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A Model for the Cooperative Binding of Eukaryotic Regulatory Proteins to Nucleosomal Target Sites

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The mechanism by which gene regulatory proteins gain access to their DNA target sequences in chromatin is not known. We recently showed that nucleosomes are intrinsically dynamic, transiently exposing their DNA to allow sequence-specific protein binding even at buried sites. Here we show that this dynamic behaviour provides a mechanism for cooperativity (synergy) in the binding of two or more proteins to sites on a single nucleosome, even if those proteins do not interact directly with each other in any way. As a consequence of this cooperativity, two proteins binding to the same nucleosome facilitate each other's binding and also control the level of occupancy at each other's sites. This model, with no adjustable parameters, accounts quantitatively for recent reports of cooperative (synergistic) binding to nucleosomes *in vitro*. We assess the potential importance of this new cooperativity for gene regulation *in vivo* by comparing its magnitude to free energies of cooperative protein–protein direct contacts having known significance for gene regulation. Possible roles for nucleosome dynamics in eukaryotic gene regulation, and key remaining questions, are discussed.

Keywords: synergy; gene regulation; chromatin; DNA; transcriptional activation

Introduction

An important step in eukaryotic gene regulation is the binding of a regulatory protein to a specific DNA target site. Surprisingly, at this time it is not known how eukaryotic regulatory proteins gain access to their DNA target sequences in chromatin, the natural context of eukaryotic DNA *in vivo*. The available evidence (see Polach & Widom, 1995) suggests that, *in vivo*, critical DNA regulatory sequences will in some cases be occluded by organization into nucleosomes, the subunits of chromatin (Lutter *et al*., 1979; Lutter, 1981; Richmond *et al*., 1984; Arents *et al*., 1991; Arents & Moudrianakis, 1993). Nevertheless, these sites must be accessible for protein binding. Remarkably, studies *in vitro* have demonstrated that many regulatory proteins do have the capability of binding to their target sites even when those sites are positioned in nucleosomes in such a manner as to appear to be inaccessible (Archer *et al*., 1991; Taylor *et al*., 1991; Perlmann, 1992; Cote *et al*., 1994; Imbalzano *et al*., 1994; Kwon *et al*., 1994; Pazin *et al*., 1994; Tsukiyama *et al*., 1994; Wechsler *et al*., 1994). In certain cases, binding is dependent on ATP hydrolysis and may be an ''active'' process, whereas in many other cases binding is a spontaneous process even in purified systems, with no requirement for additional energy sources.

While the ability of many proteins to bind to occluded nucleosomal target sites is not in dispute, how they were able to bind was unexplained. Moreover, previous studies had also uncovered many examples of proteins which reportedly ''could not'' bind to nucleosomal target sites (e.g. Taylor *et al*., 1991; Hayes & Wolffe, 1992; Lee *et al*., 1993; Wechsler *et al*., 1994). It was not possible to predict which proteins ''could'' bind and which ''could not'', nor could one predict the extent to which organization of the target sites in nucleosomes would affect the observed binding affinity.

We recently proposed a model for a mechanism through which regulatory proteins could gain access to their target sites even when those target sites are initially occluded by their organization in nucleosomes (Polach & Widom, 1995). In this model, nucleosomes are dynamic structures, transi-

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ently exposing their DNA. Regulatory proteins gain access to their target sites in the exposed state, and bind with a net free energy given by the sum of the free energy cost of the required nucleosome-conformational change plus the free energy for binding to naked DNA: $\Delta G^{o}_{net} = \Delta G^{o}_{conf} + \Delta G^{o}_{naked\ DNA}$. Expressed in terms of affinities, this expression means that regulatory proteins will bind to nucleosomal target sites with an apparent dissociation constant ($K_d^{apparent}$) which is equal to their dissociation constant for naked DNA ($K_d^{naked\ DNA}$) divided by the position-dependent equilibrium constant for site exposure (K_{eq}^{conf}), given by $K_{eq}^{conf} = \exp(-\Delta G^{o}_{conf} / RT)$. A sensitive assay based on the kinetics of restriction enzyme digestion revealed this dynamic behavior and provided quantitative measurements for ΔG^{o}_{conf} as a function of position within the nucleosome. The free energy cost of exposing a site must, at some level, depend on properties specific to each case, such as the size and shape of the protein, the translational and rotational positions of the target site within the nucleosome, or bending of the DNA that accompanies protein binding, since these directly affect the amount of DNA that must be exposed off the surface of the histone octamer to allow binding. Nevertheless, our values for ΔG^{o}_{conf} together with values taken from the literature for $K_d^{naked\ DNA}$ for a variety of proteins lead to predicted values for $K_d^{apparent}$ for the binding of these proteins to nucleosomal target sites that are generally in good agreement with experimental measurements (Polach & Widom, 1995).

In that study, we noted that a simple extension of our site-exposure model provides a possible mechanism through which the binding of multiple proteins to target sites on the same nucleosome could be inherently cooperative. This possibility occurs as a consequence of those proteins having in common the same nucleosome as a competitor for binding. Recent results have now appeared from another laboratory (Adams & Workman, 1995) that appear qualitatively to exhibit this behavior that we anticipate (with one exception). Those investigators observe cooperative binding of several unrelated proteins to sites on a single nucleosome, and conclude that the binding of proteins to nucleosomal target sites is inherently cooperative (Adams & Workman, 1995).

Here, we analyze those results in terms of a general model for cooperativity in the binding of proteins to nucleosomal target sites. We derive the relationships between the free energy of cooperativity, the free energy of nucleosome conformational transitions, and the free energies of protein binding; and, we show how these quantities may be measured by experiment. We obtain numerical solutions of the coupled equilibria to illustrate important general features of the model. We show that this model, with no adjustable parameters, accounts quantitatively for the experimental observations of cooperative binding (Adams & Workman, 1995). The application of these ideas to eukaryotic gene regulation *in vivo* is discussed.

Results

Models

Our model is illustrated schematically in Figure 1(a). N represents a nucleosome that contains buried within it binding sites for two proteins, X and Y. X and Y may bind individually by the site-exposure mechanism, yielding XN and NY, respectively; subsequent binding of the second protein to the singly occupied nucleosome yields XNY. Let ΔG_1^o be the net free energy cost for breaking the set of histone-DNA contacts (represented here by a pair of arrows) necessary to expose the binding site for X. Let ΔG_2^o be the net free energy cost of breaking any additional histone-DNA contacts as necessary to expose the binding site for Y, once X has already bound. The net free energy cost for exposing Y's site in the absence of bound X is given by $\Delta G_1^o + \Delta G_2^o$.

This model illustrates that the existence of the histones as a common-competitor can introduce a favorable cooperativity or synergy between the binding of X and Y, so that the binding of the second (or subsequent) protein can occur with larger negative free energy change than would otherwise obtain. This coupling arises from the possibility (as shown in the drawing) that, once protein Y has bound, the binding of protein X can take place without having to pay the energetic penalty for site exposure which otherwise would be required. Similarly, the ability of X to bind facilitates the binding of Y, since at least some of the final free energy penalty for the required conformational change is already paid. These processes are linked in a thermodynamic cycle, so the same binding cooperativity (synergy) necessarily applies regardless of the order in which the proteins bind.

The cooperativity inherent in the model of Figure 1(a) is not, however, necessarily a feature of nucleosomes. This point is illustrated in Figure 1(b),(c). Figure 1(b) illustrates a hypothetical case in which site exposure at the two sites is mechanically unlinked: in this Figure, binding sites simply twist out independently of each other. An alternative model of this class might allow a loop of DNA that includes a recognition site to slide up past the edge of the octamer surface, thereby exposing that site while leaving other sites unaffected. In any case, the Figure demonstrates the feasibility in principle of binding-site independence. In another alternative, if a nucleosome is positioned such that at least one site faces outward, and if the protein that binds there is sufficiently small so that binding may occur without site exposure, then in this case too cooperativity will not be observed. Note however that nucleosome positioning is inherently statistical, not ''precise'' (Polach & Widom, 1995; P. T. Lowary and J.W., unpublished), so in a population of equivalent cells alternative nucleosome positionings will sometimes occur in which the same site is buried.

Figure 1(c) shows a third possible outcome, in which binding events at the two sites are mechanically coupled, but in a manner that yields a reduced cooperativity relative to that inherent in the model of Figure 1(a). This Figure illustrates a particular example in which a looped structure is formed, as has been postulated for intermediates in nucleosome transcription (Studitsky *et al*., 1995). In this case, binding at site Y does not concomitantly expose site X, although it may substantially reduce the remaining energetic penalty that must be paid in order for site X to be fully accessible.

Free energy diagram

A free energy diagram corresponding to model 1(a) (Weber, 1992) is illustrated in Figure 2. $\Delta G^o(X)$ is the standard free energy change for the process $X + N \rightarrow XN$; $\Delta G^o(Y)$ is the standard free energy change for the process $N + Y \rightarrow NY$; $\Delta G^o(Y/X)$ is the standard free energy change for binding Y with X already bound, $XN + Y \rightarrow XNY$; and $\Delta G^o(X/Y)$ is the standard free energy change for binding X with Y already bound, $X + NY \rightarrow XNY$.

In the absence of any interaction between the binding of X and of Y, the net free energy change for the process $X + N + Y \rightarrow XNY$ would be given by $\Delta G^o(X) + \Delta G^o(Y)$, which is indicated in the Figure. However, simply because these sites are present in the same nucleosome, binding events at one site can interact with those at another, such that the binding of X or Y to a nucleosome that is already occupied by Y or X, respectively, can be more favorable or less favorable than the binding of X or Y on its own, by an amount we refer to as the coupling free energy, δG_{xy}. The Figure illustrates a case in which the coupling free energy is negative, so that the free energy of the complex XNY is reduced below $\Delta G^o(X) + \Delta G^o(Y)$ by the amount δG_{xy}. As seen in Figure 2, δG_{xy} is the amount by which $\Delta G^o(X/Y)$ differs from $\Delta G^o(X)$, and equivalently, the amount by which $\Delta G^o(Y/X)$ differs from $\Delta G^o(Y)$.

Thermodynamic analysis

A simple argument allows one to relate the various free energies of Figure 2 to those of the model in Figure 1(a). The model supposes, as a

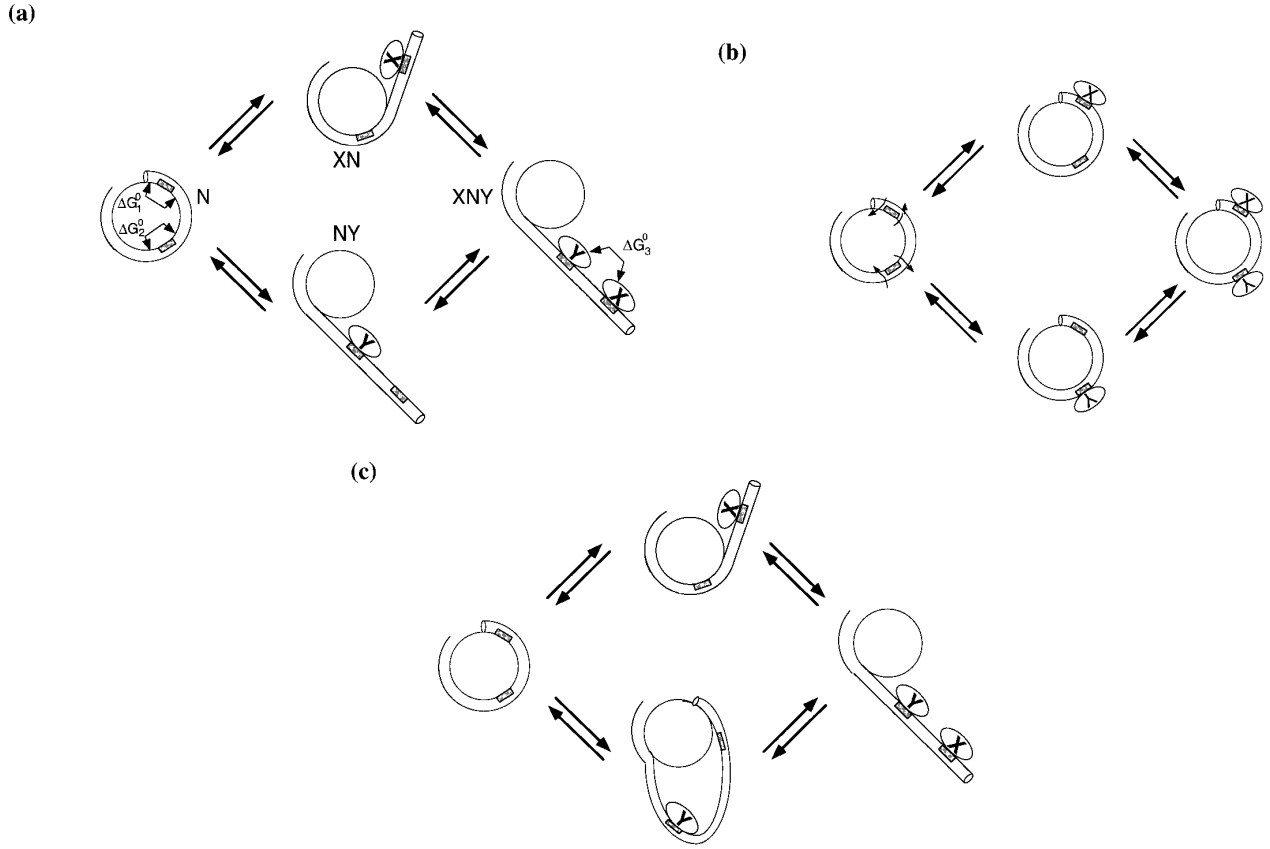

Figure 1. Models for the binding of multiple regulatory proteins to nucleosomes. Three potential outcomes for the binding of proteins X and Y to sites on a single nucleosome are shown schematically here, (a) A fully cooperative model, suggested by our mechanism for site exposure. The binding of X and Y are linked in a thermodynamic cycle such that the binding of either one facilitates the binding of the other. (b) A non-cooperative model, where binding of the 2 proteins is unlinked. (c) An intermediate case; it is less-cooperative than the system shown in (a), since residual histone-DNA contacts must still be broken to allow the binding of the X even in the presence of bound Y.

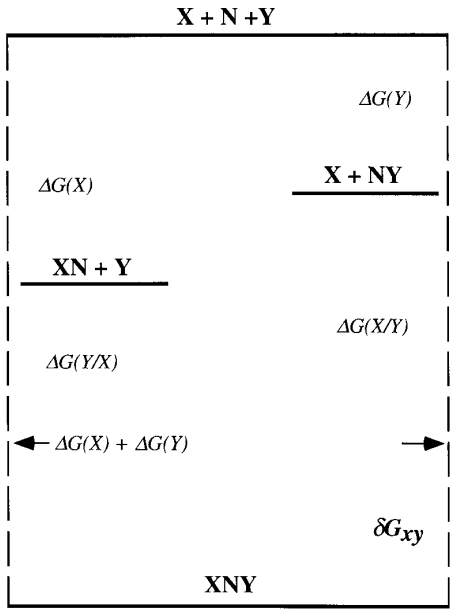

Figure 2. Free energy diagram for cooperative binding. Free energy changes for the binding of each protein (X and Y) to the nucleosome (N) are shown. Increased height in the diagram represent greater Gibbs free energies. Formation of XN + Y has standard free energy $\Delta G^o(X)$. Subsequent binding of Y occurs with free energy change $\Delta G^o(Y/X)$. Formation of X + NY occurs with standard free energy of $\Delta G^o(Y)$, and subsequent binding of X occurs with standard free energy $\Delta G^o(X/Y)$. If the binding were non-cooperative, formation of XNY would have a free energy equal to $\Delta G^o(X) + \Delta G^o(Y)$, indicated on the Figure. The Figure shows that the system may be cooperative, such that the free energy of XNY is decreased below the sum $\Delta G^o(X) + \Delta G^o(Y)$ by an amount known as the the coupling energy, δG_{xy} (Weber, 1992).

limiting case, that sufficient DNA is exposed such that the rates and equilibria for the binding of a regulatory protein to an exposed nucleosomal target sequence or to a naked DNA target sequence are identical. Hence,

$$\Delta G^o(X) = \Delta G_1^o + \Delta G^o(X \text{ to naked DNA}), \quad (1)$$

$$\Delta G^o(X/Y) = \Delta G^o(X \text{ to naked DNA}) \quad (2)$$

$$\Delta G^o(Y) = \Delta G_1^o + \Delta G_2^o + \Delta G^o(Y \text{ to naked DNA}), \quad (3)$$

and

$$\Delta G^o(Y/X) = \Delta G_2^o + \Delta G^o(Y \text{ to naked DNA}). \quad (4)$$

From Figure 2, the relationship of these quantities to the coupling free energy is:

$$\delta G_{xy} = \Delta G^o(X/Y) - \Delta G^o(X), \quad (5)$$

or, equivalently,

$$\delta G_{xy} = \Delta G^o(Y/X) - \Delta G^o(Y). \quad (6)$$

Combining equations (1), (2), and (5), or (3), (4), and (6), leads to the remarkable conclusion that

$$\delta G_{xy} = -\Delta G_1^o: \quad (7)$$

the coupling free energy equals the negative of the free energy of site exposure for the outer-more site or, in other words, the existence of a free energy penalty for site exposure of the outer-more site that is mechanically coupled to binding at the inner-more site is the fundamental origin of the cooperativity inherent in this model.

Relation to ΔG^o_{conf} from the site-exposure model

Comparison of our Figure 1(a) with the site exposure model leads to another remarkable conclusion. Consider a system in which only one protein, which we shall call X, can bind to a nucleosomal target site. The free energy of the nucleosome-conformational change associated with exposure of that site, previously referred to as ΔG^o_{conf}, is evidently identical to the free energy that we refer to here as ΔG_1^o, the free energy associated with breaking the set of histone DNA contacts necessary to expose X's site. But, we showed above that ΔG_1^o is also equal to the coupling free energy, δG_{xy}. This means that, in our earlier study, we already obtained experimental measurements of the coupling free energy, for a variety of positions throughout the nucleosome.

Note that the operative definitions of ΔG_1^o and ΔG_2^o in the present model depend on the locations of the binding sites for the pair of proteins, X and Y. If X always implies the outer-more protein of the pair, then ΔG_1^o (defined as the cost of breaking the contacts necessary to allow the binding of X) corresponds to ΔG^o_{conf} for a single protein binding at that site (although protein-specific effects may exist, see above).

Experimental measurement of key parameters

The free energies $\Delta G^o(X)$ and $\Delta G^o(Y)$ relate to the apparent dissociation constants for binding of X or Y to nucleosomal target sites according to:

$$\Delta G^o(X) = -RT \ln K_d^{apparent}(X) \quad (8)$$

and

$$\Delta G^o(Y) = -RT \ln K_d^{apparent}(Y). \quad (9)$$

As shown in our earlier study (Polach & Widom, 1995), these quantities can be determined experimentally, either by direct binding studies, or indirectly, through measurement of the equilibrium constant for site exposure.

In the context of model 1(a), the value of $\Delta G_1^o (= -\delta G_{xy})$ can be determined by experiment through measurement of the binding free energy for X in the presence and absence of saturating Y using equation (5), or through measurement of the binding free energy for Y in the presence and absence of saturating X using equation (6). Alternatively, according to this model, ΔG_1^o can be determined through experimental measurement of the affinity for X alone, to nucleosomal and naked DNA target sites using equation (1); or equivalently,

as discussed above, ΔG_1^o can be equated to the free energy of site exposure ΔG_{conf}^o measured in our earlier studies of restriction enzyme site accessibility.

ΔG_2^o can be determined through measurement of the binding free energy for Y in the presence of saturating X and for Y to naked DNA, according to

$$\Delta G_2^o = \Delta G^o(Y/X) - \Delta G^o(Y \text{ to naked DNA}) \quad (10)$$

or, together with prior measurement of ΔG_1^o, through measurement of the free energy for Y binding to nucleosomes in the absence of X and for Y binding to naked DNA, according to

$$\Delta G_2^o = \Delta G^o(Y) - \Delta G^o(Y \text{ to naked DNA}) - \Delta G_1^o. \quad (11)$$

Numerical simulations

We used the computer program BIOEQS (Royer & Beechem, 1992; Royer 1993) to examine the behavior of the model of Figure 1(a) for various reasonable values of the parameters. The program is supplied with values for $\Delta G^o(X)$ and $\Delta G^o(Y)$ and for the coupling free energy δG_{xy}. The total concentration of nucleosomes is fixed at tracer quantities (well below any K_d for binding by X or Y). Then, in separate calculations, one of the pair ($[X]_{total}$ or $[Y]_{total}$) is held fixed at various values, while the other is titrated through a range of values. Calculations were carried out for three values of δG_{xy}: (1) $\delta G_{xy} = 0$, which corresponds to the case of no cooperativity, or, equivalently, to the case of a cooperative system in which one of the two proteins simply is not present; and (2, 3) $\delta G_{xy} = -3$ kcal mol^{-1} and $\delta G_{xy} = -6$ kcal mol^{-1}, which correspond approximately to the range of values for ΔG_{conf}^o measured in our earlier work (Polach & Widom, 1995).

We carried out calculations to examine four key aspects of the model. Figure 3(a) examines how the presence of protein Y, at a concentration well below its intrinsic affinity (so that on its own, very little Y would be bound to its nucleosomal target site), may affect the binding of protein X, as quantified by the fractional saturation of X's site in a titration with increasing $[X]_{total}$. In the absence of cooperativity ($\delta G_{xy} = 0$), the presence of Y in the solution necessarily has no effect on binding by X; the titration curve for X that results (square symbols) is that owing to the intrinsic affinity of X for its nucleosomal target site. However, when there does exist free energy coupling, and the second protein (Y in this case) is present, quite different behavior obtains For $\delta G_{xy} = -3$ kcal mol^{-1} (triangles), $K_d^{apparent}(X)$ decreases from 1.2×10^{-7} M to 7.0×10^{-9} M; at -6 kcal mol^{-1} (circles), it decreases to 1.2×10^{-10} M. Evidently, because of the cooperativity, the mere presence of Y in solution greatly facilitates the binding of X, in this example shifting the apparent K_d for X to lower concentration by a factor of $\sim$10 to 1000.

Figure 3(b) reveals a second important point; it considers the same system, again titrated with increasing $[X]_{total}$, but now focusing on binding events at Y's site. When $\delta G_{xy} = 0$, a small amount of Y (set by the relative values of [Y] and $K_d(Y)$) is bound, but this fractional saturation of Y's site is independent of [X]. By contrast, when $-\delta G_{xy} > 0$, the calculations show that F_Y is strongly dependent on [X]: in other words, because of the cooperativity, [X] controls the occupancy of Y's site.

Figure 3(c),(d) represent the converse cases. Note that X and Y are not arbitrarily interchangeable labels, since we define X to be the outer-more of the pair, and Y to be the inner-more, relative to the two ends and the nucleosomal dyad; for particular fixed affinities for naked DNA, $K_d(X)$ (for nucleosome binding) is suppressed substantially less than $K_d(Y)$. Figure 3(c) shows that the presence of X, again at a concentration well below its intrinsic affinity, greatly facilitates the binding of Y. When $\delta G_{xy} = -3$ kcal mol^{-1}, $K_d^{apparent}(Y)$ decreases from 9×10^{-7} M to 8×10^{-8} M; and for $\delta G_{xy} = -6$ kcal mol^{-1}, $K_d^{apparent}(Y)$ decreases to 6×10^{-10} M. Figure 3(d) shows that, in the same system, [Y] controls the occupancy of X's site.

Analysis of experimental data

Investigators in another laboratory have recently carried out several experimental tests for possible cooperativity in the binding of proteins to nucleosomal target sites (Adams & Workman, 1995). Nucleosomes were constructed that contain sites for three unrelated eukaryotic regulatory proteins, GAL4, USF, and NF-κB; variant nucleosomes were constructed in which the nucleosomal locations of the binding sites for particular protein pairs were interchanged. Binding experiments were monitored using gel electrophoretic mobility shift assays or by quantitative DNase I footprinting, to assess interactions between binding events at the multiple sites. Their results are phenomenological in nature, the studies were not interpreted in the context of a particular model. Nevertheless, clear evidence of cooperativity was observed in several cases, leading those investigators to the conclusion that such binding can be inherently cooperative.

In this section we examine whether our model provides an explanation for their results. We compare fractional site occupancies, that were measured directly and reported in that study (Adams & Workman, 1995), or that we extracted approximately from the published primary data of that study, with the predictions of our model, which we evaluate numerically.

In order to relate our model to their experimental observations, three quantities must be specified: $\Delta G^o(X)$, $\Delta G^o(Y)$, and δG_{xy}. We obtain these quantities from their primary data by direct analysis of the appropriate experiment as described in the following paragraphs. Importantly, recall that our analysis implies that values for δG_{xy} can also be

obtained from our earlier, and independent, measurements of ΔG^{o}_{conf}. We evaluated the predictions of our model using values for δG_{xy} that we derived from their data and, separately, with those derived from our earlier measurements.

We emphasize that we are not treating these key quantities as adjustable parameters to be determined by some least-squares procedure that optimizes the agreement with our model! Rather, we are extracting them directly from the primary data, as specified by the equations.

We determine $\Delta G^{o}(X)$ and $\Delta G^{o}(Y)$ from the appropriate K_d values, according to equations (8) and (9). The K_d values were derived from the primary data in two ways. (1) In some cases (for the inner-more of a protein pair, which we refer to as Y), the authors report the ^{32}P counts present in super-shifted material (in a gel mobility-shift assay) relative to the counts in total ^{32}P-labeled nucleosomal template, for nucleosomes titrated with Y only (in the absence of any X). This measurement corresponds to the fractional saturation of binding by Y, $\equiv F_Y$. Values for $K_d^{apparent}$ are calculated from these data according to the standard equation for simple binding, rearranged to express K_d in terms of measured F_Y and specified $[Y] \approx [Y]total$:

$$K_d^{apparent}(Y) = \left(\frac{1}{F_Y} - 1\right)[Y]. \qquad (12)$$

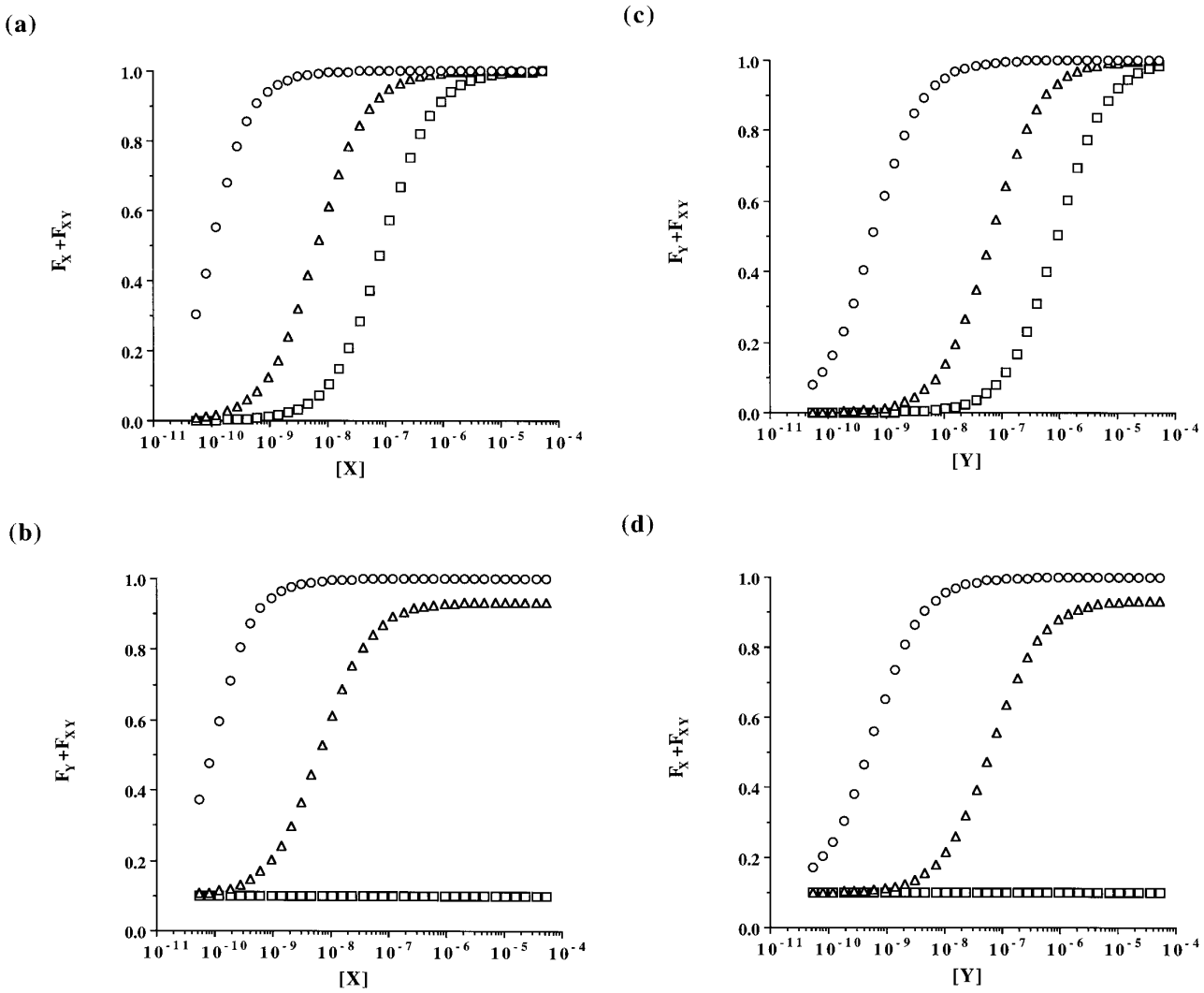

Figure 3. Simulated titration curves. The results from four simulated titrations are shown here; the parameters used are as follows: 100 pM nucleosomes, 10 nM X (in [Y] titrations), 100 nM Y (in [X] titrations), $K_d(X)$ = 100 nM, $K_d(Y)$ = 1 μM. Titrations are shown with coupling free energies δG_{xy} = 0 kcal mol^{-1} (□); 3 kcal mol^{-1} (△) and 6 kcal mol^{-1} (○); titrations from 50 pM to 50 μM. F_X and F_Y are the fraction of particles having bound X only, and bound Y only, respectively; F_{XY} is the fraction of doubly occupied particles. (a) The effect of the presence of a small (but non-zero) [Y] on a titration with [X], observing the fractional occupancy of X's site (= $F_X + F_{XY}$). (b) The fractional occupancy of Y's site (= $F_Y + F_{XY}$) in the same titration. (c) and (d) The converse cases. (c) The fractional occupancy of Y's site in a titration with [Y] in the presence of small (but non-zero) [X]; and (d) the fractional occupancy of X's site in this same titration.

Table 1. Theoretical and experimental parameters for simulated titrations

Simulation	[X] (M)	[Y] (M)	K_d(X) (M)	K_d(Y) (M)	K_d(Y / X) (M)	ΔG^o(X) (kcal mol^{-1})	ΔG^o(Y) kcal (mol^{-1})	ΔG^o(Y / X) (kcal mol^{-1})	δG_{XY} calculated
Figure 4(a)	2.1×10^{-6}	Varied	2.0×10^{-7}	1.0×10^{-5}	9.0×10^{-8}	9.3	6.9	9.8	2.9
Figure 4(b)	4.5×10^{-6}	Varied	4.5×10^{-6}	1.1×10^{-5}	1.2×10^{-7}	7.4	6.9	9.6	2.7
Figure 4(c)	1.2×10^{-6}	Varied	1.2×10^{-6}	8.1×10^{-6}	9.0×10^{-9}	8.2	7.0	11.1	4.1
Figure 4(d)	2.1×10^{-6}	Varied	2.0×10^{-7}	1.1×10^{-5}	1.2×10^{-7}	9.3	6.9	9.6	2.7
Figure 5(a)	Varied	9.0×10^{-8}	2.0×10^{-7}	1.0×10^{-5}	9.0×10^{-8}	9.3	6.9	9.8	2.9
Figure 5(b)	Varied	9.0×10^{-8}	2.0×10^{-7}	8.1×10^{-6}	9.0×10^{-9}	8.2	7.0	11.1	4.1

Experimental values taken from Adams & Workman (1995) are listed along with free energies calculated from that data. δG_{XY} values listed here were calculated from data for the binding of Y in the presence and absence of saturating (or near saturating) X, as described in the text.

(2) In other cases gel electrophoretic mobility shift assays are shown for entire titrations, with appropriate titration points provided so as to adequately constrain possible values of K_d. In these cases, K_d is estimated by direct examination of the gel: we estimate from the photographs provided which [X] (or [Y]) (i.e. which gel track) resulted in F_{XY} being the closest to the value $F_{XY} \approx 0.5$.

The coupling free energy, δG_{xy}, is calculated from titrations of Y in the presence and absence of X using equation (6), or from titrations of X on nucleosomes and on naked DNA according to equation (1). Coupling free energies calculated in these two ways were in general agreement for those cases in which the sites for X were similarly located. As will be seen, the coupling free energies calculated in these ways from their data are also, in most cases, in reasonable agreement with the values for ΔG^o_{conf} obtained in our earlier study.

We extracted quantitative data (Table 1) sufficient to allow comparisons between their experimental results and our model for six independent systems. The results from these comparisons are shown in Figures 4 and 5. We define F_{XY} equal to the fractional simultaneous occupancy of the binding sites for X and Y.

Figure 4(a) to (d) plots measurements of F_{XY}, obtained for four separate experimental systems in particular conditions (Adams & Workman, 1995), together with titration curves predicted by our model appropriate for those experiments. The predicted titration curves were evaluated using the computer program BIOEQS (Royer & Beechem, 1992; Royer, 1993). For these four experiments, we obtained values for ΔG^o(X) and ΔG^o(Y) from the appropriate primary data, as described above.

We evaluated predicted titration curves using these values of ΔG^o(X) and ΔG^o(Y), together with three different values for δG_{xy}. For each experiment, one simulation evaluated the result expected if there were no cooperativity, the case when $\delta G_{xy} = 0$ kcal mol^{-1}. A second simulation was carried out using the value for δG_{xy} which we determined for that system from their primary data, as described above. Finally, a third simulation was carried out using the best estimate for δG_{xy} obtained from our earlier study of site exposure. For this, we calculated an average of $\Delta G^o_{conf}(= -RT \ln K^{conf}_{eq})$ using the values obtained with restriction enzymes in our earlier study, for sites at locations comparable to the location of X in the new studies by Adams & Workman (1995). In each of their four constructs, the site for X is centered at $\sim$20 bp from one end ($\sim$150 bp constructs), reasonably close to a set of sites probed in our construct I (Polach & Widom, 1995).

Figure 4(a) to (d) represents experiments in which [X] is held constant at concentrations large compared to $K_d^{apparent}$(X) (the affinity for X binding on its own for each nucleosomal construct); the system is titrated with increasing [Y], and the fractional formation of the doubly occupied nucleosome is determined experimentally. In panel (a), X is GAL4 and Y is USF; in panel (b), X is USF and Y is GAL4; in panel (c), X is NF-κB and Y is USF; in panel (d), X is GAL4 and Y is NF-κB.

Points that we could extract from their data are plotted along with our predicted curves. In all four experiments, gel electrophoretic mobility assays are provided for complete titrations; we estimate from the photographs provided which [Y] (i.e. which gel track) resulted in F_{XY} being the closest to the value $F_{XY} \approx 0.5$; these points are shown as filled squares. We attach to such points, for their particular [Y], error bars which correspond to the range $F_{XY} = 0.5 \pm 50\%$; we consider this to be a conservative estimate of the uncertainty in judging band intensities between shifted and unshifted bands within the gel track for that particular [Y]. In two of the four experiments, the fraction of the total ^{32}P counts in nucleosomal template present as the doubly occupied complex is reported by the authors for a particular [Y]. These points are plotted as filled triangles. Particular datapoints of this kind imply a particular new $K_d^{apparent}$(Y) (which is now [X]-dependent, and is valid only for the particular conditions of that experiment), in accord with equation (12). Given that quantity, we can then estimate, for that particular experiment, the [Y] for which $F_{XY} = 0.5$; these points are plotted as open triangles. The good agreement between the location of the open triangles and the filled squares suggests that our estimates of [Y] for which $F_{XY} = 0.5$, which we obtained by inspection of the gels, are probably much better than represented by the conservatively chosen error bars.

The predictions of our model for the three different values of δG_{xy} are shown as three curves

in each panel. The dotted curves show the behavior predicted by the model if there were no cooperativity. The continuous curves show the behavior predicted using the δG_{xy} extracted from the primary data for each specific system, as described above. The broken curves show the behavior predicted if δG_{xy} = 2.5 kcal mol^{-1}, the value deduced from our earlier measurement of ΔG^{o}_{conf} for similar locations (Polach & Widom, 1995).

Several points emerge. Most importantly, the data are in remarkably good agreement with our model, using either the δG_{xy} values that we derived for each of their systems, or using the estimate obtained from our earlier work. Evidently, our model does

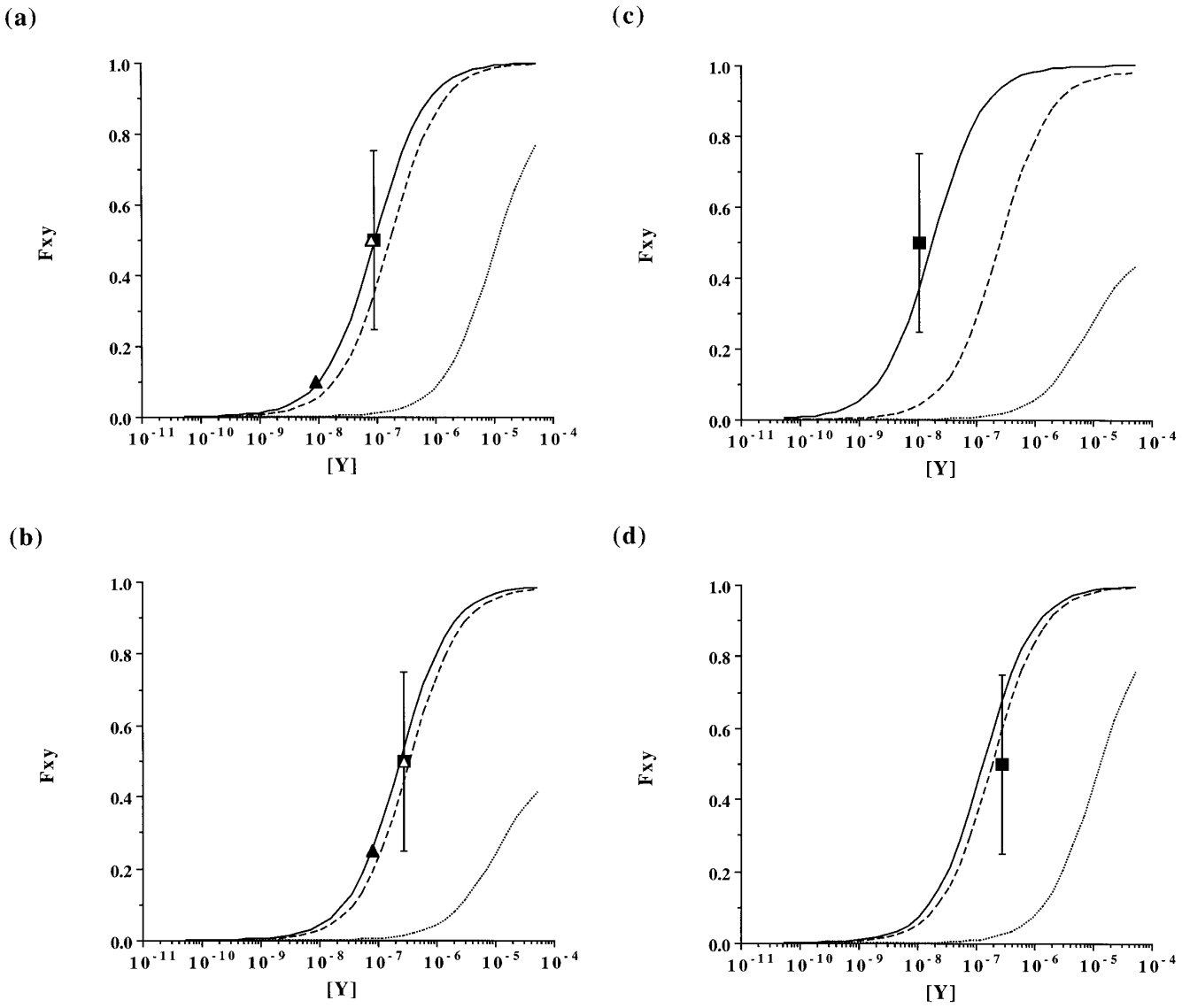

Figure 4. Comparison of predictions with experimental results. Datapoints extracted from the work of Adams & Workman (1995) are superimposed on the predictions of our model 1(a), using no adjustable parameters. For each system, three simulated titrations are shown: - - -, δG_{xy} = 0 kcal mol^{-1}; ——, δG_{xy} calculated from the primary data of Adams & Workman (values given below); – – –, δG_{xy} = 2.5 kcal mol^{-1}, calculated from K^{conf}_{eq} measured with restriction enzymes (Polach & Widom, 1995; see the text). All simulations were carried out for nucleosomes present at trace concentrations. Other conditions for each experiment are as described below. (▲ ■, Δ) Experimental or extrapolated datapoints (see the text for details); (▲), experimental data point calculated from results expressed as percent supershifted counts; (■), experimental datapoint deduced from autoradiograms of gel mobility shift experiments; (△), extrapolated datapoint based on K_d calculated from the corresponding ▲. (a) A titration of USF (Y) in the presence of saturating GAL4 (X); 2.1 μM X, K_d(X) = 200 nM K_d(Y) = 10 μM, δG_{xy} = 2.86 kcal mol^{-1}. (b) A titration of GAL4 (Y) in the presence of USF (X); 4.5 μM X, K_d(X) = 4.5 μM, K_d(Y) = 11.0 μM, δG_{xy} = 2.72 kcal mol^{-1}. (c) A titration of USF (Y) in the presence of NF-κB (X); 1.2 μM X, K_d(X) = 1.2 μM K_d(Y) = 8.1 μM, δG_{xy} = 4.09 kcal mol^{-1}. (d) A titration of NF-κB (Y) in the presence of GAL4 (X); 2.1 μM X, K_d(X) = 200 nM, K_d(Y) = 11.0 μM, δG_{xy} = 2.71 kcal mol^{-1}. Error bars on (■) represent our conservative estimate for our uncertainty in F_{XY}.

account for the behavior of the system when nucleosomes are titrated with multiple DNA binding proteins *in vitro*. It is noteworthy that, for three of the four specific systems, the δG_{xy} values estimated from our earlier work are very close to those that we derive for each specific system from the primary data provided by Adams & Workman (1995) (Table 1). We consider this agreement to be a reflection of the generality of the model. Reasons that some differences in behavior specific to each protein are to be anticipated are discussed above (and see Discussion).

It is evident that the dotted curves do not accord with the data. The system is not well-described by variants of our model such as Figure 1(b) in which there is no cooperativity. The agreement of the present system-specific δG_{xy} values with those from our earlier study means that there is little coupling free energy unaccounted for. This means that variants of our model such as illustrated in Figure 1(c), are not contributing significantly to the behavior of these four systems, within the experimental uncertainty.

Reciprocal cooperativity

A striking finding of the study by Adams & Workman (1995) was that, in one case, cooperativity appeared not to be reciprocal. For systems in which X is GAL4 or NF-κB and Y is USF, they note that reciprocal cooperativity is observed between NF-κB and USF. However, while they find that GAL4 facilitates the binding of USF as seen above, USF appeared to lack an anticipated effect on the binding of GAL4. They consider the dependence of F_{XY} in an [X]-titration carried out in the presence of low levels of [Y], for X being GAL4 or NF-κB, and Y being USF (in both cases). They find that, given a particular [USF], whereas F_{XY} approaches 1 (we estimate from their data that it approaches $\sim$0.8 to 0.9) in the limit of high [NF-κB], F_{XY} approaches little over 0.5 in the limit of high [GAL4] (and with the same [USF]). They conclude, for this latter case, that [USF] is by chance $\approx K_d^{apparent}$ for USF binding to GAL4-bound nucleosomes, and that USF binding is not reciprocally stimulating the binding of GAL4.

If the same states are reached regardless of the order in which the molecules bind, which seems most likely, this eventuality could not occur, because the two binding events would then be linked in a thermodynamic cycle: USF must have the same effect on GAL4 as GAL4 has on USF.

Figure 5(a),(b) examines how those experimental results may be understood. As above, the parameters needed for our analysis $\Delta G^o(X)$, $\Delta G^o(Y)$, and δG_{xy}, are extracted from the appropriate primary data presented by those authors. Figure 5(a) shows the predicted behavior for F_{XY} in the titration with increasing [NF-κB]. F_{XY} increases with [NF-κB] but eventually reaches a plateau value of $F_{XY} \approx 0.9$ (in qualitative agreement with their observations and in good agreement with a particular experimental datapoint). Figure 5(b) shows the predicted behavior in the titration with increasing [GAL4], which is carried out at the same [USF], and with the same location of USF's binding site. We find that F_{XY} is predicted to plateau at a value $F_{XY} \approx 0.55$, in remarkable agreement with their observations.

Why should the same [USF] (playing the role of Y, at the same location) allow a significantly smaller maximum value for F_{XY} in this latter case? The reason for the discrepancy is the difference in cooperativity free energy, δG_{xy}, for the two different

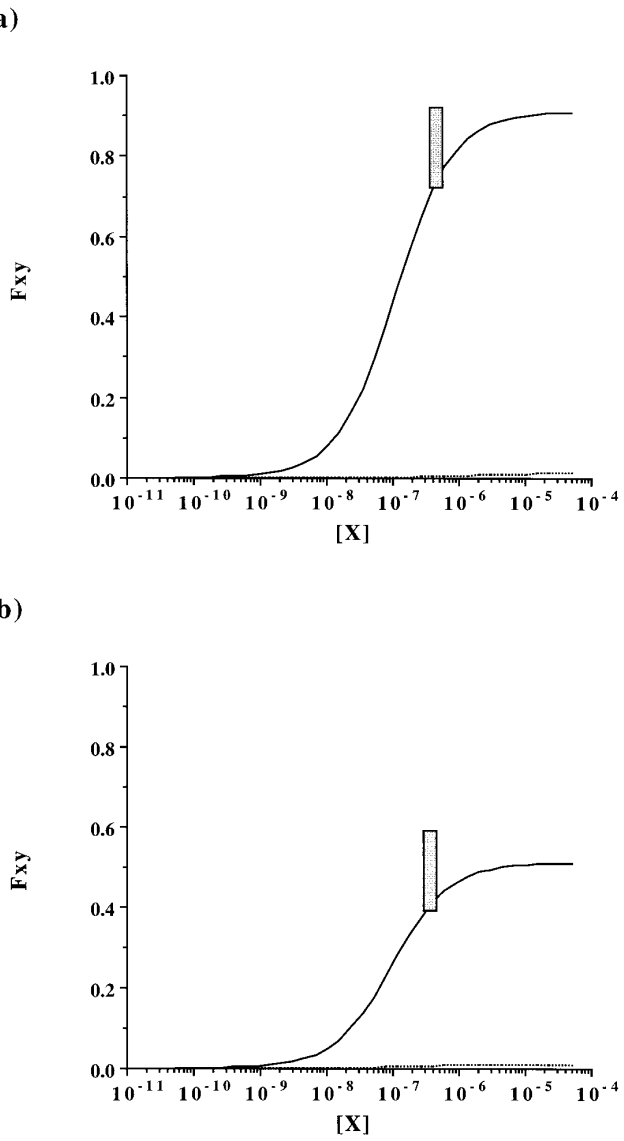

Figure 5. Tests of reciprocal cooperativity. (a) Titration of NF-κB (X) in the presence of USF (Y); 90 nM Y, $K_d(X) = 1.2$ μM, $K_d(Y) = 8.1$ μM, $\delta G_{xy} = 4.09$ kcal mol^{-1}. (b) Titration of GAL4 (X) in the presence of USF (Y); 90 nM Y, $K_d(X) = 200$ nM, $K_d(Y) = 10$ μM, $\delta G_{xy} = 2.86$ kcal mol^{-1}. The shaded rectangles represent particular datapoints extracted from Adams & Workman (1995). They are shown as regions rather than points because the reported data only specified approximate values; the span of the rectangle approximates a range in F_{XY} that is consistent with the data available for that [X].

proteins playing the roles of X in these two experiments. The δG_{xy} measured for NF-κB binding to the X site in these particular constructs is significantly greater than that for GAL4 (Table 1). This increased coupling free energy for NF-κB (Figure 5(a)) drives F_{XY} to higher values than can be achieved with the reduced value of δG_{xy} appropriate for GAL4 (Figure 5(b)), for the particular [USF] and $K_d^{apparent}$(USF-only) examined in these particular experiments.

Also plotted in Figure 5(a),(b) are the predictions for these two systems for the case of no cooperativity ($\delta G_{XY} = 0$, dotted lines). These curves represent the behavior of the system if the expected (reciprocal) cooperativity were not present. The effects of the cooperativity that does exist can be seen by comparing (within each panel) the continuous and dotted curves. Thus, even in the system of Figure 5(b), there is plain evidence of cooperativity; in the absence of the cooperativity F_{XY} would have reached a much lower maximal value than the value $F_{XY} \approx 0.5$ obtained experimentally.

In summary, rather than representing a system-specific failure of reciprocal cooperativity, these data for both systems are in remarkable agreement with the prediction of our model with its inherent cooperativity fully, and reciprocally, realized.

Discussion

One chief conclusion of this study is that the cooperativity inherent in our site-exposure model of Figure 1(a) evidently does occur *in vitro*. Remarkably, this model, with no adjustable parameters, accounts quantitatively for the results from a set of experimental studies on diverse particular systems (Adams & Workman, 1995). Moreover, we showed previously that the precursor of this model, which considers individual binding by proteins, accounts quantitatively for the behavior of a large number of proteins (see Polach & Widom, 1995). Evidently, we have a good quantitative understanding and predictive capability for protein binding to nucleosomal DNA target sites *in vitro*.

Significant questions do remain even at this level of the analysis. We have not yet explored whether or how cooperative interactions may persist across the nucleosomal dyad. We summarized above various reasons that may cause the actual nucleosome-binding affinity and cooperativity exhibited for particular proteins to vary somewhat on a case-by-case basis; also we discussed various ways in which the simple experimental system explored here differs from the natural system of chromatin *in vivo*. All of these effects need to be further explored and quantified.

Additional modes of cooperativity

Many systems are known in which two proteins bind cooperatively (synergistically) even to naked DNA. This cooperativity may arise from favorable direct protein-protein contacts that may be present in the DNA-bound state, or it may arise from a variety of other less-direct mechanisms. In any case, these mechanisms may also operate when the DNA is initially organized in nucleosomes. This possibility is indicated by ΔG_3^o in Figure 1(a), which represents the free energy of such cooperative interactions on naked DNA. Our model implies that the net or apparent cooperativity will be the sum of δG_{XY} from nucleosome-competition plus this additional ΔG_3^o.

Protein-specific effects

The data of Figures 4 and 5 (and Table 1) provide one striking example of protein-specific effects on the coupling free energies. In otherwise equivalent systems, we find that GAL4 (as X) provides 2.86 kcal mol^{-1} of coupling free energy in a system with USF (as Y), whereas binding of NF-κB (as X) at a site essentially equivalent to the GAL4 site yields 4.09 kcal mol^{-1} of coupling free energy with USF (as Y). USF and NF-κB are not known to interact directly in any meaningful way, so we cannot attribute the difference in these two coupling free energies to a specific interaction energy ΔG_3^o. Interestingly, the known structures suggest that the difference may be due to substantial differences in the size and shape of the corresponding protein-DNA complexes as discussed above. Compared to the structure of the GAL4-DNA complex (Marmorstein *et al*., 1992), the structure of the NF-κB p50-DNA complex (Ghosh *et al*., 1995; Muller *et al*., 1995) reveals that the NF-κB p50 protein dimer surrounds the DNA with four large domains that project radially away from the DNA surface, each of which is larger than the two GAL4 DNA-binding domains in the GAL4 dimer. It is likely that substantially more DNA needs to be uncoiled or otherwise exposed off of the histone surface in order for NF-κB to bind to a nucleosomal NF-κB site, than is required for the binding of the short DNA-binding fragments of GAL4 to an equivalently positioned nucleosomal GAL4 site. In the context of our uncoiling picture for the mechanism of site exposure, such additional uncoiling occurs with greater free energy cost (larger ΔG_{conf}^o), and hence yields a greater negative coupling free energy when it occurs.

New mechanisms for biological regulation

The cooperative behavior described here provides a basis for two new mechanisms of biological regulation *in vivo*. First, two DNA-binding proteins can, in principle, facilitate each other's binding to nucleosomal target sites *in vivo* even if these proteins do not touch or interact directly in any way. Second, cells can in principle vary the DNA binding-site occupancy by an arbitrary protein either by varying the nuclear concentration of that protein or, alternatively, by varying the concentration or activity (e.g. by phosphorylation) of any

other protein that binds to a site within the same nucleosome, even if these two proteins do not directly interact.

Cooperativity from non-specific DNA binding proteins

Another interesting property of our model is that cooperativity should also be observed when one of the two (or more) proteins binding to sites within a single nucleosome is a non-specific DNA binding protein, provided only that such proteins cannot simply adsorb to the outer surface of the nucleosomal DNA. The net coupling free energy will be an appropriately weighted average for the particular distribution of nucleosomal sites bound non-specifically by this protein. This simple picture accounts for many aspects of the behavior of the swi/snf complex proteins currently in the literature, and it can in principle apply to many other systems as well.

Benchmarks for the significance of the coupling free energies

The significance of the free energy coupling that arises from two or more DNA binding proteins having a single nucleosome as a common-competitor may be assessed by comparison to two benchmarks: thermal energies, and the free energies of conventional DNA-binding cooperativity between two proteins binding at neighboring locations, the ΔG^{o}_{3} mentioned above.

The coupling free energies measured in our earlier study as $-\Delta G^{o}_{conf}$ and considered here as $-\delta G_{XY}$ vary from $\sim$2.5 to $\sim$6 kcal mol^{-1} or more. By comparison, RT (a measure of thermal energy, where R is the gas constant and T the temperature) has the value $\sim$0.6 kcal mol^{-1} at physiological temperatures. Evidently the coupling free energies are significant in comparison to thermal energies.

Table 2 presents data from some arbitrarily chosen examples of protein-protein cooperative interactions having established significance for gene regulation. Many of these systems exhibit modest cooperativity, leading e.g. to $\sim$4 to 20-fold increases in affinity, corresponding to free energies that are substantially smaller than those arising from the nucleosome-competition mechanism reported here ($\sim$2.5 to 6 kcal mol^{-1}). We conclude that the coupling free energies revealed in the present study are significant also in comparison to these benchmarks of biologically significant cooperative interactions.

Roles for nucleosome dynamics in gene regulation

In our earlier study (Polach & Widom, 1995) we showed that one important role of nucleosome dynamics is to provide a mechanism whereby site-specific DNA binding proteins may always be able to gain access to their DNA target sites *in vivo*. No other mechanisms guaranteeing such access are currently known.

In this new study we show that the dynamic behavior of nucleosomes has the additional consequence of providing a new mechanism for the cooperative or synergistic binding of proteins. We envision two different possible roles for this cooperativity in biological regulation *in vivo*.

One possibility is that, *in vivo*, nucleosomes remain present in stable complexes with one or more regulatory proteins, and that these complexes are the states in which the biological actions of regulatory proteins are manifested. In this case, the coupling free energy δG_{XY} may be a dominant term in the equilibrium or steady-state energetics of gene regulation, since it is large in comparison both to thermal energies and to the ΔG^{o}_{3}'s typical in many systems (Table 2).

A different possible consequence of the binding of one or more regulatory proteins to target sites

Table 2. Some conventional cooperativity free energies

Protein 1	Protein 2	System	Approx. $-\Delta G^{o}_{3}$ kcal mol^{-1}	Ref.
NF-κB	Sp1	HIV-1 LTR	1.4	(a)
ATF-2	HMGI(Y)	β-IFN promoter	0.9	(b)
Oct1	OAP	IL-2 enhancer	1.4	(c)
Ubx	exd	dpp promoter	1.8	(d)
p53	TFIID	fos1wt	1.3	(e)
λ Repressor	λ Repressor	λ O_R1-O_R2	2.0	(f)
Mat a1	Mat α2	Yeast hsg operator	$\geqslant$4.3	(g)

(a) Estimated from reported values for K_d of NF-κB in presence and absence of saturating Sp1 (Perkins *et al*., 1993). (b) Estimated from reported values for K_d of ATF-2 in presence and absence of saturating HMGI(Y) (Du *et al*., 1993). (c) Estimated from ratio of off-rates for Oct 1 in presence and absence of OAP (Crabtree & Clipstone, 1994). (d) Estimated from reported values for K_d of Ubx protein in presence and absence of exd protein (Chan *et al*., 1994); [exd] was less than saturating, so this calculated ΔG^{o}_{3} is a slight underestimate. (e) Estimated from concentrations of TBP required for comparable gel-shift band-intensities in presence and absence of p53 (Chen *et al*., 1993). (f) Data of Ackers *et al*. (1982). (g) Estimated from concentrations of α2 required for comparable gel-shift band-intensities in presence and absence of al protein; this ΔG^{o}_{3} is a lower bound, because the al protein was present at a concentration such that, on its own, its binding was undetectable.

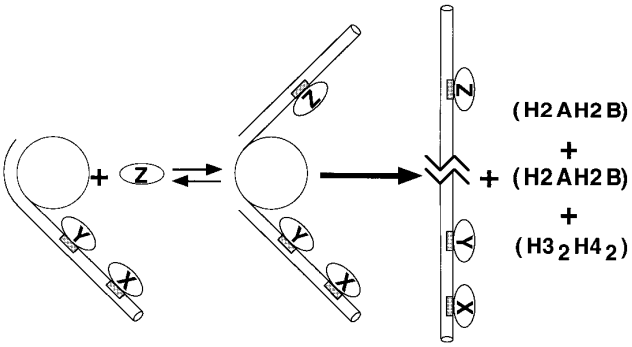

Figure 6. Possible nucleosome dissociation resulting from multiple binding events. Binding by sufficient numbers of regulatory proteins may lead ultimately to displacement of the histone octamer, which, in physiological conditions, dissociates into two H2A-H2B dimers, and one H3-H4 tetramer (van Holde, 1989). Reassociation of the nucleosome requires a quaternary reaction (three histone units plus the DNA), which is unlikely, hence binding of the regulatory proteins may drive dissociation of the histones.

within a nucleosome *in vivo* might be to displace the histone octamer along the DNA through some sliding process as has been suggested (Varga-Weisz, *et al*., 1995), or alternatively, to displace the histone octamer off of DNA altogether, as indicated schematically in Figure 6. In either of these cases, the regulatory complexes ultimately produced would involve regulatory proteins bound to naked DNA, not to a nucleosome, and the mechanisms reported here and in our earlier study would not contribute to the equilibrium or steady-state energetics, in important contrast to terms such as the ''conventional'' cooperativity, ΔG°_{3}, which would persist. Even in this case, however, the nucleosome dynamics and the corresponding energetics analyzed here may have central roles. We previously suggested that the site exposure mechanism may be an essential initial step in nucleosome sliding. Moreover, the dynamic behavior of nucleosomes and its accompanying coupling free energy δG_{XY} may contribute to both the mechanism and the driving force for displacing the histone octamer (Figure 6).

Some key questions

The present analysis focuses attention on several questions. It is particularly important to establish whether equilibrium or steady-state regulatory complexes do or do not contain nucleosomes. The available evidence suggests that they generally may not. In connection with this question, it is important to test whether nucleosome reassembly occurs spontaneously in the G_1 and G_2 phases of the cell cycle, and thus whether histones can continue to compete thermodynamically with regulatory proteins for binding even after regulatory complexes are established. Finally, it is critical to develop methods for experimentally measuring the concentration of free regulatory proteins in nuclei *in vivo*, since, in the context of current theories for site-specific binding of proteins to DNA, it is the concentration of free protein that governs the fractional occupancies of binding sites.

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References

Ackers, G. K., Johnson, A. D. & Shea, M. A. (1982). Quantitative model for gene regulation by λ phage repressor. *Proc. Natl Acad. Sci. USA*, **79**, 1129–1133.

Adams, C. C. & Workman, J. L. (1995). Binding of disparate transcriptional activators to nucleosomal DNA is inherently cooperative. *Mol. Cell. Biol.* **15**, 1405–1421.

Archer, T. K., Cordingley, M. G., Wolford, R. G. & Hager, G. L. (1991). Transcription factor access is mediated by accurately positioned nucleosomes on the mouse mammary tumor virus promoter. *Mol. Cell. Biol.* **11**, 688–698.

Arents, G. & Moudrianakis, E. N. (1993). Topography of the histone octamer surface: repeating structural motifs utilized in the docking of nucleosome DNA. *Proc. Natl Acad. Sci. USA*, **90**, 10489–10493.

Arents, G., Burlingame, R. W., Wang, B.-C., Love, W. E. & Moudrianakis, E. N. (1991). The nucleosomal core histone octamer at 3.1 Å resolution: A tripartite protein assembly and a left-handed superhelix. *Proc. Natl Acad. Sci. USA*, **88**, 10148–10152.

Chan, S.-K., Jaffe, L., Capovilla, M., Botas, J. & Mann, R. S. (1994). The DNA binding specificity of ultrabithorax is modulated by cooperative interactions with extradenticle, another homeoprotein. *Cell*, **78**, 603–615.

Chen, X., Farmer, G., Zhu, H., Prywes, R. & Prives, C. (1993). Cooperative binding of p53 with TFIID (TBP): a possible mechanism for transcriptional activation. *Genes Dev*. **7**, 1837–1849.

Cote, J., Quinn, J., Workman, J. L. & Peterson, C. L. (1994). Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science*, **265**, 53–60.

Crabtree, G. R. & Clipstone, N. A. (1994). Signal transmission between the plasma membrane and nucleus of T Lymphocytes. *Annu. Rev. Biochem.* **63**, 1045–1083.

Du, W., Thanos, D. & Maniatis, T. (1993). Mechanisms of transcriptional synergism between distinct virus-inducible enhancer elements. *Cell*, **74**, 887–898.

Ghosh, G., Van Duyne, G., Ghosh, S. & Sigler, P. B. (1995). Structure of NF-κB p50 homodimer bound to a κB site. *Nature*, **373**, 303–310.

Hayes, J. J. & Wolffe, A. P, (1992). Histones H2A/H2B inhibit the interaction of transcription factor IIIA with *Xenopus borealis* somatic 5S RNA gene in a nuclesome. *Proc. Natl Acad. Sci. USA*, **89**, 1229–1233.

Imbalzano, A. N., Kwon, H., Green, M. R. & Kingston, R. E. (1994). Facilitated binding of TATA-binding protein to nucleosomal DNA. *Nature*, **370**, 481–485.

Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E. & Green, M. R. (1994). Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex. *Nature*, **370**, 477–481.

Lee, D. Y., Hayes, J. J., Pruss, D. & Wolffe, A. P. (1993). A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell*, **72**, 73–84.

Lutter, L., Klug, A., Levitt, M. & Crick, F. H. C. (1979). Periodicity of deoxyribonuclease I digestion of chromatin. *Science*, **204**, 855–858.

Lutter, L. C. (1981). DNase II digestion of the nucleosome core: precise locations and relative exposures of sites. *Nucl. Acids Res.* **9**, 4251–4265.

Marmorstein, R., Carey, M., Ptashne M. & Harrison S. C. (1992). DNA recognition by GAL4: structure of a protein-DNA complex. *Nature*, **356**, 408–414.

Muller, C. W., Rey, F. A., Sodeoka, M., Verdine, G. L. & Harrison, S. C. (1995). Structure of the NF-κB p50 homodimer bound to DNA. *Nature*, **373**, 311–317.

Pazin, M. J., Kamakaka, R. T. & Kadonaga, J. T. (1994). ATP-dependent nucleosome reconfiguration and transcriptional activation from preassembled chromatin templates. *Science*, **226**, 2007–2011.

Perkins, N. D., Edwards, N. L., Duckett, C. S., Agranoff, A. B., Schmid, R. M. & Nabel, G. J. (1993). A cooperative interaction between NF-κB and Sp1 is required for HIV-1 enhancer activation. *EMBO J.* **12**, 3551–3558.

Perlmann, T. (1992). Glucocorticoid receptor DNA-binding specificity is increased by the organization of DNA in nucleosomes. *Proc. Natl Acad. Sci. USA*, **89**, 3884–3888.

Polach, K. J. & Widom, J. (1995). Mechanism of protein access to specific DNA sequences in chromatin: A dynamic equilibrium model for gene regulation. *J. Mol. Biol.* **254**, 130–149.

Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D. & Klug, A. (1984). Structure of the nucleosome core particle at 7 Å resolution. *Nature*, **311**, 532–537.

Royer, C. A. (1993). Improvements in the numerical analysis of thermodynamic data from biomolecular complexes. *Anal. Biochem.* **210**, 91–97.

Royer, C. A. & Beechem, J. M. (1992). Numerical analysis of binding data: advantages, practical aspects, and implications. *Methods Enzymol.* **210**, 481–505.

Studitsky, V. M., Clark, D. J. & Felsenfeld, G. (1995). Overcoming a nucleosomal barrier to transcription. *Cell*, **83**, 19–27.

Taylor, I. C. A., Workman, J. L., Schuetz, T. J. & Kingston, R. E. (1991). Facilitated binding of GAL4 and heat shock factor to nucleosomal templates: differential function of DNA-binding domains. *Genes Dev.* **5**, 1285–1298.

Tsukiyama, T., Becker, P. B. & Wu, C. (1994). ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature*, **367**, 535–531.

van Holde, K. E. (1989). *Chromatin*, p. 497, Springer-Verlag, NY.

Varga-Weisz, P. D., Blank, T. A. & Becker, P. B. (1995). Energy-dependent chromatin assembly and nucleosome mobility in a cell-free system. *EMBO J.* **14**, 2209–2216.

Weber, G. (1992). *Protein Interactions*, p. 293, Chapman and Hall, NY.

Wechsler, D. S., Papoulas, O., Dang, C. V. & Kingston, R. E. (1994). Differential binding of c-Myc and Max to nucleosomal DNA. *Mol. Cell. Biol.* **14**, 4097–4107.

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