Effect of thyrotropin on ^{32}P -labelled histones H1 and H3 in specific populations of nucleosomes in the thyroid

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ABSTRACT

Thyrotropin (TSH) increases the labeling of histones H1 and H3 in thyroid slices incubated with $^{32}\text{P}_i$. We have prepared nuclei from control and TSH-treated thyroid slices, digested them with micrococcal nuclease, and extracted specific populations of nucleosomes by salt fractionation. Mononucleosomes, derived from the most nuclease-sensitive regions of chromatin, appeared to be selectively enriched in ^{32}P -labeled H1 and H3. However, we were able to detect TSH enhancement of H1 and H3 labeling only in nucleosome multimers derived from less nuclease-sensitive chromatin.

Recent studies have indicated that transcriptionally competent regions of chromatin may be more susceptible to micrococcal nuclease digestion than inactive regions. Our results therefore suggest that H1 and H3 may be actively phosphorylated in transcriptionally competent chromatin; however, they suggest either that hormone-dependent phosphorylation of H1 and/or H3 does not confer transcriptional competence, or that not all transcriptionally competent chromatin is nuclease sensitive.

INTRODUCTION

The fundamental building block of eukaryotic chromatin, the nucleosome, consists of nearly two turns of double-stranded DNA wound around a histone "core" - an octamer of two molecules each of histones H2A, H2B, H3 and H4 (1). Extending from one nucleosome to the next is a "linker" region of DNA, to which H1 is bound; it is widely believed that H1 plays a role in stabilizing higher orders of chromatin structure (1-3). Several polypeptide hormones stimulate the phosphorylation of H1 *in vivo* (4), while *in vitro* studies have shown that phosphorylation at the hormone-dependent site (serine 37/38) affects the ability of H1 to bind to DNA (5-8).

Thyrotropin (TSH) increases the labeling of histone H1 (9-11) and histone H3 (10) in thyroid slices incubated with $^{32}\text{P}_i$. The thyroid therefore provides a particularly interesting model in which to investigate the effects of polypeptide hormone action on chromatin. In this report, we describe the effects of TSH on the distribution of phosphorylated H1 and H3 in specific nucleosomal

populations prepared by micrococcal nuclease digestion of nuclear chromatin followed by low-speed centrifugation (12-17). In some experiments, further nucleosome fractionation was carried out by salt extraction (18) of the pellet obtained after this initial centrifugation step.

MATERIALS AND METHODS

Phosphorylation of Thyroid Slices

Batches of calf thyroid slices (0.5-2 g) were incubated in modified Krebs-Ringer bicarbonate buffer containing 40 μ M phosphate and 2 mg/ml dextrose ("low-phosphate KRB"), as previously described (10). After a 15-min preincubation, slices were incubated for one hr in 2-4 ml of low-phosphate KRB containing 0.9-1.1 mCi $^{32}\text{P}_i$ (carrier-free) and 0.5 mM methylisobutylxanthine (MIX). TSH was then added to a final concentration of 100 mU/ml, and incubation continued for 2 hr. The vials were then placed on ice; the slices were rinsed in 5 ml of iced low-phosphate KRB containing 0.5 mM MIX, and transferred immediately to a 5-10 ml of homogenization buffer (see below).

Preparation of Nuclei and Digestion with Micrococcal Nuclease

Nuclei were prepared by a modification of the method of Chevallier and Philippe (19). Each batch of washed thyroid slices was homogenized at 4C in 5-10 ml of buffer A (5 mM HEPES, 80 mM KCl, 1.8 mM MgCl_2 , 0.9 mM CaCl_2 , 0.5 mM phenylmethylsulfonylfluoride, pH 7.6), containing 2.1M sucrose. Homogenates were filtered through cheesecloth and centrifuged at 19,500 rpm ($g_{\text{max}} = 46,850$) for 60 min at 4C. Nuclear pellets were resuspended in buffer A containing 0.25M sucrose on ice, generally in 1 ml volume. Nuclei (1.5-5.0 mg DNA/ml) were incubated with 150 U/ml of micrococcal nuclease (Worthington or Boehringer-Mannheim) at 37C for one min and then placed on ice for an additional 9 min. Digestions were stopped by the addition of ethylene glycol bis- $[\beta\text{-aminoethylether}]\text{-N}$, N'-tetraacetic acid (EGTA) to a final concentration of 1.1 to 1.2 mM.

Nucleosome Fractionation

Following nuclease digestion, each preparation was centrifuged at 3,000 rpm ($g_{\text{max}} = 1,750$) for 15 min at 4C, and the supernatants ("80 mM salt extracts") were removed. In some studies, the 80 mM salt extracts were compared simply with whole digests and with the unextracted material remaining in the pellets. In these experiments, the pellets were resuspended in buffer A containing 0.25M sucrose and 1.2 mM EGTA, to a volume equal to that of the original whole digests.

In some experiments, nucleosome multimers of increasing size were extracted by a modification of the procedure described by Sanders (18). Pellets from the initial 3,000 rpm centrifugation were resuspended in buffer A containing 0.25M sucrose, 1.2 mM EGTA and 200 mM chloride (80 mM KCl plus 120 mM NaCl). After 20-30 min on ice, the suspensions were centrifuged as above, the supernatants ("200 mM salt extracts") removed, and the pellets resuspended in buffer A containing 0.25M sucrose, 1.2 mM EGTA and 300 mM chloride (80 mM KCl plus 220 NaCl). These steps were repeated with 400 mM chloride (80 mM KCl plus 320 mM NaCl). The pellets from this extraction ("final pellets") were resuspended in buffer A containing 0.25M sucrose and 1.2 mM EGTA.

Separation of nucleosome-bound from unbound protein was achieved by ultracentrifugation. Salt extracts (0.3 ml) of nuclease-treated nuclei were layered onto 4.1 ml of buffer A containing 10% (w/v) sucrose and the corresponding chloride concentrations, and centrifuged at 59,000 rpm ($g_{max} = 480,000$) for 2 hr at 4°C. The pellets were resuspended in electrophoresis buffer (see below) for analysis.

DNA Determinations and DNA Gel Electrophoresis

Total DNA was measured in each extract (20). For size analysis, the DNA in the various fractions was extracted with chloroform:isoamyl alcohol (24:1) and precipitated with 2 vol of absolute ethanol at -20°C overnight (21). After centrifugation and washing once with absolute ethanol, precipitates were dissolved in 50 mM Tris, 20 mM sodium acetate, 2 mM EDTA and 5% sucrose (pH 7.8). Aliquots were electrophoresed on 3.5% polyacrylamide slab gels (22) at 75 to 150 V for 3 to 4 hr, using bromophenol blue as tracking dye. Gels were stained with 2 µg/ml ethidium bromide in water for 30 min, and photographed on a UV light box.

Protein Determination and Protein Gel Electrophoresis

We and others (23,24) have found that the technique employed by Sanders (18) to prepare histones for analysis (precipitation with ethanol at -20°C) does not always give quantitative yields, particularly when histones are present at low concentrations. We have also observed incomplete recovery when low concentrations of histone H1 are precipitated with trichloroacetic acid (TCA). We therefore avoided the use of histone precipitation in the preparation of samples for gel electrophoresis.

Acid-soluble proteins were extracted from the various fractions by adjusting to 500 mM chloride and 0.4N HCl. After centrifugation at 4°C, supernatants were neutralized with NaOH and made 0.06M in Tris, 2% in SDS, 10% in

glycerol and 5% in β -mercaptoethanol. Aliquots were run on SDS-acrylamide (18%) gels (25) for approximately 20 hr (50 V for 16 hr and 120 V for the duration). Cytochrome c was used as tracking dye. After fixing in methanol:acetic acid:water (5:1:5) for 3 to 4 hr, gels were stained in 0.04% Coomassie Brilliant Blue R-250 in methanol:acetic acid:water (5:1:5) overnight and then destained in 40% methanol. Standards containing pure histone H1 (Worthington) and H4 (Boehringer-Mannheim) were run on each gel. Protein concentrations were determined densitometrically using a Corning Model 500 Scanner. For autoradiography, gels were sealed in Saran Wrap^R, placed on Kodak XR2 or XAR5 film and exposed for 1 to 10 days. Stained bands were then cut out of the gels, and the radioactivity in each was determined by Cerenkov radiation.

Histone H1 was clearly resolved into two major bands on our SDS-acrylamide gels. However, the distance between these bands was generally too small to allow us to separate them physically, and so they were cut out of the gels as single blocks. The H2A + H2B pair was also treated as a single band, for similar reasons.

In some experiments, acid extracts of various fractions were dialyzed overnight against 0.9N acetic acid containing 40% sucrose, and analyzed by electrophoresis on acetic acid-urea gels (26).

RESULTS

Preparation of Various Nucleosomal Populations by Extraction with Increasing Concentrations of Salt

The pattern of nucleosomal DNA elution from micrococcal nuclease-digested calf thyroid nuclei was similar to that found in rat liver under comparable conditions by Sanders (18). Unlike Sanders, we obtained some DNA (about 6% of total), very largely in the form of mononucleosomes, in our initial low-salt extracts. Monomers, dimers and small amounts of larger fragments were eluted at 200 mM chloride; the monomers eluted in this fraction were, on average, slightly larger than those extracted at 80 mM chloride. A spectrum of oligomers (mostly dimers to octamers) was obtained at 300 mM chloride, whereas only larger fragments were found at 400 mM and in final pellets. The amounts of the various histones in each fraction closely paralleled the amount of DNA, except in the 80 mM salt extracts which were about 50% depleted of H1. Under the digestion conditions employed, we were unable to detect any effect of TSH on the pattern of DNA or histone elution.

Patterns of ^{32}P -Histone Labeling in the Various Nucleosomal Populations, and Their Response to TSH

The effects of TSH on the patterns of ^{32}P -labeling were first examined in whole nuclear digests, and in supernatants ("80 mM salt extracts") and pellets prepared by low-speed centrifugation of digested nuclei. Fig. 1 shows radioautographs of acid extracts of these fractions run on SDS-acrylamide gels. In the acid extracts of whole nuclear digests, we found a prominent band of radioactivity corresponding to the stained H1 bands. Heavily labeled bands were also found corresponding to stained H3 and H2A + H2B, and in positions corresponding to molecular weights 21-22,000, 25-27,000, 40-42,000 and 50-54,000. TSH stimulated the ^{32}P -labeling of H1 (by 250%; $p < 0.02$, by two-tailed t-test) and H3 (by 150%; $p < 0.05$), but we were unable to detect significant changes in the labeling of other proteins.

Figure 1. Effect of TSH on the phosphorylation of acid-extractable proteins in whole nuclear digests ("W.N."), 80 mM salt extracts ("80") and pellets ("P") prepared by 3,000 rpm centrifugation of nuclear digests. Batches of thyroid slices (1.2-1.5g) were incubated for 2 hr with 1.1 mCi ^{32}P , with or without 100 mU/ml TSH. Nuclei were prepared and digested with micrococcal nuclease as described in the text. 80 mM salt extracts and pellets were then prepared, and acid extracts of these fractions analyzed by SDS-acrylamide gel electrophoresis and autoradiography. C = Control, T = TSH-treated. Radioautograph shows TSH-stimulation of histone H1 and H3 labeling in whole digests and pellets, but not in 80 mM salt extracts. The band of phosphorylated histones H2A and H2B is designated "H2" and two other reference phosphoproteins are designated "A" and "B".

Although the 80 mM salt extracts contained less than 7% of total histone protein, much of the radioactivity in the gel slices corresponding to the stained H1, H2A + H2B and H3 bands appeared in this fraction (16-45%, 43- 56% and 48-71%, respectively, of the activity in the whole nuclear digests). No significant TSH-dependent changes were apparent. In contrast, the pellets contained relatively small amounts of ^{32}P -labeled phosphoprotein, but displayed marked TSH-dependent increases in H1 and H3 labeling. ^{32}P activity in H1 was increased by 330% ($p < 0.01$) and in H3 by 175% ($p < 0.05$).

To compensate for differences in the amounts of radioactivity taken up by different batches of slices, the ratio of ^{32}P in a given phosphoprotein to that in other phosphoprotein bands was determined. The three denominators chosen (A, MW 50-54,000; B, MW 40-42,000; and the H2A + H2B pair) showed no significant alteration with TSH. TSH-dependent increases in H1 and H3 labeling were again clearly evident in whole nuclear digests and in the pellets, but no significant increases could be detected in the 80 mM salt extracts (Table I).

Table I: Effect of TSH on the Ratio of ^{32}P in Histones H1 and H3 to ^{32}P in Other Phosphoproteins in Whole Nuclear Digests, 80 mM Salt Extracts and Pellets from Low-Speed Centrifugation

	Histone H1			Histone H3		
	Whole Nuclear Digest	80 mM Salt Extract	Pellet	Whole Nuclear Digest	80 mM Salt Extract	Pellet
Ratio to A						
Control	0.57±.06	0.33±.03	1.12±.07	0.24±.02	0.27±.02	0.35±.05
TSH	1.54±.10 ^d	0.39±.05	4.35±.17 ^d	0.46±.04 ^b	0.31±.04	0.88±.09 ^b
Ratio to B						
Control	0.62±.02	0.34±.03	1.23±.07	0.26±.01	0.29±.03	0.39±.05
TSH	1.55±.09 ^d	0.39±.02	3.90±.45 ^b	0.46±.03 ^b	0.32±.02	0.78±.09 ^a
Ratio to H2A+B						
Control	0.86±.05	0.60±.04	0.94±.03	0.36±.01	0.50±.04	0.29±.02
TSH	1.82±.11 ^d	0.64±.01	2.48±.10 ^d	0.54±.03 ^c	0.53±.05	0.50±.05 ^a

Incubation conditions, sample preparation and gel electrophoresis as in Fig.1. Mean ± SEM for the ratio of ^{32}P in H1 and H3 bands to that in denominator phosphoprotein bands A (MW 50-54,000), B (MW 40-42,000) and the H2A+H2B pair. a: $p < .05$, b: $p < .02$, c: $p < .01$, d: $p < .005$ vs control by two-tailed t test. All determinations in quadruplicate.

Acid-urea gel electrophoresis confirmed our observations of ^{32}P -labeling of histones H1 and H3, and of TSH-dependent increases in the 3000 rpm pellets (data not shown).

In subsequent experiments, we examined the effect of TSH on the distribution of ^{32}P -labeled H1 and H3 by salt fractionation of the pellets remaining after initial centrifugation (Fig. 2). Stimulation of H1 and H3 labeling was clearly evident in whole nuclear digests, in the 300 mM and 400 mM salt extracts and in the final pellets.

Fig. 3 shows the pattern of ^{32}P distribution in the histone H1 bands in the various salt extracts. TSH increased ^{32}P incorporation in the 300 mM and 400 mM salt extracts (by 254%, $p < 0.005$, and 466%, $p < 0.002$) and in final pellets (by 103%, $p < 0.02$). No significant TSH-dependent changes in H1 labeling could be detected in the 80 mM and 200 mM salt extracts.

Total ^{32}P activity in the histone H3 band was greatest in the initial (80 mM) salt extracts; it decreased progressively in the higher salt extracts, the final pellets containing only 1/10 that in the initial extracts. TSH in-

Figure 2. Effect of TSH on the phosphorylation of acid-extractable proteins in the various salt extracts. Batches of thyroid slices (1.8-1.9 g) were incubated for 2 hr with 1.1 mCi $^{32}\text{P}_i$, with or without 100 mU/ml TSH. Nuclei were prepared, digested and salt-fractionated as described in the text; acid extracts of the various fractions were then prepared and analyzed by SDS-acrylamide gel electrophoresis and autoradiography. A = Control, B = TSH-treated. In each, the right hand lane shows an extract of a whole nuclear digest ("W.N."); lanes 1-5 show the various salt extracts, and final pellets. The band of phosphorylated histones H2A and H2B is designated "H2".

Figure 3. Pattern of ^{32}P distribution (cpm per ml of extract) in the histone H1 bands in the various salt extracts after micrococcal nuclease digestion of nuclei prepared from calf thyroid slices incubated with (open bars) and without TSH (hatched bars). Incubation conditions, sample preparation and gel electrophoresis as in Fig. 2.

creased the activity significantly in the 400 mM salt extracts (by 160%, $p = 0.02$) and in the final pellets (by 170%, $p < 0.01$). Increases in the 300 mM extracts (by 100%) and in whole nuclear digests (by 40%) did not reach statistical significance. No TSH-dependent stimulation of H3 labeling was observed in the 80 mM or the 200 mM salt extracts.

We determined the ratios of ^{32}P in the H1 and H3 bands to ^{32}P in the histone H2A + H2B band in each salt extract (Table II), to compensate for differences in the amounts of radioactivity taken up by different batches of slices. TSH-dependent increases in H1 and H3 labeling were again clearly evident in the 300 mM and 400 mM extracts and in the final pellets, but no significant changes were found in the 80 mM and 200 mM extracts.

Finally, we determined the ratios of total ^{32}P activity to total protein in the histone H1 and H3 bands in each fraction (Table III). As would be expected on the basis of the high activities and the relatively small amounts of histones found in the 80 mM salt extracts, the ratios were very high in these fractions; again, no hormone-dependent effects were apparent. The TSH-dependent increase in H1 labeling remained significant in the 300 mM and 400 mM extracts, but not in the final pellets. Although TSH-dependent changes in H3 la-

Table II: Effect of TSH on the Ratio of ^{32}P in H1 and H3 to ^{32}P in the H2A + H2B Pair in the Various Salt Extracts

	80 mM	200 mM	300 mM	400 mM	Pellet	Whole Nuclei
H1 H2A+B						
Control	0.57±.04	0.58±.04	0.48±.02	0.64±.02	0.83±.07	0.72±.02
TSH	0.71±.08	0.80±.09	1.81±.18 ^c	3.00±.28 ^d	1.59±.12 ^b	1.76±.10 ^d
H3 H2A+B						
Control	0.53±.08	0.46±.04	0.30±.04	0.26±.04	0.27±.05	0.50±.02
TSH	0.56±.07	0.57±.04	0.62±.07 ^a	0.65±.11 ^a	0.73±.09 ^a	0.70±.09

Incubation conditions, sample preparation and gel electrophoresis as in Fig. 2. Mean $\pm$ SEM for the ratio of ^{32}P in H1 or H3 to ^{32}P in the denominator H2A+H2B band. a: $p < 0.05$, b: $p < 0.02$, c: $p < 0.01$, d: $p < 0.005$ vs control by two-tailed t test.

beling were still apparent, they no longer reached statistical significance.

Separation of Nucleosome-Bound from Unbound Phosphoprotein

Histone H1 may be dissociated from nucleosomes between 300 mM and 400 mM salt (18), and phosphorylation reduces the strength of H1-DNA binding, particularly in this range (7,8). To determine whether histone H1 and phosphorylated H1 were nucleosome-bound, samples of 300 mM and 400 mM extracts from control and TSH-treated slices were centrifuged through 10% sucrose containing

Table III: Specific Activities (cpm/ μg protein) of Histone H1 and H3 in the Various Salt Extracts, and the Effect of TSH

	80 mM	200 mM	300 mM	400 mM	Pellet	Whole Nuclei
H1						
Control	1006±199	129±29	16.8±2.5	18.3± 3.1	49.7±12.4	48.2±8.7
TSH	1141±194	138±24	58.0±9.7 ^a	83.3±13.4 ^b	106±16	111±15 ^a
H3						
Control	309±99	63±7.9	10.9±2.1	7.7±1.4	7.9±0.5	36.2±2.9
TSH	261±44	58±8.9	17.4±2.3	17.5±3.5	23.3±3.6	45.5±4.3

Incubation conditions, sample preparation and gel electrophoresis as in Fig. 2. Mean $\pm$ SEM; a: $p < 0.05$, b: $p < 0.02$ vs control by two-tailed t test.

the same concentrations of salt that were present during extraction. The pellets obtained contained nucleosomal DNA and core histones; H1 and phosphorylated H1 were found in pellets from the 300 mM, but not the 400 mM salt extracts.

DISCUSSION

In view of the central role played by histones in maintaining the nucleosomal and higher order structures of chromatin (1-3), it seems possible that their hormone-dependent phosphorylation could modulate chromatin organization and function. Our present study represents an initial attempt to investigate hormone-dependent histone phosphorylation at the nucleosomal level, taking advantage of the fact that TSH enhances the ^{32}P -labeling of both H1 (which interacts with linker DNA) and H3 (which is an element of the nucleosomal core).

We have employed mild conditions to prepare specific populations of nucleosomes, and it is possible that some phosphorylation and/or dephosphorylation of histones may have taken place during these procedures. We believe, however, that the TSH-dependent increases we have observed in ^{32}P -labeling of H1 and H3 were *in vivo* responses, since they were similar to those found in previous studies in which we analyzed acid extracts of whole thyroid slices by two-dimensional gel electrophoresis (10).

We emphasize that the methods we have employed do not enable us to determine absolute levels of histone phosphorylation. TSH-dependent increases in ^{32}P -labeling could have resulted from increased phosphorylation or decreased dephosphorylation, or both. Further, we cannot exclude the possibility that some of the ^{32}P activity in our histone fractions was due to the presence of highly phosphorylated contaminants, or to histones labeled by ADP-ribosylation.

Our nuclear digests were initially fractionated by low-speed centrifugation into supernatants ("80 mM salt extracts") and pellets (12-17). Supernatants contained mononucleosomes which were partially depleted in histone H1. These monomers were probably derived from the most nuclease-sensitive regions of chromatin (12,13). One possible explanation for their H1-depletion is that particularly nuclease-sensitive chromatin is, in fact, relatively deficient in H1: selective removal of H1 from chromatin *in vitro* increases the rate of its digestion (27-29). Alternatively, H1 binding sites may have been lost by digestion of linker DNA (30), since monomer DNA in the 80 mM salt extracts appeared smaller than that in the 200 mM extracts. It might be expected that displaced H1 would bind to larger oligonucleosomal fragments, and so be found in the pellet fraction. In agreement with Sanders (18), we found that histone

H1 (and ^{32}P -labeled H1) was bound to oligonucleosomes in 300 mM chloride, but was displaced at higher salt concentrations.

Several recent studies have suggested that transcriptionally competent or active regions of chromatin are more susceptible to micrococcal nuclease digestion than inactive regions (12-18,31,32). It therefore seems possible that some or all of the nucleosomes in our 80 mM extracts may have been derived from transcriptionally competent or active chromatin.

When our various salt extracts were analyzed by SDS-acrylamide gel electrophoresis, the H1 and H3 bands in the 80 mM (and 200 mM) fractions were found to contain relatively high ratios of ^{32}P activity to total histone. We cannot rule out the possibility that this was due, in part, to the presence of highly phosphorylated contaminants in these bands, but our data are compatible with the results of other investigations in which nucleosomes containing relatively highly phosphorylated histones were found to be preferentially released from chromatin by micrococcal nuclease digestion (33,34). These observations suggest that histone phosphorylation may be particularly active in transcriptionally competent chromatin.

We were unable to detect any TSH effect on the apparently active labeling of the H1 and H3 in our 80 mM and 200 mM salt extracts. While this suggests, at first sight, that the phosphorylation of these histones in nuclease-sensitive chromatin is unaffected by TSH, there may be alternative explanations. One is that TSH-stimulated increases may have been masked by the relatively high "basal" levels of ^{32}P activity in the low-salt extracts. A second is that the more nuclease-sensitive regions of chromatin may contain relatively high levels of a specific phosphatase that acts selectively on hormone-dependent sites. Finally, in the case of H1 (but not H3), it is possible that phosphorylation at the hormone-dependent site makes this histone particularly susceptible to displacement from mononucleosomes during or after digestion.

TSH-dependent increases in ^{32}P -labeling of H1 and H3 were clearly apparent in the 300 mM and 400 mM salt extracts and final pellets, so we cannot escape the conclusion that hormonal effects were not confined to the most nuclease-sensitive regions of chromatin. We have found no evidence that any of the relatively nuclease-resistant fractions was derived from a particularly TSH-sensitive or insensitive region of chromatin.

Gurley et al. (35) have described two types of cell cycle-dependent H1 phosphorylation, one occurring in interphase (H1_I) and the other in metaphase (H1_M). A third type of phosphorylation (hormone-specific) takes place exclusively on serine 37/38 (4,36). We cannot exclude the possibility that some of the ^{32}P -

labeling observed in our experiments may have been attributable to H1_I or H1_M phosphorylation. However, the data obtained by Lamy et al. (9) clearly indicate that TSH-stimulated labeling of H1 in thyroid slices takes place selectively on the hormone-specific site.

Dramatic increases in histone H3 phosphorylation, paralleling increases in H1_M phosphorylation, take place at mitosis (4,35); however, we believe that the TSH-stimulated increases in H3 labeling that we have observed reflect hormone-specific effects on interphase chromatin, and not stimulation of cell division. TSH-stimulation of H3 labeling closely parallels hormone-specific labeling of H1, and can be detected after only 70 min of TSH treatment (10). Lamy et al. (9) failed to observe increased H1_M phosphorylation after treatment of thyroid slices with TSH for one hr, and we know of no studies in which mitogenic effects of this hormone have been detected within this time period.

Hormone-specific phosphorylation of H3 has so far received little attention, and it is not yet known which sites are affected. At least two enzymes are able to phosphorylate H3 in vitro - a cAMP-dependent protein kinase (at serine residue 10) (37), and a chromatin-bound, cAMP-independent, H3-specific kinase (at threonine residue 3) (38) - but neither has yet been shown to be active in vivo.

It has been proposed that histone modifications, including phosphorylation, may result in specific changes in chromatin structure and function (36). There is evidence that H1 phosphorylation at the hormone-dependent site, serine 37/38, decreases the strength of H1-DNA binding (5-8), and that H3 phosphorylation affects its binding both to DNA (38) and to its nucleosomal pair histone H4 (37); however, it is not yet clear whether these changes may affect transcription. Our observation of hormone-specific increases in ³²P-labeling of H1 and H3 in relatively nuclease-resistant fractions of chromatin suggests either that hormone-dependent phosphorylation of these histones does not (at least in itself) confer transcriptional competence, or that not all transcriptionally competent regions of chromatin are nuclease sensitive.

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