REVIEW: TRANSCRIPTION

Going the Distance: A Current View of Enhancer Action

Elizabeth M. Blackwood and James T. Kadonaga*

ABSTRACT In eukaryotes, transcription of genes by RNA polymerase II yields messenger RNA intermediates from which protein products are synthesized. Transcriptional enhancers are discrete DNA elements that contain specific sequence motifs with which DNA-binding proteins interact and transmit molecular signals to genes. Here, current models regarding the role of enhancers in the regulation of transcription by RNA polymerase II are presented.

Enhancers were originally identified as cis-acting DNA sequences that increase transcription in a manner that is independent of their orientation and distance relative to the RNA start site (*1*). The eukaryotic genome itself best illustrates the positions from which enhancers can activate transcription. For example, the wing margin enhancer of the *Drosophila cut* locus resides 85 kb upstream of its promoter, whereas the murine immunoglobulin Hμ core enhancer lies within the second intron of the transcription unit. The T cell receptor α-chain gene enhancer resides up to 69 kb downstream of the promoter. Moreover, in the phenomenon of transvection, chromosome pairing allows an active enhancer on one chromosome to activate transcription from an allelic promoter on the other chromosome (*2*). In spite of tremendous progress in our understanding of transcriptional regulation, there remain many questions regarding the function of enhancers. In this review, we present current views on the mechanisms by which enhancers regulate gene expression.

Enhancers, Promoters, and Boundary Elements. Transcriptional control regions often contain multiple, autonomous enhancer modules that vary from about 50 bp to 1.5 kbp in size. Each of these modules appears to be designed to perform a specific function, such as the activation of its cognate gene in a specific cell type or at a particular stage in development. A gene might thus contain many such enhancer modules, each of which contributes, in a somewhat cumulative manner, to the overall spatial and temporal regulation of the gene. For the purposes of this review, we will refer to an independent enhancer module as an enhancer.

Enhancers are also distinct from other transcriptional elements such as core promoters and boundary (also termed insulator) elements. Core promoters comprise DNA sequence motifs within −40 to +40 nucleotides relative to the RNA start site [such as the TATA box, TFIIB recognition element (BRE), initiator (Inr), and the downstream promoter element (DPE)] that, in the appropriate combinations, are sufficient to direct transcription initiation by the basal RNA polymerase II transcriptional machinery (*3*). Immediately upstream of the core promoter (from about −50 to −200 bp relative to the RNA start site), there are typically multiple recognition sites for a subgroup of sequence-specific DNA-binding transcription factors, which include Sp1, CTF (CCAAT-binding transcription factor; also called nuclear factor–I, or NF-I), and CBF (CCAAT-box–binding factor; also called nuclear factor–Y, or NF-Y). Collectively, we will refer to the core promoter and the promoter-proximal region as the promoter.

Boundary elements are DNA segments (from about 0.5 to 3 kbp) that are thought to function as transcriptionally neutral DNA elements that block, or insulate, the spreading of the influence of either positive DNA elements (such as enhancers) or negative DNA elements (such as silencers, or heterochromatin-like repressive effects) (*4*, *5*). A schematic view of enhancers, promoters, and boundary elements is depicted in Fig. 1.

Enhancer-Promoter Selectivity. Cognate enhancer-promoter interactions are important not only when an enhancer must select the correct promoter over a large distance, but also when an enhancer must activate only one of multiple promoters in its immediate vicinity. Two possible mechanisms by which enhancer-promoter selectivity might be achieved are shown (Fig. 2). First, there could be specific interactions between enhancer-binding proteins and factors that interact with the promoter (Fig. 2A). Second, transcriptional boundary elements could be used to block undesired enhancer-promoter interactions (Fig. 2B). In fact, both of these mechanisms could be used.

The autoregulatory element 1 (AE1) enhancer in *Drosophila* provides an example of preferential interactions between an enhancer and a core promoter. In its natural context, this enhancer is equidistant from both the *Sex combs reduced* (*Scr*) and *fushi tarazu* (*ftz*) promoters (as in Fig. 2A), but it selectively activates *ftz* expression. The *Scr* and *ftz* genes differ in their core promoter elements. The *ftz* promoter contains a TATA box, whereas the *Scr* promoter lacks a TATA box but contains initiator and downstream promoter element sequences. In synthetic test constructions, the

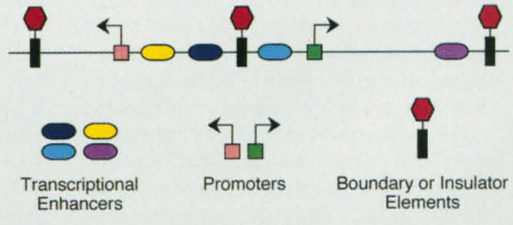

Fig. 1. DNA elements that affect transcription by RNA polymerase II. The horizontal line depicts a segment of the genome. Promoters (arrows) comprise core promoter motifs (such as the TATA box, TFIIB recognition element, initiator, and downstream promoter element) that specify the site of transcription initiation as well as recognition sites for sequence-specific DNA-binding activators (such as Sp1 and CCAAT-binding transcription factor) that reside in the vicinity (within about 150 nucleotides) of the RNA start site. Transcriptional enhancers (ovals) each contain arrays of recognition sites for sequence-specific DNA-binding factors (there are hundreds of different types of such regulatory factors in cells). The different colors of the enhancers represent their different biological functions. For example, one enhancer might activate its cognate gene in the brain, whereas another enhancer might activate the same gene in the liver. When located between an enhancer and a promoter, boundary (also termed insulator) elements (hexagons) impair the ability of the enhancer to activate transcription from the promoter. It is thought that boundary elements might function to demarcate regulatory domains.

Department of Biology and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093–347, USA.

*To whom correspondence should be addressed. E-mail: jkadonaga@ucsd.edu

AE1 enhancer can activate transcription from a TATA-less promoter in the absence of a competing TATA-containing promoter. In the presence of both TATA-containing and TATA-less promoters, however, the AE1 enhancer preferentially activates transcription from the TATA-containing promoter (*6*). Thus, components of the promoter are important for productive and specific enhancer-promoter interactions (*7*).

The properties of a boundary element are exemplified by sequences from the *Drosophila gypsy* retrotransposon, which acts as an insertional mutagen. This *gypsy* boundary or insulator element comprises multiple binding sites for the Suppressor of Hairy-wing [Su(Hw)] protein (*4*). When inserted between an enhancer and a downstream promoter, the *gypsy* insulator blocks the ability of the enhancer to activate transcription from the promoter. The *gypsy* insulator does not, however, appear to affect the intrinsic activity of the enhancer, because the enhancer remains active in the upstream direction (as in Fig. 2B) (*8*). In addition, when a transgene is flanked by *gypsy* boundary elements, the resulting construction is insulated from activating (due to integration near an enhancer) or repressing (due to integration near silencers or heterochromatin) position-effects. Thus, the *gypsy* insulator appears to function as a neutral boundary element that blocks the spreading of both positive and negative transcriptional effects (*9*).

Locus Control Regions and Transcriptional Competence. Early studies of the regulation of the β-globin locus suggested that the pathway leading to transcriptional activation involves the initial conversion of the inactive locus to a "preactivated" (competent) state [as detected by an increase in sensitivity to digestion by deoxyribonuclease I (DNase I)] from which the genes are subsequently activated (*10*). This multistep model in which transcriptional competence precedes transcriptional activation remains consistent with the current data (Fig. 3). Notably, the analysis of the transcriptional elements that regulate the β-globin locus led to the identification of a locus control region (LCR) (*11*). The human β-globin LCR corresponds to several (four or five) DNase I–hypersensitive sites that are distributed throughout a 15-kbp region that is located upstream of the genes. Like enhancers, the LCR contains multiple binding sites for sequence-specific transcriptional activators, but unlike typical enhancers, the LCR first acts at a stage in development before the genes are transcribed and renders all five genes in the locus competent but transcriptionally inactive. Once in this competent state, each of the genes is further regulated by stage-specific enhancers or repressors (*12*).

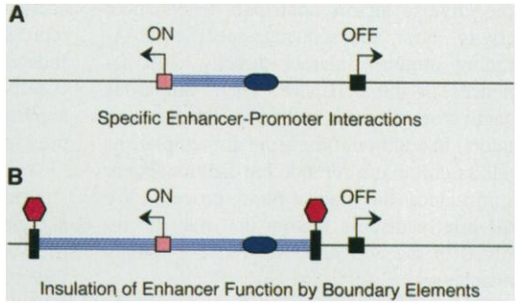

Fig. 2. Two possible mechanisms for cognate enhancer-promoter interactions. Enhancers (ovals), promoters (arrows), and boundary elements (hexagons) are as in Fig. 1. (**A**) Specific protein-protein interactions between regulatory factors that are bound at the enhancer and at the promoter could establish activation of the correct promoter. These interactions may also require the participation of coactivator molecules that act as intermediary adapters. (**B**) Boundary elements could define the range of enhancer activity and prevent interactions with inappropriate promoters.

What is the nature of the transcriptionally competent state? Studies of the chicken β-globin locus have revealed that the establishment of the competent state correlates with an increase in histone acetylation and general sensitivity to digestion by DNase I (*13*). These effects are likely to be related, because hyperacetylation of the histones leads to unfolding of the chromatin that should facilitate the general accessibility of factors (for example, DNase I or transcription factors) to the DNA (*14*).

"On or Off" Versus Progressive Models for Enhancer Function. The analysis of transcriptional activation by enhancers on a single-cell basis (by using immunofluorescence or fluorescence-activated cell sorting to determine reporter gene activity) has led to the observation that enhancers can increase the probability that a gene will be transcribed in any particular cell while not affecting the level of transcription in the cells in which the gene is active (Fig. 4A) (*15*). This on or off response is in contrast to a uniform and progressive response of a gene to enhancer activity (Fig. 4B), which has also been observed (*16*). Notably, in experiments where transcriptional activity is measured with populations of cells (such as in standard chloramphenicol acetyltransferase reporter assays), it is not possible to discern whether enhancers are functioning by "on or off" or progressive mechanisms.

What might be the basis for the on or off phenomenon? Enhancers could affect only the competency of the chromatin template, and promoter strength could determine the subsequent level of transcription. Alternatively, enhancers could promote the assembly of productive and stable transcription complexes in a repressive chromatin environment. If such a complex is formed, then it could overcome the chromatin-mediated transcriptional repression, but if the assembly of the transcription complex is incomplete, then the gene would remain in a repressed state.

Consistent with both views is the observation that in the absence of an enhancer, there is a small fraction of cells in which there are high levels of transcription. In such cells, rarely occurring irregularities or discontinuities in chromatin structure might permit the spontaneous formation of productive and stable transcription complexes.

Although the on or off mechanism for enhancer function has been observed in some experimental systems, the on or off strategy does not afford much regulatory flexibility and thus may not be useful for the transcription of all genes. For example, an extended off period for any one of the many essential genes in a unicellular organism would be lethal.

The Nature of Enhancer Activity. The current data suggest that a composite of mul-

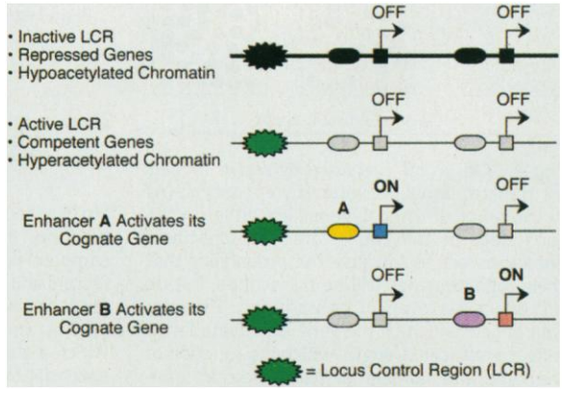

Fig. 3. A model in which transcriptional competence precedes gene activation. In this multistep model for transcriptional activation, the inactive locus becomes transcriptionally competent as the locus control region (LCR) is activated, and subsequent transcription from each promoter (arrows) is directed by its cognate enhancer (ovals). Inactive DNA elements are depicted in black or gray, whereas the active forms of the DNA elements are shown in color. LCRs and enhancers both contain multiple binding sites for sequence-specific transcriptional activators. Unlike enhancers, however, LCRs act before genes are transcribed and convert an entire locus (which may consist of multiple genes) from a highly repressed state to a transcriptionally competent state.

tiple, diverse factors contribute to enhancer activity. First, the sequence-specific DNA-binding proteins interact directly with sequences in the enhancer. Then, numerous coactivators interact with the DNA-bound factors. In addition, the chromatin template as well as chromatin remodeling factors appear to influence the transcription process. We will briefly discuss factors that may be involved in the activation of transcription by enhancers.

Protein-protein contacts. There are likely to be direct protein-protein contacts between enhancer-associated factors and components of the basal transcription machinery that are formed by DNA looping (*17*). These interactions probably contribute to the formation of a large transcription complex at the promoter from which transcription can be readily initiated and reinitiated.

Covalent modification of proteins. Enzymatic activities in transcription factors and cofactors, such as those that catalyze protein phosphorylation or acetylation, can modify the properties and activities of proteins. For example, the repeated heptapeptide sequences in the COOH-terminal domain of RNA polymerase II are extensively phosphorylated in the early stages of transcription. Also, the acetylation of histones appears to reduce the repressive nature of chromatin (*13*, *14*, *18*). Indeed, many transcriptional activators and coactivators possess histone acetyltransferase activity, whereas some transcriptional corepressors exhibit histone deacetylase activity (*18*). However, histones are not the sole substrates of these acetyltransferases and deacetylases. For example, the p53 tumor suppressor protein and the basal transcription factors TFIIE and TFIIF have been found to be acetylated (*19*). Thus, activators and coactivators can potentially acetylate or deacetylate both histones and transcription factors to regulate the transcription process.

Chromatin structure. The packaging of DNA into chromatin appears to promote long-distance interactions among DNA-bound factors as a consequence of the compaction of the DNA. For example, in biochemical studies, long-distance activation of transcription was found to occur more readily with chromatin templates than with nonchromatin templates (*20*). In addition, enhancers may function, at least in part, to counteract repression by chromatin or chromatin-associated proteins (*20*, *21*).

Nucleosome remodeling. Protein complexes that can alter chromatin structure and increase the mobility of nucleosomes are likely to facilitate the function of transcription factors in the context of the chromatin template. These adenosine 5′-triphosphate (ATP)-utilizing chromatin remodeling factors include SWI-SNF, NURF, RSC, ACF, and CHRAC (*22*). The yeast SWI-SNF complex, for example, appears to be a transcriptional activator in vivo and an ATP-utilizing chromatin remodeling factor in vitro. Some of these chromatin remodeling complexes may act globally to increase the mobility of nucleosomes throughout the genome, whereas other remodeling complexes might be specifically targeted to genes during transcriptional activation.

Superhelical tension. It is possible that enhancer activity might be transmitted via superhelical tension in the DNA (*23*). Under certain conditions, for example, higher levels of transcription can be achieved in vitro with negatively supercoiled DNA templates than with relaxed or linear DNA templates. However, the SV40 enhancer can activate transcription when it is located on a hairpin tail that is attached to a double-stranded circle containing the promoter (*24*). Thus, the enhancer can function when it is topologically unlinked from the promoter. In addition, transcription in yeast is unimpaired by local relaxation of the chromatin template upon cleavage by HO endonuclease (*25*). Thus, transient superhelical tension is not strictly required for transcriptional activation.

Nuclear localization. It has been postulated that enhancers target their cognate promoters to specialized domains of the nucleus containing high local concentrations of transcription factors (*26*). Several observations suggest the importance of nuclear architecture. First, the nuclear matrix appears to be the principal site of active transcription. Second, euchromatic genes reside in nonrandom positions within the interphase nucleus, whereas both telomeres and centromeres are apposed to the nuclear periphery. Third, a relation between nuclear location and transcriptional competence is supported by the finding that insertion of satellite DNA at a euchromatic locus not only directs the localization of the normally euchromatic gene to the "heterochromatic compartment," but also results in position-effect variegation (a form of heterochromatic transcriptional repression).

A Facilitated Tracking Model for Enhancer Function. How might enhancer-binding proteins and their associated coactivators establish a productive interaction with the cognate promoter? Looping between the enhancer and promoter has been an attractive and popular hypothesis (*17*). In theory, tethering of cis elements by an interval of freely mobile DNA can increase the probability of their interaction. As the distance between the enhancer and promoter is lengthened, however, the advantage of the two elements being linked in cis is decreased. Thus, from a theoretical and perhaps naïve standpoint, the likelihood of forming small loops (which might be viewed as short jumps or small steps along the DNA) seems to be higher than the chance of forming large loops.

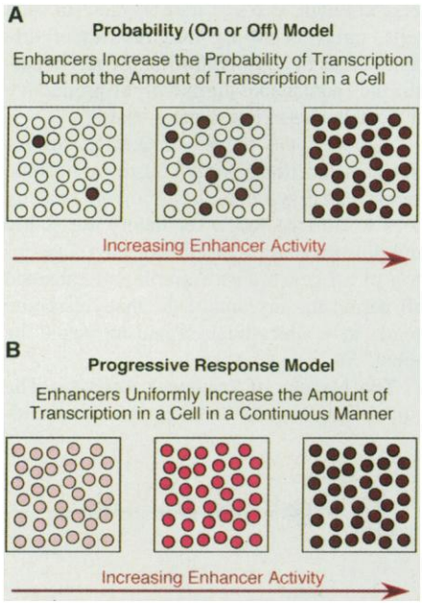

Fig. 4. "On or off" versus progressive models for transcriptional activation by enhancers. (**A**) In the on or off model, genes are either in the "on" state or the "off" state. Transcriptional enhancers act to increase the probability that their cognate genes will be transcribed, but do not affect the levels of transcription. The fraction of cells in which the gene is activated may reflect enhancer strength, which is a function of the type and number of its associated transcription factors. (**B**) In the progressive (or graded) model, genes are uniformly activated by enhancers, and the amount of transcription is proportional to the strength of the enhancer.

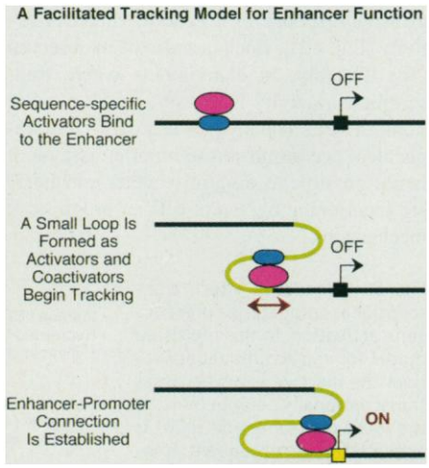

Fig. 5. A facilitated tracking model for enhancer function. In this model, an enhancer-bound complex (large oval) containing DNA-binding factors and coactivators "tracks" via small steps (and perhaps scanning) along the chromatin until it encounters the cognate promoter, at which a stable looped structure is established. Potential changes in the structure of the chromatin template, such as the acetylation of histones by transcriptional coactivators and unfolding of the chromatin fiber, are depicted by the green coloration of the template.

DNA scanning is an alternative mechanism for establishing enhancer-promoter contact. In a simple scanning model, enhancer-binding factors would bind to their recognition sequences and then move continuously along the DNA until they encountered their cognate promoter. A scanning mechanism is, for example, consistent with the enhancer-blocking properties of boundary or insulator elements. On the other hand, a scanning mechanism would not explain how an enhancer could activate transcription from a tailed hairpin that extends outwardly from a double-stranded circle (*24*) or how an enhancer on one chromosome could activate transcription from an allelic promoter on another paired chromosome, as in transvection (*2*).

A "facilitated tracking" mechanism for enhancer function incorporates elements from each of these proposed modes of enhancer action. In this model, an enhancer-bound complex containing DNA-binding factors and coactivators "tracks" via small steps (and perhaps scanning) along the chromatin until it encounters the cognate promoter, at which a stable looped structure is formed (Fig. 5). An important component of the tracking mechanism is the chromatin structure of the template. For example, coactivators such as CBP or p300, which possess histone acetyltransferase activity, may recognize and modify the chromatin substrate and thereby facilitate enhancer-promoter communication and alter a repressive chromatin structure. In addition, ATP-utilizing chromatin remodeling factors might facilitate the interaction of DNA-binding factors with the enhancer as well as the tracking of the proposed factor-coactivator complex along the chromatin template.

This facilitated tracking mechanism is consistent with the broad range of phenomena associated with enhancer function, which include long-distance and orientation-independent transcriptional activation, the action of boundary elements, and transvection. For example, the "small steps" that occur in tracking would be blocked by insulator elements but would enable the transfer of the proposed factor-coactivator complex from one chromosome to another paired chromosome to mediate transvection.

Transcriptional enhancers are both fascinating and biologically important. In the future, there will continue to be many exciting discoveries that will provide new insight into the molecular mechanisms of enhancer action as well as the biological role of enhancers. At present, it is evident that the understanding of these phenomena will require the integration of many diverse cellular processes.

References and Notes

1. For early reviews on transcriptional enhancers, see G. Khoury and P. Gruss, *Cell* **33**, 313 (1983); E. Serfling, M. Jasin, W. Schaffner, *Trends Genet.* **1**, 224 (1985).
2. For a review of transvection, see C.-t. Wu, *J. Cell Biol.* **120**, 587 (1993).
3. For recent reviews of basal transcription by RNA polymerase II, see G. Orphanides, T. Lagrange, D. Reinberg, *Genes Dev.* **10**, 2657 (1996); R. G. Roeder, *Trends Biochem. Sci.* **21**, 327 (1996); C. P. Verrijzer and R. Tjian, *ibid.*, p. 338; S. T. Smale, *Biochim. Biophys. Acta* **1351**, 73 (1997).
4. For reviews of boundary elements, see G. Felsenfeld, *Cell* **86**, 13 (1996); T. I. Gerasimova and V. G. Corces, *Curr. Opin. Genet. Dev.* **6**, 185 (1996); P. K. Geyer, *ibid.* **7**, 242 (1997).
5. For reviews of heterochromatin and position-effect variegation, see G. H. Karpen, *Curr. Opin. Genet. Dev.* **4**, 281 (1994); S. Henikoff, *Genetics* **138**, 1 (1994); S. C. Elgin, *Curr. Opin. Genet. Dev.* **6**, 193 (1996).
6. S. Ohtsuki, M. Levine, H. N. Cai, *Genes Dev.* **12**, 547 (1998).
7. For other examples of enhancer-promoter specificity, see X. Li and M. Noll, *EMBO J.* **13**, 400 (1994); C. Merli, D. E. Bergstrom, J. A. Cygan, R. K. Blackman, *Genes Dev.* **10**, 1260 (1996).
8. See, for example, H. Cai and M. Levine, *Nature* **376**, 533 (1995).
9. For other examples of boundary elements, see R. Kellum and P. Schedl, *Cell* **64**, 941 (1991); *Mol. Cell. Biol.* **12**, 2424 (1992); J. H. Chung, M. Whiteley, G. Felsenfeld, *Cell* **74**, 505 (1993); K. Hagstrom, M. Muller, P. Schedl, *Genes Dev.* **10**, 3202 (1996).
10. H. Weintraub and M. Groudine, *Science* **193**, 848 (1976); J. Stalder, M. Groudine, J. B. Dodgson, J. D. Engel, H. Weintraub, *Cell* **19**, 973 (1980); H. Weintraub, H. Beug, M. Groudine, T. Graf, *ibid.* **28**, 931 (1982).
11. For recent reviews on locus control regions, see S. M. Jane and J. M. Cunningham, *Int. J. Biochem. Cell Biol.* **28**, 1197 (1996); D. I. Martin, S. Fiering, M. Groudine, *Curr. Opin. Genet. Dev.* **6**, 488 (1996); R. Hardison *et al.*, *Gene* **205**, 73 (1997); C. Bonifer, U. Jagle, M. C. Huber, *J. Biol. Chem.* **272**, 26075 (1997); D. Kioussis and R. Festenstein, *Curr. Opin. Genet. Dev.* **7**, 614 (1997).
12. However, the LCR may not necessarily act independently to establish the competent state [M. Reitman, E. Lee, H. Westphal, G. Felsenfeld, *Mol. Cell. Biol.* **13**, 3990 (1993)].
13. T. R. Hebbes, A. L. Clayton, A. W. Thorne, C. Crane-Robinson, *EMBO J.* **13**, 1823 (1994).
14. M. Garcia-Ramirez, C. Rocchini, J. Ausió, *J. Biol. Chem.* **270**, 17923 (1995); see also K. Luger, A. W. Mäder, R. K. Richmond, D. F. Sargent, T. J. Richmond, *Nature* **389**, 251 (1997).
15. See, for example, H. Weintraub, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5819 (1988); M. C. Walters *et al.*, *ibid.* **92**, 7125 (1995); M. C. Walters *et al.*, *Genes Dev.* **10**, 185 (1996).
16. W. Magis, S. Fiering, M. Groudine, D. I. Martin, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13914 (1996); L. G. Guy, R. Kothary, L. Wall, *Nucleic Acids Res.* **25**, 4400 (1997); D. No, T. P. Yao, R. M. Evans, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3346 (1996).
17. J. C. Wang and G. N. Giaever, *Science* **240**, 300 (1988); K. Rippe, P. H. von Hippel, J. Langowski, *Trends Biochem. Sci.* **20**, 500 (1995); M. Ptashne and A. Gann, *Nature* **386**, 569 (1997).
18. For recent reviews of histone acetylation and deacetylation and transcriptional regulation, see J. E. Brownell and C. D. Allis, *Curr. Opin. Genet. Dev.* **6**, 176 (1996); A. P. Wolffe and D. Pruss, *Cell* **84**, 817 (1996); G. A. Hartzog and F. Winston, *Curr. Opin. Genet. Dev.* **7**, 192 (1997); M. J. Pazin and J. T. Kadonaga, *Cell* **89**, 325 (1997); M. Grunstein, *Nature* **389**, 349 (1997); K. Struhl, *Genes Dev.* **12**, 599 (1998).
19. W. Gu and R. G. Roeder, *Cell* **90**, 595 (1997); A. Imhof, X.-J. Yang, V. V. Ogryzko, Y. Nakatani, A. P. Wolffe, H. Ge, *Curr. Biol.* **7**, 689 (1997).
20. P. J. Laybourn and J. T. Kadonaga, *Science* **257**, 1682 (1992); R. T. Kamakaka, M. Bulger, J. T. Kadonaga, *Genes Dev.* **7**, 1779 (1993); M. C. Barton and B. M. Emerson, *ibid.* **8**, 2453 (1994).
21. S. Majumder, M. Miranda, M. L. DePamphilis, *EMBO J.* **12**, 1131 (1993); R. Bagga and B. Emerson, *Genes Dev.* **11**, 629 (1997).
22. For recent reviews of chromatin remodeling complexes, see G. A. Hartzog and F. Winston, *Curr. Opin. Genet. Dev.* **7**, 192 (1997); J. T. Kadonaga, *Cell* **92**, 307 (1998); R. E. Kingston, C. A. Bunker, A. N. Imbalzano, *Genes Dev.* **10**, 905 (1996); T. Krude and S. C. R. Elgin, *Curr. Biol.* **6**, 511 (1996); C. L. Peterson, *Curr. Opin. Genet. Dev.* **6**, 171 (1996); T. Tsukiyama and C. Wu, *ibid.* **7**, 182 (1997); C. Wu, *J. Biol. Chem.* **272**, 28171 (1997); M. J. Pazin and J. T. Kadonaga, *Cell* **88**, 737 (1997).
23. For review, see L. A. Freeman and W. T. Garrard, *Crit. Rev. Eukaryotic Gene Expr.* **2**, 165 (1992).
24. S. E. Plon and J. C. Wang, *Cell* **45**, 575 (1986).
25. C.-P. Liang and W. T. Garrard, *Mol. Cell. Biol.* **17**, 2825 (1997).
26. See, for example, D. A. Jackson and P. R. Cook, *EMBO J.* **4**, 919 (1985); A. K. Csink and S. Henikoff, *Nature* **381**, 529 (1996); E. Milot *et al.*, *Cell* **87**, 105 (1996); A. I. Lamond and W. C. Earnshaw, *Science* **280**, 547 (1998).

27. We thank T. Palmer, J. Tyler, and R. Kobayashi for helpful comments on the manuscript and figures. Research in the laboratory of J.T.K is supported by grants from the National Institutes of Health (GM 41249 and GM 46995) and the National Science Foundation (MCB 9631121).