Genes Coding for Histone Proteins in Man Are Located on the Distal End of the Long Arm of Chromosome 7

Abstract. *Tritium-labeled complementary RNA's to two cloned sea urchin DNA sequences, one coding for histones H1, H2B, and H4 and the other for H2A and H3, were hybridized in situ to high resolution human chromosomes. Evidence is presented showing that the histone genes in man are localized in bands q32-36 on the long arm of chromosome 7.*

The technique of in situ hybridization provides a method for the localization of genes on chromosomes. In man, it has been successfully employed to show that messenger RNA (mRNA) transcribed from multiple copy genes shows sequence homology to DNA of Giemsa-stained negative bands (*1*), that 18*S* and 28*S* ribosomal RNA (rRNA) cistrons are located in the secondary constrictions of the short arms of chromosomes 13 to 15, 21, and 22 (*2*, *3*), and 5*S* rRNA is synthesized at the distal end of the long arm of chromosome 1 (1q42-43) (*4*). A major obstacle to the localization of other specific gene sequences has been the lack of probes of sufficient purity. With the development of techniques for cloning genes in bacterial plasmids and λ bacteriophages, probes free from other hybridizable sequences can now be obtained in large quantities and radioactively labeled to high specific activities (*5*).

The first DNA sequences successfully cloned include the histone genes of sea urchin. This success is due to ease in separation from bulk DNA by physical methods (*6*) and the availability of embryonic histone mRNA for identification of recombinant clones (*7*). In view of the evolutionary stability of these sequences, as evidenced by the ability of sea urchin mRNA to cross-hybridize with DNA from several eukaryotes including man (*8*), cloned sea urchin histone genes have been useful probes for molecular analysis in a number of heterologous species (*9*). In this work, ^{3}H-labeled complementary RNA's (cRNA's) to two histone-coding Eco RI restriction endonuclease fragments from *Strongylocentrotus purpuratus*, previously inserted in the *Escherichia coli* plasmid pSC101 (*10*), were hybridized in situ to human chromosome preparations. The results showed highly significant labeling on the distal end of the long arm of chromosome 7 (bands q32 to 36), suggesting localization of the histone genes in man to this chromosome segment.

The two fragments used represent adjacent segments in sea urchin native DNA and together comprise a tandemly repetitive 6.6-kbp (kilobase pairs) histone gene unit (*11*, *12*). One sequence, 4.6 kbp in length, contains the coding regions for H1, H2B, and H4; the other 2-kbp segment includes the H2A and H3 genes (*13*). In both fragments, the protein-coding regions are separated by spacer DNA's which are species-specific (*6*, *12*). Complementary RNA's to the two purified histone DNA fragments were synthesized with *E. coli* RNA polymerase, the reaction mixtures contained ^{3}H-labeled adenosine, cytidine, and uridine triphosphates (ATP, CTP, and UTP) (20 Ci/mmole each; New England Nuclear) which resulted in a specific activity for each probe of approximately 1×10^8 dis/min per microgram of RNA (*14*).

The in situ hybridization technique used (*13*) allows the simultaneous visualization of radioactive grains and G-banding patterns of high resolution chromosomes obtained by amethopterin synchronization of cultured lymphocytes (*15*). Slide preparations 1 to 5 days old were treated with ribonuclease A [100 μg/ml in double strength (2×) SSC; SSC is 0.15*M* NaCl and 0.015*M* sodium citrate] for 60 minutes at 37°C. Chromosomal DNA was denatured by immersion in 70 percent formamide (Eastman) in 2×SSC, *p*H 7.2, at 70°C for 2 minutes and dehydrated immediately by treatment with 70, 80, 90, and 100 percent ethanol. ^{3}H-Labeled cRNA (1 μg/ml) in 50 percent formamide in 2×SSC was applied to each slide, and hybridization was carried out at 37°C for 10 hours. The slide preparations were then placed in 50 percent formamide in 2×SSC at 39°C, rinsed extensively in 2×SSC at 39°C, and treated with ribonuclease (30 μg/ml) in 2×SSC for 60 minutes at 37°C. Slides were coated with Kodak NTB_2 emulsion (diluted 1:1), exposed at 4°C for 16 or 26 days under desiccating conditions, and treated with Kodak Dektol at 15°C for 2 minutes. The chromosome spreads were Giemsa-banded with Wright stain for 5 to 6 minutes, then destained and restained one or two times to improve the quality of G-banding patterns (*3*). Grain locations were recorded quantitatively to ascertain size, position, and staining intensity of every band of early mitotic chromosomes (*15*, *16*). Only grains lying on or touching a chromosome were considered in the analysis.

An early metaphase cell hybridized with ^{3}H-labeled cRNA to the H1, H2B, H4 probe (Fig. 1) illustrates the typical pattern of preferential labeling over the distal end of the long arm of chromosome 7 (arrow) while the rest of the cell shows low background radioactivity. Analyses of a large number of early metaphases (193) hybridized with cRNA to the H1, H2B, H4 genes and exposed for 16 days showed an average of 4.60 chromosomal grains and 4.56 cytoplasmic grains (Table 1). Of these cells, 20.7 percent were labeled on the distal end of the long arm (q32-36) of one chromosome 7 and 2.1 percent on both chromosomes 7, such that 5.4 percent of the total chromosomal grains were located within this segment (Fig. 1b, upper row). This segment represents only 0.97 percent of the total length of the chromosome complement and the label observed was found to be statistically significant ($\chi^2 = 182.27$; $P = .001$). Cells (143) hybridized to the same probe but exposed for a longer period of time (26 days) exhibited on the average 11.87 chromosomal grains and 10.56 cytoplasmic grains. A high proportion were labeled on 7q32-36 (30.1 and 3.5 percent on one and two chromosomes 7, respectively) (Table 1). This label represents 3.1 percent of the total chromosomal grains, which is again statistically significant ($\chi^2 = 81.91$; $P = .001$) when compared to that expected on the basis of percent of total chromosome length. In experiments in which cRNA to the H2A, H3 segment was hybridized, slides

Table 1. In situ hybridization of ^{3}H-labeled cRNA's of the sea urchin histone genes to human chromosomes.

Histone genes	Autoradiography (days)	Mitoses (No.)	Cells labeled 7q32-36		Average number of grains per cell	
			Number	Percent	Chromosomal	Cytoplasmic
H1, H2B, H4	16	193	44	22.9	4.60	4.56
	26	143	48	33.6	11.87	10.56
H2A, H3	26	145	40	27.6	8.90	5.83

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were exposed for 26 days. We analyzed 145 early metaphase cells; these showed an average of 8.90 chromosomal and 5.82 cytoplasmic grains, and 27.6 percent of these had one chromosome 7 labeled on q32-36 while none of the cells showed both chromosomes 7 labeled on this segment (Table 1). These results again correspond to 3.1 percent of all chromosomal grains being located in this region.

Chromosomal grains other than those on region 7q32-36 were evenly distributed over the rest of the chromosome complement except in a limited number of cells from certain experiments, where chromosomes segments 6p21-22, 13q22-32, and 19q13 exhibited a relatively high number of grains. On further analysis, however, this labeling was found not to be consistent with both probes and was insignificant in the total analysis from different experiments (χ^2, 0.79 to 2.02; P, .50 to .10). This contrasted with the data obtained for segment 7q32-36, which was repeatedly shown to be highly significant ($P = .001$).

The distribution of grains throughout chromosome 7 from 100 early metaphases hybridized under the experimental conditions described above (H2A, H3 probe, 26 days; H1, H2B, H4 probe, 16 and 26 days) is illustrated in Fig. 1c. With each probe, the segment corresponding to bands q32 to 36 contains most of the label, while the rest of the chromosome exhibits relatively few grains scattered throughout. Because each of the bands of 7q32-36 measures from 0.22 to 0.60 μm in early metaphase (Fig. 1c) and the resolution of tritium is also in this range (*17*), no attempt was made to analyze for preferential labeling within these bands.

Since highly elongated late prophase chromosomes have, on the average, two times the length of early metaphase chromosomes (*15*), a preliminary analysis was made on 25 prophases found, in the course of this study, to be labeled at the distal end of the long arm of chromosome 7. We observed 81.5 percent of the grains on the Giemsa-negative bands q32, 34, and 36 (seven, six, and nine grains, respectively) and 18.5 percent on the G-positive bands q33-35 (three and two grains, respectively) (Fig. 1b, lower row). Although the results suggest preferential labeling of Giemsa-negative bands, further study of a large number of these more rarely observed cells is needed.

Our observation that ^{3}H-labeled cRNA's to two nonoverlapping DNA histone segments result in significant label on the same chromosome region suggests that hybridization is specific for the human histone genes, since the intervening spacer sequences of sea urchin do not cross-hybridize with each other (*11*).

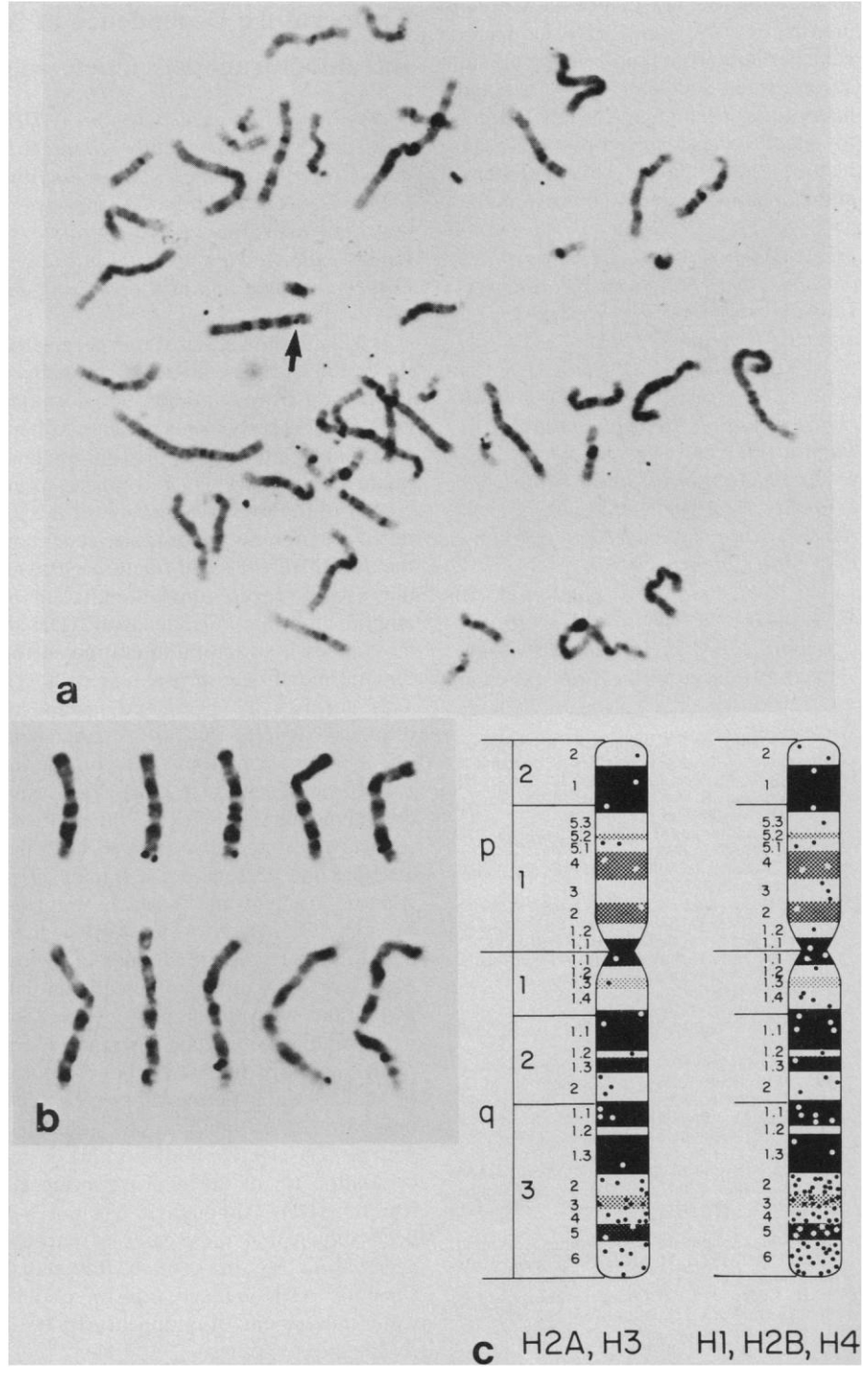

Fig. 1. (a) Human early metaphase cell hybridized in situ with ^{3}H-labeled cRNA (milligrams per milliliter) to sea urchin histone genes H1, H2B, and H4, exposed to autoradiographic emulsion for 16 days, and G-banded, showing label on the distal end of the long arm of one chromosome 7 (arrow). (b) Selected G-banded human early metaphase (upper row) and late prophase (lower row) chromosomes 7 showing preferential hybridization of ^{3}H-labeled cRNA to sea urchin H1, H2B, and H4 histone DNA to the distal end of the long arm (7q32-36). (c) Schematic representation of an early metaphase chromosome 7. (Left) Distribution of label of 100 early metaphases hybridized in situ with ^{3}H-labeled cRNA to sea urchin histone genes H2A and H3 and exposed for 26 days; (right) 200 early metaphases hybridized with ^{3}H-labeled cRNA to H1, H2B, and H4, with half exposed for 16 days and half for 26 days. Bands 7q32, 33, 34, 35, and 36 have an average length of 0.35, 0.22, 0.24, 0.27, and 0.60 μm, respectively. Complementary RNA to *Escherichia coli* was used as control and showed no labeling on 7q32-36.

These results are also supported by the recent identification of chromosome 7 in man as the preferential hybridization site of ^{125}I-labeled human H4 mRNA (*18*). Moreover, these data imply that the genes for more than one of the five major histones are clustered in one chromosomal region. This is not surprising since in both sea urchin and *Drosophila* the five histone genes are arranged in a tandemly arrayed repeat unit (*11*, *12*, *19*); in *D. melanogaster* histone genes are located in region 39D-E on chromosome 2 (*19*, *20*); and hybridization of histone mRNA with restriction endonuclease-cleaved DNA human placenta suggested clustering of the genes in a unit of at least 10×10^6 daltons (*21*). Since there are approximately 40 copies of each histone gene per human haploid genome (*22*) and chromosome segment 7q32-36 contains many times the amount of DNA needed to encode these sequences (*23*), nonhistone DNA may be interspersed among histone-coding regions in man.

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23. If each human histone linear repeat unit is 10×10^6 daltons, and the unit is repeated 40 times per haploid genome (*22*), approximately 4×10^8 daltons of DNA would be needed for these sequences. Since the human haploid genome contains 1.8×10^{12} daltons of DNA [B. Lewin, *Gene Expression* (Wiley, New York, 1974), vol. 2, p. 7] and segment 7q32-36 comprises 0.97 percent of the total chromosome complement length, 1.75×10^{10} daltons of DNA may be contained in 7q32-36, of which up to 97 percent may be nonhistone sequences.
24. Supported by NIH grant GM 26800 and National Foundation grant 1-568 (to J.J.Y.) and by NIH grant GM-17995 and a grant from the American Cancer Society (to L.H.K.). Correspondence should be addressed to J.J.Y.

22 February 1979; revised 11 May 1979

Temperature Dependence of ADH-Induced Water Flow and Intramembranous Particle Aggregates in Toad Bladder

Abstract. *Antidiuretic hormone (ADH)-induced luminal intramembranous particle aggregates and hormonally stimulated water flow in toad urinary bladder are reduced simultaneously with a reduction in temperature. When water movement is factored by the aggregation response, the apparent activation energy for this process decreases from 12.1 ± 1.6 to 3.0 ± 2.3 kilocalories per mole. The data are consistent with the view that the particle aggregates contain sites for transmembrane water movement and that these sites behave as pores.*

It is well known that water permeability of toad urinary bladder is enhanced in response to antidiuretic hormone (ADH). This response is mediated by intracellular adenosine 3′,5′-monophosphate (cyclic AMP) (*1*) and appears principally to involve an alteration of the luminal membrane of granular-type epithelial cells (*2*). Freeze-fracture electron microscopy reveals that stimulation of amphibian urinary bladder with ADH also induces a structural alteration in the luminal membrane of granular cells (*3*). This consists of aggregated intramembranous particles that are organized in linear arrays at multiple sites on the inner (protoplasmic) fracture face and complementary grooves on the apposed (exoplasmic) face. It occurs in both the absence and presence of a transbladder osmotic gradient and it can be induced by stimulation with cyclic AMP (*4*). In quantitative terms the number of aggregates per area of granular cell luminal membrane and the cumulative area of membrane occupied by aggregates are related linearly to ADH-induced levels of bladder water permeability (*3*, *4*) and both are either inhibited or enhanced with either selective inhibition (*5*) or accentuation (*6*) of the hydro-osmotic effect of ADH. Although it has not yet been shown that these sites of particle aggregation are or contain the actual sites for ADH-induced transmembrane water movement, this appears to be a reasonable hypothesis.

We studied this hypothesis by assessing the dependence of the aggregation and water permeability responses to ADH on temperature. In addition, by normalizing ADH-stimulated water movement for the effect of temperature on membrane morphology, which until now had not been fully realized, and by calculating the activation energy (E_A) for this process from the Arrhenius equation, we considered the question of whether ADH-stimulated water permeation of the luminal membrane of the granular cell involves aqueous channels ("pores") or a solubility-diffusion process.

Two series of experiments were performed. In the first, paired hemibladders ($N = 6$) from large female Dominican toads (*Bufo marinus*) were prepared as sacs on the ends of glass tubes. All bladders were washed inside (mucosal) and out (serosal) with Ringer solution (111 m*M* NaCl, 3.5 m*M* KCl, 2.5 m*M* $NaHCO_3$, 1.0 m*M* $CaCl_2$, *p*H 7.6 to 8.2, 220 mOsm per kilogram of H_2O), then filled with Ringer solution diluted 1:5 with distilled water and suspended in an aerated Ringer bath. Bladders were permitted to stabilize for 30 minutes while transbladder electrical potential (PD) was monitored with calomel electrodes and a Keithley electrometer (610 C). If the PD was less than 20 mV at the end of this period for either hemibladder of a pair, the experiment was terminated. Otherwise, the mucosal contents of both

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