below.

TABLE 1. *Nuclear binding of receptor–steroid complexes in whole cells and cell-free systems*

		Apparent concentration of nuclear acceptor sites (pmol/mg of DNA)*	
Tissue	Steroid	Whole cells†‡	Cell-free system‡
HTC cell	Dexamethasone	1.29 (0.64–2.40)	1.60 (1.00–2.00)
Uterus	Estradiol	1.39 (0.73–2.05)	1.60 (0.70–2.60)

HTC cells were incubated with [³H]dexamethasone and uteri with [³H]estradiol. Receptor–steroid complexes bound in the washed nuclear fractions of these tissues were determined. In the cell-free systems, nuclear-binding of receptor–steroid complexes was measured at several concentrations of the complex and the saturation values were obtained as reported (16, 17).

* Mean (and range) of at least seven experiments.
† Data from ref. 10.
‡ Data from ref. 17.

Abbreviation: HTC cells; hepatoma tissue-culture cells.
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TABLE 2. *Receptor–dexamethasone complexes (RD) bound to nuclei of intact HTC cells do not inhibit cell-free nuclear binding*

Treatment of cells with [^{3}H]-dexamethasone	Nuclei incubated with:	Nuclear [^{3}H]RD complex (pmol/mg of DNA)		
		From intact cell binding (A)	After cell-free binding (B)	Added during cell-free binding (B − A)
+	—	0.78	—	—
+	Buffer, 0°	0.60	—	—
+	Nonradioactive RD, 0°	0.58	—	—
+	[^{3}H]RD, 0°	0.59	1.45	0.86
−	[^{3}H]RD, 0°	0	0.86	0.86
+	Buffer, 20°	0.58	—	—
+	Nonradioactive RD, 20°	0.55	—	—
+	[^{3}H]RD, 20°	0.57	1.49	0.92
−	[^{3}H]RD, 20°	0	0.89	0.89

Steroid-free HTC cells were used to prepare control nuclei and cytosol. Other cells were treated with [^{3}H]dexamethasone, washed nuclei were obtained, and nuclear-bound receptor–[^{3}H]dexamethasone (*RD*) complexes were determined immediately. Portions of these "prebound" nuclei and the control nuclei (0.2 mg of DNA) were incubated at 0° for 2 hr with activated cytosol complexed with [^{3}H]dexamethasone, and then were assayed for [^{3}H]RD. Parallel portions of "prebound" nuclei were incubated at 0° for 2 hr with buffer or activated cytosol containing nonradioactive dexamethasone to determine the amount of [^{3}H]RD bound in the intact cell that remained after the period of cell-free binding. This value (*column A*) was subtracted from the total nuclear-bound [^{3}H]RD after the cell-free binding (*column B*). Similarly, prebound and control nuclei were compared at 20°. The values represent means of duplicate determinations differing by less than 15%; samples contained between 7,500 and 19,000 cpm.

Nuclear-Binding in Intact Cells. HTC cells were incubated in growth medium at 37° for 0.5–1.0 hr with nonradioactive dexamethasone (0.1–1 μM) or with 0.05 μM [^{3}H]dexamethasone (9–12 Ci/mmol). Nuclear fractions prepared from these cells contain 75–80% of the cellular receptor–dexamethasone complexes (10). When [^{3}H]dexamethasone was used, nuclear-bound complexes were estimated after correction for nonspecific (low affinity) binding by using parallel cultures containing 500- to 1000-fold excess of nonradioactive dexamethasone in addition to the [^{3}H]steroid (6, 8, 10).

Uteri from 23- to 27-day-old Buffalo rats were incubated at 37° for 1 hr with 0.01 μM [^{3}H]estradiol (85 Ci/mmol), a concentration sufficient for maximal nuclear binding (17). Nuclear-bound receptor–estradiol complexes were estimated in washed nuclei by the method used for the HTC cells (17).

Cell-Free Nuclear Binding. Nuclei and cytosol were prepared from HTC cells and receptor–[^{3}H]dexamethasone complexes were formed by incubating the cytosol for 1.5 hr at 0° with 0.05 μM [^{3}H]dexamethasone. So that the glucocorticoid complex would bind to isolated nuclei, the cytosol was then "activated" by incubation with 0.15 M NaCl at 20° for 30 min. Free dexamethasone and salt were removed by gel filtration through Sephadex G-25, equilibrated with homogenization buffer [20 mM *N*-tris(hydroxymethyl)methylglycine (pH 7.4)–2 mM $CaCl_2$–1 mM $MgCl_2$]. The macromolecular fraction was then incubated with HTC cell nuclei (0.2–1.0 mg of DNA) at 0° for 2 hr (sufficient for equilibrium binding) in homogenization buffer containing 0.25 M sucrose (final volume: 0.8 ml). The nuclei were washed in the same buffer, suspended in water, and assayed for radioactivity and DNA. Under these conditions, nuclear-bound tritium represents bound receptor–steroid complexes (16).

For uterus, a similar procedure was used except that homogenization buffer was replaced by NaCl–tricine buffer [0.15 M NaCl–10 mM *N*-tris(hydroxymethyl)methylglycine (pH 7.6)] and the cytosol was bound with 0.01 μM [^{3}H]estradiol.

Binding of the glucocorticoid receptor to HTC cell nuclei was also studied by incubating nuclei and cytosol together at 20°; activation and nuclear binding occur simultaneously (18).

Release of Nuclear-Bound Receptor–Steroid Complexes by NaCl. HTC cell nuclei (0.2–1.0 mg of DNA) containing receptor–dexamethasone complexes were incubated at 0° for 30 min in homogenization buffer (0.5 ml) containing NaCl (up to 0.5 M). The nuclei were removed, washed twice in homogenization buffer, and assayed for receptor–[^{3}H]dexamethasone complexes.

Glycerol Gradient Analysis of Receptor–Steroid Complexes. NaCl (0.35 M) was used to extract the receptor–[^{3}H]dexamethasone complexes bound to HTC cell nuclei in the cell-free system and in intact cells. The extracts (0.2 ml) were layered on 5-ml linear glycerol gradients (10–30%) containing 0.35 M NaCl and 0.1 mM dithiothreitol in homogenization buffer. The gradients were centrifuged at 64,000 rpm for 18 hr at 2° in a Spinco SW65 rotor. Fractions (0.2 ml) were collected and assayed for radioactivity. Bovine-serum albumin (Plasma Fraction V, Armour Co.), acetylated with [^{14}C]acetic anhydride (21), was included in each tube as a marker.

RESULTS AND DISCUSSION

Nuclei isolated from HTC cells incubated with [^{3}H]dexamethasone contain receptor–[^{3}H]dexamethasone complexes (Table 2) (10). These nuclear-bound complexes do not, how-

TABLE 3. *Cell-free binding of [^{3}H]receptor–dexamethasone (RD) complexes to nuclei from HTC cells incubated with and without nonradioactive dexamethasone*

Added [^{3}H]RD (nM)	[^{3}H]RD complex bound in cell-free system (pmol/mg of DNA)	
	Control nuclei	"Prebound" nuclei
0.28	0.18	0.20
0.85	0.62	0.67
2.0	1.21	1.33

Steroid-free cells were used to prepare control nuclei and cytosol, which was incubated with [^{3}H]dexamethasone. The activated cytosol (0.1–0.7 ml) was incubated for 2 hr at 0° with either the control nuclei or "prebound" nuclei from cells treated with 1μM nonradioactive dexamethasone. Nuclear-bound [^{3}H]RD was determined. Values are means of duplicate determinations differing by less than 15%. Samples contained between 1100 and 9000 cpm.

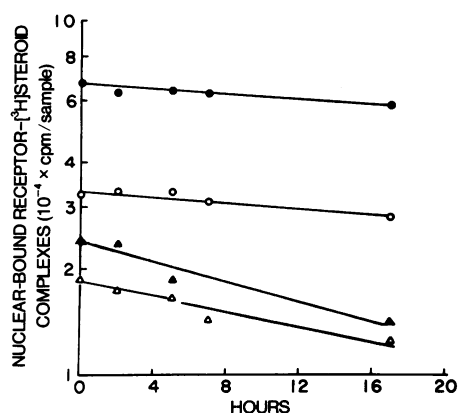

FIG. 1. Stability of nuclear receptor–steroid complexes. Washed HTC cell nuclei, bound with receptor–[^{3}H]dexamethasone complexes in intact cells (●) or in the cell-free system at 0° (O), were resuspended at 0° in homogenization buffer containing 0.25 M sucrose. At intervals, duplicate samples were removed, washed, and assayed for retained complex. Similar experiments were done with uterine nuclei bound with receptor–[^{3}H] estradiol complexes in intact cells (▲) or in the cell-free system at 0° (△).

ever, prevent binding of receptor–[^{3}H]dexamethasone complexes in the cell-free system at 0°. In this cell-free system, these "prebound" nuclei retained as much receptor–steroid complex as did control nuclei from steroid-free cells (Table 2). The lack of effect of complexes bound to the nuclei of intact cells on further cell-free nuclear-binding was also observed when nuclei were incubated with receptor–dexamethasone complexes at 20° (Table 2). At this temperature, specific nuclear binding of the complex takes place even without preliminary activation of the cytosol (16, 18). The data in Table

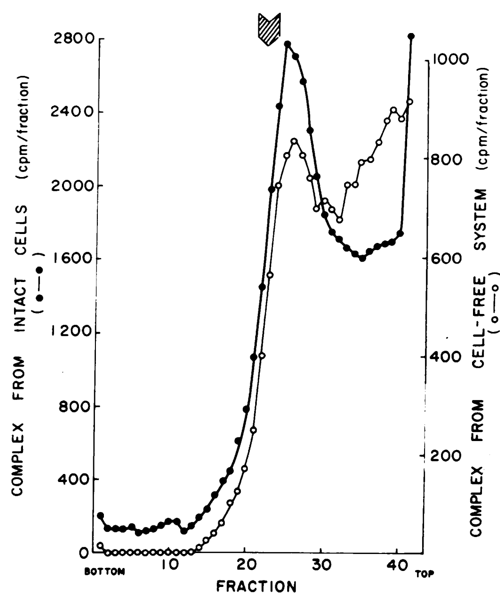

FIG. 2. Glycerol gradient analysis of receptor–dexamethasone complexes extracted from HTC cell nuclei. HTC cell nuclei bound with receptor–[^{3}H]dexamethasone complexes in intact cells (●) or in the cell-free system at 0° (O) were extracted with 0.35 M NaCl, and the extracts were centrifuged on glycerol gradients. The *arrow* indicates the position of the marker, [^{14}C]bovine-serum albumin, used in each gradient.

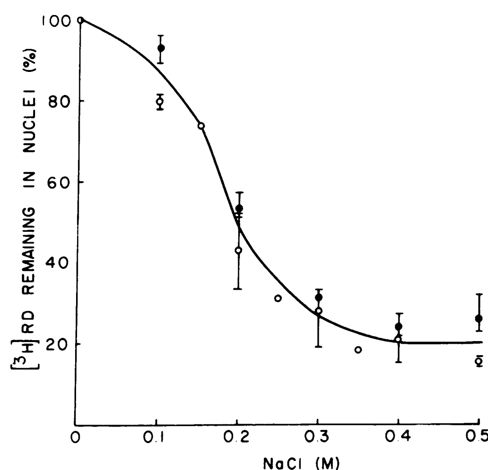

FIG. 3. Release of receptor–[^{3}H] dexamethasone (*RD*) complexes from HTC cell nuclei by NaCl. Washed HTC cell nuclei containing [^{3}H]RD complexes bound in the intact cell (●) or in the cell-free system at 0° (O) were resuspended in 0.5 ml of homogenization buffer containing NaCl (0–0.5 M). After 30 min at 0°, the nuclei were washed and assayed for retained radioactivity. Data and range of four experiments are shown. Before NaCl treatment, nuclear binding was 2000–8000 cpm per sample.

3 illustrate this phenomenon in another way. Cells were exposed to a concentration of nonradioactive dexamethasone far greater than that required to saturate the cytoplasmic receptors (8, 10). Nuclei from these cells, as well as control nuclei, were incubated with increasing concentrations of receptor–[^{3}H]dexamethasone complexes. Clearly, the treatment of the cells with steroid did not significantly alter the cell-free binding characteristics of their nuclei. It should be noted that exposure of control nuclei to a saturating concentration of receptor–dexamethasone complex in the cell-free system does prevent further binding of the complex (data not shown). It is clear that the absence of a difference in the cell-free binding of "prebound" and control nuclei was not due to release of receptor–steroid complexes from the "prebound" nuclei before or during the cell-free binding. Such complexes remained

TABLE 4. *Receptor–estradiol complexes (RE) bound to nuclei of intact uteri do not inhibit cell-free nuclear binding*

Treatment of uteri with [^{3}H]estradiol	Nuclei incubated with:	Nuclear [^{3}H]RE complex (pmol/mg of DNA) From intact cell binding (A)	After cell-free binding (B)	Added during cell-free binding (B − A)
+	—	1.61	—	—
+	Buffer, 0°	1.52	—	—
+	Nonradioactive RE, 0°	1.58	—	—
+	[^{3}H]RE, 0°	1.55	2.42	0.87
−	[^{3}H]RE, 0°	0	0.78	0.78

Conditions are the same as in Table 2 except that nuclei (0.4 mg of DNA) from uteri and [^{3}H]estradiol were used. The uterine cytosol containing 10 nM [^{3}H]estradiol was activated at 20° for 30 min, then gel filtered and incubated with nuclei at 0°. Samples contained between 27,000 and 84,000 cpm.

bound in the nuclei, even after incubation at 0° or 20°, whether the nuclei were suspended in buffer or in "activated" cytosol equilibrated with nonradioactive steroid (Fig. 1, Table 2).

In seven experiments where the cell-free nuclear binding capacity of "prebound" and control nuclei have been compared, the amount of receptor–[^{3}H]dexamethasone complex bound (on a DNA basis) was variable (Table 1). Yet, within a single experiment, duplicate samples were always within 10–15% and the ratio: (Complexes bound by "prebound" nuclei)/(Complexes bound by control nuclei) = 0.94 ± 0.05 (SEM). Preliminary studies suggest that this relationship also holds true for another glucocorticoid-sensitive tissue, rat thymus (data not presented).

We have obtained similar results in the uterine system. Table 4 shows that in the cell-free system at 0°, nuclei from uteri incubated with [^{3}H]estradiol bind no less receptor–[^{3}H]estradiol complex than do control nuclei.

Despite these results, the nuclear acceptor sites in intact cells and in isolated nuclei share many characteristics. Besides those cited in the *Introduction*, further similarities have been found. First, isolated nuclei containing receptor–steroid complexes bound in the cell-free system or in intact cells release their bound radioactivity at similar rates (Fig. 1). Secondly, the receptor–[^{3}H]dexamethasone complexes extracted with 0.35 M NaCl (see below) from HTC cell nuclei bound in the cell or in the cell-free system are indistinguishable on glycerol gradients. Both have the same sedimentation coefficient (about 4 S) (Fig. 2). Finally, the extent to which receptor–[^{3}H]dexamethasone complexes are released from HTC cell nuclei at different NaCl concentrations is independent of whether receptor–steroid complexes are added in intact cells or in extracts (Fig. 3). In either case, maximal release was observed at 0.35 M NaCl and most of the eluted radioactivity was macromolecular (ref. 17 and Fig. 2), suggesting disruption of the nucleus-complex association rather than the receptor–steroid interaction.

Thus, despite the fact that the nuclear acceptor sites active in whole cells do not appear to be those involved in cell-free binding, the two sets of sites must be quite similar. There are several conceivable explanations for this apparent paradox. For instance, the true binding capacity of nuclei in the cell may greatly exceed the number of receptor–steroid complexes formed in the cell. If this were the case, only a subset of these acceptor sites appears to be available for cell-free binding, since the capacity of isolated nuclei is clearly limited. Thus, in this situation it would not be expected that complexes bound in the cell would noticeably inhibit cell-free binding.

Another class of explanations involves the possibility that the acceptor sites detected in the nuclei of whole cells are chemically similar to, but topographically different from, those in isolated nuclei. This situation could arise in two ways. It is conceivable that the cytoplasmic receptors themselves are so altered during isolation that when they are exposed to isolated nuclei they no longer recognize the "correct" nuclear acceptors but bind to other sites. However, the finding that chromosomal proteins are prone to exchange and rearrangement during chromatin (and nuclear) isolation (22, 23) suggest a second mechanism whereby this topographical difference could arise. The receptor–steroid complexes might bind initially in the cell to the "correct" receptor sites but then be translocated to other regions, thus freeing the acceptors for cell-free binding of more complexes. Alternatively, rearrangement could expose new sites for cell-free binding without affecting complexes bound in the intact cell. These possibilities are being tested, but if either of the latter phenomena are involved, they pose problems in studies of not only the early events in steroid-hormone action, but also of chromatin structure and function.

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