



A Chromatin Switch

JOHN W. BODNAR[†] AND MARGARET K. BRADLEY[‡]

[†] *Chemistry Department, U.S. Naval Academy, Annapolis, MD 21402 and* [‡] *55 Park Street, Charlestown, MA 02129, U.S.A.*

(Received on 18 January 1995, Accepted in revised form on 25 March 1996)

Cellular and molecular biological approaches that study eukaryotic gene regulation have led to separate models which describe structure and mechanism with differing precision. Using principles of combinatorial and cooperative interactions inherent in both models, we have extended concepts derived from a “genetic switch” for prokaryotes to a chromatin switch for eukaryotes composed of DNA, transactivators, nucleosomes and the nuclear matrix. We present a consensus model for gene regulation that uses a simple Monte Carlo method for simulating condensation and extension of chromatin. Such a chromatin switch can be modulated by known biochemical and molecular modifications, and the transactivator binding sites or enhancers within DNA domains can be organized into a hierarchy to control cell cycling and differentiation.

© 1996 Academic Press Limited

Introduction

Today in the study of eukaryotic gene regulation we are much like the blind men studying the elephant. Data is being accumulated at an enormous rate, but none of the individual scientific disciplines can see the whole. As suggested by Maddox (1993, 1994) perhaps we can begin to assemble all the viewpoints into a coherent whole by rereading the literature (be it from molecular, cellular or development biology) reflecting on common themes across disciplines, aiming to quantitate models by simple calculations using basic principles of mass action and equilibrium.

We present consensus models for eukaryotic gene regulation derived from emerging cellular and molecular models in Fig. 1(a) (Bodnar, 1988; Cook, 1991; Felsenfeld, 1992; Frankel & Kim, 1991; Garrard, 1990; Lewin, 1990; Lin *et al.*, 1990; Pugh & Tjian, 1990; van Holde, 1989; Villarreal, 1991). Supporting data from cellular biology has defined structures very well down to the limits of what can be seen by microscopy. Further refinements using

electron microscopy and cell fractionation require destruction of the cell producing problems of interpretation. On the other hand molecular biology and biochemistry have defined many of the chemical interactions associated with cell function in solution. Current investigations involve injecting and attempting to track the fate of DNA within a single cell or animal embryo, as well as isolating and reconstituting subcellular structures. To interpret the results we must close the gap in our model building between the cellular and molecular viewpoints. We propose the chromatin switch [see Fig. 1(b)] whose operational properties we have explored using chemical principles and a simple Monte Carlo calculation.

In the cell-based model for gene regulation the nucleus has a structural scaffold or matrix that organizes DNA into chromatin and higher order structures to package about one meter of DNA into each human cell. The DNA is stably attached to the nuclear matrix in loops that are on average 50000 base pairs long. Between the matrix attachment regions (MARs) the DNA in inactive genes is condensed by nucleosomes. During gene activation the chromatin structure is disrupted and the DNA is bound to the matrix at additional sites within the

[†] Author to whom correspondence should be addressed.
E-mail: Bodnar@Brass.NADN.Navy.Mil

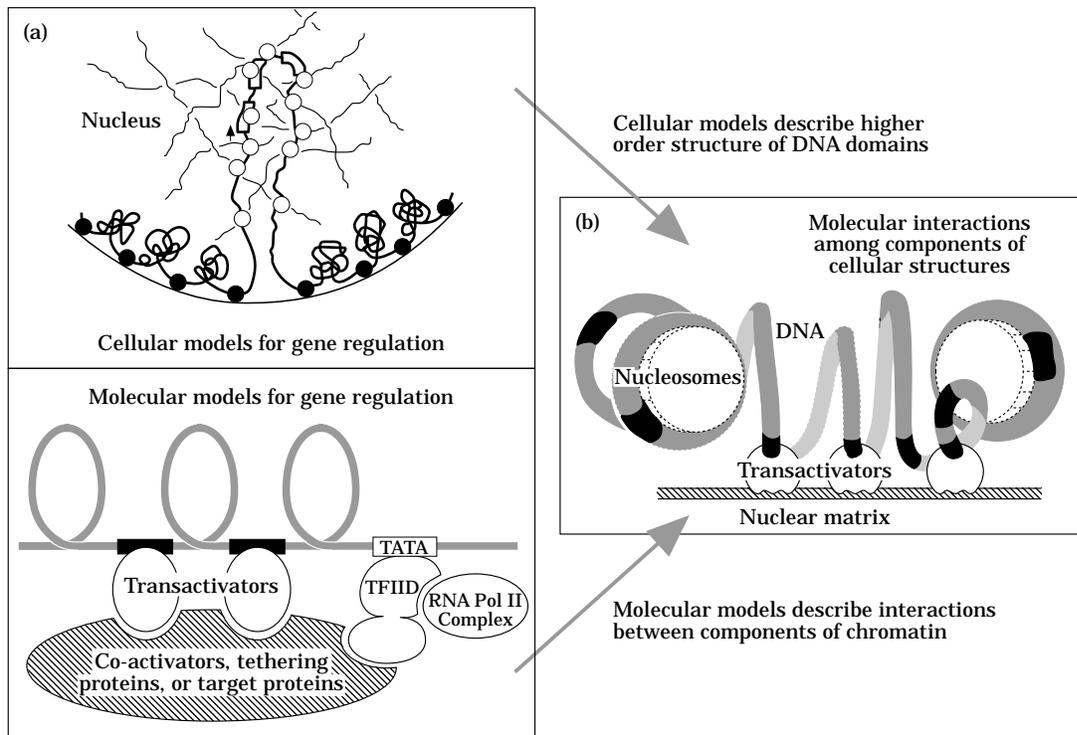


FIG. 1. Cellular, molecular and integrated models for eukaryotic gene regulation. Cellular approaches have led to a consensus cellular model for the organization of DNA domains associated with nuclear matrix structures (a) (Bodnar, 1988; Cook, 1991; Garrard, 1990; van Holde, 1989; Villarreal, 1991). At the same time molecular approaches have led to a consensus model for the dynamics of molecular interactions among the components of chromatin (a) (Felsenfeld, 1992; Frankel & Kim, 1991; Lewin, 1990; Lin *et al.*, 1990; Pugh & Tjian, 1990). We propose that the two models describe the same cellular structures and have integrated them by drawing details about function from the molecular models (b).

loops. An active or extended chromatin conformation (DNase-sensitive regions) allows access to DNA for transactivators and for transcriptional machinery. Methods of cellular analysis show the several different states of chromatin but provide little evidence for the molecular mechanisms by which those structures can be changed.

In the molecular-based model for gene regulation the process of activation depends on specific transactivator-DNA interactions that ultimately allow RNA polymerase to transcribe the DNA of a specific gene. To activate genes transactivators bind cooperatively to specific DNA sequences near a promoter displacing nucleosomes and mediating additional DNA-protein interactions by looping out DNA between binding sites. Such interactions involve co-activators, tethering or target proteins, all of which increase the potential for cooperative interactions among DNA binding sites. Experiments *in vitro* have shown how certain DNA-protein interactions can modulate transcription by a factor of ten to a hundred; however, genes in their cellular environment may be activated by a factor of a thousand or more during cellular differentiation. Thus, molecular processes

describe how cooperative combinations of DNA-protein and protein-protein activate and inactivate genes, but not how that happens in a coordinated manner to several genes within the human genome.

An integrated model must, therefore, describe both cellular and molecular components involved in the process of gene regulation in the nucleus, especially in an environment mediated by signal transduction initiated by neighboring cells through the plasma membrane. To do that we need to find and describe a stable chromatin switching mechanism.

Results and Discussion

A CHROMATIN SWITCH

By examining both the cellular and molecular models for gene activation one can see that the common principle is cooperativity. Molecular models indicate that nucleosomes condense chromatin into higher order structures by cooperative interactions—with the DNA with other nucleosomes and with additional proteins such as histones H1 and H5. Additionally, transactivators can work cooperatively

by binding to each other or to “tethering” proteins. Cellular models indicate that the nuclear matrix acts as a structure to organize DNA through multiple interactions. Although current evidence cannot discriminate whether the nuclear matrix is fibrillar, granular, or even merely an aggregate of transactivators (or maybe a combination of all of these), whatever their form the solid state structures provide cooperativity by assembling chromatin components to function more effectively. We believe the key to switching eukaryotic genes lies in having two independent cooperative systems competing with each other—nucleosomes to inactivate chromatin and transactivators plus the nuclear matrix to activate chromatin.

A switch has two stable states—off and on. To build a chromatin switch one uses the structural components from the cell including DNA, transactivator proteins, nucleosomes and the nuclear matrix. To operate the switch one uses combinatorial and cooperative interactions of DNA with proteins as well as the structures they form to condense and block access to chromatin, or to extend it and make it accessible to transcription factors. In particular, nucleosomes bind DNA non-specifically but can assemble cooperatively into higher order structures to stabilize condensed (inactive) chromatin. On the other hand, the nuclear matrix can serve as a solid state catalyst to mediate cooperativity in interactions between transactivator proteins and DNA thus stabilizing extended (active) chromatin. We tested the operation of a switch by simulating bistable chromatin (condensed and extended) using known binding constants. A Monte Carlo method was used for determining the fate of chromatin as nucleosomes and transactivators competed to stabilize each of the two states (see legend in Fig. 2).

We assumed the following: (1) Transactivator proteins bind DNA specifically at enhancer sites. (2) Transactivators also bind directly or indirectly to the nuclear matrix. (3) Nuclear matrix is always, at least minimally, associated with chromatin. (4) Nuclear matrix is a granular or fibrillar structure, or an aggregate of tethering, target and co-activator proteins, with sufficient mass to act as a solid state catalyst.

In our first attempt to simulate a chromatin switch we accounted for only cooperativity in the transactivator/nuclear matrix system. We started with eukaryotic DNA in a stable condensed chromatin structure and increased the effective transactivator concentration as would occur by new synthesis, nuclear transport or activation through phosphorylation or ligand binding. Transactivators would be

able to bind only DNA sites accessible within the chromatin structure (Becker, 1994). Those sites would be adjacent to rare matrix attachment sites present in fully condensed DNA [see assumption (3) above]. We calculated the probabilities that transactivator would bind and secure each DNA site to the nuclear matrix [see legend in Fig. 2 and assumption (2)]. As in Ptashne’s analysis of prokaryotic DNA-protein interactions (Ptashne, 1986) we added a cooperativity factor to the reaction based on a higher probability of transactivator binding adjacent sites [see assumption (4)].

We generated a sigmoidal curve (not shown) and the slope occurred over a wide range of transactivator concentration similar to that seen in Ptashne’s analysis of prokaryotic DNA. This implied that the change from condensed to extended chromatin went through several intermediate forms suggesting a switch, but an inefficient one.

Also, this stimulation did not reflect the continual competition between transactivators and nucleosomes to bind DNA and change its structure that occurs in eukaryotes. It must be considered that once nucleosomes dissociate they can rebind DNA, and nucleosomal structure could inhibit binding of even high affinity transactivators (Lee & Archer, 1994). In order for chromatin to be extended increases in transactivator binding, thus cooperativity, must reach a critical point at which nucleosomal structure is disrupted. To incorporate the competition we needed to add the cooperativity inherent among nucleosomes. We proposed that if the number of transactivators binding the DNA exceeded a threshold value, then the cooperative interactions of nucleosomes would be disrupted.

In the next simulation we used a threshold value for the cooperativity factor in the calculations (Fig. 2). The sigmoidal curve for extending eukaryotic chromatin became nearly vertical implying that chromatin could be stable in either its condensed or extended form with no intermediate conformation. An effective switch for long segments of DNA was formed.

Thus, chromatin switches from one to another state, but what keeps the switch from continually flipping on and off when the transactivator concentration is near the threshold? We recalculated the binding curve, this time starting from fully extended DNA by decreasing the transactivator concentration. Transactivator cooperativity, greatest in extended chromatin, prevents condensation by nucleosomes until the transactivator concentration has decreased to a new threshold value. Then a new positive feedback loop is set up of increasing nucleosome

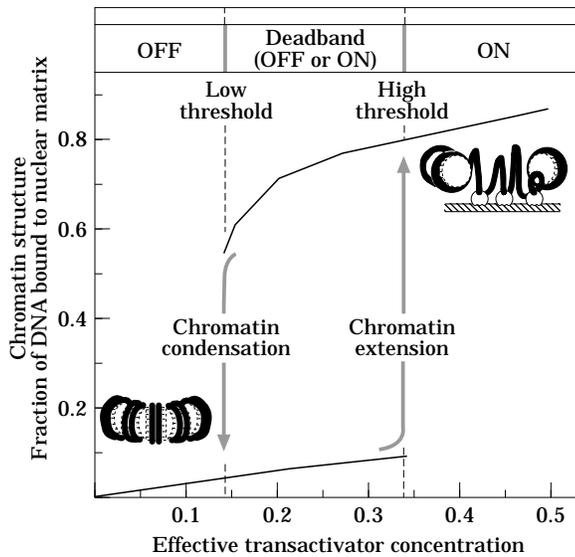


FIG. 2. Simulation of a chromatin switch. A computer model was used to calculate binding curves in a DNA-nucleosome-transactivator-nuclear matrix system. Two separate sigmoidal curves representing chromatin structure were produced by calculating the effects of increasing then decreasing transactivator concentration. In each case chromatin structure was maintained until a threshold value was reached (see Method below) at which point cooperative binding of one set of components successfully competed with the cooperativity of the other switching between stable chromatin states—condensed (OFF) and extended (ON). In the deadband between thresholds either condensed or extended chromatin was stable dependent on the previous equilibrium state.

Method: The Monte Carlo method can simulate nonlinear systems to define equilibria by rates of competing processes. Changes in state for individual sites are calculated in small time steps using a probability of changing state proportional to the ratio of the on- and off-rates, and recalculated until equilibrium is reached. Multiple sites on a DNA segment could be in one of three states: unbound, bound by free transactivator, and bound by nuclear matrix-bound transactivator. All calculations assumed an on- and off-rate of transactivators with the nuclear matrix and an on- and off-rate of transactivators (both free and matrix-bound) with DNA using values consistent with the literature. For neighboring sites we assumed a cooperativity factor (CF) where a matrix-bound site increased on-rates of nearest sites by CF, second nearest sites by CF/2, third nearest sites by CF/4 and no cooperativity beyond that. Our initial calculations, as for Ptashne (1989), assumed constant values of CF. Successful calculations of the chromatin switch assumed that CF varied with chromatin structure: CF = 5 if fully condensed, CF = 80 if fully extended and CF increased linearly from five to 80 as the chromatin changed from condensed to extended.

cooperativity and decreasing transactivator cooperativity. This value was lower than that required for extension. The two binding curves plotted together created a deadband with a low and a high threshold (Fig. 2). With that a chromatin switch could still be flipped on by increasing transactivator concentration to the high threshold, but it would remain on as long as the concentration remained within the deadband above the low threshold. Thus, a continuously

changing transactivator concentration is converted into a bistable chromatin switch.

Our calculation of chromatin conformation vs. transactivator concentration indicated that cellular structure provides a framework for cooperative molecular functions in chromatin with characteristics inherent in mechanical switches (or electronic logic elements). In the switching process chromatin is either condensed into higher order structures or extended along the nuclear matrix. In our simulation we used only one, but a series of different transactivators could act in combination to extend chromatin dependent on the distribution or clustering of their binding sites along the DNA. Regions of chromatin would respond differently to the same transactivator according to whether they were near or far from the threshold for switching. If distant regions of DNA were near the threshold they could be activated coordinately with one transactivator. Networks of chromatin switching within a nucleus can work by mechanisms similar to gene switching in a test tube, yet be many orders of magnitude more effective because the molecular interactions are cooperative (proteins binding to DNA) and the cellular interactions are competitive (extension and condensation).

MODULATING THE OPERATION OF THE CHROMATIN SWITCH

Many different biochemical modifications have been reported that modulate gene expression. We suggest that each one affects the thresholds for competing cooperative interactions in the chromatin switch. The following four examples show how a switch would be biased by the make-up of DNA sequence or by biochemical changes. First, certain DNA sequences such as bent or Z-DNA form secondary structures that disrupt formation of higher order chromatin (van Holde & Zlatanova, 1994). Transactivators that bind and stabilize DNA structure open it to additional DNA binding proteins associated with gene activation. Thus, a bias is present in a specific DNA sequence for transactivator interactions. Second, methylation of DNA throughout a domain would block some transactivator binding sites (Chomet, 1991) thus decreasing cooperative interactions in that domain. The threshold for switching that chromatin on would not be reached even by high concentrations of transactivator. Third, if an anti-oncoprotein binds a transactivator already bound to DNA, it blocks transactivator activity (i.e. Rb and myc; Adnane & Robbins, 1995) by inhibiting nuclear matrix association. Without the cooperative effect of transactivators DNA could not be extended and made accessible to transcription machinery.

Fourth, histone H1 binding to chromatin would increase the threshold necessary to disrupt nucleosome cooperativity, biasing the chromatin switch toward off. H1 phosphorylation increases that cooperativity most during G2 phase of the cell cycle, when chromatin becomes highly condensed in preparation for mitosis (Roberge *et al.*, 1990).

Rather than present more examples we invite the reader to examine known modifications to DNA, transactivators, nucleosomes or the nuclear matrix resulting in changes in gene expression. We suggest that each can be explained by its effect on the chromatin switch according to the following rule: modifications that increase nucleosome cooperativity or decrease transactivator cooperativity will bias the chromatin switch toward off, and modifications that decrease nucleosome cooperativity or increase transactivator cooperativity will bias the chromatin switch toward on.

ENHANCERS AS CHROMATIN SWITCHES

One defines the location of a chromatin switch by the nucleotide sequence of DNA. The effectiveness of a given DNA sequence as a switch depends on the number of protein binding sites it contains, the affinity of the binding sites, and the bias of the DNA toward forming or disrupting chromatin. Enhancers are short DNA sequences known to increase gene expression by virtue of their protein binding sites and structure. Some genes will be readily extended and turned on by action at a single enhancer, while others may require a combination of enhancer sites to be bound before the threshold for displacing chromatin structure or switching can be reached. We, therefore, define an enhancer as any contiguous DNA sequence that can be bound by transactivators (or that preferentially binds or excludes nucleosomes) to switch chromatin.

By integrating cellular and molecular models for gene expression we propose that MARs become the primary enhancers working at short or very long distances from promoters (Boulikas, 1993; Forrester *et al.*, 1994). Other enhancers have restrictions on their orientation and in the accessibility of the DNA to protein binding such as extension sequences (EXTs; Bodnar, 1988), locus control regions (LCRs; Reitman *et al.*, 1993), upstream control elements (UCEs; Lewin, 1990), and the more restricted basal promoter elements (PROMs; Lewin, 1990). A MAR, for example, usually has multiple closely-spaced transactivator binding sites (about one each turn of the helix) and a DNA structure that disrupts chromatin assembly. This means that MAR-associated genes can be switched on whenever MAR and

PROM transactivators are present. As a consequence it may be easier to reach the threshold for turning on the promoters neighboring these MAR-activated genes. To enhance condensation of chromatin structure, inhibitory factors may bind enhancer DNA or transactivators and not the matrix (see anti-oncoproteins above). This would block extension and allow chromatin to refold. Thus, enhancer sites have the potential to function as chromatin switches turning genes on and off.

DNA replication must be considered in the extension and refolding of chromatin since replication temporarily strips off the nucleosomes (Cusick *et al.*, 1989) and perhaps the transactivators at the DNA fork. Each S phase the short segment of newly replicated DNA directly behind the replication forks would be exposed to re-equilibrate with nucleosomes and transactivators. Biochemical modifications and changes in transactivator concentration that occurred in the last cell cycle, which were insufficient to overcome the stability produced within the deadband (Fig. 2), have this limited opportunity to flip the switch off or on. Thus, a chromatin switch found within the deadband has two equilibrium states and can be flipped kinetically during DNA replication dependent on the neighboring chromatin structure and changes in transactivator concentrations during the entire last cell cycle. Note that parent and progeny DNAs may have different patterns of nucleosome and transactivator binding and thus different fates after mitosis. We are already aware that many changes in cell structure occur in conjunction with successive DNA replication and cell division. We suggest that the reason is DNA replication-triggered chromatin switching.

BUILDING CHROMATIN SWITCHING NETWORKS

After examining the literature on regulation of cellular and viral genes we noted two types of chromatin switches which appear to have evolved to regulate genes required for cell growth and differentiation. A flipflop switch would be turned off and on repeatedly by extracellular or other signals. A commitment switch would respond once or constitutively to such signals. Together these switches would form genetic networks.

A flipflop switch would contain an UCE within a few thousand base pairs of a MAR already bound to the nuclear matrix by MAR-binding proteins. That region of DNA would be bound by nucleosomes but have no solenoid structures. When present and activated, UCE-binding transactivators could displace the nucleosomes providing access to the PROM element by a complete set of basal transcription

factors. An example of this process involving the heatshock promoter has been proposed to be ATP-dependent (Tsukiyama *et al.*, 1994). Such a chromatin switch might be turned off or on by controlling one transactivator.

A transactivator for a flipflop switch might be controlled in several ways. Synthesis of a transactivator protein could be paired with constitutive degradation (e.g. by the ubiquitin-protease pathway). If the protein is not bound at its cognitive site, it is destroyed. At each round of DNA synthesis the UCE site might be rebound by nucleosomes until new synthesis of the transactivator occurred. Alternately a constitutively synthesized transactivator protein could be activated by signal transduction. Two mechanisms include nuclear localization of the protein and phosphorylation to induce DNA binding activity. Also controlled by signal transduction, an inhibitor protein could bind and block transactivator. For example, the retinoblastoma protein inhibits a putative flipflop switch for synthesis of DNA replication factors by binding the transactivator protein E2F (Hiebert, 1993).

A commitment switch is located in a long DNA domain (50–200 kb pairs) and includes a combination of enhancers both clustered near a MAR and spread throughout. Immediate access to the DNA is provided by the MAR enhancers that anchor DNA onto the nuclear matrix. Multiple LCR and EXT enhancers are located both proximal and distal to the MAR. Transactivators for those enhancers are not readily degraded nor negatively regulated by their own synthesis. They accumulate and when they are activated (e.g. by removal of inhibitor) they may not be sufficient to switch the domain to on if their concentration is within the deadband. However, during DNA replication their concentration can compete with nucleosome assembly at the DNA fork to switch chromatin structure to on at long distances from a MAR. Once displaced nucleosome condensation can no longer compete with combinations of MAR, LCR and EXT transactivators, the UCE and PROM enhancers within the region are extended and committed to expression.

The genome of Adenovirus 2 (AD2) is an example of a genetic network containing chromatin switches fitting the descriptions above for flipflop and commitment. There is currently enough experimental data to characterize how this genetic network programs the lytic cycle of the virus in an infected host cell (see, for example, Berk, 1986; Bodnar, 1988 and references therein; Nevins, 1991; Moran, 1993). The AD2 genome is a linear 36 kb long, double-stranded DNA which enters the infected cell nucleus

with a viral transactivator called Terminal Protein attached to its 5' ends. The genome encodes three sets of genes categorized by their time of expression after infection of the host cell. The following summary outlines the activity of the chromatin switches in AD2 DNA.

Immediate early. Terminal Protein attaches the ends of the viral DNA to the nuclear matrix thereby mimicking a bound MAR in cellular DNA (Bodnar *et al.*, 1989a). Immediate early genes E1A and E1B have promoters only a few hundred base pairs from one end of the DNA (reviewed in Berk, 1986), and bent AD2 DNA sequences nearby allow cellular transactivators access to E1A and E1B enhancers (UCEs). The flipflop switch in immediate early viral chromatin switch is activated and cellular RNA polymerase transcribes the genes.

Early. More flipflop switches for turning on viral early genes (E2, E3 and E4) are activated directly or indirectly by the E1A immediate early viral proteins (Berk, 1986; Hardy *et al.*, 1989; Nevins, 1991). These same viral proteins also transactivate cellular gene promoters. Products of the early viral genes include proteins that regulate replication of AD2 DNA (i.e. Terminal Protein and viral DNA Polymerase).

Late. Viral late gene expression shows how a commitment switch works. The single AD2 late promoter is almost six thousand base pairs from the AD2 DNA left end, and its activation depends on a combination of enhancers binding both viral and cellular transactivators (Bodnar, 1988 and references therein). Until viral DNA replication takes place the DNA surrounding the late promoter can bind some but not all of the transactivators required to extend and constitutively activate the viral late gene promoter. Once committed high levels of late gene transcription produce viral capsid proteins for assembly into virion particles to complete the AD2 lytic cycle.

Other examples of commitment switches and genetic networks can be found in data derived from studies on muscle differentiation and more recently from neurogenesis and hematopoiesis (Lee *et al.*, 1995, and references therein). In these examples the transactivators involved are basic helix-loop-helix (bHLH) proteins that form dimers as well as binding DNA at LCRs or E boxes (CANNTG). A potentially active gene network requires binding of hetero- or homo-dimeric proteins allowing one to build a series of inter-related networks of gene expression. Regulation is accomplished by expression of specific inhibitors that affect the dimerization of transactivators and by inactivation of inhibitors through signal

transduction. Thus, gene networks can be set up and activated in specific cell types in a defined progression (Bodnar *et al.*, 1989b).

PERSPECTIVES

We have presented a model for gene regulation that uses a simple Monte Carlo method for simulating condensation and extension of chromatin. This simple biochemical process is dependent upon a complex arrangement of DNA sequence that forms a self-extracting program. A chromatin switch is an expansion of prokaryotic genetic switching to the eukaryotic nucleus, and it can be applied at both the molecular and cellular levels. The switch can be modulated by known biochemical and molecular modifications to DNA, nucleosomes, transactivators, and the nuclear matrix. The transactivator binding sites or enhancers within DNA domains can be organized into a hierarchy to control chromatin switching for cell cycling and differentiation.

Bridging the gap between cellular and molecular models is only the beginning of a more comprehensive model for development. A cascade of switches can respond to a combination of internal and external control of transactivators. Also, if the concentration curve for transactivator is in space rather than time, it becomes a morphogen gradient in a growing embryo. Thus, the logic of developmental gene switching resembles a digital computer program and simple embryogenesis can be modeled (Bodnar, submitted).

We thank Phillip Moen, Paul Galgano, Charles Rowell, and Luis Villarreal for helpful discussions. Support for this project was funded in part by the Naval Academy Research Council.

REFERENCES

- ADNANE, J. & ROBBINS, P. D. (1995). The retinoblastoma susceptibility gene product regulates Myc-mediated transcription. *Oncogene* **10**, 381–387.
- BECKER, P. (1994). The establishment of active promoters in chromatin. *BioEssays* **16**, 541–547.
- BERK, A. J. (1986). Adenovirus promoters and E1A transactivation. *Ann. Rev. Genet.* **20**, 45–79.
- BODNAR, J. W. (1988). A domain model for eukaryotic DNA organization: A molecular basis for cell differentiation and chromosome evolution. *J. theor. Biol.* **132**, 479–507.
- BODNAR, J. W., HANSON, P. I., POLVINO-BODNAR, M., ZEMPSKY, W. & WARD, D. C. (1989a). The Terminal Regions of Adenovirus and Minute Virus of Mice DNAs are Preferentially Associated with the Nuclear Matrix in Infected Cells. *J. Virol.* **63**, 4344–4353.
- BODNAR, J. W., JONES, G. J. & ELLIS, C. H., JR. (1989b). The domain model for eukaryotic DNA organization 2: A molecular basis for constraints on development and evolution. *J. theor. Biol.* **137**, 281–320.
- BOULIKAS, T. (1993). Homeodomain protein binding sites, inverted repeats, and nuclear matrix attachment regions along the human beta-globin gene complex. *J. Cell. Biochem.* **52**, 23–36.
- CHOMET, P. S. (1991). Cytosine methylation in gene-silencing mechanisms. *Curr. Opin. Cell Biol.* **3**, 438–443.
- COOK, P. R. (1991). The nucleoskeleton and topology of replication. *Cell* **66**, 627–635.
- CUSICK, M., WASSARMAN, P. & DEPAMPHILIS, M. (1989). Application of nucleases to visualizing chromatin organization at replication forks. *Meth. Enzymol.* **170**, 290–316.
- FELSENFELD, G. (1992). Chromatin as an essential part of the transcriptional mechanism. *Nature* **355**, 219–224.
- FORRESTER, W., VAN GENDEREN, C., JENUWEIN, T. & GROSSCHIEDT, R. (1994). Dependence of enhancer-mediated transcription of the immunoglobulin μ gene on nuclear matrix attachment regions. *Science* **265**, 1221–1225.
- FRANKEL, A. & KIM, P. (1991). Modular structure of transcription factors: Implications for gene regulation. *Cell* **65**, 717–719.
- GARRARD, W. T. (1990). *Chromosomal Loop Organization in Eukaryotic Genomes*. pp. 163–175. Berlin: Springer-Verlag.
- HARDY, S., ENGEL, D. A. & SHENK, T. (1989). An Adenovirus early region 4 gene product is required for induction of the infection-specific form of cellular E2F activity. *Genes Dev.* **3**, 1062–1074.
- HIEBERT, S. W. (1993). Regions of the retinoblastoma gene product required for its interaction with the E2F transcription factor are necessary for E2 promoter repression and pRb-mediated growth suppression. *Mol. Cell. Biol.* **13**, 3384–3391.
- LEE, H. & ARCHER, T. (1994). Nucleosome-mediated disruption of transcription factor-chromatin initiation complexes at the mouse mammary tumor virus long terminal repeat in vivo. *Mol. Cell. Biol.* **14**, 32–41.
- LEE, J., HOLLENBERG, S., SNIDER, L., TURNER, D., LIPNICK, N. & WEINTRAUB, H. (1995). Conversion of *Xenopus* ectoderm into neurons by neuroD, a basic helix-loop-helix protein. *Science* **268**, 836–843.
- LEWIN, B. (1990). Commitment and activation at pol II promoters: A tail of protein-protein interactions. *Cell* **61**, 1161–1164.
- LIN, Y.-S., CAREY, M., PTASHNE, M. & GREEN, M. R. (1990). How different eukaryotic transcriptional activators can cooperate promiscuously. *Nature* **345**, 359–361.
- MADDOX, J. (1993). The dark side of molecular biology. *Nature* **363**, 13.
- MADDOX, J. (1994). Origin of life by careful reading. *Nature* **367**, 409.
- MORAN, E. (1993). DNA tumor virus transforming proteins and the cell cycle. *Curr. Opin. Genet. Dev.* **3**, 63–70.
- NEVINS, J. R. (1991). Transcriptional activation by viral regulatory proteins. *TIBS* **16**, 435–439.
- PTASHNE, M. (1986). *A Genetic Switch: Gene Control and Phage Lambda*. Cambridge, MA: Cell Press and Blackwell Scientific Publications.
- PUGH, B. F. & TJIAN, R. (1990). Mechanism of transcriptional activation by sp1: Evidence for coactivators. *Cell* **61**, 1187–1197.
- REITMAN, M., LEE, E., WESTPHAL, H. & FELSENFELD, G. (1993). An enhancer/locus control region is not sufficient to open chromatin. *Mol. Cell. Biol.* **13**, 3990–3998.
- ROBERGE, M., TH'NG, J., HAMAGUCHI, J. & BRADBURY, E. (1990). The topoisomerase II inhibitor VM-26 induces marked changes in histone H1 kinase activity, histones H1 and H3 phosphorylation and chromosome condensation in G2 phase and mitotic BHK cells. *J. Cell Biol.* **111**, 1753–1762.
- TSUKIYAMA, T., BECKER, P. & WU, C. (1994). ATP-dependent nucleosome disruption at a heat shock promoter mediated by binding of GAGA transcription factor. *Nature* **367**, 525–532.
- VAN HOLDE, K. & ZLATANOVA, J. (1994). Unusual DNA structures, chromatin and transcription. *BioEssays* **16**, 59–68.
- VAN HOLDE, K. E. (1989). *Chromatin*. New York: Springer-Verlag.
- VILLARREAL, L. P. (1991). Relationship of eukaryotic DNA replication to committed gene expression: General theory for gene control. *Microbiol. Rev.* **55**, 512–542.