presumed or suggested functions of the unwinding proteins in *E. coli* throw any light on the specific role which the U-protein might play in meiotic cells of lily and whether the distinctive properties of the lily U-protein point in any particular direction with respect to function. The U-protein II of *E. coli*, or "rep" protein, has been assigned a role in ΦX-174 replication (Scott et al., 1977), but we doubt that a direct extrapolation of a replicative role from phage to lily meiocytes is warranted. We have already noted in the introductory section that the lily U-protein is most prominent during the interval of pairing and crossing-over and not during the interval of chromosome replication. To our knowledge, there are no other established functions of these bacterial U-proteins that would point to a specific role for the lily U-protein. Nor can any specific role be identified on the basis of the particular properties described, unless that role is defined in the general sense of unwinding being a required function for effecting recombination between DNA duplexes. There remains, of course, the still unexamined molecular issue of how the lily U-protein, which can unwind pure DNA in vitro, accomplishes unwinding in the context of nucleosome organization.

Acknowledgment

We are grateful to Dr. Morris Friedkin and Dr. E. Peter Geiduschek for their very helpful reading of the manuscript and for their many incisive suggestions.

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Asymmetry of Chromatin Subunits Probed with Histone H1 in an H1–DNA Complex†

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ABSTRACT: Treatment of nucleosomes with a low concentration of sodium dodecyl sulfate removed all proteins except histone H1 from DNA, thus confirming our previous observation on sheared chromatin. No redistribution of H1 occurred during this procedure for isolation of the H1–DNA complex. The H1–DNA complex was isolated from a nucleosome monomer, doubly labeled in its protein and DNA and fractionated according to the length of DNA, and then the distribution of H1 was analyzed quantitatively. The results indicated that the monomer consisted of two subspecies, one containing 160 base pairs of DNA and one molecule of H1, and the other containing 140 base pairs of DNA and no H1. Since no monomer with two molecules of H1 was found, it is concluded that the nucleosome core has a binding site for H1 on only one side, and thus that the nucleosome is not a dyad.

Eukaryotic chromatins are all composed of a linear array of nucleosomes (υ bodies or chromatin subunits), like a "string of pearls" (Olins & Olins, 1974; Felsenfeld, 1975). These nucleosomes consist of an octamer of histone molecules (two molecules each of H2A, H2B, H3, and H4) and 140 base pairs of DNA.

Histone H1 is found in stoichiometric amount in chromatin and it is thought to be one of the building blocks of chromatin. H1 is believed to be located at the linker DNA, because the nucleosome core does not contain this histone (Varshavsky et al., 1976; Whitlock & Simpson 1976; Shaw et al., 1976; Noll

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0006-2960/78/0417-1880$01.00/0

& Kornberg, 1977). However, it is uncertain in which part of the linker the H1 is located. If we simply assume that H1 is located in the linker region, three kinds of monomer should be produced by nuclease attack, namely, monomers with decreasing lengths of DNA having two, one, and no H1. Previous reports have shown that the monomer can be separated into two subspecies, one having H1 and longer DNA, the other having no H1 and shorter DNA. However, it seemed possible that the former subspecies might be a mixture of two further subspecies, that is, monomer with two molecules of H1 and monomer with one molecule of H1, but this could not be tested because of the difficulty of measuring the amount of H1 or DNA quantitatively in stained gel after electrophoresis. Here, we prepared chromatin from cultured cells which were doubly labeled in their protein and DNA, and then isolated nucleosome monomer. The H1-DNA complex was isolated by the technique we developed previously (Hayashi, 1975), in which the complex is also fractionated according to the length of DNA. Quantitative analyses of H1 and DNA in each fraction revealed that there was no monomer having two H1 molecules. We, therefore, concluded that a binding site for H1 is present on only one side of the nucleosome. This is the first evidence against the widely accepted idea that the nucleosome is a dyad (Weintraub et al., 1976; Finch et al., 1977).

Experimental Procedure

Cell Culture and Isotope Labeling. L-5178Y cells (derived from mouse lymphocytic leukemia cells) were grown in Fisher's medium (Nissui) supplemented with 10% horse serum (Flow Lab). They were harvested in the late log stage of growth at a cell density of 1×10^6 cells/mL. Cells were labeled for 20 h with [^{14}C]thymidine (RCC, 60 Ci/mol, 0.01 μCi/mL) and/or L-[^{3}H]lysine (RCC, 33 Ci/mmol, 1μCi/mL). For experiments on isotope redistribution, cells were labeled with fivefold higher concentrations of these isotopes.

Chromatin Subunit Preparation. Cells were washed three times with 0.9% NaCl, 3 mM $CaCl_2$, 10 mM Na-Hepes (pH 7.2), and nuclei were prepared by homogenizing the cells in a Dounce type glass-Teflon homogenizer with 1% Triton X-100, 3 mM $CaCl_2$, 10 mM Na-Hepes[1] (pH 7.2), 1 mM $PhCH_2SO_2F$, 1 mM iodoacetic acid, 1% propanol and suspended in 0.25 M sucrose, 0.4 mM $CaCl_2$, 0.2 mM EDTA, 10 mM Tris-HCl (pH 8.5) at a concentration of 10^8 nuclei/mL. The protein to DNA ratio of these nuclei was approximately 3. The nuclei were digested with staphylococcal nuclease (Worthington), and the digestion was terminated by adding 0.2 M Na-EDTA (pH 8.0) to a final concentration of 5 mM and cooling the mixture in ice. Chromatin consisting of monomer to oligomer was obtained by digestion with 40 units of the nuclease per 10^8 nuclei, for 2 min at 0 °C and then for 3 min at 37 °C (mild digestion). Polymer-sized chromatin was prepared by digestion with 10 units of the nuclease per 10^8 nuclei for 2 min at 0 °C and then for 30 s at 37 °C (slight digestion). The digested chromatin was then dialyzed against 0.2 mM EDTA (pH 8.0) overnight and insoluble materials were removed by centrifugation at 3000 rpm for 10 min.

Monomer, dimer, and trimer were separated by centrifugation in a linear gradient of 5 to 20% sucrose, buffered with 10 mM Tris-HCl (pH 9), 0.2 mM EDTA, at 25 000 rpm for 24 h at 2 °C in an RPS-25 rotor (Hitachi).

H1-DNA Preparation. H1-DNA was prepared as described previously (Hayashi, 1975) with the following improvements. A column fitted with a water-jacket (Pharmacia K16-100) was filled with Sephadex G-25 to a height of 20 cm from the bottom, and the rest of the column was filled with agarose beads (Bio-Gel A-50m, 50–100 mesh). All following operations were carried out at a temperature of 20 °C. Equilibration and elution were done at an upward flow rate of 5 mL per h, and fractions of 2 mL were collected. The buffer used contained 1 mM Tris-HCl (pH 8.5), 0.2 mM EDTA, 0.1 mM $PhCH_2SO_2F$, 0.1 mM iodoacetic acid, 0.1% propanol, and $NaDodSO_4$ as specified. The column was first washed with the buffer, and then about one-third of the column (60 mL) from the bottom was equilibrated with the same buffer containing 0.053% $NaDodSO_4$. The sample was then applied and the column was eluted with buffer containing $NaDodSO_4$.

Electrophoresis. DNA was analyzed in a slab gel ($0.6 \times 12 \times 18$ cm) of 3.5% or 5% acrylamide using the system of Maniatis et al. (1975) with modifications. The buffer used contained 0.1% $NaDodSO_4$ and electrophoresis was done at 200 V. Gels were stained with 5 μg/mL of ethidium bromide. Phage fd DNA, digested with Hap (a gift from Dr. T. Takanami, Kyoto University), served as a molecular weight marker. Protein was subjected to electrophoresis in cylindrical 12.5% gel (0.5×8 cm) as described previously (Hayashi et al., 1974).

Measurement of Radioactivity. The radioactivity of samples was measured in Aquasol-2 (NENC). After electrophoresis gels were cut up, and the sections were dissolved in 30% H_2O_2, mixed with Aquasol-2, and counted.

Results

Redistribution Experiments. Doubly labeled monomeric nucleosomes of high specific activity (see Experimental Procedure) were isolated by sucrose gradient centrifugation. A portion (0.5 A_{260} unit) of the preparation was mixed with ten times as much (5 A_{260} units) unlabeled polymer-sized chromatin, and the H1-DNA complex was prepared by gel filtration in 0.053% $NaDodSO_4$. As seen in Figure 1a, no ^{3}H radioactivity was found in the polymer fraction, and the overall elution profile of radioactivity was identical with that obtained when labeled monomer alone was applied to the column (Figure 1b). When a mixture of labeled monomer and unlabeled polymer was adjusted to 0.3 M NaCl and then dialyzed against 1 mM Tris-HCl, 0.2 mM EDTA (pH 8.5) and applied to the column, a peak of ^{3}H radioactivity, associated with polymer-sized DNA, and therefore a peak of UV absorbance, was found, obviously as the result of redistribution (Figure 1c). These observations all unambiguously prove that, under our conditions for H1-DNA preparation, no redistribution of H1 occurs.

Analysis of Monomer-Sized H1-DNA. Monomer was prepared by sucrose gradient centrifugation and subjected to gel filtration through 0.053% $NaDodSO_4$, and the fractions were examined by electrophoresis of DNA and protein (Figures 2a and 2b, respectively).

Two bands of DNA are seen in Figure 2a, the longer one, having about 160 base pairs, eluted with a peak at fraction 49, and the shorter one with 140 base pairs forming a peak between fractions 52 and 55. Figure 2b shows that the distribution of H1 in the fractions is identical with that of the longer DNA. Table I shows this fact more clearly. The ratio of ^{3}H radioactivity in H1 to ^{14}C radioactivity in the longer DNA is constant throughout the fractions. Thus the longer DNA was carrying a constant amount of H1, while the shorter one was not. In separate experiments the monomer and polymer, labeled with [^{3}H]lysine and [^{14}C]thymidine, were isolated and examined

[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-2-hydroxypiperazine-*N'*-2-ethanesulfonic acid.

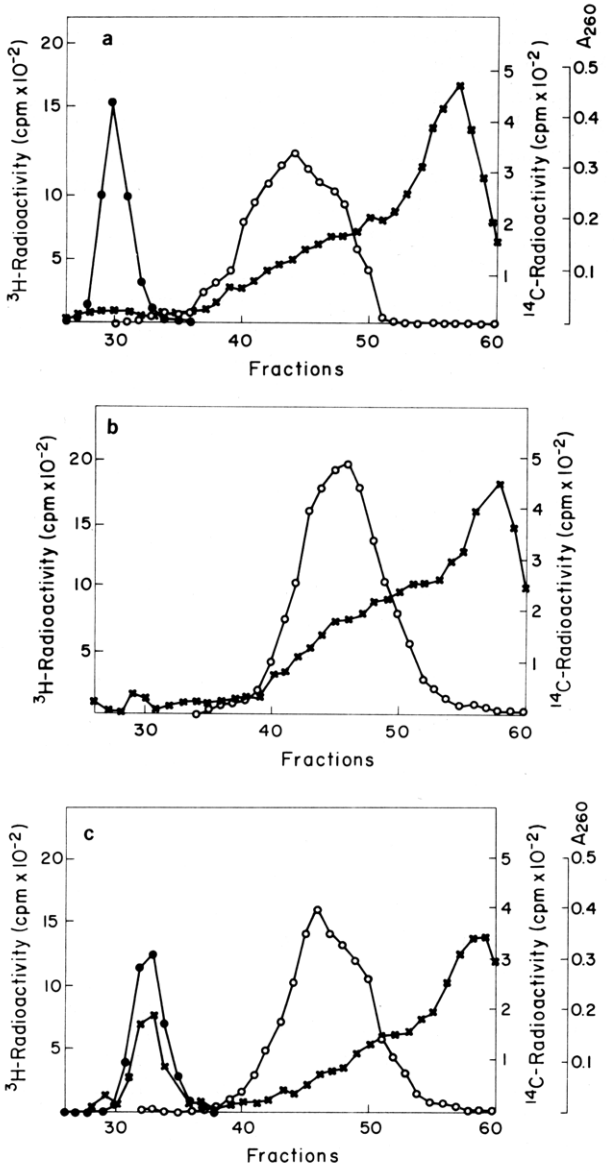

FIGURE 1: $NaDodSO_4$ gel filtration of a mixture of polymer and monomer. Monomer, labeled with L-[^{3}H]lysine and [^{14}C]thymidine, was mixed with ten times as much unlabeled polymer and subjected to $NaDodSO_4$ gel filtration (a). The monomer alone was subjected to $NaDodSO_4$ gel filtration (b). The mixture was first mixed with a final concentration of 0.3 M NaCl, dialyzed to remove the salt, and then analyzed by $NaDodSO_4$ gel filtration (c). (○)^{14}C; (X) ^{3}H; ● A_{260}.

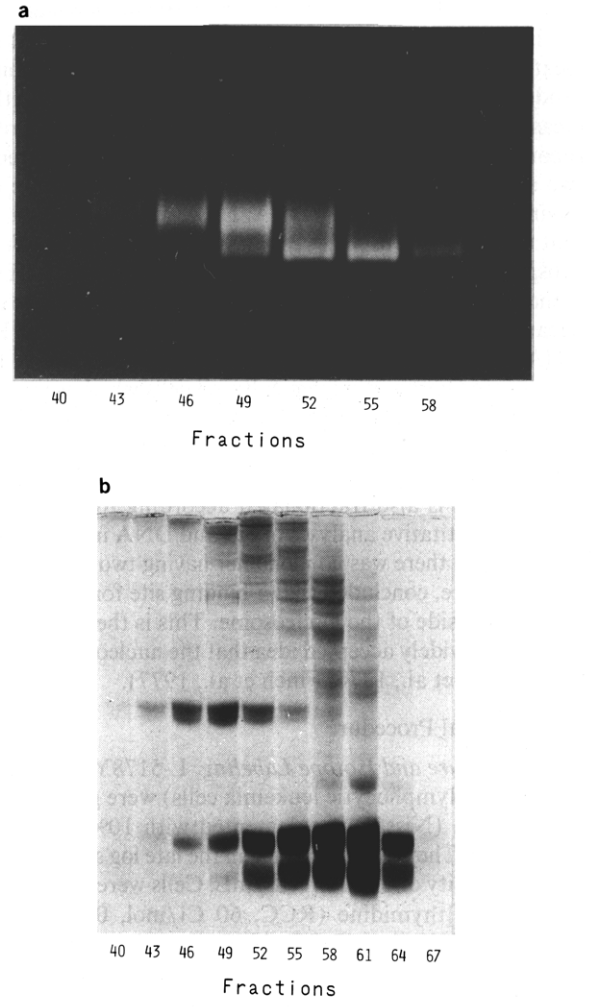

FIGURE 2: Electrophoretic analyses of fractions from the $NaDodSO_4$ gel filtration of the monomer. Monomer was subjected to $NaDodSO_4$ gel filtration and the fractions indicated were analyzed by electrophoresis for DNA (a) and protein (b).

by electrophoresis of DNA and of protein. The ^{3}H radioactivity in H1 (H_1) and in other histones (H_n) and the ^{14}C radioactivity of longer (C_l) and shorter (C_s) DNA were counted. The number of H1 molecules per longer DNA (N) was calculated by the following equation

$$\frac{H_1(\mathrm{m})}{H_n(\mathrm{m})}\frac{C_s + C_l}{C_l} = N\frac{H_1(\mathrm{P})}{H_n(\mathrm{P})}$$

where m and p stand for monomer and polymer, respectively. The above equation takes into account the fact that, on an average, one molecule of H1 is found per octameric histone. It also assumes that monomers with longer and shorter DNAs both contain the same amount of octameric histones. H_1, H_n, C_s, and C_l were determined in three independent experiments and all gave a value for N of close to 1. Therefore, it was concluded that there was one molecule of H1 per nucleosome containing DNA with 160 base pairs.

TABLE I: Association of H1 with Long DNA from Monomer.

Fraction (× 10^3 cpm)	^{3}H radioact. in H1[a]	^{14}C radioact. in long DNA[b]	Ratio ^{3}H/^{14}C
43	2.1	2.4	0.86
46	6.3	7.5	0.85
49	9.0	10.4	0.86
52	5.9	7.2	0.82
55	2.8	3.1	0.91

[a]Bands of H1 in the gels shown in Figure 2b were cut out and counted to measure the ^{3}H radioactivity in H1. [b]The ratio of long to short DNA in fractions 43 to 55 was determined by cutting out each band shown in Figure 2a. The ^{14}C radioactivity in long DNA was calculated from this ratio and the total ^{14}C radioactivity in each fraction.

This calculation, together with the results in Table I, clearly shows that no monomer has two or more molecules of H1.

Discussion

We have previously shown (Hayashi, 1975) that the H1–DNA complex can be prepared by gel filtration of sheared chromatin in a low concentration of $NaDodSO_4$. This report demonstrates that the technique was also effective for native unsheared chromatin (Noll et al., 1975). As seen in Figure 1, no redistribution of H1 occurs during isolation of the H1–DNA

complex. The finding is not consistent with that of Renz and Day who used the membrane filter assay technique and observed rapid redistribution of H1 even at low ionic strength. Possible alteration in the properties of H1 by binding to nitrocellulose may explain this discrepancy.

Using $NaDodSO_4$ gel filtration we found that monomeric nucleosomes consisted of a mixture of two species, one having one H1 and 160 base pairs of DNA, and the other having no H1 and 140 base pairs of DNA. These data clearly indicate that H1 is bound to the linker region of DNA consisting of about 20 base pairs. Several groups (Varshavsky et al., 1976; Whitlook & Simpson, 1976; Shaw et al., 1976; Noll & Kornberg, 1977) independently reached the same conclusion by separating two monomer species, one with H1 and the other without, by gel electrophoresis of intact monomer.

If we simply assume an internucleosomal location of H1, and equal or random susceptibility to nuclease attack of all the DNA regions between H1 and nucleosomes, nuclease digestion should produce three species of monomer: monomer without H1, monomer with a molecule of H1 on one side, and monomer with a molecule of H1 on each side. Our quantitative analysis of the distribution of H1 showed, however, that only the first and second species of monomer existed. Thus we propose a model for the site of binding of H1 in a beads-on-a-string structure (Figure 3). In this model, the region termed A is more susceptible to nuclease attack than region B, probably because A is a longer naked stretch of DNA. Attack on two neighboring A regions produces a nucleosome with one molecule of H1 and further attack on a less susceptible region B produces a nucleosome without H1. Prolonged digestion is known to produce monomers with only 140 base pairs on DNA (Sollner-Webb & Felsenfeld, 1975; Noll et al., 1975), a fact supporting the present model.

The most important feature of this model is that H1 is bound asymmetrically to the nucleosome. This strongly suggests that the nucleosome core does not have twofold symmetry.

Although no direct evidence was obtained on whether the neighboring nucleosomes (plus H1) are oriented in the same direction or not, we expect the arrangement to be "head to tail", for the following reason. If there is a considerable portion of adjacent pairs of nucleosomes arranged "head to head", there will be three kinds of internucleosomal regions: i.e., those without H1, those with one H1, and those with two H1. Digestion of the chromatin with nuclease would then cause preferential production of dimer or trimer at some stage. However, even on varying the ionic strength and concentrations of divalent cations, no conditions were found to give preferential production of dimer or trimer. The "head to tail" arrangement necessarily means that nucleosomes (plus H1) recognize strand asymmetry of DNA. The polarity of the beads-on-a-string structure as proposed in this study may play an important role in asymmetrical reading of DNA at transcription.

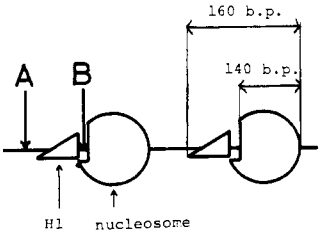

FIGURE 3: Proposed model of the location of H1 along the "beads on a string" structure.

Acknowledgment

The authors thank Dr. T. Sugimura, Director of National Cancer Center Research Institute, for helpful discussions and encouragement.

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