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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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pair *lac* reconstitutes were dialyzed exhaustively against 10 mM Tris,[1] pH 8, and 0.1 mM EDTA, and then carrier nucleosomes were added to achieve an A_{260} of 10. In a separate reaction tube, 1 mL of 0.1 M sodium borate, pH 10, was added to 5 mg of dimethyl suberimidate. Immediately, 100 μL of the *lac* reconstitute was added to the dimethyl suberimidate solution with rapid mixing. Cross-linking was carried out for 40 min at room temperature. Sodium dodecyl sulfate–polyacrylamide gel analysis indicated that no cross-linked species smaller than an octamer was produced. The cross-linked reconstitute was fractionated on a 5–20% sucrose gradient in an SW41 rotor for 12 h at 35 000 rpm. Particles sedimenting at 11 S were pooled and dialyzed against repressor binding buffer for analysis.

Method of Data Analysis

We have shown in the preceding paper (Chao et al., 1980) that the sedimentation rates of *lac* operator-containing particles in sucrose gradients are increased by repressor at concentrations sufficient to induce substantial repressor–operator binding. The higher the affinity of the operator-containing particle for repressor, the lower the concentration of repressor which is required to induce substantial repressor–operator binding and hence a significant increase in sedimentation rate of the operator-containing particle. For the work to be described below, we have derived a quantitative method of data analysis in order to obtain precise K_{diss} values and to analyze heterogenous systems.

The equilibrium dissociation expression for the repressor–operator interaction is

$$K_{diss} = \frac{[\text{repressor}][\text{operator}]}{[\text{repressor·operator}]} \equiv \frac{[R][O]}{[R{\cdot}O]} \qquad (1)$$

This expression shows that a concentration of free repressor equivalent to the dissociation constant of the complex gives operator which is bound by repressor an average of 50% of the time. Since we assume that the repressor–operator interaction is at equilibrium during centrifugation [see Chao et al. (1980)], it follows that a concentration of repressor in the gradient equivalent to the dissociation constant of the complex should give rise to a single peak of *lac* operator sedimenting halfway between the characteristic uncomplexed and fully complexed positions. In principle, it is thus possible to determine K_{diss} directly by ascertaining the repressor concentration required to yield such a "half-shift" in operator sedimentation. Experimentally this is difficult, however, since it is necessary to know the corresponding "full-shift" in order to deduce the half-shift, and full repressor binding of operator is approached only asymptotically as the repressor concentration is increased. We have therefore derived an expression based on eq 1 which relates the increase in sedimentation distance of an operator-containing population to the repressor concentration in the gradient. For single component systems this allows K_{diss} to be determined directly from a linear plot of the data.

Single-Component System. We define D as the distance sedimented by the operator (in naked restriction fragment or as restriction fragment–histone complexes) in an isokinetic sucrose gradient. ΔD is the additional increment of distance sedimented by the operator in the presence of repressor. The association and dissociation rates for the repressor–operator interaction are rapid compared to our centrifugation time. Therefore, equilibrium is maintained during sedimentation and the entire population of *lac* operators responds uniformly to increasing concentrations of repressor in the gradient (Chao et al., 1980). ΔD_{max} is defined as the ultimate distance increment which is approached asymptotically as the repressor concentration goes to infinity. In practice, ΔD_{max} is approachable only for species which bind repressor very tightly and therefore nearly completely at experimentally acceptable concentrations of repressor. We will express D, ΔD, and ΔD_{max} in distance units, defined for our system as the number of (0.1 mL) fractions sedimented under specific conditions.

We now define the ratio $\Delta D'$ which is the proportion of operators bound by repressor under a given set of conditions:

$$\Delta D' = \frac{[R{\cdot}O]}{[O] + [R{\cdot}O]} = \frac{\Delta D}{\Delta D_{max}} \qquad (2)$$

In the absence of repressor, ΔD and, hence, $\Delta D'$ are 0. On the other hand, at very high repressor concentrations where the operator population approaches complete binding, ΔD approaches ΔD_{max} and, hence, $\Delta D'$ goes to 1. At intermediate repressor concentrations the ratio varies between 0 and 1. For example, as described above, at a repressor concentration equal to the dissociation constant (K_{diss}), half of the operators are repressor bound and the sedimenting peak of operators becomes half-maximally shifted, giving a $\Delta D'$ of 0.5.

Rearranging eq 1 gives

$$[O] = K\frac{[R{\cdot}O]}{[R]} \qquad (3)$$

Substituting eq 3 into eq 2 then gives

$$\Delta D' = \frac{[R{\cdot}O]}{K\dfrac{[R{\cdot}O]}{[R]} + [R{\cdot}O]} = \frac{1}{\dfrac{K}{[R]} + 1} \qquad (4)$$

and taking the reciprocal

$$\frac{1}{\Delta D'} = \frac{K}{[R]} + 1 = \frac{K'}{[R]'} + 1 \qquad (5)$$

where K' and R' are expressed in the convenient operational units of micrograms of repressor per milliliter. We have then substituted eq 2 into eq 5 to obtain

$$\frac{\Delta D_{max}}{\Delta D} = \frac{K'}{[R]'} + 1 \qquad (6)$$

which upon rearranging gives

$$\frac{1}{\Delta D} = \frac{K'}{\Delta D_{max}}\,\frac{1}{[R]'} + \frac{1}{\Delta D_{max}} \qquad (7)$$

This equation conveniently is plotted as $1/\Delta D$ vs. $1/[R]'$ which is a straight line. The Y intercept is $1/\Delta D_{max}$ and the slope is $K'/\Delta D_{max}$. Thus, for a single-component system, both ΔD_{max} and K' for the repressor–operator interaction can be calculated precisely from an appropriate set of data.

Multicomponent System. Equation 7 is linear only for single-component systems. Heterogeneity in a population with respect to K' results in a nonlinear relationship such that a plot of $1/\Delta D$ vs. $1/[R]'$ is concave downward. K' values may be extracted from such plots by curve fitting.

When a mixture of operator-containing particles which vary in K' is exposed to repressor at a given concentration, each component gives rise to a characteristic ΔD. However, as will become clear under Results, ΔD usually cannot be measured separately for each component because the individual sedimentation profiles are not adequately resolved. Therefore, in

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

the case of a multicomponent sytem, we must measure the composite term ΔD_{av} which is the number-average increment in sedimentation for all of the operator-containing components in the gradient:

$$\Delta D_{av} = \sum_{i=1}^{i=n} \Delta D_i f_i \quad (8)$$

where f_i represents the fractional contribution of each component to the original mixture. Equation 8 states simply that the average increase in sedimentation distance for a heterogeneous population is equal to the sum of the contributions to that increase of all species present. The contribution of each component to the overall increase is expressed as the increase in sedimentation distance for that component times the proportion of the total population which that component comprises.

In order to deduce K' values for the various components by curve fitting to eq 7, it is necessary to use assumed values for K'_i in constructing the trial curves. With these values $1/\Delta D_{av}$ can be calculated for any [R]′ by using eq 6 rearranged as

$$\Delta D = \frac{\Delta D_{max}}{\frac{K'}{[R]'} + 1} \quad (9)$$

and substituted into the reciprocal of eq 8 to give

$$\frac{1}{\Delta D_{av}} = \sum_{i=1}^{i=n} \left(\frac{\Delta D_{max,i} f_i}{\frac{K'_i}{[R]'} + 1} \right)^{-1} \quad (10)$$

Trial plots of $1/\Delta D_{av}$ vs. $1/[R]'$ are then prepared for various sets of assumed K'_i values in the search for a best fit to the data. The minimum number of components (n) and the fractional contribution of each (f_i) are estimated from gradients of appropriate [R]′ in which the individual components are resolved (see Results). The values for $\Delta D_{max,i}$ are obtained from separate experiments on single-component systems as described below.

Results

Repressor Affinity for lac Restriction Fragments. The double-reciprocal method of data presentation described in the previous section is illustrated in Figure 1 for the case of naked *lac* restriction fragments. Two operator-containing restriction fragments were used, one 203 and the other 144 base pairs in length. In separate experiments, these were sedimented in the presence of repressor as described previously (Chao et al., 1980), and the distance sedimented, D, was determined as a function of repressor concentration. Experimentally, D is determined by integrating the area under the peak and then calculating the average peak position in the gradient. The results for both restriction fragments are plotted in Figure 1 as $1/\Delta D$ vs. $1/[R]'$ according to eq 7. From the slope of the resulting line, we calculate a single dissociation constant, K', of 0.09 μg/mL. Extrapolation to the ordinate gives a ΔD_{max} of 5.5 for completely repressor-bound restriction fragment. The same K' is obtained when the data for each restriction fragment are analyzed separately.

We emphasize that the K' values obtained in our system are used for comparative purposes only (see below). We note then, in this context, that the apparent repressor–operator binding constant which may be calculated from our data is lower than those obtained by other procedures (Lin & Riggs, 1975; Gilbert & Müller-Hill, 1967). Our gradients use a slightly different buffer, lack dimethyl sulfoxide, are subject to the forces of sedimentation, and use much shorter lengths of DNA. All of these factors may contribute to the observed difference. However, the reliability of our system for measuring relative binding constants is illustrated by the linearity of the plot in Figure 1 which reveals the predicted concentration dependence for the repressor–operator interaction.

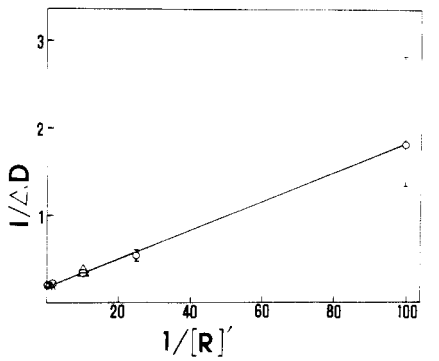

FIGURE 1: Graphical analysis of repressor binding to *lac* DNA restriction fragments. ^{32}P-End-labeled *lac* DNA was incubated and then sedimented in the presence of various concentrations of repressor. The increase in sedimentation distance (ΔD) relative to a control gradient containing no repressor was determined for each repressor concentration [R]′, and the data were plotted as shown. The error bars represent an uncertainty of ±0.2 fraction which was estimated from many control experiments in which nonoperator (calf) nucleosomes were sedimented in the presence of repressor and the variability in measured peak position was determined. The line is a least-squares fit to the data. The coordinates of the points plotted in the figure are as follows: for 203 base pair *lac* DNA (○), (0.38, 0.20), (0.83, 0.20), (2.0, 0.22), (10, 0.33), (25, 0.54), and (100, 1.8); for 144 base pair *lac* DNA (△), (0.38, 0.20), (1.0, 0.20), (10, 0.33), and (10, 0.38); for 144 or 203 base pair *lac* DNA treated with formaldehyde (×), (2.0, 0.20) and (2.0, 0.20).

Repressor Affinity for 203 Base Pair lac Nucleosomes. In the preceding paper we reported that repressor binds specifically to *lac* nucleosomes prepared by complexing the 203 base pair *lac* restriction fragment with histones (Chao et al., 1980). Indeed, sedimentation in the presence of 1.2 to 1.3 μg/mL repressor gave rise to a peak shift of similar magnitude for both the *lac* DNA and the *lac* nucleosomes, raising the possibility that histone binding to 203 base pair *lac* DNA may actually have little effect on the affinity for repressor.

In order to examine this inference quantitatively, we have determined the K' for the repressor–lac nucleosome interaction. *lac* nucleosomes were sedimented in the presence of various concentrations of repressor, and the resulting data were analyzed by plotting $1/\Delta D$ vs. $1/[R]'$ as shown in Figure 2A. An example of a series of parallel gradients from which some of the sedimentation data plotted in Figure 2 were obtained is shown in Figure 3. A straight line fitted to the points of Figure 2A by the method of least squares yields a K' of 0.2 μg/mL and, as for naked DNA, a ΔD_{max} of 5.5 fractions. The identical ΔD_{max} values for nucleosomes and DNA, though unexpected on the basis solely of changes in molecular weight, can be accounted for by the different changes in partial specific volume ($\bar{v}$) which accompany the binding of repressor to the different particles. However, we wish to focus here on the respective K' values for the nucleosomes and DNA. These K' values (0.2 and 0.09 μg/mL, respectively) are remarkably similar (see Table I for a comparison to other values) and show clearly that the binding of histones to the 203 base pair *lac* restriction fragment has little effect on the affinity of subsequent *simultaneous* [see below and Chao et al. (1980)] binding of the *lac* repressor.

The ability of the 203 base pair *lac* nucleosomes to bind repressor with an affinity close to that of naked *lac* DNA

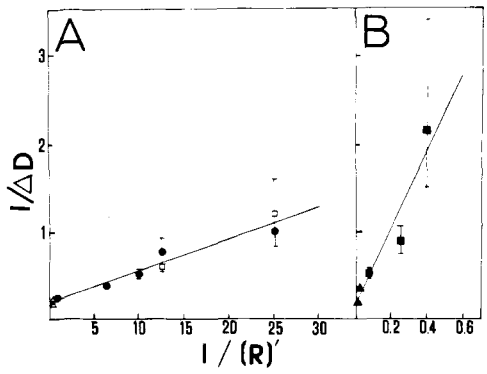

FIGURE 2: Graphical analysis of repressor binding to 203 base pair *lac* nucleosomes. (A) Un-cross-linked *lac* nucleosomes. Anlaysis was carried out as for Figure 1 except that *lac* nucleosomes were used. Similar symbols represent gradients run in parallel in the same rotor. The line is a linear least-squares fit assuming a single component system. Any assumption involving two or more components which differ in K' (see Method of Data Analysis) results in a poorer fit to the data. We have also analyzed the same data by means of plotting ΔD vs. $\Delta D/[R]'$ according to the equation

$$\Delta D = -K'\frac{\Delta D}{[R]'} + \Delta D_{max} \qquad (11)$$

which is a rearrangement of eq 7 and gives rise to a different weighting of the experimental points. This plot was linear, again indicating homogeneity or near homogeneity. (B) Cross-linked *lac* nucleosomes. Analysis was carried out as for part A except that cross-linked *lac* nucleosomes were used. Similar symbols represent gradients run in parallel in the same rotor. The line is a modified least-squares fit to the data derived by requiring the line to intersect the $1/\Delta D$ axis at the same value as in part A and then obtaining the best fit to the data subject to this constraint. This approach was used because the steepness of the slope and the low number of data points precluded a reliable estimation of $1/\Delta D_{max}$ by extrapolation. However, our conclusions do not depend on the particular method of data treatment. Note that the scale for the $1/[R]'$ axis is considerably expanded relative to that in part A.

Table I: Apparent Equilibrium Dissociation Constants for *lac* DNA and *lac* DNA-Histone Complexes

lac species		K' values (μg/mL) un-cross-linked	cross-linked
144 base pair	(x?)	≃100	≃100
core particle	(y?)	≃250	≃250
	(z?)	≃1	≃100
203 base pair nucleosome (A and B)		0.2	25
naked *lac* DNA		0.09	≃0.09

implies either that the *lac* nucleosomes contain *lac* operator in a state which *intrinsically* permits high-affinity binding by repressor or that *lac* nucleosomes are able to *conform* to a state which has high affinity for repressor. Therefore, we wished to determine whether the 203 base pair *lac* nucleosomes contain operator whose intrinsic state of histone–DNA association permits the high-affinity repressor binding. We have shown previously that *lac* nucleosomes which have been "fixed" by cross-linking still bind repressor specifically (Chao et al., 1980). Therefore, we could test this possibility by measuring the repressor affinity of cross-linked *lac* nucleosomes.

Figure 2B shows, for 203 base pair *lac* nucleosomes, that immobilization of the particle by cross-linking reduces the affinity for repressor drastically. Thus, whereas the slope of the plot for un-cross-linked *lac* nucleosomes gives a K' of 0.2 μg/mL (Figure 2A), that for cross-linked *lac* nucleosomes is greater by more than 100-fold, giving a K' of 25 μg/mL (Figure 2B). (Note that the scale for $1/[R]'$ in Figure 2B is

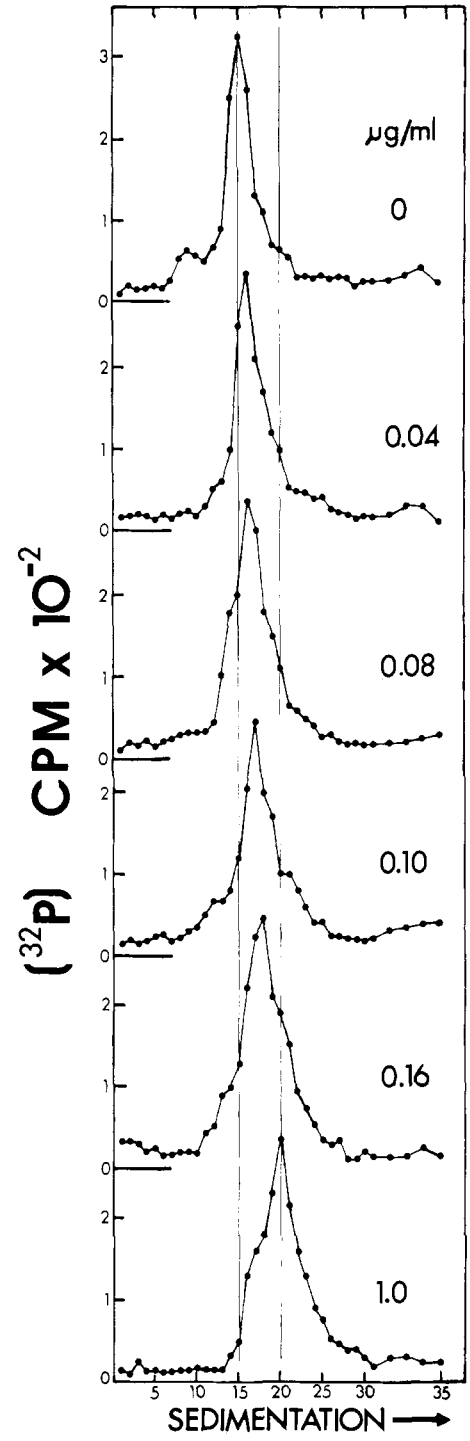

FIGURE 3: Sedimentation analysis of repressor binding to 203 base pair *lac* nucleosomes. ^{32}P-End-labeled 203 base pair *lac* DNA was reconstituted with histones and then incubated and sedimented on parallel gradients in the presence of the indicated concentration of repressor. Fractions 1–30 were 0.1 mL and fractions 31–35 were 0.2 mL. Determination of the average peak position at each repressor concentration relative to the position in the absence of repressor allows the construction of double-reciprocal plots. The data points derived in this way from the above figure are plotted as filled circles in Figure 2A.

greatly expanded relative to that for Figure 2A.) We emphasize again that although the binding to cross-linked *lac* nucleosomes is of greatly reduced affinity (Figure 2B), it is nevertheless operator specific (Chao et al., 1980).

The reduced affinity for repressor imposed on *lac* nucleosomes by formaldehyde cross-linking cannot be explained trivially by a direct chemical effect of formaldehyde on the

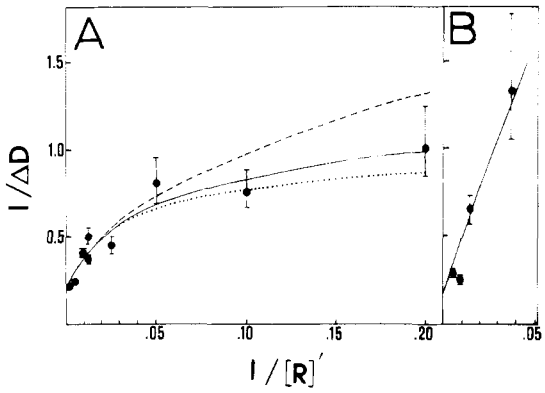

FIGURE 4: Graphical analysis of repressor binding to 144 base pair *lac* core particles. (A) Un-cross-linked *lac* core particles. Analysis was carried out as for Figures 1 and 2 except that *lac* core particles were used. The curves shown were obtained by using eq 10 as described in the text. (—) Best-fit curve; obtained for K' values of 1.2, 100, and 250 μg/mL for the three components in the mixture. (– – –) Curve for K' values of 3.6, 100, and 250 μg/mL. (···) Curve for K' values of 0.4, 100, and 250 μg/mL. The dashed and dotted curves illustrate the degree of deviation from the experimental points caused by relatively minor variations in the values chosen for the smallest K'. Two- to three-fold variations in the larger K' values similarly cause the curve to deviate strikingly from the experimental points (not shown). (B) Cross-linked *lac* core particles. Analysis was carried out as for part A except that cross-linked core particles were used. The curve shown was obtained by using eq 10 for three components with K' values of 100, 100, and 250 μg/mL (see the text). The use of significantly different values for any of these K' gives rise, as for the curve of part A, to large deviations from the experimental points.

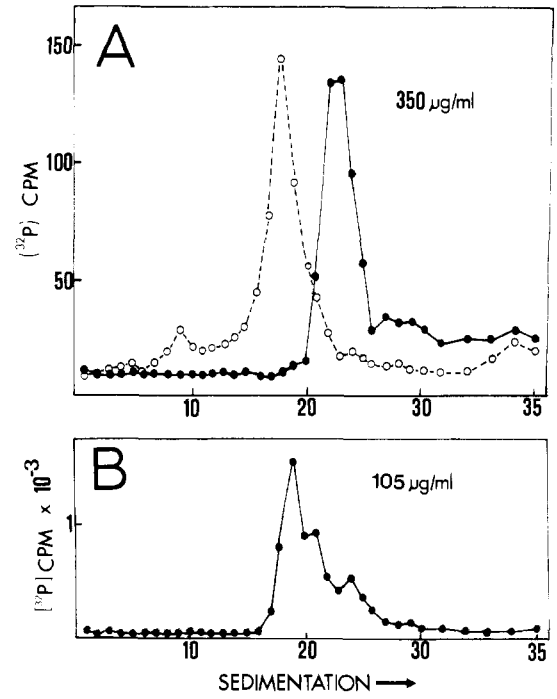

FIGURE 5: Sedimentation analysis of repressor binding to 144 base pair *lac* core particles. ^{32}P-End-labeled core particles were analyzed for repressor binding, as in Figure 3, at the indicated concentrations of repressor (solid lines). Parts A and B were separate experiments. For clarity, the control gradient containing no repressor is shown only for one of the runs (dashed line).

operator. As controls we have subjected both 144 and 203 base pair naked restriction fragments to the same conditions of formaldehyde cross-linking as were used for the nucleosomes and then challenged them with repressor at 0.5 μg/mL. Essentially complete binding of repressor occurred in both cases. A plot of these data points in Figure 1 shows that there is no evidence for a decrease in repressor affinity of the formaldehyde-treated DNA. Additional experiments, described later, argue further against the possibility that the observed reduction in K' is due to operator damage in the cross-linked *lac* reconstitutes. These results suggest that it is the immobilization, per se, induced by cross-linking which is responsible for the decrease in repressor affinity.

This lowered binding affinity of the cross-linked 203 base pair *lac* nucleosomes is reminiscent of the behavior of 144 base pair *lac* core particles which we have shown require a rather high concentration of repressor to achieve significant repressor binding (Chao et al., 1980). Therefore, we wished to determine the binding constant for 144 base pair *lac* core particles so that the comparison to cross-linked 203 base pair *lac* nucleosomes could be made.

Repressor Affinity for 144 Base Pair lac Core Particles: A Multicomponent System. *lac* core particles were sedimented through gradients containing various concentrations of *lac* repressor, and the shifted peaks were analyzed as described above. Strikingly, the resulting plot of $1/\Delta D$ vs $1/[R]'$ is curved rather than linear (Figure 4A). This shows that the population of 144 base pair *lac* core particles is heterogeneous with respect to affinity for repressor (see Method of Data Analysis). A more complex analysis is therefore required in order to determine the K' values for the individual components of the mixture.

The heterogeneity in affinity for repressor of the population of 144 base pair *lac* reconstitutes is illustrated independently by the data of Figure 5. Panel A shows that in the absence of repressor, or in the presence of a high concentration of repressor (350 μg/mL) when the core particles are largely repressor bound, they sediment as fairly symmetrical peaks. In contrast, at an intermediate concentration of repressor (Figure 5B), the sedimenting peak of *lac* core particles is clearly trimodal. Thus, the peak as a whole does not shift steadily to higher sedimentation values with increasing repressor concentration as occurs with free DNA or for the 203 base pair *lac* nucleosomes (see Figure 3). Rather, discrete subpeaks appear at the intermediate repressor concentrations, reflecting minor subpopulations of more readily bound *lac* core particles which interact preferentially with the repressor. The presence of three distinct repressor binding populations in these preparations has been confirmed in this manner in three separate experiments.

We have estimated the dissociation constants (K'_i) for the three components of Figure 5B by curve fitting of eq 10 to the data points of Figure 4A. In using eq 10 we have assumed ΔD_{max} to be 5.5, the value measured for *lac* nucleosomes and *lac* DNA (see above). Also, we have used estimated values of 60, 20, and 20% for the proportions of the total population represented by each of the three components of Figure 5B (i.e., f_i = 0.6, 0.2, and 0.2). Figure 5 and similar data have further provided useful constraints which were applied in selecting the trial K'_i values for the curve fitting. Thus, the component of intermediate affinity must have a K' value near 105 μg/mL since it is approximately half-shifted at this concentration (Figure 5B). Also, the major (low-affinity) component must have a K' value between 105 and 350 μg/mL since it is shifted only slightly at the former concentration and very substantially at the latter. For the purpose of constructing trial curves, therefore, we began by assuming K'_i values of 100 and 250 μg/mL, respectively, for these two components and then varied K'_i only for the third component. The resulting best fit (solid curve, Figure 4A) was obtained by using a K' of about 1 μg/mL for the minor highest affinity component. Since this curve fits the data very well, we have not attempted to refine

further our estimates of K'_i for the two components of lower affinity (see Figure 4 legend).

The results of curve fitting thus yield approximate K'_i values of 250, 100 and 1 μg/mL for the three components which comprise the population of 144 base pair *lac* core particles. Thus, consistent with our previous report (Chao et al., 1980), it is evident that the bulk (i.e., the 80% with $K' \geq 100$ μg/mL) of the 144 base pair *lac* core particle preparation binds repressor with much lower affinity than the 203 base pair *lac* nucleosomes. However, about one-fifth of the core particle preparation differs from the rest and does bind repressor with high affinity (see Table I).

The shape of the fitted curve in Figure 4A is quite sensitive to the value chosen as K' for the component of highest affinity. This is illustrated by the dashed line of Figure 4A which was calculated by using a K' for the highest affinity component of only 3 times the best-fit value. We also show a dotted line which corresponds to one-third the best-fit value for this K'. Figure 4A illustrates that although a K' of up to 2 or 3 would be consistent with our data, even such maximum estimates for this minor high-affinity component yield K' values much lower than those of the other two components in the mixture. Thus, while the low affinity components differ from each other in K' by at most a few-fold, the high-affinity component differs from them by a further 2 orders of magnitude. We emphasize, however, that all of the 144 base pair *lac* core particle components are operator specific in their binding of repressor (Chao et al., 1980).

The above quantitative conclusions are consistent with the sedimentation profile in Figure 5B. From Figure 5B it is evident that the highest affinity component must have a considerably lower K' than the other two since 105 μg/mL repressor is sufficient to induce for it, but not for the others, a shift which is very close to the ΔD_{max}. Since ΔD_{max} is approached only asymptotically as the repressor concentration is increased, this shows that 105 μg/mL is very far above the K' of the high-affinity component.

The above estimates of binding constants for individual components among the 144 base pair *lac* core particles allow us to return to the question of comparison with the cross-linked 203 base pair *lac* nucleosomes. However, now we ask, more precisely, whether the cross-linked 203 base pair *lac* nucleosomes and the *low-affinity* 80% of the *lac* core particle preparation are similar. If, as suggested earlier, it is the immobilization per se of cross-linking which causes the decrease in affinity for repressor of *lac* nucleosomes, then perhaps the *lac* core particle species of low affinity resemble the cross-linked *lac* nucleosomes in also being inflexible for some reason. This can be tested by determining the effect of cross-linking on the 144 base pair *lac* core particles. The low-affinity core particle components should experience little or no further reduction in affinity as a consequence of cross-linking if they already resemble the cross-linked 203 base pair *lac* nucleosomes.

Cross-Linking Does Not Reduce the Repressor Affinity of the Low-Affinity lac Core Particles. We have shown above that 80% of the *lac* core particle population is of K' between 100 and 250 μg/mL. This may be compared to the results of Figure 6 in Chao et al. (1980) in which cross-linked core particles were sedimented in the presence of 200 μg/mL repressor. Inspection of that figure reveals that 200 μg/mL repressor increases the sedimentation distance by more than a half-shift (i.e., $\Delta D > \Delta D_{max}/2$) for the cross-linked *lac* core particles. Therefore, the "average" K' for the population must be less than 200 μg/mL. This suggests that cross-linking may

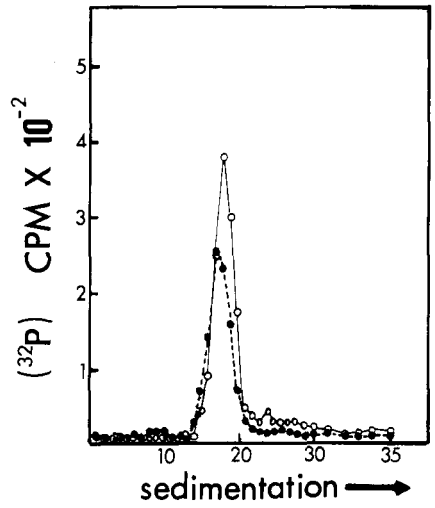

FIGURE 6: Sedimentation analysis of repressor binding to formaldehyde-cross-linked 144 base pair *lac* core particles. Analysis was carried out as for Figure 5 in parallel gradients containing no repressor (●) or 26 μg/mL repressor (○). Any component of $K' < 3$ μg/mL should be shifted five or more fractions at 26 μg/mL repressor.

indeed have no effect on the repressor affinity of the majority of *lac* core particles which are of low affinity in the uncross-linked state.

We have analyzed the repressor binding properties of cross-linked *lac* core particles graphically as well by plotting $1/[R]'$ vs. $1/\Delta D$ (Figure 4B). The most striking feature of the plot, by contrast to Figure 4A, is its linearity, indicating the apparent loss of heterogeneity. In order to interpret this result, we must also consider the effect of cross-linking on the high-affinity component among the *lac* core particles since the entire core particle population was cross-linked. Individual profiles of cross-linked *lac* core particle preparations sedimented in the presence of repressor differ from those of uncross-linked preparations in being relatively symmetrical with no evidence of a high-affinity component migrating ahead of the main peak (e.g., Figure 6). This suggests that cross-linking selectively affects the high-affinity component of the preparation. This inference is consistent with Figure 4B since a reduction in repressor affinity of only the highest affinity component should yield a fairly homogeneous preparation in which all components are of the low-affinity type. This interpretation is supported quantitatively by the curve shown in Figure 4B. That curve was calculated by using eq. 10 and all of the same parameters as for Figure 4A *except* that, by analogy to the 203 base pair *lac* nucleosomes, the highest affinity *lac* core particle component was assigned the greatly increased K' value of 100 μg/mL (reflecting the sharp decrease in affinity-caused selectively by cross-linking). Clearly, the calculated curve fits the observed data very well. We therefore conclude, as the simplest interpretation of the data, that formaldehyde cross-linking of 144 base pair *lac* core particles has little or no effect on the low-affinity *lac* core particle components but selectively reduces the affinity for repressor of the one minor component which normally binds repressor most tightly.

Thus, it appears that the similarity of the low-affinity 144 base pair *lac* core particle component to the cross-linked 203 base pair *lac* nucleosome is significant. Moreover, the *high*-affinity core particle component appears to resemble the *un*-cross-linked *lac* nucleosome. These groupings are suggestive of a general relationship which can be summarized by stating that cross-linking reduces repressor affinity only for species with a high *initial* repressor affinity (i.e., for all 203 base pair

lac nucleosomes as well as for the $K' \simeq 1$ *lac* core particle; see Table I).

High-Affinity Binding Requires a Conformational Change in the Nucleosome Core. The simplest explanation which accounts for all of the data which we have presented is that repressor binding to *lac* reconstitutes can be facilitated by a conformational change. Only species of reconstitute which can undergo this change are capable of binding repressor with high affinity. Blocking this change by fixing with formaldehyde prevents *high-affinity* binding (Figure 2 and 4) but not *specific* binding per se (Chao et al., 1980). The low-affinity components in the 144 base pair *lac* core particle preparation (i.e., those of $K' \simeq 100$ and 250 μg/mL) presumably are unable to undergo the facilitating conformational change and for this reason are unable to bind repressor tightly. Moreover, since they are intrinsically unable to undergo this conformational change, immobilization by cross-linking with formaldehyde is without measurable effect (see above). We note parenthetically that the lack of a formaldehyde effect on certain *lac* reconstitutes substantiates our conclusion that the striking effect on other reconstitutes is not a trivial chemical effect of formaldehyde on the operator.

In order to investigate the nature of the postulated conformational change, and in order to eliminate any remaining uncertainties concerning possible trivial chemical effects of formaldehyde, we have begun additional studies using a different cross-linking agent, dimethyl suberimidate. All of the cross-linking experiments described so far were conducted using formaldehyde. However, formaldehyde forms both histone–histone and histone–DNA cross-links (Jackson, 1978). In contrast, dimethyl suberimidate forms only histone–histone cross-links and does not cross-link histones to DNA (Stein, 1979). Thus, repressor binding experiments conducted on nucleosomes cross-linked with dimethyl suberimidate could reveal the relative importance to the conformational change of mobility about histone–histone contacts and mobility about histone–DNA contacts.

Figure 7 shows the results of a preliminary experiment which demonstrates that dimethyl suberimidate cross-linking, like formaldehyde cross-linking, prevents high-affinity binding of *lac* repressor to *lac* nucleosomes. Dimethyl suberimidate cross-linked 203 base pair *lac* nucleosomes were sedimented in the presence (dashed line) or absence (solid line) of 1.3 μg/mL repressor. Clearly, there is not measurable repressor binding at this concentration. Parallel control gradients (not shown) of un-cross-linked *lac* nucleosomes gave a peak shift of about five fractions as expected for this concentration of repressor based on Figure 3. We therefore conclude that dimethyl suberimidate cross-linking disallows high-affinity binding by repressor (see also the legend to Figure 7). As additional controls, we have verified that *lac* DNA can be reisolated from dimethyl suberimidate cross-linked material by phenol extraction and that this DNA binds repressor comparably to untreated DNA. This shows that the DNA of *lac* nucleosomes is neither damaged nor linked to the histones by dimethyl suberimidate, and we conclude that histone–histone cross-linking alone is sufficient to block the conformational transition which facilitates high-affinity binding by repressor. We therefore infer that the particular formaldehyde cross-links which are principally responsible for decreasing repressor affinity also are those of the histone–histone type.

We have already pointed out that the formaldehyde cross-linking results cannot have a trivial explanation based on direct damage to the operator by formaldehyde since the same treatment of naked restriction fragment does not result in a reduction of repressor affinity (see Figure 1). The suberimidate cross-linking results (above) now serve to rule out trivial explanations based on *in*direct damage to the operator as well. Thus, previously, the formal possibility existed that, for unspecified reasons, the operator environments in only some types of reconstitute (i.e., those with high affinity for repressor) rendered the operators particularly susceptible to chemical damage by formaldehyde. The completely different cross-linking mechanism of dimethyl suberimidate (Stein, 1979; Jackson, 1978; Means & Feeney, 1971), but its similar blockage of high affinity repressor binding (Figure 7), renders such possibilities extremely unlikely.

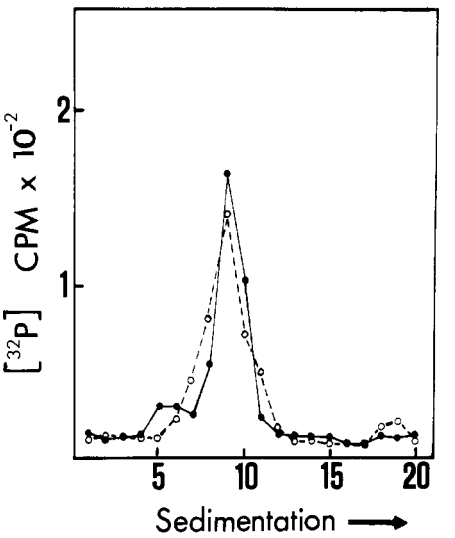

FIGURE 7: Sedimentation analysis of repressor binding to dimethyl suberimidate cross-linked 203 base pair *lac* nucleosomes. Analysis was carried out as for Figure 3 except that suberimidate-cross-linked *lac* nucleosomes and a higher concentration of repressor were used. Fractions of 0.2 mL (twice the usual size) were collected. The parallel gradients contained no repressor (●) or 1.3 μg/mL repressor (○). Clearly, there is no detectable repressor-induced shift in the sedimentation of the suberimidate-cross-linked *lac* nucleosomes. Our uncertainty in the measurment of relative peak positions is ±0.2 fraction when 0.1-mL fractions are collected (see the legend to Figure 1). Since the fractions in the above gradient were twice the usual size, we assume that, in this case particularly, a shift of 0.2 fraction would readily be detected. Since, in fact, no shift is detected at 1.3 μg/mL repressor, we deduce from eq 1 and 2 that the K' for suberimidate-cross-linked 203 base pair *lac* nucleosomes must be greater than 16 μg/mL (corresponding to a shift of less than 0.2 fraction in this gradient).

Discussion

Nucleosome Cores Can Change Conformation To Facilitate Binding of Repressor. We have measured the affinity for specific binding of *lac* repressor to operator contained in restriction fragments which have been complexed with histones. The restriction fragments, 203 and 144 base pairs in length, approximate nucleosomes or core particles, respectively, when complexed with histones. Our analysis reveals that the *lac* DNA–histone reconstitutes fall into two discrete classes with respect to affinity for repressor: a high-affinity class and a low-affinity class.

All *lac* reconstitutes containing the 203 base pair *lac* restriction fragment are of the high-affinity type. However, preparations of 144 base pair *lac* reconstitutes contain species which fall into both the high- and low-affinity classes. The repressor–operator dissociation constants for species in the high-affinity class average more than 2 orders of magnitude less (tighter binding) than those for species in the low-affinity class (see Table I).

Cross-linking converts all high-affinity species into the low-affinity type. However, members of the low-affinity class are unaffected by cross-linking in their affinity for repressor. We have shown previously that all species, cross-linked or not, bind repressor specifically and quantitatively (Chao et al., 1980). Thus, the high-affinity species differ principally in that they support tight binding by virtue of some property which can be eliminated by cross-linking. Since cross-linking does not lead to chemical damage of the operator (see Results), we infer that the relevant property is the ability of these particles to change conformation.

This conformational change is not *simply* the release of the operator segment of DNA from histone binding upon interaction with repressor [see Simpson (1979)]. This follows from the observation that dimethyl suberimidate can block the conformational change although it cannot cross-link DNA to histones. Thus, whatever the nature of the final conformationally altered state which renders the operator more fully available to repressor, the process leading to this new conformation apparently requires mobility about histone–histone contacts within the nucleosome core.

In preliminary experiments we have attempted to determine whether the conformational change in the histone core leads to changes in the association of histones and DNA which can be detected by digestion with nucleases. In several experiments, patterned after ones reported previously (Chao et al., 1979, 1980), we have found that partial digestion of ^{32}P-end-labeled 203 base pair *lac* nucleosomes with DNase I or *Escherichia coli* exonuclease III yields the same collection of bands both in the absence of repressor and in the presence of sufficient repressor to give essentially complete high-affinty binding. It therefore appears that the altered conformational state of *lac* nucleosomes imposed by repressor does not involve gross changes in the locations of the sites of histone–DNA association. Consequently, lateral movement of the histones along the DNA, as by "jumping" (Stein, 1979) or "sliding" (Williamson & Felsenfeld, 1978; Wasylyk et al., 1979) mechanisms, appears unlikely.

Operator Recognition and Binding within lac Nucleosomes and Core Particles. From our data, we infer a two-stage model for the high-affinity repressor–nucleosome interaction. The first stage would involve recognition and binding to a subset of operator contacts. Cross-linked reconstitutes as well as the two low-affinity *lac* core particle species can engage *only* in this first-stage binding mode. Binding is specific, but the complex remains in a relatively high energy state and the dissociation constant is high. For the *lac* nucleosomes as well as the high-affinity *lac* core particles, however, the high-energy state of the first stage of binding is relieved in a second stage by a conformational change which allows more complete utilization of the repressor–operator contacts. The concept of functionally competent subsets of specific contacts has precedent in that both the tryptic headpiece and the tryptic core of *lac* repressor can bind operator specifically (Ogata & Gilbert, 1978; Matthews, 1979). Moreover, the binding of repressor via a subset of contacts has been demonstrated directly by Schmitz & Galas (1979) for the case of repressor binding to preformed polymerase–operator/promoter complexes.

What might account for the inability of repressor to utilize fully its association contacts during the first stage of binding? Tightly bound repressor requires the operator to have a particular pitch (Wang et al., 1974) and perhaps also a slight bend (Hirsh & Schleif, 1976). One factor then is probably the existence in nucleosomal DNA of the inappropriate pitch and curvature for tight binding. Furthermore, the DNA of nucleosomes is arranged in compact supercoils so that during the first stage of binding there is likely to be steric hindrance from parallel layers of DNA [see Prunell et al. (1979) for a recent discussion relevant to this point]. The histones themselves presumably also offer some steric interference. The relative importance of each of these factors depends on both the rotational and linear orientations of the operator on the nucleosome (see below). The interference of each of these factors with repressor binding could be relieved by an appropriate conformational change.

We note that even after cross-linking, differential affinity for repressor persits among the reconstitutes (see Table I). This suggests either that cross-linking does not block completely the ability of the high-affinity species to change conformation or that the various cross-linked species differ in the inherent accessibilities of their operators to repressor. Although the operator faces generally out for all reconstitutes (Chao et al., 1980), its precise orientation or environment may differ slightly among species. The differences need only be small since Goeddel et al. (1978) have shown that the perturbation of contacts at only a single base is sufficient to increase the dissociation constant by more than a factor of 10.

Does the Position of the Operator on the Histone Core Govern the Ability To Change Conformation? Our analysis has shown that, despite compositional homogeneity, there exist among the 144 base pair *lac* core particles important structural distinctions which give rise to striking differences in repressor binding affinity. We have shown previously that the 144 base pair *lac* reconstitute is a mixture of probably three DNA–histone arrangments in each of which the histone core is bound to the DNA in a different register with respect to sequence (Chao et al., 1979). Clearly, as a consequence, these arrangements differ in the location of the operator DNA sequence on the histone core (see Figure 8). We will show below that it is probably these different locations of the operator on the histone octamer which give rise to the different affinities for repressor. Thus, each of the peaks in the trimodal sedimentation profile of Figure 5B may correspond to one of the particular arrangements of histones and restriction fragment which we have already characterized (see below and the legend to Figure 8).

Figure 8A summarizes the placements of the *lac* operator for all the major *lac* reconstitute arrangements. The horizontal lines are linear representations of the path followed by DNA in nucleosomes. Each path is shown with two symmetrically located operator positions reflecting the presumed pseudosymmetry of the nucleosome. Calibrations in base pairs for both core particle and chromatosome paths are presented (see Figure 8 legend). For ease of visualization, the shaded member of each operator pair in Figure 8A is illustrated also in the three-dimensional representation of Figure 8B.

From Figure 8A we observe that two of the three 144 base pair *lac* core particle arrangements are similar to each other in their placement of the operator. The third is similar to one of the 203 base pair *lac* nucleosome arrangements in the placement of the operator. Since all *lac* nucleosomes bind repressor tightly, whereas only one of the three *lac* core particle species binds repressor tightly (see Table I), we speculate that the single *lac* core particle component which resembles the *lac* nucleosomes in repressor binding characteristics is also the component which resembles the nucleosomes in operator placement. This tight binding operator placement would be arrangement "Z" in Figure 9 of Chao et al. (1979). In a preliminary attempt to verify this attractive possibility, we have

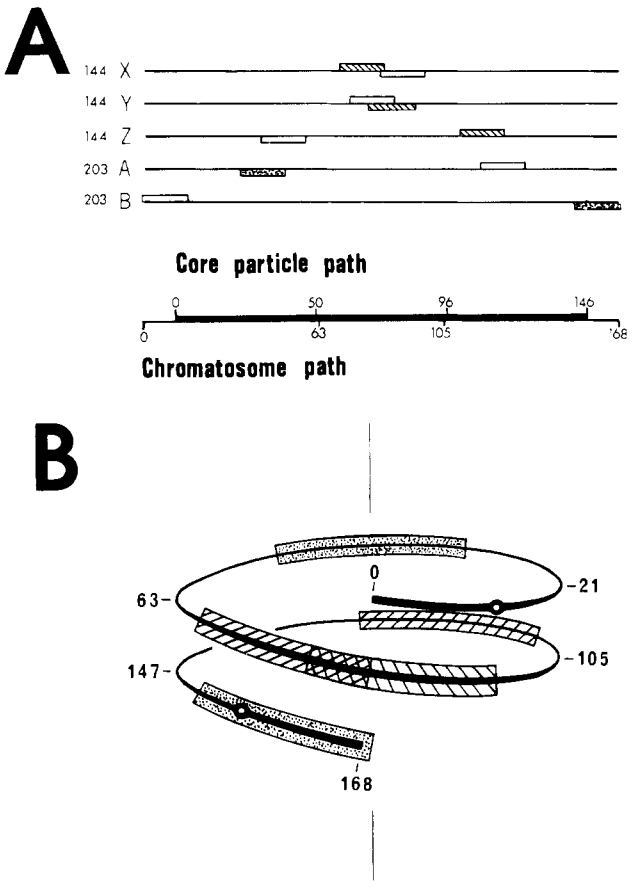

FIGURE 8: *lac* operator positions in *lac* reconstitutes. (A) Linear representation. (B) Helical representation. The histone octamer (no H1) prescribes a defined path of at least 168 base pairs in length along which DNA is bound in nucleosomes (Weischet et al., 1979). All of the lines in this figure, including the helix, represent this path and not the DNA itself. In part A the path is calibrated with reference to both this 168 base pair length (Weischet et al., 1979), termed "chromatosome" (Simpson, 1978), and the more common length of 146 base pairs characteristic of core particles [e.g., Bryan et al. (1979)]. For simplicity, the core particle path is assumed to be situated symmetrically within the chromatosome path (Simpson, 1979). The ends of the core particle path are designated by nodes in the helix of part B. However, our conclusions concerning domains do not depend on the exact relationship between the two paths. We have shown previously that *lac* restriction fragments exhibit sequence preference in their association with histones. Part A of this figure illustrates the resulting operator locations along the DNA path for each of the major *lac* reconstitute DNA–histone arrangements (i.e., species or components) which exist. The assignments shown (X, Y, Z, A, and B) were deduced from Figures 7 and 9 of Chao et al. (1979) based on recent estimates of the respective DNA path lengths [e.g., Weischet et al. (1979) and Bryan et al. (1979)]. Each assignment is represented as a symmetric pair of positions reflecting the presumed symmetry of the histone octamer [see Felsenfeld (1978)]. For clarity, the shaded member only of each pair in part A is illustrated in part B. For example, the operator location at position 168 in part B represents also the symmetrically disposed but unillustrated location at position 0. The coordinates within the chromatosome path of the operator locations shown in part B and their symmetrically related counterparts (in parentheses) shown as open boxes in part A are as follows: 34–50 (119–135) and 153–169 (–1–16) for the 203 base pair *lac* nucleosome and 112–128 (41–57), 80–96 (73–89), and 69–85 (84–100) for the 144 base pair *lac* core particle. For the purposes of this figure, we chose to illustrate as operator the 17 base pair sequence shown by Bahl et al. (1978) to be the minimum required for operator function. This corresponds to nucleotides 49–65 from the rightward 5′ end of both of our *lac* restriction fragments [which differ only at the left end; see Chao et al. (1979)]. However, we note that at least 27 base pairs of *lac* operator become intimately associated with repressor upon complex formation with DNA as evidenced by protection of operator–repressor complexes from DNase I digestion (Gilbert & Maxam, 1973; Schmitz & Galas, 1979).

characterized by exonuclease digestion [see Chao et al. (1979)] the three individual components from the gradient shown in Figure 5B. Because of cross-contamination, the results so far have not been conclusive but are consistent with the above assignment and do confirm clearly that the components in each peak are indeed distinct.

From Figure 8A we observe that the two major operator placements in the 203 base pair *lac* nucleosomes are in quite different locations—one at the end of the chromatosome path and the other well within the core particle path. Yet the plots in Figure 2 show no evidence of heterogeneity (see also the legend to Figure 2). Thus, the *exact* operator position appears to be relatively unimportant within this region of the nucleosome. Since both operator positions clearly support tight binding by repressor, they apparently fall within a "tight binding domain" on the nucleosome core; recall that only one of the three *lac* core particle operator positions also falls within this domain.

The above correlations raise the possibility that *lac* nucleosomes are divided into functional domains which govern the ability to undergo conformational changes in order to facilitate repressor binding. Thus, we may account for our data by a model in which the conformationally induced availability of a particular DNA sequence depends on the domain of that same sequence within the nucleosome. For *lac* operator particles the flanking domains or "wings" of the nucleosome support tight binding of repressor whereas the central domain, though allowing specific binding, cannot sustain a conformational change which would accomodate tight binding of repressor to a resident operator.

Whether or not our domain model is correct in detail, it is clear that operator position is a crucial determinant of repressor binding affinity. There are two related ways in which operator position could determine the ease of changing conformation. First, in only certain positions may sufficient operator–repressor contacts be made during stage I binding so as to provide adequate energy to drive the conformational change. This is consistent with the observation that different cross-linked species vary in affinity for repressor (see Table I and earlier discussion) and with evidence suggesting that the DNA of the core particle may vary in accessibility and structure along its binding path [see Simpson & Whitlock (1976) and Simpson (1979)]. The second way in which operator position could determine the ease of changing conformation could be operative if particular locations on the nucleosome core present especially low energy barriers to the conformational transition. Thus, only when the operator resides at these locations would the strain imposed by repressor binding be relieved by the conformational change. These two possibilities are, of course, not mutually exclusive.

Nucleosome Core Domains and the Conformational Change. The relationship between the repressor binding domains we have proposed and the histones of the nucleosome core can be deduced by comparison of Figure 8A with the linear "map" of the histones on core DNA determined by histone–DNA cross-linking (Mirzabekov et al., 1978). The tight binding operator domains in the nucleosome wings, at which repressor induces a conformational change, correspond to the primary H2A–H2B binding regions of nucleosomal DNA. The low affinity operator domain in the central region corresponds to the primary H3–H4 binding region on the DNA. We regard this correspondence as significnt because the histone core itself has been shown to be divided also into separate structural H2A–H2B and H3–H4 domains (Eickbush & Moudrianakis, 1978; Ruiz-Carrillo & Jorcano, 1979;

Martinson & True, 1979). Thus, the delineation of nucleosomal domains which we have proposed on the basis of a functional criterion (i.e., the ability of the nucleosome cores to change conformation in response to repressor binding) corresponds to the distinction between separate histone domains proposed on the basis of independent structural criteria.

Since our "functional" binding domains in nucleosomes were inferred based on differing abilities to change conformation, it may be significant that most of the studies leading to identification of the comparable structural domains of the histone core also were based upon monitoring conformational changes in response to stress. Thus, for the solubilized histone octamer itself, diverse destabilizing solution conditions elicit the common response of dissociation into separate H2A–H2B and H3–H4 containing complexes (Eickbush & Moudrianakis, 1978; Ruiz-Carrillo & Jorcano, 1979; Martinson & True, 1979). Similarly, native chromatin responds to stress (when exposed to low ionic strength) by changing conformation in a way which maintains H2A–H2B contacts while rupturing contacts between H2B and H4 (Martinson et al., 1979). Taken together, these reults support the concept, derived from our present repressor binding studies, that nucleosomes undergo conformational transitions which rely on the differential behavior of separate domains within the histone core.

There are several possible implications associated with our repressor binding domain model of nucleosome function. For example, the greater ease of inducing a conformational change in nucleosomal wings may be related to the processes of transcription and replication in that it is the wings of the nucleosome which are first encountered by the polymerases. Also, propagated conformational changes in chromatin may initiate within the wings of one nucleosome and then proceed along chromatin by successive perturbations at the wings of adjacent nucleosomes. Finally, perhaps the primary recognition events in chromatin, involving regulatory proteins, depend upon whether the appropriate DNA sequence is contained in the nucleosome wings and whether its binding face is outward.

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