lac Operator Nucleosomes. 1. Repressor Binds Specifically to Operator within the Nucleosome Core†

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ABSTRACT: We have shown that the *lac* repressor can recognize and bind specifically to the *lac* operator contained in short restriction fragments which have been complexed with the four core histones to form artificial nucleosomes and core particles. These *lac* reconstitutes have been well characterized, and it is apparent that the operator DNA itself is associated fully and normally with the octameric histone cores. The binding of repressor to these reconstitutes is operator dependent since nucleosomes lacking the operator sequence fail completely to bind repressor under our conditions. Moreover, binding is abolished by IPTG (isopropyl thiogalactoside), further demonstrating operator specificity. Nevertheless, sedimentation studies show that repressor binding does not involve displacement of the histone octamer. Thus, the *lac* repressor and the histone octamer bind simultaneously to the same DNA. *lac* reconstitutes, in which the DNA has been cross-linked to the histones with formaldehyde, also support simultaneous specific binding by *lac* repressor. Since all particles among the reconstitues, cross-linked or not, bind repressor quantitatively, we infer that the repressor binding surface of the operator DNA always faces generally outward rather than inward toward the histone core. It is likely to be this feature of *lac* operator particle structure, dictated in an unknown manner by DNA sequence, that allows the simultaneous binding of histones and repressor to the same DNA region.

Eukaryotic DNA is complexed tightly with histones to form nucleosomes, the fundamental repeating structural unit of chromatin [for a recent review, see Felsenfeld (1978)]. Even actively transcribed sequences apparently remain packaged in nucleosome-like structures while nevertheless supporting transcription. Thus, it seems likely that regulatory sequences in DNA also are packaged as nucleosomes. We have therefore initiated experiments designed to determine the effect of the nucleosomal DNA conformation on the availability of specific nucleotide sequences for recognition and binding by regulatory proteins. As a model system, we are examining the effect on *lac* repressor recognition and binding of packaging its cognate DNA sequence, the *lac* operator region of *Escherichia coli*, into nucleosomes.

The nucleosome consists of a histone core (an octamer comprised of two each of H2A, H2B, H3, and H4) around which is wrapped ~190 base pairs of DNA (Felsenfeld, 1978; Spadafora et al., 1978; Christiansen & Griffith, 1977) in approximately two tight superhelical turns (Prunell et al., 1979; Bryan et al., 1979; Crothers et al., 1978). By use of micrococcal nuclease, various subnucleosomal particles have been produced [e.g., Bakayev et al. (1977)]. Of these, the "core particle" has received the greatest attention because it is the most stable of the digestion intermediates. The nucleosomal core particle consists of 146 base pairs of DNA wound probably in 1.75 superhelical turns around the histone octamer [Prunell et al., 1979; Simpson & Künzler, 1979; Bryan et al., 1979; Rhodes, 1979; but see Crothers et al. (1978)]. A particle called the "chromatosome" (Simpson, 1978), which probably contains 168 base pairs of superhelical DNA (Weischet et al., 1979), has also been described. It has been suggested that this, rather than the core particle, more accurately represents the complete elementary repeat unit of chromatin (Simpson, 1978; Weischet et al., 1979).

An important feature of nucleosome structure is that the DNA is wrapped on the *outside* of the core histone octamer (Felsenfeld, 1978). Thus, nucleosomal DNA is accessible to the DNA "binding" protein, DNase I,[1] over essentially all of its length [see Felsenfeld (1978) and Prunell et al. (1979)]. The mechanism by which DNase I recognizes, binds to, and then cleaves the DNA has been the subject of intensive study [see Sollner-Webb et al. (1978) and Lutter (1979)].

Eukaryotic systems have not yet provided detailed information on the nature of regulatory protein–DNA recognition mechanisms. There are, therefore, only prokaryotic precedents to rely upon. Study of the *lac* repressor, λ repressor, catabolite activator protein, and *E. coli* RNA polymerase have revealed several features of specific DNA–protein complexes. These are as follows: (1) the DNA remains mostly helical (Saucier & Wang, 1972; Maniatis & Ptashne, 1973; Wang et al., 1974; Goeddel et al., 1978; Schmitz & Galas, 1979); (2) the protein makes contact with the major groove of DNA (Humayun et al., 1977; Majors, personal communication) and sometimes with the minor groove as well (Gilbert et al., 1976; Johnsrud, 1978; Goeddel et al., 1978); (3) the protein binds exclusively (Goeddel et al., 1978; Majors, personal communication) or predominantly (Siebenlist & Gilbert, 1980) to only one face of the double-helical DNA. None of these features of recognition is necessarily excluded with nucleosomal DNA, since the DNA is helical and remains exposed on one side over essentially all of its length.

Previously we have reported the reconstitution of *lac* operator containing DNA in vitro with core histones from calf thymus to form artificial nucleosomes and core particles (Chao et al., 1979). The use of a 144 base pair restriction fragment containing *lac* operator yields an artificial "*lac core particle*". The use of a 203 base pair restriction fragment (of which the 144 base pair piece is a subfragment) yields an artificial "*lac*

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[1] Abbreviations used: IPTG, isopropyl thiogalactoside; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; DNase I, pancreatic deoxyribonuclease.

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nucleosome". Here we report that *lac* repressor, whose binding involves contacts to both DNA grooves but only one face of the double helix (Goeddel et al., 1978; Schmitz & Galas, 1979), is able to recognize and bind specifically to both *lac* nucleosomes and *lac* core particles.

Materials and Methods

Preparation of DNA. Plasmid containing the 203 base pair *lac* restriction fragment was prepared as described (Chao et al., 1979). In some instances, plasmid DNA was purified by elution through a Sepharose 2B column in lieu of CsCl buoyant density centrifugation. Plasmid-containing fractions were pooled and concentrated to ~1 mg/mL by precipitation with ethanol. The DNA was then cleaved with restriction endonuclease *Eco*RI, and the *lac* insert was purified either by polyacrylamide gel electrophoresis (Chao et al., 1979) or by sucrose gradient sedimentation (J. D. McGhee and G. Felsenfeld, personal communication). The 144 base pair *lac* subfragment was obtained by restricting the ^{32}P-end-labeled 203 base pair *lac* fragment with endonuclease *Hha*I from *Haemophilus haemolyticus*, followed by gel purification as described previously (Chao et al., 1979). "Random sequence" calf thymus DNA for use in control experiments was extracted from kinased ^{32}P-labeled nucleosomes isolated as described by Martinson et al. (1979). The nucleosomal DNA was fractionated on an 8% polyacrylamide gel, and DNA of 150 ± 10 base pairs was recovered from the gel by the elution procedure of Maxam & Gilbert (1977).

Reconstitution with Core Histones. Reconstitution of restriction fragments was accomplished by using the rapid-mixing procedure described previously (Chao et al., 1979) in which tracer amounts of labeled *lac* fragment and unlabeled carrier nucleosomal DNA were reconstituted with acid-extracted histones. The reconstituted nucleosomes were freed of H1 and most nonhistone proteins by sucrose gradient centrifugation in 0.6 M NaCl. Particles sedimenting at ~11 S were pooled and dialyzed overnight at 4 °C into repressor binding buffer. The concentration of carrier in the pooled fractions was adjusted to at least 60 μg/mL, if necessary, by addition of unlabeled calf thymus nucleosomes before dialysis into repressor binding buffer.

By a number of criteria, including DNase I digestion and sedimentation (Chao et al., 1979), as well as zero-length cross-linking [unpublished results; see Martinson & True (1979)], this reconstitution procedure yields core particles which resemble native core particles isolated by micrococcal nuclease digestion of calf thymus nuclei. All four histones are present in equimolar amounts (Martinson et al., 1979).

Preparation of Repressor. Repressor, prepared by the method of Rosenberg et al. (1977), was a gift of A. Riggs and R. Dickerson.

Repressor Binding Studies. A tracer amount of ^{32}P-end-labeled test DNA was incubated with repressor and then assayed for repressor binding by monitoring its sedimentation rate as a function of repressor concentration on isokinetic sucrose gradients. The labeled DNA was either naked or complexed with histones and was either a *lac* restriction fragment or random sequence calf thymus DNA as specified in the text. A typical binding reaction was conducted in 100 μL of repressor binding buffer (100 mM KCl, 0.1 mM EDTA, 3 mM $MgCl_2$, and 10 mM Tris, pH 7.5) containing ~60 μg/mL nonradioactive carrier calf thymus nucleosomes or nucleosomal DNA as appropriate. *lac* repressor was added to the sample and incubated for 10 min at 37 °C before being layered on a sucrose gradient which contained repressor at the same concentration.

Isokinetic sucrose gradients were formed by layering the following volumes and concentrations of sucrose (in repressor binding buffer) in SW60 polyallomer tubes: 0.29 mL of 19% (w/v) sucrose; 0.5 mL each of 17.9, 16.8, 15.5, 14.0, 12.4, 10.6, and 8.5% sucrose. These volumes and concentrations of sucrose were calculated to be isokinetic for nucleosomes at a temperature of 20 °C by using a computer program made available to us by V. Schumaker and assuming a particle density of 1.5 gm/cm^3. For the repressor-containing gradients, the top four layers of sucrose solution contained repressor at the appropriate concentration. The layered gradients were stored for 4–5 h prior to centrifugation in order to allow diffusion between layers. (For the experiments reported here, this step was carried out at 4 °C as a cautionary measure to preserve repressor activity. Recently we have found that incubation at room temperature yields the same results.) Centrifugation was at 60 000 rpm for 3–3.5 h at a gradient temperature of 15–20 °C. Fractions of 0.1 or 0.2 mL were collected in Croan cups and monitored for radioactivity in DNA by Cerenkov counting. Background (10–15 cpm) was not subtracted. Analysis of the A_{280} profile of a control gradient showed that the concentration of repressor remained constant throughout the region of the tube traversed by operator and that repressor did not aggregate and pellet even at the highest concentrations used.

DNase I Digestion. Digestion of the 144 base pair *lac* core particle was performed as described previously (Chao et al., 1979). The products of digestion were denatured by heating for 2–3 min at 100 °C in deionized formamide and electrophoresed on 12% polyacrylamide–7 M urea gels for 3.5 h at 250 V. The gels were autoradiographed with a Du Pont Quanta II intensifier on Cronex 4 X-ray film at −70 °C for 2–3 days.

DNase I digestion of repressor-bound 144 base pair *lac* core particles was conducted in the presence of 200 μg/mL repressor. For Figure 5, repressor was preincubated with the reconstitute in 10 mM Tris, 0.1 mM EDTA, and 5 mM $MgCl_2$ for 10 min at 37 °C with DNase I at a final concentration of 5 μg/mL. Our standard repressor binding buffer has also been used with essentially identical results.

Formaldehyde Cross-Linking. Cross-linking of *lac* reconstitutes by formaldehyde was performed as described by Simon et al. (1978). Reconstitutes pooled from the 0.6 M NaCl gradients were dialyzed first against 10 mM sodium phosphate buffer, pH 6.7, and 0.1 mM EDTA and then against the same buffer containing 1% formaldehyde for 16 h at 4 °C. The cross-linked material was then dialyzed exhaustively (~1 day) against repressor binding buffer and analyzed for repressor binding properties. We have confirmed that these particles remained cross-linked following the dialysis to remove formaldehyde by determining that the DNA was no longer recoverable by sodium dodecyl sulfate–phenol extraction and that it did not migrate in DNA gels.

Results

Method for Analyzing Repressor–Operator Binding. We have used a sedimentation method to detect binding of *lac* repressor to operator-containing restriction fragments. The basic experimental protocol is to sediment ^{32}P-end-labeled *lac* DNA (or reconstitutes) through an isokinetic sucrose gradient containing a constant concentration of *lac* repressor. In the absence of repressor, the *lac* DNA or reconstitutes sediment at a characteristic rate. However, if repressor is present in the gradient, it binds to *lac* operator, and the DNA (or reconstitute)–repressor complexes sediment faster. Since the rates of association and dissociation are rapid relative to the

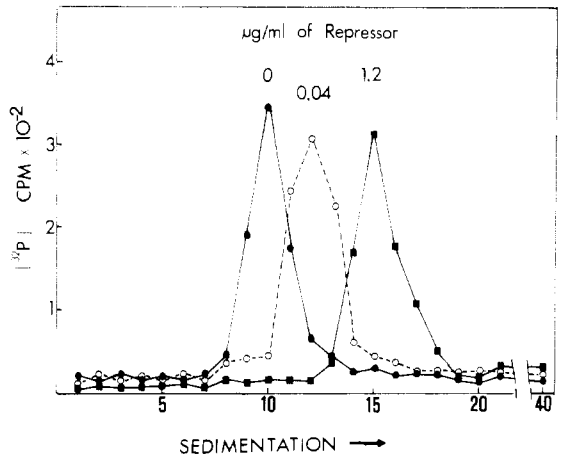

FIGURE 1: Sedimentation analysis of repressor binding to 203 base pair *lac* DNA. Tracer amounts of ^{32}P-labeled *lac* DNA plus carrier DNA isolated from nucleosomes were incubated with varying concentrations of *lac* repressor and sedimented in parallel sucrose gradients as described under Materials and Methods. The incubation mixtures and gradients contained the indicated concentrations of repressor. Fractions were 0.1 mL and the fraction numbers are given on the abcissa. Fractions 22–39 contained only background levels of radioactivity.

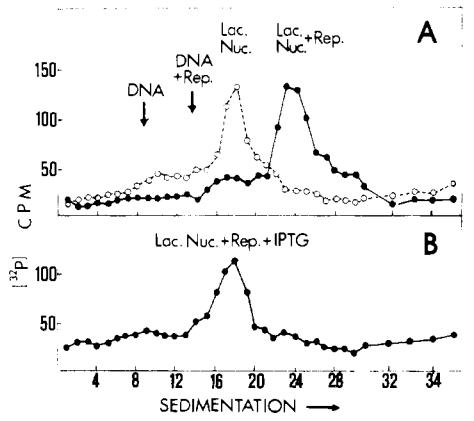

FIGURE 2: Repressor binding to 203 base pair *lac* nucleosomes. (A) Reconstituted *lac* nucleosomes were incubated and sedimented in the absence or presence of 1.3 μg/mL repressor. Fractions 1–30 were 0.1 mL and fractions 31–35 were 0.2 mL. The arrows indicate the sedimentation positions of naked 203 base pair *lac* DNA and repressor-bound *lac* DNA in parallel gradients. (B) the *lac* nucleosomes were incubated in the presence of repressor as described above, IPTG was then added to 5 mM, and sedimentation was conducted in the presence of both IPTG and repressor in parallel with the gradients of part A.

time of centrifugation (Riggs et al., 1970), we assume that the operator DNA is at equilibrium with respect to repressor binding during the centrifuge run. Therefore, the presence of repressor in the gradient should yield a single more rapidly sedimenting peak of end-labeled operator DNA. The greater the concentration of repressor in the gradient, the greater will be the increase in average sedimentation rate of the operator-containing species. As the limit of fully repressor-bound operator is approached, the sedimentation rate will simply approach that of the repressor–operator complex. On the other hand, a species which has no affinity for repressor will be unaffected in its sedimentation rate by any concentration of repressor in the gradient.

Figure 1 demonstrates the application of this method to the 203 base pair operator restriction fragment. Three representative gradients containing different concentrations of *lac* repressor are shown. It can be seen that the DNA–repressor complexes which are formed in the presence of 1.2 μg/mL repressor sediment quantitatively about five fractions further in these gradients than the free DNA. Moreover, at an intermediate repressor concentration (0.04 μg/mL), a single partially shifted peak is observed as predicted. In the presence of isopropyl thiogalactoside (IPTG), a gratuitous inducer of *lac* repressor, no peak shift is observed, confirming the operator specificity of the interaction (data not shown).

This result demonstrates that the method is capable of detecting operator–repressor complexes. Therefore, we used this method to probe binding of repressor to operator contained within reconstituted nucleosomes.

Repressor Binds to lac Nucleosomes. The 203 base pair restriction fragment containing the *lac* operator was reconstituted with histones to give artificial *lac* nucleosomes. Figure 2A illustrates the results of an experiment in which these 203 base pair *lac* nucleosomes were sedimented in the presence or absence of 1.3 μg/mL *lac* repressor. Remarkably, the sedimenting peak of *lac* nucleosomes is quantitatively shifted in the presence of repressor, reflecting efficient complex formation much the same as for the naked restriction fragment. The sedimentation rate of the repressor–nucleosome complex of Figure 2A clearly is substantially greater than that of the repressor–DNA complex of Figure 1, indicating that repressor binding has not resulted in displacement of the histone octamer.

These data demonstrate directly that *lac* repressor can recognize and bind to 203 base pair nucleosomes containing the *lac* operator. Figure 2B shows that repressor binding is abolished in the presence of the gratuitous inducer, IPTG (Lin & Riggs, 1975), confirming that the interaction with *lac* nucleosomes is operator specific. Moreover, the sedimentation rate of the *lac* nucleosomes is not altered by the specific binding and subsequent release of the *lac* repressor, confirming the continued presence of the histones.

Repressor Binds Specifically to lac Core Particles. The 144 base pair *lac* core particles also bind repressor specifically; Figure 3A shows that in the presence of 200 μg/mL *lac* repressor, a substantial shift in sedimentation of the 144 base pair *lac* core particles occurs. We note that this shift depends on a higher concentration of *lac* repressor than the 1.3 μg/mL used for the 203 base pair *lac* nucleosomes (Figure 2A). Figure 3C shows that at a lower repressor concentration only a slight shift is observed for the *lac* core particles.

Three lines of evidence show that, despite the higher concentration of repressor required, this binding to 144 base pair *lac* core particles is operator specific and is *not* the result of nonspecific binding. First, Figure 4 demonstrates the application of this method to reconstituted calf nucleosomes containing 150 ± 10 base pair *non*operator DNA. *No* shift in sedimentation is observed, even though the repressor concentration in the gradient is 400 μg/mL. In contrast, Figure 3A, as well as additional experiments [see Chao et al. (1980)], show that repressor causes a large increase in the sedimentation rate of all *lac* core particles at a concentration (200 μg/mL) which is half of that which is without any effect on the nonoperator nucleosomes. Thus, binding is specific for particles which contain the *lac* operator.

The second demonstration of operator specificity for repressor binding to the *lac* core particles is illustrated in Figure 3B. There it is shown, by comparison to Figure 3A, that the specific inducer, IPTG, abolishes the repressor–core particle interaction.

In a final demonstration of binding specificity, we have established by direct DNase I protection experiments that repressor is bound specifically to the operator region of the

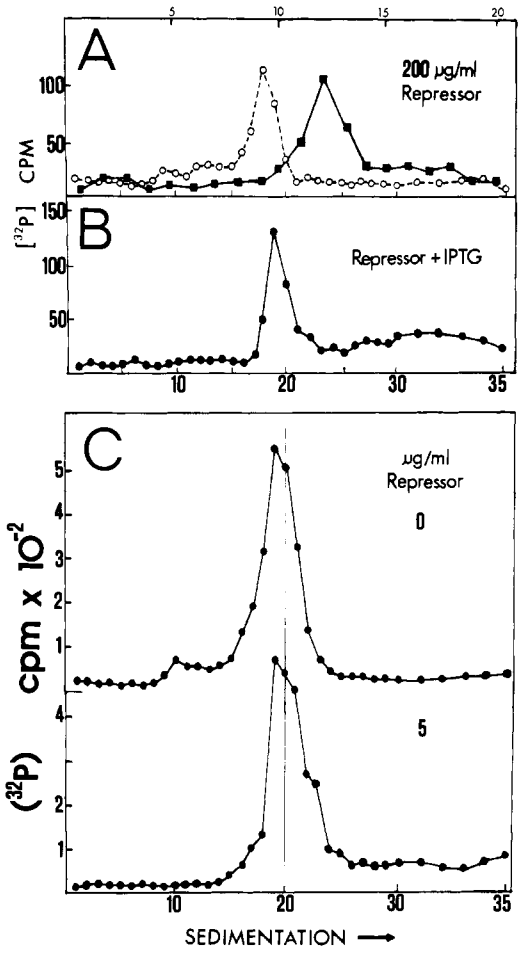

FIGURE 3: Repressor binding to 144 base pair *lac* core particles. (A and C) *lac* core particles were assayed for repressor binding in the presence of the indicated concentration of repressor as described for Figure 2. Fraction numbers for the repressor-containing gradient of this panel are given by the smaller numerals on the upper scale since 0.2-mL fractions only were collected for this gradient. (B) Repressor binding was reversed by addition of 100 mM IPTG as described for Figure 2, and sedimentation was conducted in parallel with the gradients of part A. The unusually high concentration of IPTG used was made necessary by the high concentration (200 μg/mL) of repressor present. This concentration of inducer has no effect on nonspecific DNA binding in our system (data not shown).

lac core particles. Figure 5 shows partial DNase I digestion patterns of singly end-labeled *lac* core particles [see Chao et al. (1979) for a detailed analysis of the DNase I digestion patterns of *lac* core particles]. It can be seen that the patterns, plus and minus repressor, are very similar, the main difference being that position 60 is relatively much less intense (though not completely absent; see legend to Figure 5) when digestion is conducted in the presence of repressor. Of the bands produced by DNase I digestion of *lac* core particles, the one at position 60 is the only one which represents cleavage within the operator. This result, therefore, demonstrates, in agreement with the data presented above, that repressor binding to the 144 base pair *lac* core particle is operator specific since repressor specifically diminishes cleavage within this sequence by DNase I. Moreover, the high sedimentation rate of the repressor-containing complex in Figure 3 shows (refer to Figure 2) that the histone octamer is not displaced [see Stein (1979)] as a result of repressor binding.

Repressor Binds Specifically to lac Nucleosomes and Core Particles Cross-Linked by Formaldehyde. To substantiate that repressor and histones can bind simultaneously to operator DNA, we have studied the binding of repressor to reconstitutes

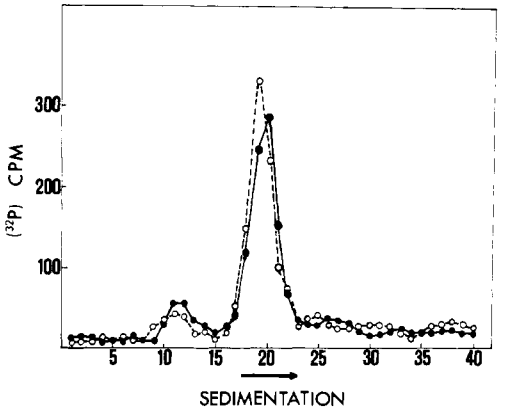

FIGURE 4: Sedimentation of nonoperator nucleosomes in a very high concentration of *lac* repressor. ^{32}P-Labeled calf thymus DNA 140–160 base pairs long was reconstituted with histones and then incubated and sedimented in the absence of repressor (●) or in the presence of 400 μg/mL repressor (○) as described under Materials and Methods. Note that the slight apparent peak shift due to experimental error is in the direction opposite to that expected for interaction with repressor.

in which the histones have been cross-linked to the DNA with formaldehyde. Figure 6 shows that the cross-linked *lac* core particles exhibit a substantial shift in sedimentation rate in the presence of 200 μg/mL *lac* repressor much as do non-cross-linked *lac* core particles (see Figure 3A). Similar results have been obtained for cross-linked *lac* nucleosomes (Figure 7). These quantitative shifts occur at a concentration of repressor which is insufficient to induce *any* detectable shift for nonoperator nucleosomes (Figure 4). Thus, covalent attachment of operator DNA to the histone core does not prevent operator-dependent repressor binding.

Discussion

lac Repressor Can Recognize and Bind Specifically to lac Operator Contained within a Nucleosome Core. The data which we have presented show that DNA within nucleosomes and core particles can be recognized and bound specifically by a regulatory protein. Two restriction fragments of different length, both containing the *lac* operator, have been complexed with histones and then challenged with repressor. When assembled with histones, a 203 base pair restriction fragment yields what we have called a "*lac* nucleosome" while a 144 base pair fragment gives a "*lac* core particle". Both the *lac* nucleosome and the *lac* core particle bind repressor specifically. Even when the DNA is cross-linked to the histones with formaldehyde, specific and quantitative repressor binding occurs for all the *lac* reconstitutes under conditions for which no detectable binding occurs to nonoperator, *un*-cross-linked, calf thymus nucleosomes. We therefore conclude that *lac* nucleosomes and nucleosome cores, which resemble closely native eukaryotic nucleosomes and core particles (Chao et al., 1979), allow recognition and sequence specific binding to their DNA of the regulatory protein, *lac* repressor.

This finding is consistent with current views concerning the structure of both histone–DNA and repressor–DNA complexes. Recent evidence indicates that the *lac* repressor binds predominantly along one side of the DNA double helix rather than surrounding the helix exterior (Goeddel et al., 1978). Similarly, it is now widely believed that the histones of nucleosomes provide primarily a surface over which the DNA is wrapped, thus leaving one side of the double helix largely exposed (Felsenfeld, 1978; Carter, 1978; Burton et al., 1978; Trifonov & Bettecken, 1979; Mirzabekov & Rich, 1979; Prunell et al., 1979; McGhee & Felsenfeld, 1979). It is

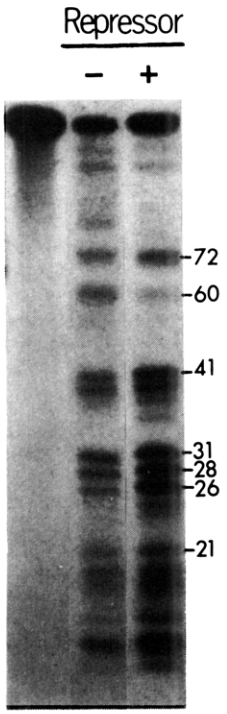

FIGURE 5: DNase I digestion of 144 base pair *lac* core particles bound by repressor. *lac* core particles were digested with DNase I in the presence or absence of repressor as described under Materials and Methods. The leftmost lane in the figure is a control sample which lacked DNase I and repressor. Digestion times were 5 min (minus repressor) and 2 min (plus repressor). The DNA lengths indicated in the figure were estimated with a precision of ±1 base from a calibration curve based on denatured *lac* restriction fragments of known sequence ranging in size between 21 and 207 bases. The "minimal" *lac* operator sequence as delineated by Bahl et al. (1978) occupies positions 49–65 on both of our *lac* fragments. Notice that in the presence of repressor the intensity of the band at 60 bases is diminished although it does not disappear completely. The experiments of Figure 3 demonstrate, using the same repressor concentration as used here, that *lac* core particles interact quantitatively with repressor (i.e., all of them are repressor bound for a significant fraction of time). However, since the operator is constantly becoming dissociated and then reassociated with repressor, a small amount of DNase I cleavage at the operator still occurs. We presume that if much higher concentrations of repressor were used, the frequency of cleavage at position 60 (i.e., within the operator) could be made vanishingly small [see Chao et al. (1980)]. Other more general differences in band intensities between the lanes +repressor and −repressor also are apparent and are under investigation.

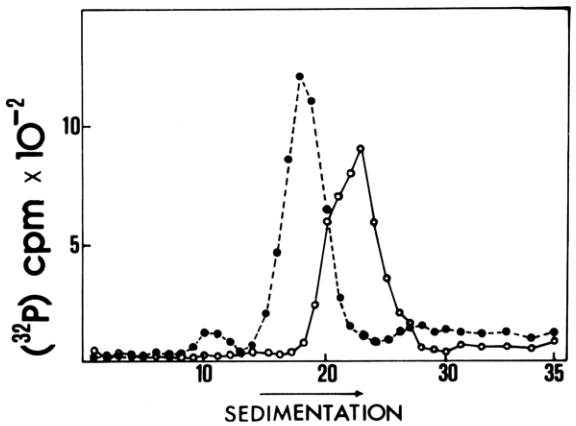

FIGURE 6: Repressor binding to formaldehyde cross-linked 144 base pair *lac* core particles. Reconstituted 144 base pair *lac* core particles were cross-linked with formaldehyde as described under Materials and Methods and then assayed for repressor binding by sedimentation. The incubation mixtures and gradients contained (– – –) no repressor or (—) 200 μg/mL repressor. Fractions 1–30 were 0.1 mL and fractions 31–35 were 0.2 mL.

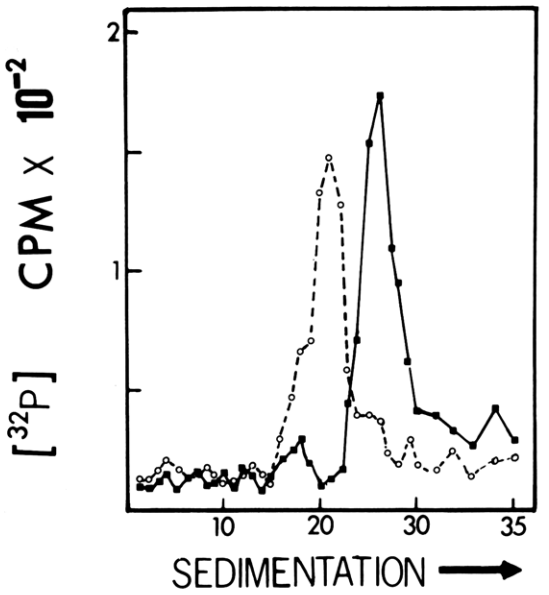

FIGURE 7: Repressor binding to formaldehyde cross-linked 203 base pair *lac* nucleosomes. Reconstituted 203 base pair *lac* nucleosomes were cross-linked and sedimented in the presence or absence of repressor as described for Figure 6.

therefore reasonable to expect that the DNA may bind to the histone octamer on one side while accommodating repressor recognition and binding to the other. This would be analogous to the simultaneous nonspecific binding of repressor to both sides of the DNA double helix reported by Zingsheim et al. (1977).

Histones Assemble lac Operator DNA So That Its Repressor Binding Face Is Always Accessible to Repressor. Since the repressor binds predominantly to only one side of the DNA double helix, the ability of repressor to bind operator should be sensitive to the screw orientation of the operator on the surface of the nucleosome. That is, the degree to which the repressor binding face of the operator is directed *away* from the surface of the histone core should be an important determinant of repressor binding affinity. The fact that both the 203 and the 144 base pair *lac* reconstitutes bind repressor specifically and quantitatively, even when cross-linked by formaldehyde, suggests that the repressor binding surface must, to some degree, be facing "out" for all particles in both types of reconstitute.

The DNase I digestion data of Figure 5 are consistent with the inference that the *lac* operator faces out in our reconstitutes. Recall from this figure that the DNase I band of 60 bases is markedly reduced in relative intensity by prior binding of repressor to *lac* core particles. Since the *lac* DNA in the reconstituted core particles is singly end labeled, the identity of the phosphodiester bond cleaved at position 60 can be determined by reference to three-dimensional models of the *lac* operator. Inspection of such models [see Goeddel et al. (1978) and Schmitz & Galas (1979), their positions 13 and 8, respectively, are equivalent to our position 60] reveals that this position is within the repressor binding surface of the operator DNA. Therefore, not only does protection of position 60 by repressor confirm the operator specificity of repressor binding to *lac* core particles (see Results) but also the initial accessibility of this position to DNase I confirms the inference that the repressor binding surface of operator DNA faces away from the histone core in the reconstitues. Unfortunately, a more precise analysis of this type currently is premature because it remains unclear what the precise steric and sequence parameters are which govern DNase I cleavage of nucleosomal

DNA (Sollner-Webb et al., 1978; Lutter, 1979; Simpson & Künzler 1979; Bryan et al., 1979; Rhodes, 1979) and knowledge of the steric requirements of DNase I *relative* to those of repressor is lacking.

Considering that in principle all faces of the DNA double helix should be able to bind histone, it would appear improbable that all particles among our reconstitutes should happen to have the repressor binding surface of the operator facing generally away from the histone core so as to be available for repressor binding. However, we have shown that the *lac* restriction fragments which we are using reconstitute with histones in a sequence-specific way (Chao et al., 1979). Since DNA binds to the histone cores on one side of the helix only, sequence preference should determine not only the linear position of the DNA with respect to the core as we have already shown (Chao et al., 1979) but also which face is in and which is out. There may be a short region of *lac* DNA which is predominantly responsible for the observed sequence preference of our *lac* restriction fragments for histone cores. If so, this region may invariably organize the DNA in such a way that the same side of the helix faces mostly out. This putative organizing region of sequence may be, but need not be, the operator itself.

Different lac Reconstitutes Bind Repressor Differently. Although both lac nucleosomes and *lac* core particles can bind repressor quantitatively and specifically, out data show that important differences exist among the *lac* reconstitutes. Thus, *lac* core particles are shifted quantitatively in sedimentation by repressor at 200 μg/mL but not by repressor at 5 μg/mL (Figure 3). In contrast, *lac* nucleosomes are shifted quantitatively at less than 2 μg/mL repressor (Figure 2). Therefore, *lac* nucleosomes have some intrinsic property which allows substantial binding at repressor concentrations much lower than those required for binding to *lac* core particles. Naked 144 and 203 base pair restriction fragments do not differ from each other in affinity for repressor (Chao et al., 1980) so the property of the 203 base pair *lac* nucleosomes which accounts for this high repressor affinity must reside in the way the DNA is packaged, not in the DNA per se.

This property is *not* a lack of histone binding to the operator in the 203 base pair *lac* nucleosomes. It can be deduced from our previous results, in which the positions of the histone core on the restriction fragment were determined (Chao et al., 1979), that the two predominant positions of the histone core along the 203 base pair DNA fragment place the operator [as delimited by Bahl et al. (1978)] within the 168 base pair "chromatosome" region (Simpson, 1978; Weischet et al., 1979) of the reconstituted nucleosome. Moreover, one of these predominant positions of the operator is within the actual core portion of the reconstituted particle. Since the total DNA binding capacity of the histone octamer may in fact be as much as 190 base pairs (Spadafora et al., 1978; Christiansen & Griffith, 1977), it is particularly clear that the operator region of the DNA in our 203 base pair *lac* nucleosomes is, in all cases, fully bound by histone.

Since our evidence indicates that the *lac* operator is firmly bound to the histone core in all of the reconstitutes, the highly enhanced binding characteristic of the 203 pair *lac* nucleosomes compared to the core particles is not a trivial consequence of the operator happening to reside in a "spacer" domain. In the following paper (Chao et al., 1980), we use a quantitative method for analyzing repressor–reconstitute affinities, in conjunction with cross-linking, to determine the structural basis for the ability of *lac* nucleosomes to bind repressor so tightly.

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lac Operator Nucleosomes. 2. *lac* Nucleosomes Can Change Conformation To Strengthen Binding by *lac* Repressor†

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ABSTRACT: We have shown previously that *lac* repressor binds specifically and quantitatively to *lac* operator restriction fragments which have been complexed with histones to form artifical nucleosomes (203 base pair restriction fragment) or core particles (144 base pair restriction fragment). We describe here a quantitative method for determining the equilibrium binding affinities of repressor for these *lac* reconstitutes. Quantitative analysis shows that the operator–histone reconstitutes may be grouped into two affinity classes: those with an affinity for repressor close to that of naked DNA and those with an affinity 2 or more orders of magnitude less than that of naked DNA. All particles in the *lac nucleosome* preparations bind repressor with high affinity, but the *lac core particle* preparations contain particles of both high and low affinities for repressor. Formaldehyde cross-linking causes all high-affinity species to suffer a 100-fold decrease in binding affinity. In contrast, there is no effect of cross-linking on species of low affinity. Therefore, the ability of a particle to be bound tightly by repressor depends on a property of the particle which is eliminated by cross-linking. Control experiments have shown that chemical damage to the operator does not accompany cross-linking. Therefore, the property sensitive to cross-linking must be the ability of the particle to change conformation. We infer that the particles of low native affinity, like cross-linked particles, are of low affinity because of an inability to facilitate repressor binding by means of this conformational change. Dimethyl suberimidate cross-linking experiments show that histone–histone cross-linking is sufficient to preclude high-affinity binding. Thus, the necessary conformational change involves a nucleosome histone core event. We find that the ability of a particle to undergo a repressor-induced facilitating conformational change appears to depend on the position of the operator along the DNA binding path of the nucleosome core. We present a general model which proposes that nucleosomes are divided into domains which function differentially to initiate conformational changes in response to physiological stimuli.

Previously we have shown that *lac* repressor can recognize and bind specifically to the *lac* operator contained in restriction fragments which have been complexed with the four core histones to form artifical *lac* nucleosomes and core particles (Chao et al., 1980). Both the 144 base pair *lac* core particles and the 203 base pair *lac* nucleosomes have been well characterized and shown to resemble native nucleosomes by a variety of tests [see Chao et al. (1979)]. The evidence for specific repressor binding includes sensitivity to inducer and quantitative binding under conditions where binding to nonoperator nucleosomes is undetectable. Moreover, sedimentation studies indicated that repressor and histones bind simultaneously to operator DNA. This was shown most clearly by the demonstration that fixing the histones to the DNA using formaldehyde did not prevent repressor binding.

In the studies reported below, we have investigated in quantitative terms the affinities for repressor of the *lac* reconstitutes. Our analysis has revealed that high-affinity repressor binding can be accommodated by the nucleosome via a conformational change within its core. Various correlations suggest that the position of the operator in the core may determine whether or not particles can undergo this facilitating change in conformation.

Materials and Methods

Restriction fragments 203 base pairs and 144 base pairs long containing the *lac* operator were prepared, complexed with histones, and challenged with repressor as described previously (Chao et al., 1980). The assay for repressor binding (Chao et al., 1980) consists of sedimenting the restriction fragments which have been ^{32}P end labeled through a constant concentration of repressor. Complex formation is detected as a repressor-dependent increase in the rate of sedimentation of the ^{32}P-labeled operator-containing particles. Repressor prepared by the method of Rosenberg et al. (1977) was a gift of A. Riggs and R. Dickerson. Formaldehyde cross-linked *lac* reconstitutes were prepared as described previously (Chao et al., 1980).

Cross-linking by dimethyl suberimidate was carried out by the procedure described by Stein (1979). Labeled 203 base

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