Selective Interaction of 5′-Bromodeoxyuridine Substituted DNA with Different Chromosomal Proteins†

Joel S. Gordon,‡,* Graeme I. Bell, Harold C. Martinson,§ and William J. Rutter

ABSTRACT: Chromosomal proteins selectively interact with 5′-bromodeoxyuridine (BrdUrd) substituted DNA relative to unsubstituted DNA. The relative affinities of chromosomal proteins for BrdUrd-DNA and unsubstituted DNA were measured by both thermal chromatography on hydroxylapatite and selective retention on nitrocellulose filters. Certain chromosomal proteins have a high affinity for hydroxylapatite; thus, during thermal chromatography of chromatin, the single-stranded DNA component percolates across a bed of adsorbed proteins as it elutes. We have measured the relative affinities of BrdUrd-DNA and normal DNA for chromosomal proteins by chromatographing appropriate mixtures on hydroxylapatite. The results show that, under these conditions, the histone components, rather than the nonhistone chromatin proteins, retard the BrdUrd-substituted DNA. In addition, the individual histones vary in the degree of their affinity for BrdUrd-DNA in the order H3 > H4 > H2A > H2B > H1. We have used the property that protein–DNA complexes have a preferential affinity for nitrocellulose filters over naked DNA to measure the selective binding of BrdUrd-DNA and unsubstituted DNA's to both histone and nonhistone chromosomal proteins at low temperatures. The histones selectively retained BrdUrd-DNA on filters in the order H4 > H2A > H3 > H2B > H1. Using this assay, the nonhistones displayed greater selectivity toward BrdUrd-DNA than the histone fraction. We interpret these results to mean BrdUrd-containing DNA has a specific affinity for certain chromosomal proteins. The selective interaction of chromosomal proteins with BrdUrd-DNA may be the basis for selective inhibition of cytodifferentiation by the thymidine analogue, BrdUrd.

The thymidine analogue 5′-bromodeoxyuridine (BrdUrd[1]) selectively inhibits the synthesis of cell-specific proteins during cytodifferentiation (Bischoff and Holtzer, 1970; Stellwagon and Tomkins, 1971; Weintraub et al., 1972; Schlitz et al., 1973; Ingram et al., 1974; Walther et al., 1974; see review by Rutter et al., 1973), activates certain latent viruses (Hampor et al., 1971; Lowy et al., 1971), and induces the appearance of at least one enzyme, alkaline phosphatase, in certain cells (Koyama and Ono, 1971, 1972; Githens et al., 1976). The molecular mechanism of BrdUrd action is yet to be elucidated. The bulk of the evidence is consistent with the view that BrdUrd selectively affects genetic expression as the result of its incorporation into DNA (see Rutter et al., 1973, for review), although possible alternative or additional sites of action (Schubert and Jacob, 1970), such as the cell membrane (Brown, 1971), have not been ruled out. Although BrdUrd is a potent procaryotic mutagen (Jones and Dove, 1972), the inhibition of cytodifferentiation in eucaryotes by BrdUrd is highly selective and is not augmented by ultraviolet irradiation. Thus, the effects of BrdUrd cannot be solely due to its mutagenic effect. BrdUrd could also act by altering the affinity of DNA for regulatory proteins.

Lin and Riggs (1972) have shown that the lac repressor has at least a tenfold greater affinity for BrdUrd substituted over unsubstituted operator DNA. The physical properties of chromatin containing BrdUrd-substituted DNA differ from those of chromatin-containing unsubstituted DNA. We have previously shown that BrdUrd substitution into DNA dramatically alters its elution profile in chromatin which is subjected to thermal chromatography on hydroxylapatite (David et al., 1974). In contrast, there are comparatively minor differences when purified DNA and BrdUrd-DNA are similarly chromatographed (David et al., 1974), or if the thermal stabilities of substituted and unsubstituted chromatin or DNA are measured by increases in hyperchromicity (David et al., 1974; Simpson and Seale, 1974; Augenlicht et al., 1974; Lapeyre and Bekhor, 1974). Simpson and Seale (1974), Augenlicht and co-workers (1974), and Lapeyre and Bekhor (1974) have recently reported a substantial change in the ORD spectrum of BrdUrd-substituted DNA in chromatin that is not manifest in purified DNA.

In this report, we show that when chromatin-containing BrdUrd in a single strand is thermally chromatographed on hydroxylapatite only the BrdUrd containing strand is retarded. This, therefore, does not result from mere stabilization of the DNA helix but rather from an increased affinity of the BrdUrd-DNA for chromosomal proteins which remain firmly adsorbed on the hydroxylapatite. This effect is dependent

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[1] Abbreviations used are: BrdUrd, 5′-bromodeoxyuridine; Tdr, 2′-deoxythymidine; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; EDTA, (ethylenedinitrilo)tetraacetic acid; ORD, optical rotatory dispersion. T_e, the temperature at which 50% of the DNA is eluted from hydroxylapatite columns; HTC cell, hepatoma tissue culture cell. The histone nomenclature used is H1 = I = F1; H2A = IIb1 = F2a2; H2B = IIb2 = F2b; H3 = III = F3; H4 = IV = F2a1.

primarily on the histone fractions, each of which has a different selective affinity for BrdUrd-DNA. We have also measured the retention of protein DNA complexes on nitrocellulose filters at low temperatures. Under these conditions, a selective interaction of BrdUrd-DNA with both histone and nonhistone proteins has been detected. The experimental results indicate that there are differences in the degree of selective interactions of BrdUrd-DNA with different chromosomal proteins.

Materials and Methods

Materials. Unless specified, all reagents were Baker analytical grade. The radioisotopes were purchased from New England Nuclear Co. All solutions were made with deionized distilled water.

Cell Culture. The hepatoma tissue culture (HTC) cells (supplied by the laboratory of Dr. Gordon Tomkins) were maintained in continuous logarithmic phase growth with a generation time of 22 h (Samuels and Tomkins, 1970). Replacement of thymidine by BrdUrd in each DNA strand was effected by growing the cells in constant darkness, in a medium containing 15 μg/ml of BrdUrd for an integral number of generations. Isopycnic centrifugation in the analytical ultracentrifuge gave a density of 1.700 g/ml for unsubstituted DNA and 1.750 g/ml for hybrid DNA from cells grown for one generation in BrdUrd. After two generations in BrdUrd, one-half the DNA banded at the hybrid density, while the other half banded at 1.798 g/ml, which corresponds to 80% substitution in both strands.

Unless otherwise specified, radioactive unsubstituted DNA was prepared from cells grown for one generation in 0.05–0.1 μCi/ml of methyl[^{3}H]thymidine (sp act. 600 Ci/mmol). The substituted DNA was labeled by growing the cells for one generation in 0.02 μCi/ml of [^{14}C]BrdUrd (final sp act. of 0.4 mCi/mM). The cells were collected and washed as described by Baxter and Tomkins (1970).

Preparation of Chromatin and DNA. Nuclei were prepared from HTC cells and disrupted in hypotonic solution as described by David et al. (1974). Unless indicated otherwise, chromatin was prepared by vigorously disrupting the freshly prepared nuclei in 15–20 volumes of 0.01 M Tris-HCl, pH 7.9, with a motorized Potter-Elvehjem Teflon–glass tissue grinder and centrifuging at 13 000g for 10 min. The sediment was washed, as above, two or more times, resuspended in 4–5 volumes of 0.01 M Tris-HCl, pH 7.5, and sonicated at 40% of maximum output for six 15-s pulses with a Biosonik microprobe (Bronwill Scientific). The insoluble material, representing less than 5% of the 260-nm absorbing material, was removed by centrifugation at 10 500g for 10 min. "Purified" chromatin was prepared from isolated nuclei by the technique of Marushige and Bonner (1966).

Chromatin or nuclei preparations, containing equal weights of substituted and unsubstituted DNA, were combined and sheared in a French pressure cell at 12 000 lb/in.^{2} (8.3×10^7 N/m^2). Storage of chromatin for 3 days at 0 °C or 1 month at −20 °C (in 20% glycerol) did not affect the results.

Histone-depleted chromatin was prepared by the procedure of Spelsburg et al. (1971). Sonicated chromatin (5–10 A_{260} units) was brought to a final volume of 5.5 ml in 2.0 M NaCl, 5.0 M urea, and 50 mM sodium acetate, pH 5.8, in cellulose nitrate centrifuge tubes that had been previously rinsed twice with 1% sodium dodecyl sulfate, once in 50 mg/ml of bovine serum albumin, then extensively in deionized distilled H_2O, and air dried. Unextracted-chromatin controls were diluted to 4.5 ml in 0.01 M Tris-HCl, pH 8.0, and layered over 1.0 ml of 1.1 M sucrose, 0.01 M Tris-HCl, pH 8.0. Both the unextracted and histone-depleted chromatins were centrifuged for 19 h at 45 000 rpm in a Beckman SW 50.1 rotor and the resulting pellets were resuspended in the bottom 0.5 ml of the supernatant by sonication. The suspension was dialyzed overnight against 100 volumes of 0.012 M potassium phosphate, pH 6.8, in dialysis tubing that had been sequentially prerinsed in sodium dodecyl sulfate, bovine serum albumin, and water, as above. The chromatin suspension was resonicated and the insoluble material was removed by centrifugation at 13 000g for 10 min. The protein:DNA ratio of the salt- and urea-extracted chromatin was 0.7–1.0, while that of the control chromatin was 2.0–2.1. We have found that this procedure extracts about 85% of the chromatin proteins that are soluble in 0.4 M HCl and 20% of the acid-insoluble proteins. Comparison of DNase I digested chromatins by electrophoresis, in 15% acrylamide gels containing sodium dodecyl sulfate, demonstrated that each histone species was extracted to the same degree.

DNA were prepared by the method of Marmur (1961), as previously described (David et al., 1974), and sheared in a French pressure cell at 12 000 lb/in.^{2}. Unless otherwise stated, a mixture containing equal amounts of substituted and unsubstituted DNA was sheared for analysis. DNA was quantitated by the method of Burton and Peterson (1957) and protein by the method of Lowry et al. (1951).

Thermal Chromatography. Thermal chromatography of chromatin and DNA was performed by a modification of the procedure of McConaughy and McCarthy (1972) as described in David et al. (1974).

Preparative and Analytical Centrifugations of DNA. The CsCl buoyant density determinations were performed as described by Mandel et al. (1968). Reference DNA of the bacterium *Micrococcus luteus* (1.731 g/ml) was used as a density marker. The degree of substitution was determined from the buoyant density by the method of Flory and Vinograd (1973).

Preparative alkaline Cs_2SO_4 gradients were run in the following manner. Chromatin samples containing 50 000–100 000 cpm of labeled DNA were diluted to 5.5 ml with a Cs_2SO_4 solution to a final density of 1.635 g/ml in 0.1 M NaOH. These solutions were centrifuged in cellulose nitrate tubes that had been prewashed in sodium dodecyl sulfate and bovine serum albumin, as detailed above, for 48–72 h at 100 000g in an SW 50.1 rotor at 22 °C. Subsequently, 150-μl fractions were collected. Fifty microliters of alternate fractions were spotted on Whatman 3MM filters and then washed, dried, and counted in a protosol (New England Nuclear) omnifluor–toluene scintillator. The appropriate fractions of DNA were then pooled and dialyzed overnight against three changes of 0.012 M KPO_4, pH 6.8, in preparation for thermal chromatography.

Preparation of Chromatin Proteins. Nonhistone proteins were isolated by chromatography on hydroxylapatite (MacGillivray et al., 1972) from 0.5% Triton-X100 washed nuclei prepared from the frozen livers of adult female Sprague-Dawley rats by the procedure of Goldberg et al. (1976). The fractions eluting at 0.05 M KPO_4 and absorbing at 230 nm, which contained the bulk of the nonhistones (MacGillivray et al., 1972), were pooled, dialyzed overnight against deionized distilled water, lyophilized to dryness, and resuspended in 5 ml of 5 M urea, 2 M NaCl, 10 mM Tris-HCl, pH 8.0. Each fraction was then stored at −75 °C until used.

Total calf thymus histones (Worthington Biochemical) were fractionated into individual species as described by Martinson and McCarthy (1975).

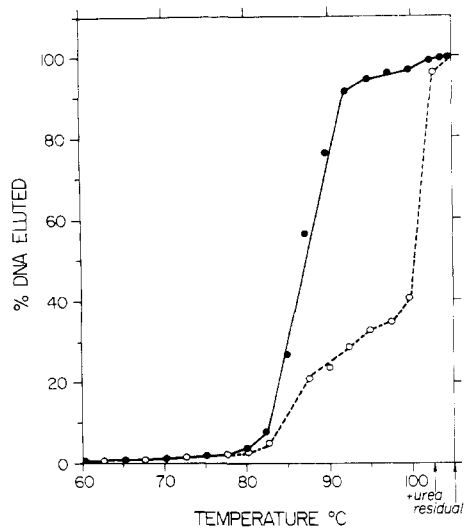

FIGURE 1: The elution of the substituted and unsubstituted strands of chromatin containing hybrid density DNA. Chromatin, isolated from HTC cells grown for four generations in [^{3}H]thymidine, and then one generation in 5×10^{-5} M [^{14}C]BrdUrd, and, thus, containing hybrid density DNA in which the unsubstituted strand (●-●) was labeled with [^{3}H]thymidine and the substituted strand (○- - -○) was labeled with [^{14}C]BrdUrd, was chromatographed as follows: 20-35 A_{260} units of sheared chromatin or nuclei were brought to 0.12 M KPO_4, pH 6.8, and applied to hydroxylapatite (Clarkson, Inc.) columns and eluted with 5 ml of 0.12 M KPO_4, pH 6.8, at every 2.5 °C increment in temperature from 60 to 100 °C. The columns were then eluted (urea) at 100 °C with 8 M urea, 0.24 M KPO_4, pH 6.8, and 10 mM EDTA. Uneluted (residual) radioactivity was recovered by dissolving the hydroxylapatite in 10% Cl_3CCOOH and filtering through glass-fiber filters.

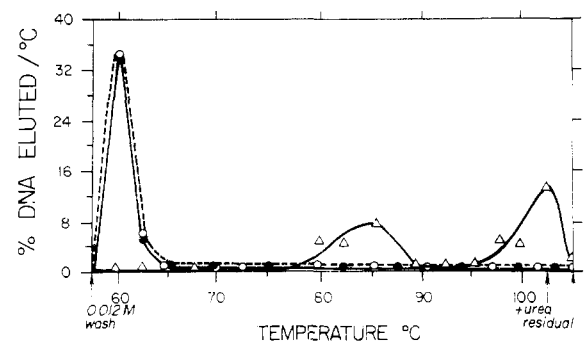

FIGURE 2: Thermal chromatography of DNA isolated from heated chromatin. A solution of sheared chromatin containing [^{14}C]BrdUrd-substituted DNA in 0.01 M Tris-HCl, pH 8.0, was divided into two equal portions. One sample was heated by gradually increasing its temperature from 60 to 95 °C over a 2-3-h interval. Over 95% of the DNA from the heated (○- - -○) and unheated (●-●) samples, recovered as a single fraction (1.845 ± 0.005 g/ml) after centrifugation to equilibrium in alkaline $CsSO_4$ gradients, was dialyzed against 0.012 M KPO_4, pH 6.8, at 4 °C overnight. These samples, along with a sample of untreated chromatin-containing [^{3}H]BrdUrd-DNA (△—△), were applied to parallel hydroxylapatite columns equilibrated with 0.12 M KPO_4, pH 6.8, at 60 °C and then washed with 5.0 ml of the same buffer. The columns were then eluted with 0.12 M KPO_4, as described in Figure 1.

Generation of Nucleoprotein Complexes. Nucleoproteins were reconstituted under equilibrium conditions following the procedures of Shih and Bonner (1970). Equal amounts of [^{14}C]BrdUrd- and [^{3}H]Tdr-DNA were mixed and sheared in a French pressure cell at 12 000 psi. The DNA preparation was then mixed with the protein to be assayed in 1 ml of 2 M NaCl, 5 M urea, 1 mM methylamine, and 30 mM Tris-HCl, pH 8.0. For testing total and histone-depleted chromatin proteins, two chromatin samples containing equal amounts of [^{14}C]BrdUrd- and [^{3}H]Tdr-DNA were mixed and sheared in the French pressure cell and then dissociated in 2 M NaCl, 5 M urea, 1 mM methylamine, 30 mM Tris-HCl, pH 8.0, in the presence of an equal-part mixture of purified [^{14}C]BrdUrd- and [^{3}H]Tdr-DNA which had been similarly sheared. The mixtures were sequentially dialyzed, using dialysis tubing with a 6000-8000 dalton exclusion limit (Spectrum Med. Indust.), for 3-h intervals against 20 volumes of 0.6, 0.4, 0.3, 0.15, and 0.015 M NaCl all in 5 M urea, 1 mM methylamine, and 30 mM Tris-HCl, pH 8.0, and, finally, twice for 12 h against 0.012 M KPO_4, pH 6.8.

Determination of Protein-Bound DNA by Nitrocellulose Disk Filtration. The effect of different protein fractions on retention of DNA by nitrocellulose filters was determined essentially by the technique of Riggs et al. (1970). Twenty-five-millimeter diameter Schliecher-Schuell B6 filter disks were wetted for 20 min in 0.4 M KOH and then washed four times in 0.012 M KPO_4, pH 6.8. Two-hundred-fifty microliters of sample was applied to filters under a slight vacuum, after which the filters were washed twice with 500 μl of 0.012 M KPO_4, pH 6.8. The initial ratio of [^{14}C]BrdUrd to [^{3}H]Tdr was determined by spotting 100-μl aliquots of nonfiltered samples on disks. The filters were then dried at 60 °C for 45 min and counted in omnifluor-toluene. The degree of selective retention of [^{14}C]BrdUrd-DNA, determined as the ratio of ^{14}C:^{3}H on the filtered disks divided by the ratio of ^{14}C:^{3}H on the unfiltered disks, is hereafter referred to as the selectivity.

Results

The Thermal Chromatography of Chromatins Containing Substituted and Unsubstituted DNA. It has been shown previously (David et al., 1974) that the interaction of BrdUrd-substituted DNA with some component of lysed nuclei gives rise to enhanced retention of the BrdUrd-DNA by hydroxylapatite during thermal chromatography. Tightly bound chromosomal components are involved, since removal of the nuclear sap and membranes (i.e., 50% of the protein) by sedimentation of the chromatin through 1.7 M sucrose (Marushige and Bonner, 1966) has no effect on the thermal elution profile (data not shown). Substitution of BrdUrd into but a single strand of the DNA is sufficient to maximally retard elution of the BrdUrd-DNA, since the same degree of retardation of BrdUrd-DNA is found for the BrdUrd-DNA upon chromatography of chromatin isolated from cells grown for either one or two generations in 5×10^{-5} M BrdUrd (data not shown).

If BrdUrd substitution resulted in the stabilization of the double-stranded structure of DNA in chromatin, then both strands of a hybrid density DNA in chromatin should elute simultaneously. When the chromatin containing such hybrid DNA labeled with [^{3}H]thymidine in one strand and [^{14}C]-BrdUrd in the other was thermally chromatographed on hydroxylapatite, the BrdUrd-substituted strand eluted at higher temperatures than the unsubstituted strand (Figure 1). The unsubstituted DNA strand eluted at the same temperature as the unsubstituted DNA in control chromatin. Thus, the retarded elution of BrdUrd-DNA during the thermal chromatography of chromatin resulted from an increased affinity of single-stranded BrdUrd-DNA for some chromosomal component which is retained on the hydroxylapatite.

The retarded elution of BrdUrd-DNA upon thermal chromatography of chromatin could be explained by the artifactual formation of covalent bonds between BrdUrd-DNA and a chromosomal component during heating. This does not seem

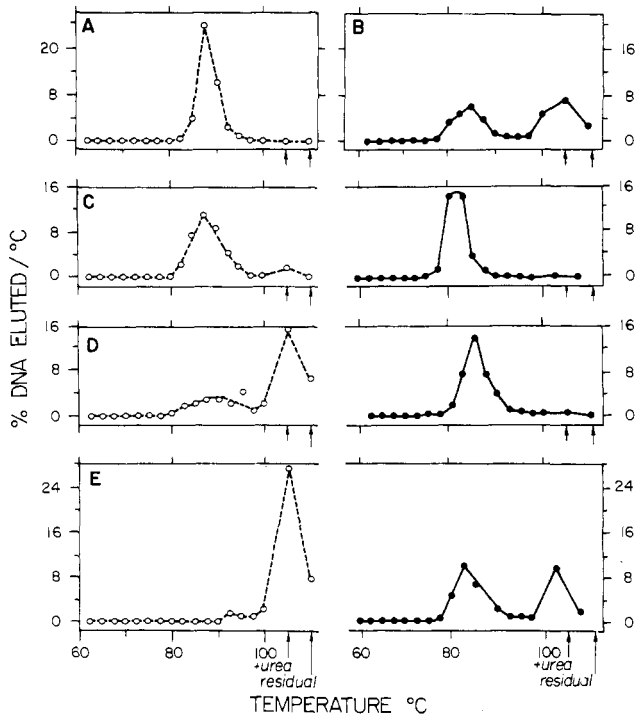

FIGURE 3: Competitive relationship of free [^{3}H]BrdUrd-DNA with [^{14}C]-unsubstituted DNA in chromatin during thermal chromatography. Varying amounts of purified ^{3}H-labeled BrdUrd-DNA (O- - -O) were chromatographed either in the absence (A) or the presence (C, D, E) of chromatin containing ^{14}C-labeled unsubstituted DNA (●-●). The ratios of pure DNA to chromatin DNA were (C) 10.0, (D) 1.0, (E) 0.1. The elution of the ^{14}C-unsubstituted DNA in chromatin chromatographed in the absence of added DNA was also monitored (B).

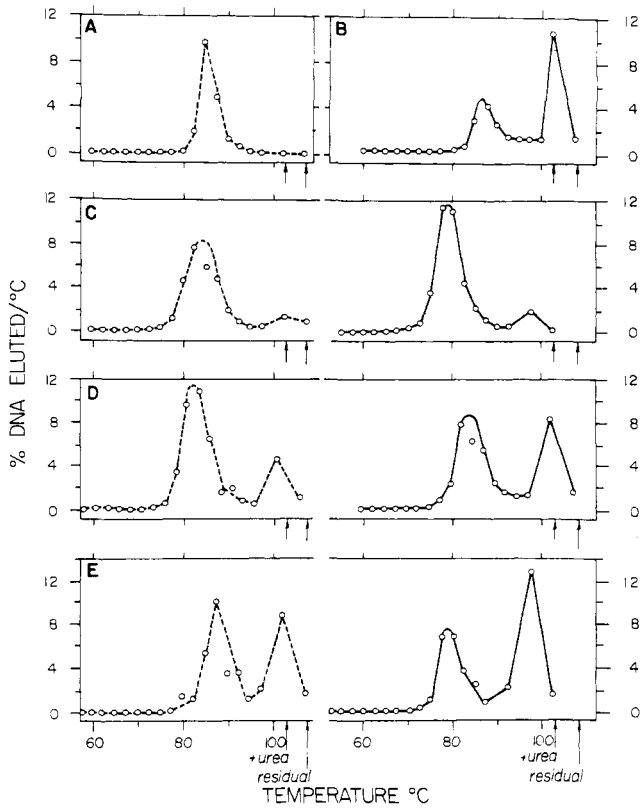

FIGURE 4: Competitive relationship of free [^{3}H]BrdUrd-DNA with [^{14}C]Brd-Urd-DNA in chromatin. Varying amounts of purified ^{3}H-labeled BrdUrd-DNA (O- - -O) were chromatographed either in the absence (A) or the presence (C, D, E) of chromatin containing ^{14}C-labeled BrdUrd-DNA (O-O). The ratios of pure DNA to chromatin DNA were (C) 10.0, (D) 1.0, (E) 0.1. The elution of [^{14}C]BrdUrd-DNA in chromatin chromatographed in the absence of added DNA was also monitored (B).

to be the case, since BrdUrd-DNA, isolated by relatively gentle techniques from extensively heated chromatin, is not retained on hydroxylapatite columns. This was seen when a sample of chromatin containing [^{14}C]BrdUrd-DNA was heated in 0.01 M Tris by gradually increasing temperatures to 95 °C, as is done during thermal chromatography. Ninety to one-hundred percent of the BrdUrd-DNA in the heated chromatin sample was recovered on an alkaline Cs_2SO_4 gradient at the same density as the BrdUrd-DNA in an unheated chromatin. The BrdUrd-DNA from both samples are not retarded and behave as single-stranded BrdUrd-DNA on thermal chromatography (Figure 2).

Added free BrdUrd-DNA appears to compete with the unsubstituted DNA in chromatin during thermal chromatography (Figure 3C-E). Thus, as seen in Figures 3 and 4, purified [^{3}H]BrdUrd-DNA elutes with a T_e (the temperature at which half the DNA is eluted) of 85 °C when chromatographed alone, while it is partially retarded when chromatographed in the presence of chromatin. Progressively more added BrdUrd-DNA is retarded as the proportion of added DNA to chromatin is decreased (Figures 3C-E and 4C-E) and, as shown in Figure 4, the added free BrdUrd-DNA is essentially in equilibrium with the BrdUrd-DNA of chromatin (Figure 4C-E). Thus, as the ratio of exogenous DNA to chromatin DNA is increased, an increasing proportion of both of the labels elute as the free DNA (Figure 4). This presumably reflects a stoichiometric interaction of DNA with chromatin components. Similar exchange is also observed at low ionic strengths (data not shown) when 0.01 M $CsPO_4$, pH 6.8, (Martinson, 1973) is the eluant, suggesting that it is occurring in response to the conditions of thermal chromatography and not to ionic strength alone.

The Effects of Various Proteins on the Thermal Chromatography of BrdUrd-DNA. The proteins of chromatin are bound much more tightly by hydroxylapatite than is the DNA, as seen in Figure 5, where 95% of the proteins in either BrdUrd or control chromatin remain bound to the hydroxylapatite column at 100 °C. Thus, pure BrdUrd-DNA elutes below 90 °C (Figures 3A and 4A), while a large proportion of chromatin BrdUrd-DNA remains bound at 100 °C together with the proteins. This suggests that the chromatin proteins, which are retained on hydroxylapatite, preferentially retard the elution of BrdUrd-DNA. This possibility is confirmed in the following experiments.

As shown in Figure 6, selective removal of most of the histones greatly diminishes the retardation of BrdUrd-DNA on hydroxylapatite columns (see Panels 6b and 6c). Ninety percent of BrdUrd-substituted DNA in histone-depleted chromatin elutes like purified substituted DNA, compared to only 30% in unextracted chromatins.

The dominant role of histones in the preferential retardation of BrdUrd-DNA during thermal chromatography is further substantiated in Figure 7, which documents the effect of various protein fractions on the elution of substituted and unsubstituted DNA. Even at a 6:1 ratio of nonhistone proteins to DNA, none of the unsubstituted DNA and only 10% of the BrdUrd-substituted DNA elutes at 100 °C with urea. In contrast, calf thymus histones retard BrdUrd-DNA (Figure 7) and the degree of retardation depends on the ratio of histone to DNA. For instance, when a mixture of equal parts of substituted and unsubstituted DNA are chromatographed with

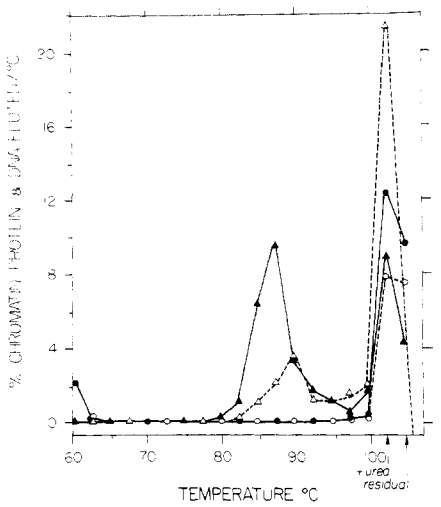

FIGURE 5: The elution of chromatin proteins during thermal chromatography. Chromatin containing unsubstituted ^{3}H-labeled DNA and [^{14}C]BrdUrd-DNA was thermally chromatographed, as described in Figure 1 on separate hydroxylapatite columns. Triplicate aliquots of the suspended 10% trichloracetic acid precipitate of each fraction were assayed by the technique of Lowry et al. (1951) to follow the elution of the chromatin proteins associated with the unsubstituted DNA (●-●) and the chromatin proteins associated with the substituted DNA (○- - -○). The elution of the unsubstituted (▲-▲) and substituted DNA (△- - -△) were followed as described in Figure 1.

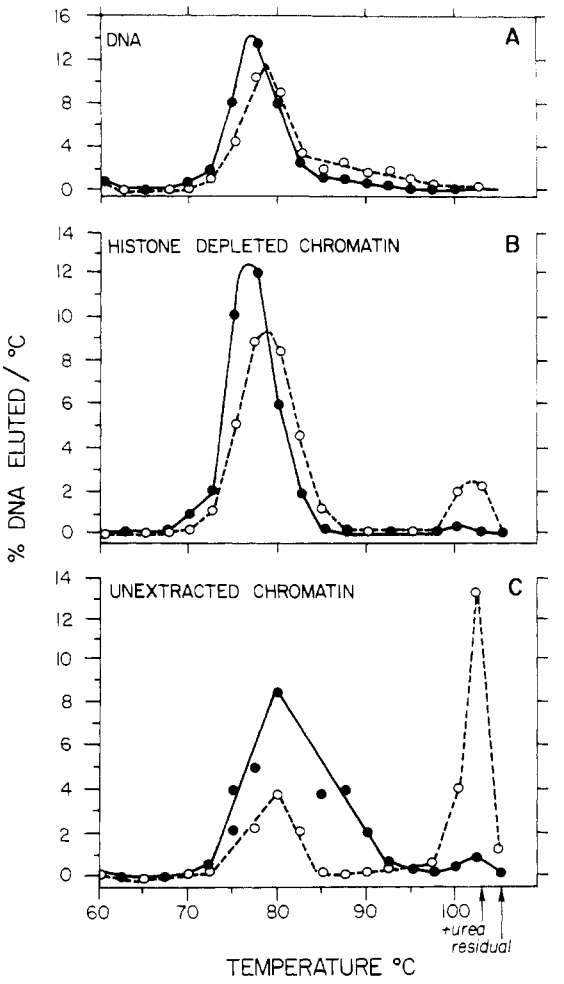

FIGURE 6: The thermal chromatography of histone-depleted chromatin. The elution of unsubstituted ^{3}H-labeled DNA (●-●) and BrdUrd substituted ^{14}C-labeled DNA (○- - -○) was compared during the thermal chromatography of (A) pure DNA preparations, (B) chromatin preparations in which over 80% of the histones were extracted with 5 M urea and 2 M NaCl at pH 5.8, as described under Materials and Methods, and (C) unextracted chromatin preparations in which the normal histone complement is present.

an amount of total histones equal to 3, 1.5, and 0.75 times the weight of DNA, 75, 60, and 10%, respectively, of the BrdUrd-DNA is recovered in the fraction eluting at high temperatures.

Further experiments were carried out in order to determine the role of each of the five individual histones in the selective retention of BrdUrd-DNA during thermal chromatography. Each histone species has a different affinity for BrdUrd-DNA relative to unsubstituted DNA (Figure 8). H3 shows the greatest selective affinity for BrdUrd-DNA and H1 the least in this assay. More than 84% of the BrdUrd-DNA and only 16% of the unsubstituted DNA is recovered in the high-temperature eluting fraction when H3 is reconstituted with an equal weight of a 1:1 mixture of both DNAs. H1, on the other hand, has nearly an equal affinity for unsubstituted and substituted DNA (Figure 8), even at a histone to DNA ratio of 3:1 (data not shown). Thus, as shown in Figure 8, a major proportion (and in other experiments all) of the unsubstituted DNA is always eluted at low temperatures, irrespective of the histone tested (Figure 8), whereas in the series H1, H2B, H2A, H3, and H4, a progressively lesser proportion of the substituted DNA is eluted at low temperatures. Consequently, the ratios of the percent substituted DNA to the percent of unsubstituted DNA eluting in the low temperature peaks for these histones are: 0.9 for H1, 0.7 for H2B, 0.5 for H2A, 0.3 for H4, and 0.2 for H3.

Since the columns were eluted with 0.12 M KPO_4, a salt concentration at which histone H1 is known to be dissociated from DNA, the failure of H1 to bind to DNA may account for its failure to discriminate between DNAs. To insure that H1 is bound to DNA during chromatography, we repeated these experiments using 0.01 M $CsPO_4$ (Martinson, 1973) as the eluant. We observed no differences in the absolute and relative affinities of each histone for BrdUrd-DNA under these conditions (data not shown).

The Selective Retention of BrdUrd-DNA on Nitrocellulose Filters by Chromosomal Proteins. We have also investigated the selective binding of chromosomal proteins to BrdUrd-DNA at relatively low temperatures by using the nitrocellulose filter assay for protein-bound DNA similar to that employed in other studies of protein–nucleic acid interactions (Chamberlin and Berg, 1964; Yarus and Berg, 1970). It was with this assay that Lin and Riggs (1972) demonstrated an increased affinity of the lac repressor for BrdUrd-substituted DNA.

Other laboratories also have used this technique to investigate the interaction of chromosomal proteins with DNA (Sheehan and Olins, 1974; Renz, 1975; Vogel and Singer, 1975). Proteins were mixed at 2 M NaCl and 5 M urea with equal amounts of differentially labeled substituted and unsubstituted DNA at different protein:DNA ratios. The salt concentration was gradually lowered, allowing the proteins to bind selectively to DNA. The retention of substituted and unsubstituted DNA on nitrocellulose filters was then compared.

As seen in Table I, total chromatin proteins from HTC cells retain BrdUrd-DNA selectively on nitrocellulose filters. A chromosomal fraction, from which the histones were largely but not entirely removed, caused an even greater selective retention of BrdUrd-DNA.

Thus, in contrast to their behavior on hydroxylapatite,

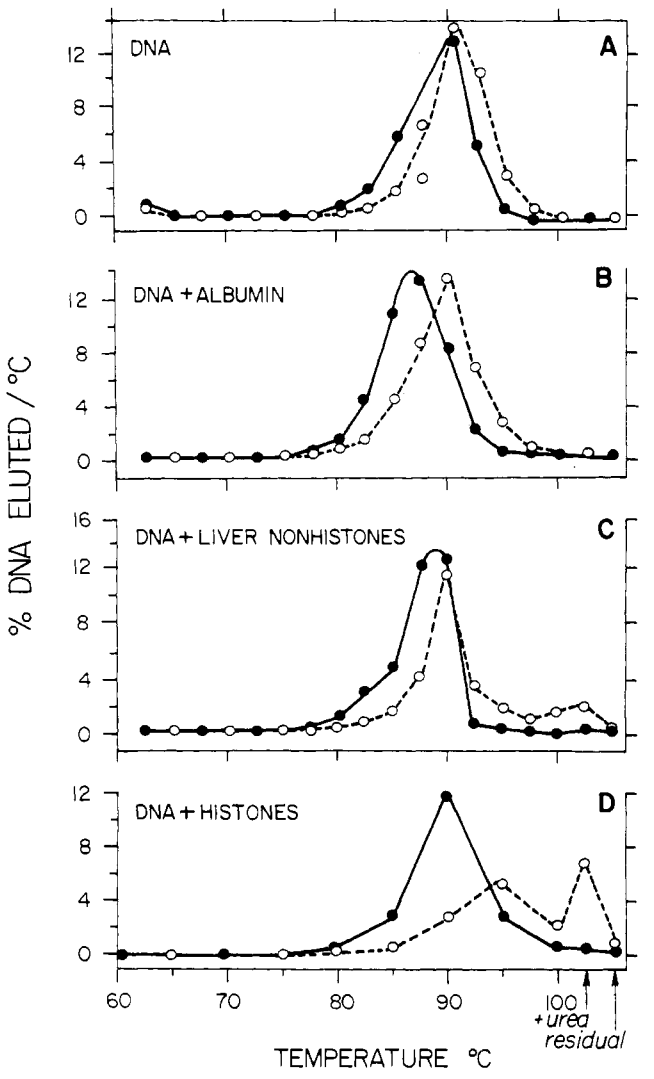

FIGURE 7: Thermal chromatography of DNA annealed with chromatin-protein fractions. ^{3}H-labeled unsubstituted (●-●) and ^{14}C-labeled BrdUrd substituted DNA (O- - -O) were chromatographed as described in Figure 1 prior to (A) and after annealing by gradient-step dialysis as described under Materials and Methods with (B) a sixfold weight of bovine serum albumin, (C) a sixfold weight of rat liver nonhistone proteins, and (D) an equal weight of calf thymus histones.

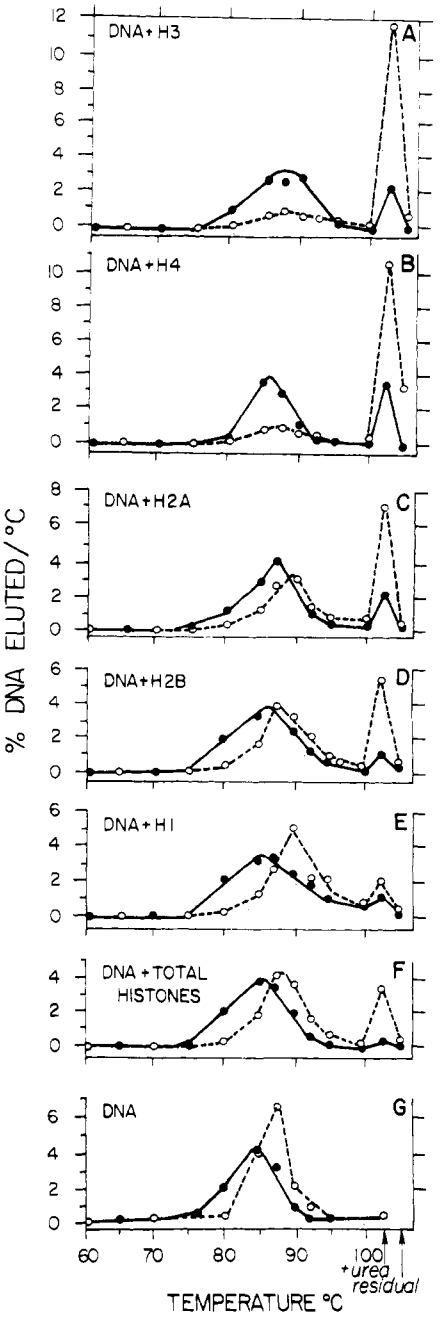

FIGURE 8: The different relative affinities of each histone fraction for BrdUrd-DNA. Unsubstituted ^{3}H-labeled (●-●) and substituted ^{14}C-labeled (O- - -O) DNA were chromatographed on hydroxylapatite, as described in Figure 1, either alone (G) or after annealing by stepwise gradient dialysis, as described under Materials and Methods, with calf thymus (A) H3, (B) H4, (C) H2A, (D) H2B, (E) H1, and (F) total calf thymus histone fractions at a 1:1 histone-DNA ratio.

nonhistones are able to cause the selective retention of BrdUrd-DNA to nitrocellulose filters. To test this further, we compared the ability of purified total histones and a nonhistone fraction from rat liver to selectively retain BrdUrd-DNA on filters (Table II). Both fractions cause the selective retention of BrdUrd-DNA. The nonhistone fraction is more effective, but neither selectively retains BrdUrd-DNA as effectively as the proteins from the histone-depleted HTC cell chromatin (Table I).

In addition, we have observed a selective retention of BrdUrd-DNA by proteins that are not associated with DNA in situ. At a protein:DNA ratio of 2:1 (w/w) the basic proteins, cytochrome *c* and lysozyme, retain BrdUrd-DNA with selectivities of 1.2 and 1.4, respectively. Crystalline bovine serum albumin does not significantly retain DNA at protein:DNA ratios below 6:1 (w/w).

Experiments with individual histones demonstrate that they selectively retain BrdUrd-DNA on filters to different degrees (Table III). Under the conditions employed in these studies, annealing with H1 causes insignificant selective retention, while the effectiveness of the other four histones decreased in the order H4 > H2A > H3 > H2B.

Discussion

The experimental results indicate that BrdUrd-DNA binds selectively to certain chromosomal proteins. The delayed elution of the substituted, compared to the unsubstituted, strand of a hybrid duplex DNA in chromatin during thermal chromatography on hydroxylapatite is explained by an increased affinity of single-stranded BrdUrd-DNA for a chromosomal component(s) that is retained on the hydroxylapatite column. In contrast, BrdUrd substitution has only a relatively

TABLE I: Selective Retention of BrdUrd-DNA Reconstituted Chromatin on Nitrocellulose Filters.[a]

Chromatin/DNA	Protein/DNA	% Total DNA Retained	Selectivity[b]
		Whole HTC Chromatin	
1.00:0	2.4:1	64 ± 6	1.7 ± 0.1[c]
4.00:1	1.9:1	44	1.5
1.50:1	1.4:1	32 ± 1	1.5 ± 0.1
0.67:1	1.0:1	18 ± 4	1.75 ± 0.05
0.25:1	0.5:1	8.6 ± 2	2.3 ± 0.5
0.11:1	0.2:1	2.7	2.5
		Histone-Depleted HTC Chromatin	
1.00:0	0.9:1	15.4 ± 0.8	3.7 ± 0.1
4.00:1	0.7:1	10.9 ± 0.3	3.8 ± 0.6
1.50:1	0.5:1	5.7	3.1
0.67:1	0.4:1	4.5 ± 0.3	3.5 ± 0.7
0.25:1	0.2:1	1.05 ± 0.05	5.0 ± 1.0

[a] Equal amounts of purified HTC cell ^{3}H-unsubstituted DNA and [^{14}C]BrdUrd-DNA were mixed with various amounts of chromatin preparations made up of an equal part mixture of HTC cell chromatin containing ^{3}H-unsubstituted and [^{14}C]BrdUrd-DNA at 2.0 M NaCl and 5.0 M urea prior to a stepwise lowering of the salt concentration and filtering through nitrocellulose filters, as described under Materials and Methods. [b] The selectivity is equal to the ratio of ^{14}C/^{3}H retained on the nitrocellulose disk after filtration divided by the ratio of ^{14}C/^{3}H on disks to which the same sample has been applied without filtration. [c] The ratios are expressed ± the average error for two to five separate determinations.

TABLE II: Selective Retention of BrdUrd-DNA on Nitrocellulose Filters by Chromatin Protein Fractions.

Protein	Protein/DNA	% Total DNA Retained	Selectivity
Histones[a]	3.00:1	100 ± 3	1.0 ± 0.1[c]
	1.50:1	60 ± 10	1.36 ± 0.08
	0.75:1	20 ± 8	1.4 ± 0.2
	0.46:1	15 ± 4	1.5 ± 0.1
	0.38:1	6 ± 3	2.1 ± 0.3
	0.19:1	3 ± 1	2.4 ± 0.6
Nonhistones[b]	6.0:1	79 ± 9	1.2 ± 0.1[d]
	3.0:1	47 ± 5	1.8 ± 0.3
	1.5:1	25 ± 8	2.5 ± 0.4
	0.38:1	4 ± 1	2.25 ± 0.05
	0.13:1	0.6	3.1

[a] Calf thymus histones were obtained from Worthington Biochemical Corp. [b] Rat liver nonhistone proteins were isolated as described under Materials and Methods. [c] The results for annealing with histones are expressed ± the average error for three to five separate determinations. [d] The results for annealing with nonhistones are expressed ± the average error for one to two separate determinations.

TABLE III: Selective Retention of BrdUrd-DNA on Nitrocellulose Filters by Different Histone Species.

Histone	% DNA Retained	Selectivity
H1	28 ± 5	1.03 ± 0.04[a]
H2B	40 ± 20	1.3 ± 0.2
H3	45 ± 20	1.4 ± 0.2
H2A	50 ± 10	1.8 ± 0.4
H4	32 ± 9	3.8 ± 0.9

[a] The results are expressed ± the average error for four separate determinations.

minor effect on the thermal stability of DNA in chromatin, as measured by the hyperchromicity (David et al., 1974; Simpson and Seale, 1974; Augenlicht et al., 1974) accompanying helix disruption.

The experiments involving reconstitution and elution from hydroxylapatite demonstrate that single-stranded BrdUrd-DNA has an increased affinity for certain chromatin proteins which retard its elution during thermal chromatography. The selective removal of the histones from chromatin abolishes this effect, which, in turn, is restored when purified DNAs are chromatographed in the presence of histones. Thus, the histones appear to be the dominant, if not the sole chromatin component, retarding BrdUrd-DNA during thermal chromatography on hydroxylapatite.

We conclude that the increased affinity of chromatin proteins for BrdUrd-DNA does not involve the formation of covalent bonds between BrdUrd-DNA and proteins during the experimental procedure for the following reasons. (1) The debromination of BrdUrd, leading to the production of free radicals which might participate in covalent-bond formation (Wataya et al., 1973; Olafsson and Bryan, 1974), requires temperatures above 125 °C (Olafsson and Bryan, 1974) and, therefore, should not occur during thermal chromatography. (2) Sedimentation of extensively heated chromatin preparations containing BrdUrd-DNA to equilibrium in alkaline Cs_2SO_4 gradients separates all of the BrdUrd-DNA from the bulk of the proteins. This BrdUrd-DNA chromatographs on hydroxylapatite as single-stranded BrdUrd-DNA. Thus, trace amounts of proteins capable of adsorbing to hydroxylapatite at high temperatures are not covalently bound to the DNA after heating and isopycnic centrifugation. (3) The exchange of histone between DNA sites during thermal chromatography (see Figures 3 and 4) argues against the participation of covalent bonds. Salt-induced exchange at low temperatures followed by covalent bond formation during chromatography almost certainly does not occur, since the same results are obtained at low ionic strengths when 0.01 M Cs_2PO_4 is used as the eluant. Also, the histones with the greatest selective affinity for BrdUrd-DNA are those which are dissociated from unsubstituted DNA at ionic strengths greater than those used here (Ohlenbusch et al., 1967). Thus, during thermal elution, substituted and unsubstituted DNA presumably bind differentially to chromatin proteins adhering to the hydroxylapatite in a manner analogous to that leading to the separation of substances by ion-exchange or affinity chromatography. (4) A selective binding of chromatin proteins to double-stranded BrdUrd-DNA has been demonstrated also by nitrocellulose filtration, which does not involve heating. Thus, we conclude that the increased affinity of BrdUrd-DNA for chromatin proteins, demonstrated by thermal chromatography, reflects a phenomenon that exists at low temperatures and probably within living cells.

Attempts to compare the results of filter binding and thermal chromatography are difficult because the molecular processes involved in the two techniques are unclear. For example, thermal chromatography on hydroxylapatite shows an increased affinity of BrdUrd-DNA for histones but not the nonhistone proteins, in contrast to the results obtained by nitrocellulose filtration. The failure of the nonhistone proteins to retard BrdUrd-DNA on thermal chromatography may result from heat denaturation of the nonhistones or a binding which is inherently heat sensitive. Alternatively, the effect may simply arise from the fact that the nonhistones are bound to

the hydroxylapatite in a manner different from that of the histones (Bernardi, 1971).

Interpretation of the results is also made difficult because nitrocellulose filters do not commonly retain 100% of the protein-bound DNA (Chamberlin and Berg, 1964; Yarus and Berg, 1970). One-hundred percent efficiency is not achieved in these experiments, since protein:DNA ratios that far exceed equimolar ratios are required to achieve 100% DNA retention. Therefore, the selective retention of BrdUrd-DNA on filters could be due to either an increased affinity or an increased number of protein binding sites. A third alternative is that the BrdUrd-DNA–protein complex could have a specific structure that may lead to more efficient retention.

Both the thermal-chromatography and filter-binding techniques demonstrate that the selective interaction of various proteins with BrdUrd-DNA differs. This is most evident when the individual histones are compared. It is notable that the relative selectivity of four of the histones for BrdUrd-DNA, as measured by both techniques, is similar (H4 > H2A > H2B > H1). The discrepancy shown by H3 is not understood but could be related to a difference in the oxidation state of its cystines (Lewis, 1976) under the two procedures. Our result, that H1 has little or no ability to distinguish between substituted and unsubstituted DNA, differs from the data of Lin and Riggs (1974), who state that the dissociation constant of an H1–BrdUrd-DNA complex is less than the dissociation constant of an H1–unsubstituted DNA complex. If the difference observed by Lin and Riggs (1974) for H1 is small relative to that of the other histones, then the apparent disagreement may merely reflect a difference in the sensitivities of the measurements.

The molecular basis for the selective interaction of chromosomal proteins with BrdUrd-DNA could involve either direct interaction with the 5′-bromine or recognition of a more far reaching structural change in the DNA. Results consistent with base recognition by histones include selective precipitation of A–T rich DNA by polylysine (Leng and Felsenfeld, 1966) and selective interaction of H1 with certain procaryotic and eucaryotic DNA (Renz, 1975). Recognition of structural differences is reasonable, as well, since the structures of substituted and unsubstituted DNA in chromatin are substantially different (Simpson and Seale, 1974; Augenlicht et al., 1974; Lapeyre and Bekhor; 1974). Moreover, the nitrocellulose-filter binding assay has been used to demonstrate a difference in the affinity of supercoiled and linear DNA for H1 (Vogel and Singer, 1975).

The selective interaction of the chromosomal proteins with BrdUrd-DNA might play a role in the selective alteration of phenotype of BrdUrd. We and others have proposed that an altered interaction of regulatory proteins with DNA could affect genetic expression (Lin and Riggs, 1972; Rutter et al., 1973; David et al., 1974). Nonhistone chromosomal proteins are likely to be involved (Paul et al., 1974; Barrett et al., 1975; Stein et al., 1975). Since the total nonhistone protein fraction was employed in these experiments, it is possible that the interaction of a minor class of (regulatory) proteins with DNA is much more substantially affected than indicated in the present experiments. The variations in the selective interactions among the histones (Figure 8 and Table III) and between the histone-depleted HTC chromosomal proteins and the rat liver nonhistones (Tables I and II) underscore this possibility. It may also be significant that the BrdUrd-DNA shows a greater selective interaction with the histones of the nucleosome (nubody, chromatin subunit). Perhaps an alteration of the binding of histones to BrdUrd-DNA within the nucleosome interferes with essential structural transition which must occur in response to regulatory signals during differentiation. Some aspects of the apparently selective nature of the biological effects of BrdUrd may also result from the purportedly nonuniform substitution of BrdUrd into DNA at low analogue concentrations (Schwartz et al., 1974; Strom and Dorfman, 1976). Numerous possibilities exist and further study of the effects of BrdUrd on gene control in eukaryots should prove fruitful in understanding the basic mechanisms of differentiation.

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References

Augenlicht, L., Nicolini, C., and Baserga, R. (1974), *Biochem. Biophys. Res. Commun. 59,* 920.

Barrett, T., Maryanka, D., Hamlyn, P. H., and Gould, H. J. (1975), *Proc. Natl. Acad. Sci. U.S.A. 71,* 5057.

Baxter, J. D., and Tomkins, G. M. (1970), *Proc. Natl. Acad. Sci. U.S.A. 65,* 709.

Bernardi, G. (1971), *Methods Enzymol. 22,* 325.

Bischoff, R., and Holtzer, H. (1970), *J. Cell Biol. 44,* 134.

Brown, J. C. (1971), *Exp. Cell Res. 69,* 44.

Burton, K., and Peterson, G. B. (1957), *Biochim. Biophys. Acta 26,* 497.

Chamberlin, M., and Berg, P. J. (1964), *J. Mol. Biol. 8,* 297.

David, J., Gordon, J. S., and Rutter, W. J. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 2808.

Flory, P. J., Jr., and Vinograd, J. (1973), *J. Mol. Biol. 74,* 81.

Githens, S., Pictet, R., Walther, B. T., and Rutter, W. J. (1976) (manuscript in preparation).

Goldberg, M. I., Perriard, J. C., and Rutter, W. J. (1976) (manuscript in preparation).

Hampor, B., Derge, J. G., Martos, L. M., and Walker, J. L. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 3185.

Ingram, V. M., Chan, L. L., Hagopian, H. K., Lipke, J. A., and Wu, L. (1974), *Dev. Biol. 36,* 411.

Jones, T. C., and Dove, W. F. (1972), *J. Mol. Biol. 64,* 409.

Koyama, H., and Ono, T. (1971), *Exp. Cell Res. 69,* 468.

Koyama, H., and Ono, T. (1972), *Biochim. Biophys. Acta 264,* 497.

Lapeyre, J., and Bekhor, I. (1974), *J. Mol. Biol. 89,* 137.

Leng, M., and Felsenfeld, G. (1966), *Proc. Natl. Acad. Sci. U.S.A. 56,* 1325.

Lewis, P. N. (1976), *Biochem. Biophys. Res. Commun. 68,* 329.

Lin, S., and Riggs, A. D. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2574.

Lin, S., and Riggs, A. D. (1974), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 33,* 1553.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265.

Lowy, D., Rowe, W., Teich, N., and Hartley, J. (1971), *Science 174,* 155.

MacGillivray, A. J., Cameron, A., Krause, R. J., Rickwood, D., and Paul, J. (1972), *Biochim. Biophys. Acta 277,* 384.

Mandel, M., Schildkraut, C. L., and Marmur, J. (1968), *Methods Enzymol. 12B*, 184.
Marmur, J. (1961), *J. Mol. Biol. 3*, 208.
Martinson, H. G. (1973), *Biochemistry 12*, 145.
Martinson, H. G., and McCarthy, B. J. (1975), *Biochemistry 14*, 1073.
Marushige, K., and Bonner, J. (1966), *J. Mol. Biol. 15*, 160.
McConaughy, B. L., and McCarthy, B. J. (1972), *Biochemistry 11*, 998.
Ohlenbusch, H. H., Olivera, B., Tuan, D., and Davidson, N. (1967), *J. Mol. Biol. 25*, 299.
Olafsson, P. G., and Bryan, A. M. (1974), *Arch. Biochem. Biophys. 165*, 46.
Paul, J., Gilmour, R. S., Fava, M. F., Birnie, G., Harrison, P., Hell, A., Humphries, S., Windass, J., and Young, B. (1974), *Cold Spring Harbor Symp. Quant. Biol. 38*, 885.
Renz, M. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72*, 733.
Riggs, A. D., Suzuki, H., and Bourgeois, S. (1970), *J. Mol. Biol. 48*, 67.
Rutter, W. J., Pictet, R. L., and Morris, P. W. (1973), *Annu. Rev. Biochem. 42*, 601.
Samuels, H. H., and Tomkins, G. M. (1970), *J. Mol. Biol. 52*, 57.
Schlitz, J. R., Mayne, R. M., and Holtzer, H. (1973), *Differentiation 1*, 97.
Schubert, D., and Jacob, F. (1970), *Proc. Natl. Acad. Sci. U.S.A. 67*, 247.
Schwartz, S. A., Horio, D., and Kirsten, W. J. (1974), *Biochem. Biophys. Res. Commun. 61*, 927.
Sheehan, D. M., and Olins, D. E. (1974), *Biochim. Biophys. Acta 353*, 438.
Shih, T. Y., and Bonner, J. (1970), *J. Mol. Biol. 48*, 469.
Simpson, R. T., and Seale, R. L. (1974), *Biochemistry 13*, 4609.
Spelsberg, T. C., Hnilica, L. S., and Ansevin, A. T. (1971), *Biochim. Biophys. Acta 228*, 550.
Stein, G., Park, W., Thrall, C., Mans, R., and Stein, J. (1975), *Nature (London) 257*, 764.
Stellwagon, R. H., and Tomkins, G. M. (1971), *J. Mol. Biol. 56*, 167.
Strom, C. M., and Dorfman, A. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73*, 1019.
Vogel, R., and Singer, M. F. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72*, 2597.
Walther, B. T., Pictet, R. L., David, J. D., and Rutter, W. J. (1974), *J. Biol. Chem. 249*, 1953.
Wataya, Y., Negishi, K., and Hayatsu, H. (1973), *Biochemistry 12*, 3992.
Weintraub, H., Campbell, G., and Holtzer, H. (1972), *J. Mol. Biol. 70*, 337.
Yarus, M., and Berg, P. (1970), *Anal. Biochem. 35*, 450.

Reactivity of Ribosomal Sulfhydryl Groups in 30S Ribosomal Subunits of *Escherichia coli* and 30S–IF-3 Complexes†

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ABSTRACT: The reaction of 30S subunits with the SH group reagent *N*-ethylmaleimide (NEM) causes the loss of approximately 60% of their synthetic activity, but has little or no effect on the ribosomal binding of initiation factor IF-3. The ribosomal binding of this factor, on the other hand, was found to significantly influence the rate and the extent to which several 30S ribosomal proteins react with radioactively labeled NEM when the reaction kinetics of individual ribosomal proteins toward NEM were compared in 30S and 30S–IF-3 complexes. Of the nine 30S ribosomal proteins which react with NEM, proteins S1, S11, S12, and S18 were found to have lower reactivities, while proteins S4 and S21 displayed higher reactivity in the presence of IF-3. The reactivity of proteins S8, S13, and S17, on the other hand, appeared to be influenced little or not at all by the presence of the factor. These results are interpreted as supporting evidence for the premise that the binding of IF-3 results in a conformational change of the 30S subunit.

Studies aimed toward the identification of the nature of the ribosomal binding site for IF-3 have suggested that this factor binds to the 16S rRNA[1] (Gualerzi and Pon, 1973; Pon and Gualerzi, 1976) in a region probably adjacent to the subunit interface (Gualerzi et al., 1973). Cross-linking experiments have indicated that, when bound to the ribosomes, IF-3 neighbors the 3′ end of the 16S rRNA (van Duin et al., 1975) as well as ribosomal proteins S1, S11, S12, S13, S14, S18, S19, and S21 (Hawley et al., 1974; Traut et al., 1974). Although data from another laboratory yield a different pattern of cross-linking, with S7 being the major cross-linked product (van Duin et al., 1975), recent immune electron microscopy data seem to confirm the localization of IF-3 in the proximity of proteins S11, S13, and S19 (Lake and Kahan, 1975).

Several lines of evidence suggest that the binding of IF-3 to the 30S ribosomal subunit causes a conformational change of the ribosomal particle (Pon and Gualerzi, 1974; Gualerzi et al., 1975). This change in conformation may be the molecular basis for the functional activity of the initiation factor in promoting ribosomal binding of mRNA (Iwasaki et al., 1968; Revel et al., 1968; Wahba et al., 1969; Sabol et al., 1973; Noll and Noll, 1974; Jay and Kaempfer, 1975) and in preventing

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[1] Abbreviation used: NEM, *N*-ethylmaleimide; rRNA, ribosomal ribonucleic acid; mRNA, messenger RNA; Tris, tris(hydroxymethyl)-aminomethane; poly(U), poly(uridylic acid).