Point of VIEW

ACS chemical biology

Allostery: DNA Does It, Too

Jonathan B. Chaires*

James Graham Brown Cancer Center, University of Louisville, 529 South Jackson Street, Louisville, Kentucky 40202

ABSTRACT Allostery is a central concept for understanding protein function and regulation. It is less well appreciated that DNA is allosteric, too, and that DNA conformational changes can by coupled to protein binding interactions on the DNA lattice. Allosteric DNA interactions are emerging as important features in the assembly of the molecular machines that regulate transcription.

Allostery is a central concept in biochemistry and molecular biology. Indeed, Jacque Monod declared allostery to be "the second secret of life" (*1*). "Allostery" is derived from the Greek *allos* ("other") and *stereos* ("space" or "shape") and is a manifestation of the thermodynamic coupling of binding reactions to conformational transitions in macromolecules. Allosteric interactions are ubiquitous in the regulation and modulation of enzymatic activity. The concept of allostery is commonly presented in introductory biochemistry textbooks and is typically presented with reference to the well-known Monod–Wyman–Changeux (MWC) and Koshland–Nemethy–Filmer models. These models feature the triggering of a "switch" between conformations of protein subunits within a multisubunit protein complex brought on by an initial binding event, a switch that alters the affinities for subsequent binding events. The view of allostery has evolved to recognize that the fundamental capacity to undergo conformational changes in response to binding may be intrinsic to all proteins and that such coupled conformational shifts may be a fundamental mechanism in regulation and communication (*2, 3*). Allostery provides a means of responding to a changing environment to modulate activity. The paper by Moretti and co-workers on page 220 of this issue shows that allostery is not unique to proteins and that DNA is allosteric, too (*4*).

It is not commonly appreciated that DNA is allosteric. DNA is often mistakenly viewed as an inert lattice onto which proteins assemble to replicate or transcribe genes. Protein binding might be guided to specific sites on the inert lattice by sequence-specific interactions utilizing recognition elements residing in the major or minor grooves, in particular specific patterns of hydrogen bond donors and acceptors along the edges of base pairs (*5*). Several exciting recent developments, including the report by Moretti, contradict this view of the static lattice and suggest that DNA may be a more active partner in its own replication and transcription through allosteric transitions in its structure that modulate protein binding. There is also a longer history of allosteric DNA that bears remembering.

As early as 1972, the intercalator ethidium was shown to act as an allosteric effector and could convert left-handed Z DNA to a right-handed form (*6*). The MWC model for allostery was invoked to explain highly cooperative binding isotherms observed for ethidium binding to Z DNA. A few years later, Crothers and co-workers showed that binding of the groove-binder distamycin induces a cooperative transition of calf thymus DNA to a new form with higher affinity for the drug and altered structural properties (*7*). Subsequently, a statistical mechanical theory was presented by the Crothers laboratory for the calculation of binding isotherms when binding is coupled to a DNA structural change (*8*). The theory was analogous to the MWC model for allosteric binding to proteins but differed in the details necessary to describe the conformational transition of the DNA lattice, as illustrated (Figure 1, panel a). The model applies equally well for protein or small-molecule binding to DNA. The Crothers model was

***Corresponding author,**
j.chaires@louisville.edu.

Published online April 18, 2008
10.1021/cb800070s CCC: $40.75

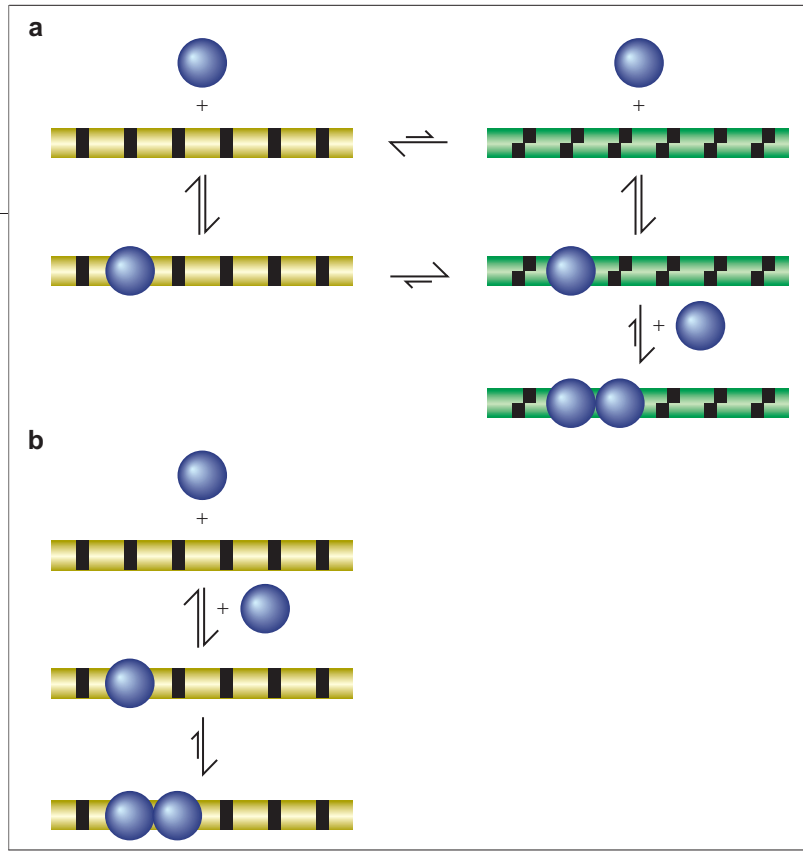

Figure 1. Types of cooperativity in protein—DNA interactions. a) Allosteric DNA. DNA can equilibrate between two conformations (yellow and green). Proteins bind to each with different affinity. Binding of the first protein shifts the DNA conformational equilibrium to a higher affinity form, facilitating the binding of subsequent proteins. Additional favorable binding energy could arise from protein—protein interactions between molecules bound to the lattice. b) Nonallosteric binding. The conformation of the DNA lattice is constant. Binding of a protein to a contiguous site next to another bound protein leads to favorable protein—protein interactions, facilitating binding of the second molecule.

subsequently used to quantitatively analyze binding isotherms for the interaction of the anticancer drug daunorubicin and its enantiomer with both left- and right-handed DNA (*9, 10*). These studies showed that binding affinity was enhanced by nearly 40-fold by coupling to the DNA conformational change, adding ~2 kcal mol^{-1} more favorable binding free energy.

A contrasting model for cooperative binding of proteins to a DNA lattice is that of McGhee and von Hippel (*11*), which describes neighbor exclusion effects. In that model, the conformation of the DNA lattice is unchanging, but positive cooperativity can arise from interactions between bound proteins. Thus, protein binding is tighter at contiguous sites because of the additional interactions between bound proteins (Figure 1, panel b). Binding to a contiguous site may contribute up to 3—4 kcal mol^{-1} of more favorable binding free energy (*12*).

The Crothers allosteric and the McGhee—von Hippel models predict binding isotherms with distinctly different shapes that can be distinguished by nonlinear curve fitting of binding isotherms to the respective models (*13*).

Another key historical concept is that of "telestability" (*14, 15*). Pioneering studies from the Wells laboratory showed that the sequence of one region of a synthetic double-helical DNA affected the physical properties of a contiguous but remote region. A manifestation of this phenomenon is that the binding affinity to a specific sequence can be altered by changes in the surrounding sequences, leading to sequence context effects. For example, the binding free energy of the drug actinomycin D for the sequence 5′-AGCT can vary by nearly 1 kcal mol^{-1}, depending on the flanking sequence surrounding the actual binding site (*16*).

The transmission of allosteric effects in DNA has been amply demonstrated by experiment, and the many examples have been reviewed (*17, 18*). Without question, the binding of proteins or small molecules to a DNA lattice can produce coupled conformational changes that alter the binding of subsequent molecules, often over long distances.

More recent studies have indicated the importance of DNA allosteric effects in the assembly of the macromolecular machines used for gene expression and replication. The "enhanceosome", for example, is a protein complex that binds to the enhancer region of a gene, either upstream or downstream of the promoter (*19*). The enhanceosome accelerates transcription of the gene. The binding and assembly of the activating proteins, some of which may be transcription factors, are cooperative, in part because of energetically favorable protein—protein interactions formed in the complex. A recent detailed structural analysis of the interferon-β enhanceosome revealed, however, that cooperative protein—protein interactions alone may not be enough to account for the high-precision assembly of the machinery (*20*). A detailed examination of an atomic model of the interferon-β enhanceosome led to the conclusion that the "paucity of local protein—protein contacts suggests that cooperative occupancy of the enhancer comes from both binding-induced changes in DNA conformation and interactions with additional components" (*20*). This study highlights a significant problem, namely, that it has been difficult, if not impossible, to separate and quantify the contribution of DNA conformational changes to the assembly process, such as construction of the enhanceosome. It is this problem that Moretti and co-workers have attacked and to which they have provided a novel and elegant solution.

Moretti and co-workers explore the cooperativity in the DNA binding interactions of Hox and Exd proteins. Exd is a transcription

factor that guides Hox to unique DNA binding sites. Exd binds to DNA with nearly 4 kcal mol^{-1} more favorable binding free energy in the presence of Ubx (a member of the Hox family). A mutant Ubx in which possible protein−protein interactions with Exd are largely eliminated still facilitates binding by >1 kcal mol^{-1}. Careful scrutiny of the known crystal structures of the complex suggests that a Ubx-induced DNA conformational change facilitates Exd binding, specifically a widening and decrease in the depth of the major groove. A hairpin polyamide molecule was designed that could bind selectively into the minor groove with transmitted effects into the major groove that mimic the changes that were seen to be induced by protein binding. The designed "wedge" molecule was found to enhance Exd binding by 1.5 kcal mol^{-1} in the absence of Ubx. The wedge thus acts as an allosteric effector to facilitate binding of the protein. The results and strategy are significant because they provide a new tool for dissecting the energetic contributions of coupled DNA conformational changes to specific protein−DNA binding interactions. Above all, the results conclusively show that DNA is allosteric, too, and that the effects of such allostery cannot be ignored.

REFERENCES

1. Pardee, A. B., and Reddy, G. P. (2003) Beginnings of feedback inhibition, allostery, and multi-protein complexes, *Gene 321*, 17–23.
2. Gunasekaran, K., Ma, B., and Nussinov, R. (2004) Is allostery an intrinsic property of all dynamic proteins? *Proteins 57*, 433–443.
3. Swain, J. F., and Gierasch, L. M. (2006) The changing landscape of protein allostery, *Curr. Opin. Struct. Biol. 16*, 102–108.
4. Moretti, R., Donato, L. J., Bresinski, M. L., Stafford, R. L., Hoff, H., Thorson, J. S., Dervan, P. B., and Ansari, A. Z. (2008) Targeted chemical wedges reveal the role of allosteric DNA modulation in protein-DNA assembly, *ACS Chem. Biol. 3*, 220–229.
5. Seeman, N. C., Rosenberg, J. M., and Rich, A. (1976) Sequence-specific recognition of double helical nucleic acids by proteins, *Proc. Natl. Acad. Sci. U.S.A. 73*, 804–808.
6. Pohl, F. M., Jovin, T. M., Baehr, W., and Holbrook, J. J. (1972) Ethidium bromide as a cooperative effector of a DNA structure, *Proc. Natl. Acad. Sci. U.S.A. 69*, 3805–3809.
7. Hogan, M., Dattagupta, N., and Crothers, D. M. (1979) Transmission of allosteric effects in DNA, *Nature 278*, 521–524.
8. Dattagupta, N., Hogan, M., and Crothers, D. M. (1980) Interaction of netropsin and distamycin with deoxyribonucleic acid: electric dichroism study, *Biochemistry 19*, 5998–6005.
9. Chaires, J. B. (1986) Allosteric conversion of Z DNA to an intercalated right-handed conformation by daunomycin, *J. Biol. Chem. 261*, 8899–8907.
10. Qu, X., Trent, J. O., Fokt, I., Priebe, W., and Chaires, J. B. (2000) Allosteric, chiral-selective drug binding to DNA, *Proc. Natl. Acad. Sci. U.S.A. 97*, 12032–12037.
11. McGhee, J. D., and von Hippel, P. H. (1974) Theoretical aspects of DNA-protein interactions: co-operative and non-co-operative binding of large ligand to a one-dimensional homogeneous lattice, *J. Mol. Biol. 86*, 469–489.
12. von Hippel, P. H. (2007) From "simple" DNA-protein interactions to the macromolecular machines of gene expression, *Annu. Rev. Biophys. Biomol. Struct. 36*, 79–105.
13. Chaires, J. B. (2001) Analysis and interpretation of ligand-DNA binding isotherms, in *Drug-Nucleic Acid Interactions* (Chaires, J. B., and Waring, M. J., Eds.), pp 3–22, Academic Press, San Diego, CA.
14. Burd, J. F., Larson, J. E., and Wells, R. D. (1975) Further studies on telestability in DNA. The synthesis and characterization of the duplex block polymers d(C20A10) - d(T10G20) and d(C20A15) - d(T15G20), *J. Biol. Chem. 250*, 6002–6007.
15. Burd, J. F., Wartell, R. M., Dodgson, J. B., and Wells, R. D. (1975) Transmission of stability (telestability) in deoxyribonucleic acid. Physical and enzymatic studies on the duplex block polymer d(C15A15) - d(T15G15), *J. Biol. Chem. 250*, 5109–5113.
16. Qu, X., Ren, J., Riccelli, P. V., Benight, A. S., and Chaires, J. B. (2003) Enthalpy/entropy compensation: influence of DNA flanking sequence on the binding of 7-amino actinomycin D to its primary binding site in short DNA duplexes, *Biochemistry 42*, 11960–11967.
17. Crothers, D. M., and Fried, M. (1983) Transmission of long-range effects in DNA, *Cold Spring Harbor Symp. Quant. Biol 47*, Part 1263–269.
18. Schurr, J. M., Delrow, J. J., Fujimoto, B. S., and Benight, A. S. (1997) The question of long-range allosteric transitions in DNA, *Biopolymers 44*, 283–308.
19. Carey, M. (1998) The enhanceosome and transcriptional synergy, *Cell 92*, 5–8.
20. Panne, D., Maniatis, T., and Harrison, S. C. (2007) An atomic model of the interferon-beta enhanceosome, *Cell 129*, 1111–1123.