CHANGES IN TEMPLATE ACTIVITY OF RAT VENTRAL PROSTATE CHROMATIN AFTER BRIEF OR PROLONGED IN VIVO EXPOSURE TO ANDROGEN: EVIDENCE FOR A SUCCESSION OF CHROMATIN "CONFORMERS"

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SUMMARY. The template activity of rat ventral prostate chromatin, estimated by incubation with E. coli RNA polymerase, was compared in preparations exposed to in vivo androgen for brief (4 hours) or protracted (72 hours) periods of time. After 72 hours, a "paradoxical" result (C>N or CT_{72}) was observed. However, N and CT_{72} chromatin contained more non-histone protein. At 4 hours, CT_4 chromatin template activity exceeded that of C by 60-80%, and this was associated with a massive (+30%) uptake of non-histone (acidic) proteins. Differences in the activities of nucleases and ribonucleoside triphosphatases did not appear to account for these results. Such findings may reflect conformational changes in prostate chromatin related to the androgen-dependent influx from the cytoplasm of non-histone "nuclear" proteins. Changes in template activity following a variety of experimental regimes can be viewed in terms of a succession of chromatin "conformers" brought about and sustained by their association with analogous non-histone proteins.

Bacterial RNA polymerases frequently are used to estimate the template activity of chromatin. However, the relationship between changes in template activity that follow various experimental protocols, and the fraction of template actually serving for in vivo transcription of RNA is not usually apparent. Although the template activity (for E. coli RNA polymerase) of prostate nuclei from testosterone-treated rats exceeded the activity of nuclei from castrates, chromatin template activities from these 2 sources exhibited no consistent differences (1). Liao and his coworkers observed that the template activity of ventral prostate chromatin from rats castrated for 3 days was somewhat reduced, and that this was probably not related to their greater nucleotidase activity (2,3). But, compared with the endogenous RNA polymerase activity of intact nuclei, chromatin template activity, measured with M. lysodeikticus RNA polymerase was 10 times greater, and nearest neighbor fre-

quency analyses of the newly synthesized RNA differed markedly (3,4).

Many studies have been performed with castrated rats exposed to testosterone for 24-72 hours. We have compared template activities of ventral prostate chromatin from castrated rats after brief (4 hour) and prolonged (72 hour) in vivo treatment with androgen. After 72 hours, a "paradoxical" result (C>N or CT_{72})was observed. Upon brief exposure to the hormone, a marked stimulation in template activity occurred (CT_4>>C), associated with a massive uptake of non-histone proteins by CT_4 chromatin. It may be useful to view these dynamic alterations of prostate template activity in terms of a series of chromatin "conformers" resulting from the androgen-induced influx of non-histone proteins.

METHODS. Male Wistar rats were maintained on Purina laboratory chow and water ad. libitum. Procedures of castration and hormone replacement, isolation of nuclei from rat N (normal), C (3 day castrates) and CT_{72} (castrates treated with testosterone propionate for 3 days) ventral prostates, and counting of radioactive RNA have been described (5). Chromatin was prepared by the method of Shaw and Huang, which does not involve sonication (6). Histones (7) were extracted by treatment of chromatin with 0.4 N H_2SO_4, and the residue considered to represent non-histone nuclear proteins (NHNP). RNA polymerase from E. coli K-12 was isolated according to the procedure of Burgess (8), and corresponded to his fraction 4. Chromatin template activity was assayed as described by Bonner et al. (7). Radioactive rat liver RNA or DNA were incubated with chromatin and cold TCA-soluble radioactivity determined. Ribonucleoside triphosphatase was assayed by incubation of ^{3}H-UTP with chromatin ± the other 3 NTP's and recovery of the radioactive products by thin layer chromatography. RNA, DNA and protein were determined by conventional procedures (9).

Chemicals were reagent grade. Biochemicals and enzymes were purchased from Sigma, Calbiochem, and Worthington, and radioisotopes from New England Nuclear; E. coli K-12 was purchased from Miles Laboratories.

RESULTS. The composition (relative to DNA = 1) of prostate chromatin from rats castrated for 3 days, and treated with testosterone propionate for 4 or 72 hours, is presented in Table 1. Little change (+10%) in the ratio of histones to DNA occurred during either time period. On the contrary, after 4 hours of treatment with androgen, half (27%) of the change of NHNP (relative to C) observed at 72 hours, had occurred. Upon brief exposure to androgen, the ratio of total protein to DNA was nearly restored to normal. Some species of chromatin non-histone protein present at 4 and 72 hours may differ. The 22% rise in the RNA to DNA ratio of CT_4 chromatin represents half of that observed at 72 hours, and is comparable to the per cent increase in NHNP.

The relation between ^{3}H-UTP incorporated into RNA, and the concentration of chromatin is presented in representative experiments of Fig. 1. The radioactive product was precipitated by cold TCA, and its synthesis, which was dependent upon all 4 nucleoside triphosphates, inhibited by actinomycin D. Chromatins from N and CT_{72} rats were invariably less efficient templates for E. coli RNA polymerase, compared to C chromatins. However, chromatin prepared from rats that received 8 mg of testosterone propionate 4 hours before sacrifice was far more active (up to +80%) than C chromatin. When these preparations were assayed for DNase, RNase and NTPase activities, differences that could explain these findings were not observed (manuscript submitted).

DISCUSSION. The content of total, non-histone (and possibly histone) proteins in chromatin from hormone-stimulated animals (N, CT_4, and CT_{72}) was greater than that of C chromatin. Although the template activity of N and CT_{72} chromatin was less than that from castrated rats, the activity of CT_4 chromatin exceeded all of them. Frequently protein-deficient chromatin assayed with exogenous heterologous RNA polymerase is more active, but this relationship does not always obtain.

Several steroid hormones increase chromatin template activity: 17β-estradiol (rat and rabbit uterus {10,11}), testosterone (rat skeletal muscle {12}), cortisol (rat liver {13}) and progesterone (chick oviduct {14}). How-

TABLE 1. Composition of N, C and CT ventral prostate chromatin from castrated rats treated with testosterone propionate for 4, or 72 hours

Chromatin	RNA / DNA	Total Protein / DNA	Histone / DNA	Non-histone Proteins / DNA
		4-Hour Chromatin[1], CT_4		
N	0.086	1.82	1.05	0.77
C	0.051	1.41	0.92	0.49
CT_4	0.062	1.73	1.01	0.62
		72-Hour Chromatin[2], CT_{72}		
N	0.090	1.79 ± 0.10	1.09 ± 0.08	0.70 ± 0.03
C	0.055	1.50 ± 0.06	0.98 ± 0.05	0.52 ± 0.04
CT_{72}	0.081	1.89 ± 0.11	1.06 ± 0.06	0.83 ± 0.12

[1] Results from 3 experiments.

[2] Results from 5 experiments.

ever, even if artefacts due to nucleases and ribonucleoside triphosphatases are rigorously excluded, the import of such results seems uncertain. Addition of NHNP to chromatin alters its template activity (15,16). It is unlikely that mammalian cytoplasmic proteins, such as the prostate dihydrotestosterone-binding proteins (17) or other non-histone proteins associated with CT chromatin, selectively modify the function of bacterial RNA polymerases templated by mammalian chromatin, which is expected to retain much of its tissue-specific character (18). Liao has already shown that the qualitative nature and quantitative amount of such chromatin-primed RNA differed markedly from the product formed by endogenous nuclear RNA polymerase assayed under similar conditions of low ionic strength (2). Bacterial RNA polymerases transcribe a highly repetitive DNA template (11,18,19). Fidelity of transcription by bacterial (and presumably mammalian) RNA polymerases is stringently controlled by many factors (such as ionic strength, "sigma" proteins, base sequences at

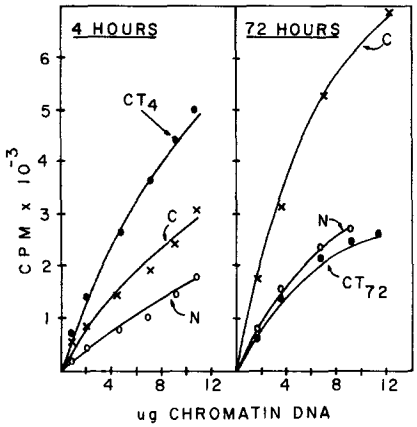

Figure 1. The incubation mixture (in a final volume of 0.25 ml) contained 10 µmoles of Tris, pH 8.0 (37°), 1 µmole $MgCl_2$, 0.25 µmole $MnCl_2$, 3 µmoles 2-mercaptoethanol, 0.1 µmole each of the 3 unlabelled ribonucleoside triphosphates, 1 µCi of the labelled nucleoside triphosphate (either ^{3}H-GTP, 5.6 Ci/mmoles, or ^{3}H-UTP, 21 Ci/mmoles), and amounts of chromatin and RNA polymerases specified (7). In some experiments the pool of GTP or UTP was increased by the addition of unlabelled compound. CT_4 chromatin (left hand panel) was incubated with 40 µg E. coli K-12 polymerase, CT_{72} chromatin with 20 µg.

operator/promoter sites, factors determining strand separation and strand selection, etc. {20}). When bacterial RNA polymerase is templated by mammalian chromatin, many of these restrictions should be absent.

The following view seems consistent with the available information. Steroid hormones trigger a massive nuclear uptake and binding of non-histone proteins in target organs that respond with marked hypertrophy (10). This is associated with profound reorganization of the nuclear chromatin, indicated by the increased binding of NHNP, and altered template activity. Much of the observed transcription mediated by added bacterial polymerase probably reflects the changing accessibility of previously unavailable template, albeit transcribed in a comparatively non-specific manner. It seems uncertain that the majority of this newly engaged template represents sites normally transcribed in quantity by mammalian polymerases (4,21,22). The hallmark of mammalian transcription must be its selective, asymmetric utilization of limited regions of the nuclear genome by at least 3 and probably 4 species of RNA polymerase (23), while transcription of mammalian template by prokaryotic

RNA polymerases should be more symmetric and less selective. Even if binding and/or initiation of heterologous RNA polymerase to chromatin does not occur at random, it must be less specific, than in the intact bacterium. The bacterial polymerase/mammalian chromatin system seems chiefly to represent a means of detecting the appearance of sites which are potentially available for transcription, but many of which may not (and are unlikely to) actually be so utilized in vivo. That this is indeed so is suggested when the transcription of chromatin by mammalian and bacterial RNA polymerases is compared (24,25).

In tissues exhibiting hormone-induced cellular proliferation, qualitatively "new" template must become engaged as "stem" cells are recruited for replication. But why should profound structural alterations, as reflected by the increased binding of NHNP and enhanced transcription with E. coli RNA polymerase, occur in chromatin from a tissue such as ventral prostate, in which limited cell proliferation occurs only after several days of hormone treatment (26)? It is possible that restricted regions of template not required for ongoing hormone-dependent function, but previously transcribed during the later maturation of the gland, became incapable of serving as transcription sites for endogenous RNA polymerase. The early events of hormone treatment may reflect changes in the conformational states of C chromatin that are necessary in order that templates for gene products required transiently during regrowth of regressed prostate cells can again become accessible to transcription by endogenous mammalian RNA polymerases.

Most of these sites are not in a form that can be immediately utilized by either mammalian or bacterial RNA polymerases. Chromatin must first undergo a series of transformations related to binding of non-histone "nuclear" proteins. Although transfer of some prostate cytoplasmic proteins to chromatin depends directly upon their association with androgen (17), once these initiating events occur, binding of most non-histone proteins to chromatin should not be intimately related to their physical association with testosterone or its metabolites. Thus, androgens induce a pleiotypic response in the prostate (27).

While many non-histone nuclear proteins are synthesized in the cytoplasm (28-31), there is evidence that some may be formed *in situ* (32,33).

The influx of proteins must include structural proteins needed to maintain chromatin in its new functional configurations (chromatin "conformers"), which permit the biochemical events of late maturation to be recapitulated. It seems most likely that at first these changes are not static, but dynamic and probably evanescent, as regressed prostate cells are altered from a repressed state to androgen-dependent function. Considerable synchronization of the events mediating cellular hypertrophy could be anticipated. Thus transcription of a series of chromatin "conformers" by heterologous bacterial RNA polymerases can be viewed as a means of "titrating" the altering accessibility of numerous template sites, many of which may well not be transcribed by mammalian RNA polymerases.

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