tured at 55°C on soil samples collected from the Sudan; some cultures exhibit obvious antibiotic behavior.

Tetracycline is effective against both Gram-negative and Gram-positive bacteria, Rickettsiae, spirochetes, and some viruses (*23, 24*). It also has an antimalarial effect on humans (*25*). While it may not have been intended as a therapeutic substance, its consumption by the Nubians would have had broad implications for their health, disease, and demographic patterns and for the evolution of R factors (*26*) within that population. It may explain the extremely low rates of infectious disease found among the X group.

The side effects of tetracycline include temporary inhibition of bone growth in infants (*25*), vitamin B depletion (*24*), and interference with phagocytic activity (*24*) and with protein synthesis (*24, 27*). Extended exposure to therapeutic dosages of tetracycline inhibits spermatogenesis; smaller dosages slow sperm mobility (*28*). The bones of the X group have not yet been studied with a view toward determining dosages, but the amount of fluorescence suggests therapeutic levels.

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Nucleosome Cores Have Two Specific Binding Sites for Nonhistone Chromosomal Proteins HMG 14 and HMG 17

Abstract. *The binding of HMG 14 (or 17) to nucleosome cores produces two major additional bands on nondenaturing polyacrylamide gels. Bound HMG 14 alters the relative densities of the end-labeled DNA fragment distribution produced by deoxyribonuclease I digestion of reconstructed poly(deoxyadenylate-deoxythymidylate) nucleosome cores. These results indicate nucleosome cores have two specific binding sites for HMG 14 (or 17).*

The nucleosome core particle, consisting of 146 base pairs of DNA (*1, 2*) and two each of the inner histones, is found throughout eukaryotes and is firmly established as constituting the first level of DNA packaging in chromatin (*3*). The high mobility group (HMG) proteins are thought to be structural proteins associated with active, or potentially active, regions of chromatin (*4*).

Various solvents can induce different nucleosome conformations (*5*). How these various states relate to nucleosome conformations in vivo is unknown. Different conformations of the nucleosome core may be used by the cell to regulate transcription or replication. DNA sequences that are capable of being transcribed are sensitive to digestion by deoxyribonuclease I (*6*). Extraction of chromatin or nucleosomes with 0.35 or 0.4*M* NaCl (salt concentrations that extract HMG proteins) destroys the sensitivity to deoxyribonuclease I, and adding back HMG 14 or 17 restores it (*7*); this indicates that HMG 14 and 17 may bind to chromatin and induce functionally significant structural changes. To investigate this possibility, we have formed complexes between nucleosome cores and HMG 14 and 17.

When nucleosome cores were titrated with HMG 14 and subjected to electrophoresis on a low-ionic strength native particle gel, the presence of bound HMG 14 produced a new set of bands with mobilities lower than that of the nucleosome core band (*8*) (Fig. 1). Two of the new bands (labeled 1 and 2 in Fig. 1) are as well defined as the original nucleosome core band (band 0). The other new bands (3, 4, 5, and 6 in Fig. 1) appear diffuse above a high background. The two types of bands representing complexes between nucleosome cores and HMG 14 indicate that there are two different modes of binding of HMG 14 to the nucleosome core. The primary, higher affinity mode gives rise to bands 1 and 2 and accounts for most of the binding when HMG 14 is added at a stoichiometry of up to two molecules per nucle-

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osome. The secondary, lower affinity mode gives rise to bands 3 to 6 and becomes significant only at stoichiometries greater than two HMG 14 molecules per nucleosome as the sites representing the primary mode of binding reach saturation.

The regular progression of lower mobility bands in Fig. 1 is highly suggestive of discrete changes in mobility resulting from incremental additions of mass or positive charge. Each successive lower mobility band could be due either to the binding of a single additional HMG 14 molecule or to the highly cooperative binding of two (or more) HMG molecules. A plot of the number of HMG molecules bound per nucleosome against the number added per nucleosome, based on quantitative densitometry, has ruled out the latter possibility. The assumption that each shift in mobility was due to the additional binding of one HMG molecule (Fig. 1) resulted in a least-squares fit of a line passing through the origin with a slope of 0.90 ± 0.05. Although this procedure allowed us to determine the number of HMG 14 molecules binding per nucleosome in each band, an accurate measure of a binding constant for either the primary or secondary mode of binding as well as the degree of cooperativity of the binding must await further work.

Chicken erythrocytes contain, in addition to HMG 14 and 17, three high-molecular-weight HMG proteins, HMG 1, 2a, and 2b (*9*). Complexes were formed between nucleosome cores and a mixture of HMG 1, 2a, and 2b (*10*). Particle gel electrophoresis of these complexes showed no additional well-defined bands similar to bands 1 and 2 in Fig. 1. Some material was present in the lower mobility region of the gel as two or three diffuse bands against a high background. These bands were similar in appearance to the bands labeled 3 to 6 in Fig. 1, which are indicative of the secondary, lower affinity mode of HMG 14 binding to nucleosome cores. The secondary mode of binding could result from nonspecific ionic interactions between the highly charged HMG proteins and the nucleosome core.

Electrophoretic patterns observed when nucleosome cores were titrated with HMG 14 and 17 together in a 1:1 ratio were similar to those observed with HMG 14 alone; that is, only two major additional bands, and not four, which would be predicted for independent binding, were observed. The pattern was what would be expected if HMG 14 and 17 bind to nucleosome cores interchangeably. The fact that HMG 14 and 17 do not bind independently of each other and appear to substitute for each other in titrating nucleosome cores suggests that they bind to the same two primary, high-affinity binding sites on the nucleosome core. This result is not surprising, since there is a high degree of homology between HMG 14 and 17 in the presumptive DNA binding regions of the two sequences (*11, 12*).

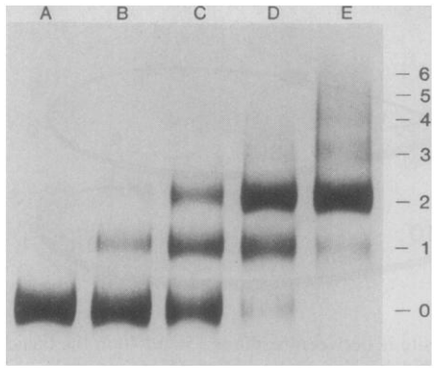

Fig. 1. Titration of nucleosome cores with HMG 14. Molar ratios of HMG 14 to nucleosome cores for tracks A to E are 0, 0.44, 0.87, 1.74, and 2.62. Numbers to the right indicate the number of HMG 14 molecules bound per nucleosome core. Nucleosome cores from chicken erythrocytes were prepared as described in (*14*), and HMG 14 from chicken erythrocytes was prepared as described in (*9*), with minor variations (*10*). The concentration of a stock solution of HMG 14 in water was determined by amino acid analysis, a molecular weight of 10,400 being assumed (*11*). The concentration of nucleosome cores was determined with an absorbance A_{260} = 20 for a DNA concentration of 1 mg/ml. Electrophoresis has been described in (*16*). Varying amounts of HMG 14 were added to cores in the presence of 0.35*M* NaCl and then diluted to 87.5 m*M* NaCl as follows. A volume of HMG 14 in 0.7*M* NaCl, 20 m*M* tris, *p*H 7.0, was added to an equal volume of nucleosome cores in 0.25 m*M* EDTA, *p*H 7.0. Samples were then diluted twofold with water and another twofold with 0.2-strength tris, borate, and EDTA (*16*), 10 percent glycerol, 0.05 percent bromophenol blue and placed directly on the gel. The gel was stained with Coomassie blue. For quantitation by densitometry it was assumed that HMG 14 stained with an intensity equal, by weight, to that of the histones.

Nucleosome cores made from reconstructed complexes of inner histones and poly(deoxyadenylate-deoxythymidylate) [poly(dA-dT)] share many structural features with native nucleosome cores (*2, 13*). Complexes formed between reconstructed poly(dA-dT) nucleosome cores and HMG 14 give the same native particle gel patterns as complexes made with native nucleosome cores. The presence of bound HMG 14 does not greatly alter the overall rate of digestion by deoxyribonuclease I (Fig. 2). The relative peak heights are altered by the presence of HMG 14; the heights of the peaks corresponding to cutting sites 4, 5, 9, and 10 are increased, whereas those corre-

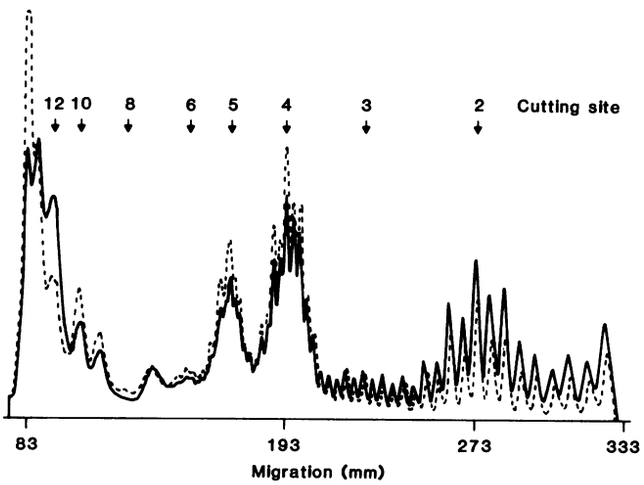

Fig. 2. Deoxribonuclease I digestion of reconstructed nucleosome cores, end-labeled with ^{32}P, complexed (- - -) with and (——) without HMG 14. Nucleosome cores, containing poly(dA-dT)·poly(dA-dT) and the four inner histones from chicken erythrocyte nuclei, were reconstructed as described by Bryan *et al.* (*2*). A concentrated solution of HMG 14 in water was added to reconstructed nucleosome cores (2.63 HMG 14 molecules per nucleosome core) in 0.4*M* NaCl, 10 m*M* tris, *p*H 7.0, and dialyzed against 0.2 m*M* EDTA, 10 m*M* tris, *p*H 7.0. Reconstructed nucleosome cores with and without bound HMG 14 were labeled at the 5′ end in the following reaction: 0.32-nmole samples were reacted with 50 m*M* tris, *p*H 7.0, 5 m*M* $MgCl_2$, 4 m*M* dithiothreitol, 0.2 nmole of ^{32}P-labeled adenosine triphosphate (2000 to 3000 Ci/mmole; New England Nuclear), and polynucleotide kinase (20 unit/ml; Miles) at 37°C for 1 hour, then chilled at 0° to 4°C. The samples were digested with deoxyribonuclease I (1 unit/ml; Worthington) for 1 minute at 20°C, and the reaction was stopped with 5 m*M* EDTA at 0° to 4°C; samples were heated to 100°C for 1 minute, then digested with proteinase K (10 μg/ml; Merck) for 1 hour at 37°C. After being heated to 100°C for 1 minute, the samples were subjected to electrophoresis on 43-cm 12 percent polyacrylamide gels (*17*). Autoradiograms were prepared at −20°C for 12 hours with Kodak XR-5 film. Scans were normalized to represent equal amounts of radioactivity in each gel track.

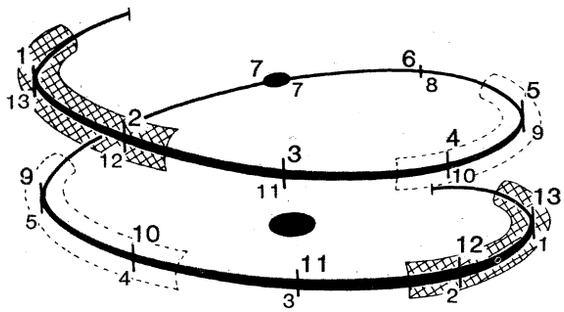

Fig. 3. Mapping of sites of altered deoxyribonuclease I accessibility onto a DNA superhelix with a pitch of 80 base pairs and model of HMG 14 binding. Nucleolysis is inhibited at cutting sites 1, 2, 12, and 13 (hatched areas) and enhanced at sites 4, 5, 6, 9, and 10 (dashed outlines) when HMG 14 is bound to reconstructed poly(dA-dT) nucleosome cores. HMG 14 has a basic NH_2-terminal portion and an acidic COOH-terminal portion (*11*). The DNA binding site is between residues 15 and 40 in the basic region of the molecule (*12*). In our model, the DNA-binding portion of one HMG 14 molecule is bound to the nucleosomal DNA at sites 1 and 2 (sites 13 and 12 on the complementary strand) and protects this region from nucleolysis. The acidic portion of the molecule is bound to the histones in the groove of the superhelix, partially neutralizing their positive charges and rendering the adjacent turn of the superhelix more accessible to deoxyribonuclease I. This would account for the enhanced digestion at sites 9 and 10 (sites 5 and 4 on the complementary strand). If one assumes the existence of a dyad axis in the nucleosome core particle, there is a symmetry-related locus at which HMG 14 binds to sites 12 and 13 (2 and 1 on the complementary strand) and loosens histone attachments to sites 4 and 5 (10 and 9 on the complementary strand).

sponding to sites 1, 2, and 12 are decreased, and others are essentially unchanged. In an end-labeling experiment, however, the frequency of cutting is not directly proportional to peak height because, for all strands cut at a given site, those also cut at another site closer to the 5′ end are effectively unlabeled and removed from the analysis. However, if the assumption is made that cutting at one site does not affect cutting at another site, the true cutting frequency can be determined by dividing the density at a given site by that density plus the densities at all sites farther from the 5′ end, as described by Lutter (*14*). When the results in Fig. 2 were analyzed in this way, we confirmed that the effect of HMG 14 binding on the frequency of cutting at each site agrees qualitatively with the direct comparison of peak heights with one exception; the presence of bound HMG 14 also reduced the frequency of cutting at site 13. Thus, digestion by deoxyribonuclease I is inhibited at sites 1, 2, 12, and 13 and enhanced at sites 4, 5, 9, and 10. These results, when combined with the model of Finch *et al.* (*15*), suggest an interesting model of HMG 14 and 17 binding to nucleosome core particles (Fig. 3).

From Fig. 2 and our analysis it is clear that the effect of bound HMG 14 depends on the location of the nucleolytic site along the length of the DNA. This in turn implies that HMG 14 binding is nonrandom with respect to location on the nucleosome surface, a finding that, together with the particle gel results presented above, supports the view that there are two specific binding sites on the nucleosome core which can accept either HMG 14 or 17.

Earlier evidence indicates that the ends of the DNA on the nucleosome core exist in an altered conformation compared to the central region of DNA (*2*). It is possible that the existence of two primary, high-affinity binding sites on the nucleosome core may be a direct result of this difference.

It has been reported that active DNA sequences are as much as two orders of magnitude more sensitive to deoxyribonuclease I digestion than inactive sequences are (*6*) and that HMG 14 and 17 are necessary for maintaining this sensitivity (*7*). It is clear from Fig. 2 that bound HMG 14 does not confer an increased sensitivity upon bulk-reconstructed poly(dA-dT) nucleosomes that is characteristic of active regions of chromatin. However, our experiments are insensitive to small subfractions of reconstructed nucleosomes—those containing more highly acelyated histones, for example—that may attain a greatly increased sensitivity to deoxyribonuclease I upon complexing with HMG 14. The relationship between the binding sites observed in our experiments and the functionally significant binding that occurs in vivo requires further investigation.

Our findings may be summarized as follows. (i) Nucleosome cores bind two HMG 14 molecules with high affinity. (ii) The same is true for HMG 17. (iii) HMG 14 and 17 do not bind independently of each other and most likely bind to the same two sites. (iv) The binding of HMG 14 to reconstructed poly(dA-dT) nucleosome cores alters the relative densities of the single-strand, end-labeled DNA fragment distribution, indicating a nonrandom phasing of binding along the length of the DNA. (v) The above findings support the view that two specific binding sites on the nucleosome core accept either HMG 14 or HMG 17.

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