sensitive to photoinactivation by ANPP than that of mitochondrial F_1 or *Escherichia coli* F_1. For example, 100 μM ANPP almost abolished the enzymatic activity of CF_1, whereas 300 μM ANPP led to 50% photoinactivation of either F_1 (Lauquin et al., 1980) or *E. coli* F_1 (Pougeois et al., 1983); in contrast, the ATPase from thermophilic bacteria PS3 (Yoshida et al., 1975) was rather sensitive to photolabeling, 85% photoinactivation being attained with 150 μM ANPP (Pougeois et al., 1983). As yet, we have no explanation for these differences in the ANPP photoinactivation of ATPase of chloroplast, bacteria, and mitochondria. There are, however, two common features for the binding of ANPP for all H^+-linked ATPases, mitochondrial, bacterial (*E. coli* and PS3), and chloroplast ATPases; full photoinactivation by ANPP of these different ATPases requires 1 mol of ANPP/mol of enzyme, and the ANPP binding site is located on the β subunit (Pougeois et al., 1982). Since these ATPases possess two or three β subunits, the fact that only 1 mol of ANPP fully inactivates the enzymatic activity suggests that the β subunits are not in the same conformational state.

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Registry No. ANPP, 74784-75-1; phosphate, 14265-44-2.

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Distribution of Thyroid Hormone Receptors, Glucocorticoid Receptors, and Growth Hormone Gene Sequences in Chromatin from Cultured Rat Pituitary Cells†

Beatriz Levy-Wilson*

ABSTRACT: Chromatin from rat pituitary tumor (GH_3) cells was fractionated into transcriptionally active and inactive domains. When the various chromatin fractions were assayed for their content of growth hormone gene sequences, it was found that these sequences were highly enriched in those chromatin fractions most sensitive to micrococcal nuclease. Fraction S_1 showed the highest enrichment in growth hormone genes and was impoverished in histones H_3 and H_4. The distribution of specifically bound thyroid and glucocorticoid receptors in chromatin fractions enriched and depleted in growth hormone gene sequences was also examined. Both thyroid and glucocorticoid receptors are enriched to different extents in transcriptionally active chromatin. Within active chromatin, both types of receptors exist in more than one molecular form.

To date, the detailed molecular mechanisms by which hormones control the expression of specific genes remain obscure. In cultured rat pituitary tumor (GH_3) cells, growth hormone production is stimulated at the transcriptional level by thyroid and glucocorticoid hormones [for reviews, see Baxter et al. (1979) and Ivarie et al. (1980)]. These hormones exert their regulatory function by means of receptor proteins which bind specifically to selected regions of the genome at which they act (Mulvihill et al., 1982). After incubation of cultured pituitary cells or nuclei isolated from them with a low concentration of radioactive triiodothyronine (T_3),[1] essentially all

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[1] Abbreviations: T_3, 3,3',5-triiodothyronine; DME, Dulbecco's modified Eagle medium; MEM, minimal essential medium; 1 × SSC, 0.15 M NaCl–0.015 M sodium citrate.

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of the specific binding observed is by the nuclear receptors for thyroid hormone (Samuels & Tsai, 1973). Similarly, when cells of glucocorticoid responsive tissues are incubated with a low concentration of [^{3}H]dexamethasone, the specific nuclear binding of this hormone is due to nuclear-bound glucocorticoid receptors (Rousseau et al., 1973; Chamness et al., 1974). Therefore, for both classes of hormones, the distribution of specifically bound radioactivity reflects the distribution of the receptors. However, the precise localization of these two types of receptors in chromatin remains to be established. Furthermore, it is not yet possible to correlate the binding of glucocorticoid and thyroid hormone receptors to chromatin with the activation or repression of specific genes.

The development in recent years of methods for fractionating chromatin into its transcriptionally active and inactive domains has helped to elucidate the structural features which distinguish these two domains [for a review, see Mathis et al. (1980)]. Thus, nucleosomes in transcriptionally active chromatin domains have been shown to contain high amounts of high mobility group proteins (Levy-Wilson et al., 1979a,b; Weisbrod & Weintraub, 1978; Egan & Levy-Wilson, 1981), of acetylated histones (Nelson et al., 1980; Levy-Wilson et al., 1979b; Vidali et al., 1978), and of phosphorylated HMG's 14 and 17 (Levy-Wilson, 1981). The DNA from these regions is undermethylated (Razin & Riggs, 1980). As an initial attempt to understand the alterations in the chromatin domains encompassing growth hormone genes promoted by these two hormones, I have examined the structure of transcriptionally active chromatin regions from GH_3 cells containing the growth hormone gene, with special regard to the kinds of proteins present and to the localization of the hormone receptors for glucocorticoids and thyroid hormone.

Materials and Methods

Cell Cultures. Cultured pituitary cellls (GH_3) (Tashjian et al., 1970) were grown in DME media supplemented with 10% fetal bovine serum. Penicillin at 100 units/mL and streptomycin sulfate at 10 μg/mL were also added. Growth was at 37 °C under 5% CO_2 in monolayer by using 75-cm^2 plastic flasks. When large numbers of cells were required, cultures were grown in Spinner flasks at 37 °C, in MEM–Joklik medium supplemented with fetal calf serum and antibiotics as indicated above for the DME media.

Cell Labeling. Some 30 h prior to labeling of the cells with radioactive hormones the growth medium of the monolayers was replaced with DME medium containing 10% fetal calf serum that had been depleted of hormones by using charcoal (Scott & Frankel, 1980) or Dowex AG-1X10. In a typical experiment some 10^8 cells were labeled with [^{125}I]T_3 at a concentration of 0.2 nM (McLeod & Baxter, 1976; Levy-Wilson & Baxter, 1976) for 2 h at 37 °C, either in the presence (to monitor nonspecific binding) or in the absence of 8 μM of unlabeled T_3.

In the experiments involving glucocorticoids, some 2×10^8 cells were labeled with [^{3}H]dexamethasone at a concentration of 20 nM (Levy-Wilson & Baxter, 1976) for 2 h at 37 °C either in the presence or in the absence of 0.4 μM unlabeled dexamethasone. The concentrations of radioactive T_3 and dexamethasone used are those required to saturate about 50–70% of the total nuclear specific binding sites for each hormone (Levy-Wilson & Baxter, 1976; Samuels et al., 1976; Higgins et al., 1973a,b).

Nuclear Isolation and Nuclease Digestions. GH_3 cells growing either in monolayer or suspension were recovered by centrifugation for 10 min at 3000 rpm on a Sorvall GSA rotor. The cell pellet was washed by resuspension in 3–4 volumes of RSB–sucrose [10 mM Tris (pH 7.5)–10 mM NaCl–3 mM $MgCl_2$–0.25 M sucrose], followed by centrifugation as described above. The clear cell pellet was resuspended in 3 mL of RSB–sucrose and lysed by the addition of Nonidet P-40 to 0.5% for 5 min at 0 °C. Nuclei were recovered by centrifugation at 4000 rpm for 10 min in a Sorvall HB-4 rotor.

The nuclear pellet was washed by gentle homogenization first in RSB–sucrose and then in RSB sucrose containing 1 mM $CaCl_2$, followed by centrifugation at 4000 rpm for 10 min. For nuclease digestions, and to prevent hormone dissociation from the receptor, nuclei were resuspended in RSB–sucrose containing 1 mM $CaCl_2$, at a concentration of $30A_{260}$/mL, and incubated with micrococcal nuclease (3 units/A_{260}) for 7 min at 0 °C.

The enzyme reactions were stopped by a low-speed centrifugation to yield a supernatant (S_1) and a initial pellet (P). Chromatin subunits released by the nuclease were solubilized from the pellet by the addition of 1 mM EDTA. These nucleosomes were further fractionated on the basis of their solubility in 0.1 M NaCl as previously described (Levy-Wilson et al., 1979a,b; Egan & Levy-Wilson, 1981).

DNA Isolation and Gel Electrophoresis. For use in hybridization experiments, DNA was isolated from the various chromatin fractions and also from whole GH_3 cell nuclei by a procedure previously described, involving proteinase K and RNase A treatment of the samples followed by phenol–chloroform extractions (Egan & Levy-Wilson, 1981).

The DNA from the pellet fraction and also that from intact nuclei were sheared by depurination to fragments of about 300 base pairs (Levy-Wilson & McCarthy, 1975). Any residual RNA was removed by treatment with 1 N NaOH for 5 min at 70 °C, followed by neutralization with HCl, dialysis, and lyophilization. Electrophoresis of the DNA fragments was performed as described before (Egan & Levy-Wilson, 1981).

Gel Electrophoresis of Proteins. Acid-soluble proteins were analyzed on Triton-containing polyacrylamide gels as described by Egan & Levy-Wilson (1981).

Hybridization Reactions. Hybridization reactions between DNA from the various chromatin fractions and the growth hormone coding sequence were performed by the Dot blot procedure (Thomas, 1980). The growth hormone containing plasmid GH800 (Seeburg et al., 1977) was labeled in vitro to high specific activity by nick translation (Maniatis et al., 1975). Following binding of the unlabeled chromatin DNA in high salt (Thomas, 1980), the nitrocellulose filters were prehybridized overnight at 42 °C in a solution containing 50% formamide, 5 × SSC, 50 mM sodium phosphate, pH 8.0, 1 × Denhardt [0.02% each of Ficoll, BSA, and poly(vinylpyrrolidone) in 0.03 × SSC], and 500 μg/mL yeast RNA. Hybridization was for 22 h at 42 °C in a solution containing 4 parts of 50% formamide, 5 × SSC, 50 mM sodium phosphate, pH 6.5, and 1 part of 50% dextran sulfate together with ^{32}P-labeled growth hormone probe (5×10^5 cpm/mL). The filters were washed as described by Thomas (1980), dried on filter paper, and autoradiographed for the desired amounts of time.

Sucrose Gradient Centrifugation Analysis of Chromatin-Bound Hormone Receptors. Samples containing some 10–15 A_{260} of fractions S_1, MN_1, MN_2, and pellet were centrifuged on 5–24% linear sucrose gradients in 10 mM Tris-HCl, pH 8.0, in the SW 41 Beckman rotor as described previously (Egan & Levy-Wilson, 1981). Fractions (0.6 mL) were collected, and the A_{260} was determined with the aid of an Isco gradient fractionator fitted with a UV monitor. In the experiments involving radioactive hormones, protein-associated

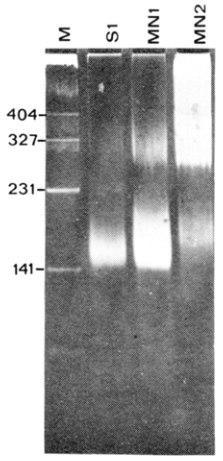

FIGURE 1: Gel electrophoresis of DNA fragments. 0.1 A_{260} of DNA, purified from fractions S_1, MN_1, and MN_2, was electrophoresed in 6% polyacrylamide gels as described previously (Egan & Levy-Wilson, 1981). M is a slot containing TaqI restriction fragments of ϕX174 DNA used as size markers.

hormone radioactivity was determined by filtration of each sample and dilution to 4 mL with 10 mM Tris, pH 8.0, through nitrocellulose filters, followed by solubilization of the filter-bound label in a toluene–Omnifluor scintillation mixture.

All determinations of label reported are specific: i.e., the counts obtained in the competitor-free minus those in the competitor-containing incubation. Under the conditions employed, nonspecific binding represented some 8% of the total T_3 binding and some 5–10% of the total dexamethasone binding.

Results

Fractionation of GH_3 Cell Chromatin. GH_3 cell nuclei, prepared as described under Materials and Methods, were fractionated into micrococcal nuclease sensitive (transcriptionally active) and resistant (transcriptionally inactive) regions as previously described (Levy-Wilson & Dixon, 1979; Egan & Levy-Wilson, 1981). Figure 1 shows the sizes of the DNA fragments of fractions S_1, MN_1, and MN_2. Fraction S_1 (4% of the input A_{260}) comprised those regions most sensitive to micrococcal nuclease and contained DNA fragments of mononucleosome size (140–200 base pairs). Fraction MN_1 (some 20% of the input A_{260}), representing those nucleosomes most sensitive to the nuclease that were released from the nuclear pellet by EDTA and were soluble in 0.1 M NaCl, contained mainly DNA of monomer length (170 base pairs) plus a small amount of dimer (320 base pairs). Fraction MN_2 (some 30% of the input A_{260}), corresponding to those nucleosomes generated by micrococcal nuclease action that were solubilized by EDTA but which remained insoluble in 0.1 M NaCl, contained a small amount of monomer DNA, plus some dimer and higher molecular weight material. The pellet fraction (46% of the input A_{260}) contained mainly large DNA fragments.

The composition of acid-soluble proteins of the various fractions was analyzed by gel electrophoresis and is illustrated in Figure 2. Fractions MN_1, MN_2, and pellet contained the expected complement of histones, while fraction S_1 was deficient in histones, particularly H_3 and H_4. The two small HMG proteins, HMG's 14 and 17, were enriched in fraction MN_1, as is the case in other mammalian cell lines (Egan & Levy-Wilson, 1981). The overall protein content of the various chromatin fractions analyzed by $NaDodSO_4$ gel electrophoresis revealed many proteins in addition to histones and HMG proteins. In particular, S_1 and MN_1 appear to share several

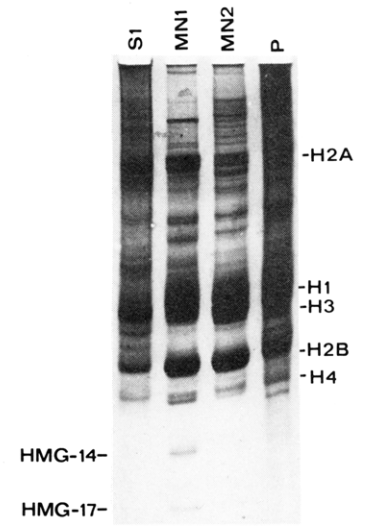

FIGURE 2: Polyacrylamide gel electrophoresis of the acid-soluble proteins from the various chromatin fractions. Electrophoresis, on Triton-containing polyacrylamide gels, was as previously described (Egan & Levy-Wilson, 1981). Proteins were extracted from 10 A_{260} of S_1, MN_1, MN_2, and the pellet.

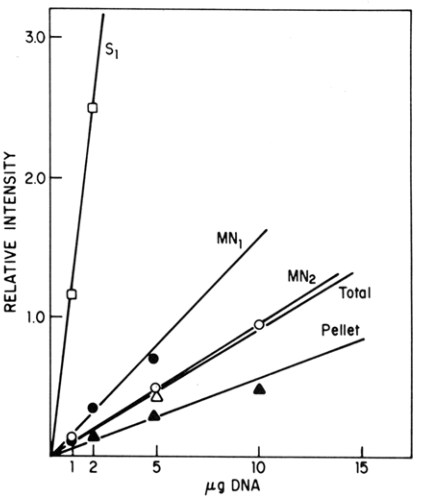

FIGURE 3: Hybridization of growth hormone cDNA sequences with DNA from the various chromatin fractions. Hybridization was performed by the Dot blot procedure as described under Materials and Methods. DNA from whole GH_3 cell nuclei was also included in the filter to provide a control. Each dot was scanned through its center with the aid of a densitometer. The relative intensity of each dot was determined by weighing each peak in an analytical balance.

proteins, ranging in size between 40 and 100 kilodaltons, that are not present in MN_2 and pellet fractions (data not shown).

Distribution of Growth Hormone Sequences in Fractionated Chromatin. To ascertain which chromatin fractions contained growth hormone genes, hybridization experiments were performed with the DNA from the various fractions and a radioactive probe representive of the growth hormone gene sequence. The probe is an 800 base-pair cDNA insert in pBR322 comprising the complete coding sequence of growth hormone (Seeburg, et al., 1977). The probe was ^{32}P-labeled by nick translation and used to challenge increasing amounts of chromatin DNA that had been immobilized on nitrocellulose filters. The kinetics and extent of the reaction among the various chromatin fractions were compared. Figure 3 illustrates the relative intensity of the dot signals in the autoradiograms. It is clear that S_1 has the highest content of growth hormone sequences, followed by MN_1. The slopes of these lines reflect the enrichment or depletion of growth hormone

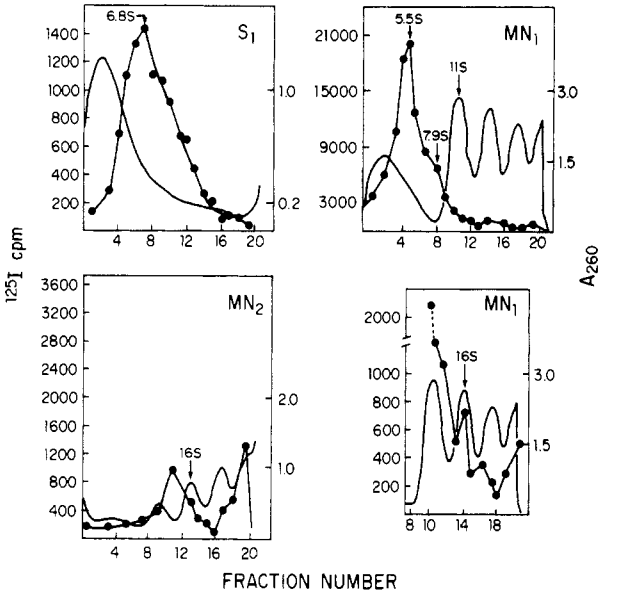

FIGURE 4: Distribution of thyroid hormone receptors in fractionated chromatin. Chromatin fractions, containing some 15 A_{260}, were sedimented on sucrose gradients under Materials and Methods. (—) = A_{260}; (●) = ^{125}I cpm. The small figure at the right bottom is an enlargement of the MN_1 region sedimenting faster than 11 S.

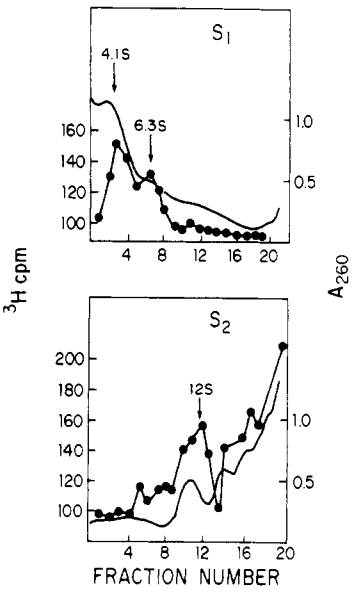

FIGURE 5: Localization of dexamethasone receptors in fractionated chromatin. Sedimentation analysis of chromatin fractions labeled with [^{3}H]dexamethasone was as described under Materials and Methods. (—) = A_{260}; (●) ^{3}H cpm.

sequences in the various fractions, relative to that of total GH_3 DNA. Thus, growth hormone sequences in S_1 are 13-fold enriched compared to those of total DNA: MN_1 is only 2-fold enriched compared to total DNA, and the pellet is depleted in growth hormone sequences. These results were very reproducible, when different batches of nuclei from GH_3 cells were used, and indicate that the growth hormone gene is exquisitively sensitive to micrococcal nuclease in GH_3 cells and therefore readily released from the chromatin.

A control experiment was performed in which total GH_3 DNA, ^{32}P-labeled by nick translation, was used to challenge the same chromatin fractions. Under our hybridization conditions, however, annealing of highly repeated (10^6 copies/genome) and moderately repeated (10^3 copies/genome) sequences dominates the reaction. Thus, satellite DNA sequences will be the first to react. Signals were strongest for the transcriptionally inactive fractions pellet and MN_2, followed by MN_1. Conversely, the concentration of repeated sequences in S_1 was quite low. This result is consistent with the fact that most of these highly repeated sequences are not transcribed and provides a control for our fractionation procedure (data not shown).

Distribution of Thyroid and Glucocorticoid Hormone Receptors within Transcriptionally Active and Inactive Domains of GH_3 Cell Chromatin. Having purified two chromatin fractions (S_1 and MN_1) enriched in their content of growth hormone gene sequences, it was important to establish the localization of both thyroid and glucocorticoid hormone receptor proteins in chromatin. Figure 4 shows the sedimentation properties of fractions S_1, MN_1, and MN_2, together with that of the ^{125}I-labeled thyroid receptor complexes. The T_3 receptor is highly enriched in fractions S_1 and MN_1, but a small amount of specifically bound receptor is also found in MN_2 (tubes 8–20). In five different sets of experiments some 70–80% of the total nuclear T_3 radioactivity was recovered in the micrococcal nuclease sensitive fractions. The pellet formation contained some 20–30% of the input labeled hormone. In control experiments performed in the absence of micrococcal nuclease, some 1–3% of the total ^{125}I radioactivity was released as soluble counts into fraction S_1.

In addition, the T_3 receptor appeared to associate with more than one kind of chromatin component. For instance, a major peak of ^{125}I radioactivity sedimenting at 6.8 S could be seen in fraction S_1. In fraction MN_1, on the other hand, a major proportion of the receptor sedimented at 5.5 S. Nevertheless, a minor but significant fraction of the receptors sedimented with mono-, di-, and trinucleosomes as shown in the enlargement of fraction MN_1 in Figure 4. In MN_2, the radioactivity cosedimented with nucleosomes. Since it is known that in another rat pituitary cell line, GH_1, thyroid receptors released from the chromatin sediment at 3.8 S (Samuels et al., 1980), it is likely that all of the chromatin-associated radioactivity sedimenting faster than 3.8 S must correspond to complexes of the receptor with various chromatin components.

Similar studies were undertaken with dexamethasone receptors, the only difference being that the salt fractionation of the nuclease-released nucleosomal particles to yield MN_1 and MN_2 was omitted to prevent release of glucocorticoid receptors from the chromatin, and all the nucleosomes released appeared in S_2. In this case, in four different experiments an average of 80% of the total nuclear [^{3}H]dexamethasone bound radioactivity was recovered in fractions S_1 and S_2, while the remaining 20% was associated with the pellet. As it is the case with thyroid receptors, only trace quantities of glucocorticoid receptors were released into S_1 following fractionation in the absence of micrococcal nuclease. The data in Figure 5 shows that the glucocorticoid receptors also appear to be associated with more than one kind of chromatin structure. For instance, in S_1, there were two peaks, one at 4.1 S and the other at 6.3 S. In the S_2, the major labeled peak was at 12 S, i.e., associated with mononucleosomes. Since the glucocorticoid receptors alone sediment at 4 S, the rapidly sedimenting forms may reflect specific association of the receptor with chromatin components. The significance of these results will be discussed below.

Discussion

Chromatin from rat pituitary tumor cells was fractionated into micrococcal nuclease sensitive and resistant domains. Growth hormone coding sequences are highly enriched in those fractions exhibiting the highest sensitivity to micrococcal nu-

clease. Our recent results using the same chromatin fractionation procedure with HeLa cells (Egan & Levy-Wilson, 1981), and a probe representative of all the transcribed genes are consistent with the results presented here.

The fraction most highly enriched in growth hormone gene sequences (S_1) was composed of structurally incomplete nucleosomes, containing monomer-sized DNA associated with histones H_2A, H_2B, and smaller quantities of H_3 and H_4. A similar observation regarding the lack of a full complement of histones in chromatin particles highly sensitive to micrococcal nuclease and enriched in the *Physarum* ribosomal genes was made by Johnson et al. (1978).

It is unlikely that S_1 consists mainly of material derived from linker regions since its DNA fragments are monomeric in size. The lack of a full complement of histones in S_1 may be either the cause or the consequence of the high nuclease sensitivity of this fraction. Since S_1 can only be obtained after nuclease digestion and is defined by its micrococcal nuclease hypersensitivity, one cannot make firm statments about the original content and configuration of proteins and DNA in this fraction.

The high mobility group proteins, HMG's 14 and 17, were enriched in MN_1, in which each was present in a half molar amount in relation to the core histones. The population of acid-soluble proteins of MN_1 was similar to that of HeLa cell MN_1, prepared in the same manner (Egan & Levy-Wilson, 1981). Furthermore, MN_1, was heterogeneous, consisting of at least three different forms of deoxyribonucleoprotein, varying in their relative content of HMG proteins. Fractions S_1 and MN_1 appear to share a series of nonhistone proteins of relatively high molecular weight and are absent, or present in reduced amounts, in MN_2 and the pellet.

The distribution of specifically bound thyroid and glucocorticoid receptors among the various chromatin fractions was examined. In fractions S_1, MN_1, and MN_2, the T_3 receptor appears to be associated with more than one kind of chromatin component. Furthermore, the majority of the receptor molecules sediment on sucrose gradients with material smaller than mononucleosomes, even though a small fraction of it is associated with mono-, di-, and trinucleosomes. The 6.8S major component of S_1 may be analogous to a 6.5S T_3 receptor–chromatin complex described as the principal form of the T_3 receptor in GH_1 cells (Samuels et al., 1980). On the other hand, the 5.5S major peak of receptor in MN_1 may correspond to 5.8S T_3 receptor–chromatin complex, predominant in rat liver. The T_3 receptor is known to be a DNA binding protein (Baxter et al., 1979), but whether it interacts both with DNA and chromosomal proteins in its different chromatin associated forms remains to be established.

Knowing the amount of specifically bound ^{125}I counts and the A_{260} of fractions S_1, MN_1, and MN_2, together with the specific activity of the ^{125}I-labeled T_3, and the amount of DNA per GH_3 cell (6 pg), it is possible to obtain an estimate of the number of molecules of thyroid hormone (T_3) receptors per cell bound specifically to these chromatin fractions. There are about 200 molecules of T_3 receptor per cell in S_1, about 1200 molecules of receptor per cell in MN_1, and about 36 molecules of T_3 receptor per cell in MN_2. The relative amount of receptors per cell is approximately 10% in S_1, 63% in MN_1, 2% in MN_2, and 25% in the pellet.

It should be understood that these numbers are tentative, since one cannot be absolutely certain whether the distribution of the receptors is influenced by the chromatin fractionation itself. For example, fractions MN_1 and MN_2 are obtained after incubating the chromatin fraction S_2 for 1 h at 0 °C with 0.1 M NaCl. This may promote the release of some of the receptors that were localized originally in MN_2 into MN_1.

Taking into account the fraction of total chromatin DNA present in the various chromatin fractions (S_1 = 10%, MN_1 = 34%, and MN_2 = 25% in this experiment), one can compare the relative enrichment of T_3 receptors among the various fractions. Thus, S_1 contains 10% of the T_3 receptor and 10% of the A_{260}, indicating no enrichment; MN_1 contains 63% of the T_3 receptor and 34% of the A_{260}, indicating a 1.8-fold enrichment; MN_2 contains 2% of the T_3 receptor and 25% of the A_{260}, indicating a 12.5-fold depletion; the pellet contains 25% of the receptor and 31% of the A_{260}, indicating a 1.2-fold depletion. The large depletion of the T_3 receptor from MN_2 is striking, assuming that no exchange and redistributions of the receptors took place. By comparison, the data from Figure 3 show no change in the relative concentration of the growth hormone gene in the MN_2 fraction. Conversely, fraction S_1 shows a 13-fold enrichment for the gene and no enrichment for the T_3 receptor. Therefore, fractions S_1 and MN_1 show no correlation between gene sequence enrichment and receptor enrichment or depletion. On the other hand, there does appear to be a direct correlation between the two in the MN_1 fraction; i.e., both the T_3 receptor and the growth hormone gene are enriched approximately 2-fold. Since the MN_1 fraction contains nearly two-thirds of the receptor, this could be significant.

The dexamethasone receptors are distributed in chromatin in at least two distinct macromolecular forms, sedimenting at 6.3S and 12S. The 12S component most likely corresponds to receptor bound to mononucleosomes. The 6.3S component of S_1 probably corresponds to a 7S form observed in the case of the estradiol receptor of rat uterus (Senior & Frankel, 1978).

In a manner analogous to that described for the T_3 receptors, we calculated the relative concentration of dexamethasone receptors in the various fractions. In this experiment, 4% of the A_{260} was released into S_1 and 78% into S_2. In this case, it was found that S_1 contained some 7000 molecules of specifically bound receptors per cell, while there were only 800 molecules of receptor bound per cell in S_2.

Thus, S_1 contained 72% of the dexamethasone receptors and 4% of the A_{260}, indicating an 18-fold enrichment; S_2 contained 8% of the receptors and 78% of the A_{260}, indicating a 10-fold depletion, and the pellet contained 20% of the receptors and 18% of the A_{260}, indicating no enrichment. The enrichment of dexamethasone receptors in S_1 correlates very well with the enrichment of growth hormone gene sequences in that fraction. The depletion of dexamethasone receptors in S_2 does not correlate with the content of growth hormone sequences.

Since neither the length of an average gene nor the number of genes in GH_3 cells is known, it is difficult to speculate about the number of receptor molecules associated with individual genes. Nevertheless, it is likely that in the case of glucocorticoids, there is a significant excess of receptors compared to the number of responsive genes. Recently, Ivarie et al. (1981) have analyzed by two-dimensional electrophoresis the patterns of protein synthesis of GH_3 cells, prior to and after treatment with glucocorticoid and thyroid hormones. These authors concluded that only 8–15 proteins or about 1% of over 1000 gene products detectable on the gels were sensitive to each hormone. Their data also indicated that these two hormones acted independently of one another in exerting regulatory effects, even though some proteins were controlled by the two hormones. The latter conclusion is supported by the work of Martial et al. (1977) and Shapiro et al. (1978), showing that glucocorticoid and thyroid hormone increase the levels of growth hormone mRNA by different mechanisms.

How can one rationalize the presence of a 35-fold excess of glucocorticoid receptor relative to thyroid receptors in the most transcriptionally active fraction S_1? A plausible hypothesis is that thyroid receptors may bind to a single site along the target gene, while glucocorticoid receptors bind to multiple sites. A similar notion has been postulated recently to explain the differing regulation of the ovalbumin and conalbumin genes by steroids in the chicken oviduct (Palmiter et al., 1981). Alternatively, most receptors may bind to specific sites in unresponsive genes or perhaps, for most genes, the response to these hormones is too small to measure.

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Registry No. Growth hormone, 9002-72-6; triiodothyronine, 6893-02-3; dexamethasone, 50-02-2.

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