Title

The transformation of the DNA template in RNA polymerase II transcription: a historical perspective

Journal

Nature Structural & Molecular Biology, 26(9)

ISSN

1545-9993

Author

Kadonaga, James T

Publication Date

2019-09-01

DOI

10.1038/s41594-019-0278-y

Peer reviewed

Nature Structural & Molecular Biology, v. **26**, in press (2019)

The Transformation of the DNA Template in RNA Polymerase II Transcription – a Historical Perspective

James T. Kadonaga

Section of Molecular Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093–0347, USA.

Correspondence should be addressed to J.T.K. (jkadonaga@ucsd.edu).

The discovery of RNA polymerases I, II, and III opened up a new era in gene expression. Here, I provide a personal retrospective account of the transformation of the DNA template, evolving from naked DNA to chromatin, in the biochemical analysis of transcription by RNA polymerase II. These studies have revealed new insights into the mechanisms by which transcription factors function with chromatin to regulate gene expression.

Fortunately science, like that nature to which it belongs, is neither limited by time nor by space. It belongs to the world, and is of no country and of no age. The more we know, the more we feel our ignorance; the more we feel how much remains unknown; and in philosophy, the sentiment of the Macedonian hero can never apply, – there are always new worlds to conquer.
Sir Humphry Davy (1778-1829)

It is difficult not to be inspired by the words of Sir Humphry Davy, whose remarkable scientific contributions include the first isolation and identification of potassium, sodium, calcium, magnesium, and other elements as well as the discovery of the curious effect that arises from the inhalation of nitrous oxide (which he termed "laughing gas"). In this brief personal perspective, I describe the stages of a small but instructive journey that follows the sense of Davy's quote. This work was concerned with the nature of the DNA template that was used for the biochemical analysis of transcription by RNA polymerase II. In the course of these studies, the more we knew, the more we realized how much more we had yet to learn.

Stage 1. Naked DNA templates and early studies with chromatin. After the discovery of RNA polymerases I, II, and III[1,2], a critical advance was the development of biochemical systems that were able to mediate promoter-directed initiation of transcription (for RNA polymerase II, see, for example, Refs. 3–7). These systems were used in conjunction with purified "naked" DNA templates, which worked well in the assays.

The exploration of the transcriptional properties of chromatin templates began soon thereafter (see, for example, Refs. 8–18). These studies demonstrated transcriptional repression by nucleosomes. In experiments involving promoter-directed transcription initiation, the pre-binding of one or more transcription factors to the template prior to nucleosome assembly was required for transcription. The inactivity of the preassembled chromatin was probably due to immobility of the nucleosomes under the conditions of the transcription reactions. [Chromatin dynamics is an important issue that is addressed below.] In the same time frame, genetic studies in *Saccharomyces cerevisiae* independently led to the conclusion that chromatin inhibits transcription. For instance, the depletion or mutation of histone H4 was

found to result in derepression of gene activity[19–21]. Thus, the early biochemical and genetic studies provided strong evidence that chromatin represses transcription, but it was also apparent that there was much to be learnt. In the following stages, I describe a journey that my lab has taken through the world of chromatin and transcription.

Stage 2. True activation vs antirepression by sequence-specific transcription factors. In the early 1980s, the discovery of sequence-specific transcription factors (ssTFs) that bind to promoter and enhancer elements led to their extensive analysis in cells and in vitro (see, for example, Ref. 22). In the biochemical studies, it appeared that many ssTFs were able to activate transcription with the existing systems that consisted of crude cell extracts (but note that this point is clarified below) and naked DNA templates. These findings led to the paradigm that ssTFs increase the rate and/or efficiency of the intrinsic transcription process ("True Activation"; Fig. 1) and that chromatin is not important for transcriptional regulation.

In contrast to this model, experiments in my lab indicated that the GAGA factor (a *Drosophila* ssTF; also known as GAF) does not increase the amount of transcription, but rather counteracts a general repressive activity[23]. We purified this repressor and found that it is histone H1[24], a non-sequence-specific nucleosome- and DNA-binding protein that is generally associated with transcriptional repression. We further observed that transcription factor Sp1 as well as GAL4-VP16[25] (a synthetic activator with the yeast GAL4 DNA-binding domain fused to the herpes simplex virus VP16 activation region) function both to counteract histone H1-mediated repression and to increase the rate and/or efficiency of transcription. We used the term "antirepression" to describe the relief of chromatin- or histone H1-mediated repression by ssTFs (Fig. 1).

We additionally found that most in vitro transcription extracts, including a widely used nuclear extract[7], contain high levels of histone H1. Hence, the previous studies with crude transcription extracts and DNA templates were actually examining the properties of ssTFs with histone H1-bound DNA templates rather than with naked DNA templates. In the absence of histone H1, we saw little or no activation by ssTFs in vitro[23,24].

These findings were controversial. First, the antirepression hypothesis suggested that chromatin is a key component in the regulation of transcription. This notion was in contrast to the prevailing model that considered chromatin to be unimportant. Second, natural chromatin consists of nucleosomes and histone H1. Thus, the antirepression model needed to be tested properly with nucleosomal templates rather than with histone H1-DNA complexes.

We therefore proceeded to test the antirepression model by using nucleosomal templates that were reconstituted with purified native core histones and polyglutamate, which functions like a core histone chaperone[26]. This method yields randomly-distributed nucleosomes that are immobile under transcription reaction conditions. Histone H1 was then incorporated into the chromatin by salt dialysis. [Note: at that time, a purified enzymatic system for the ATP-dependent assembly of periodic nucleosome arrays had not yet been developed, and ATP-driven chromatin remodeling had not yet been discovered.]

The transcriptional analysis of these nucleosomal templates revealed that Sp1 and GAL4-VP16 are each able to counteract histone H1-mediated transcriptional repression in chromatin[27]. Notably, the magnitude of transcriptional activation that was seen with histone H1-containing chromatin templates (90- and 200-fold with Sp1 and GAL4-VP16, respectively) was much higher than that seen with naked DNA templates (3- and 8-fold with Sp1 and GAL4-VP16). Moreover, threshold phenomena and long-distance (~1300 bp) activation of transcription were observed with histone H1-containing chromatin templates but not with naked DNA templates[28]. These findings supported the model that transcriptional activators function by a combination of antirepression and true activation (Fig. 1).

Overall, these early studies provided valuable information, but there was much room for improvement of the chromatin template. The next step was to incorporate nucleosome mobility and dynamics.

Interlude. Resistance to chromatin. Before proceeding to Stage 3, I should mention that chromatin was not a popular subject in the late 1980s and early 1990s. In this period, highly regarded scientists told me things like, "Doesn't 'chromatin' mean 'artifact'?" and "You're wasting your time!" It was clearly a low point for the chromatin field. When I gave talks, I would frequently receive comments on the futility of

studying the role of chromatin in transcription. I would respond by saying that we study chromatin because it is the natural state of the template in cells, and then ask, "Is that a bad thing to do?" In response, no one would say that it was bad, but they still clearly retained their disdain for chromatin. Some of the issues raised in these debates are described in the summary at the end of Paranjape et al.[29].

Eventually, to address such criticism preemptively in a somewhat lighthearted manner, I made two slides for the introductory part of my lectures (Fig. 2). Fig. 2a shows a plot over time of pro-chromatin versus anti-chromatin sentiment. In the early 1990s, we were at the low point. Optimistically, this graph was drawn as a damped harmonic oscillator that converges at the truth. Fig. 2b imagines an alternate scenario in which scientists exhibit an amplifying love or hatred of chromatin. After all, scientists are only human.

Stage 3. Periodic arrays of dynamic nucleosomes assembled with the S-190 extract. Although the preceding experiments had yielded promising results, it was nevertheless evident that the chromatin generated in vitro was an imperfect model for chromatin in cells. One obvious difference between reconstituted chromatin and natural chromatin was that bulk native chromatin consists of periodic arrays of nucleosomes, whereas chromatin reconstituted from purified components contained randomly-distributed nucleosomes. We and others had therefore sought to develop a method for the ATP-dependent assembly of periodic nucleosome arrays. At that time, the best method for ATP-dependent chromatin assembly was a *Xenopus* oocyte extract[30] that was developed in the laboratory of Abraham Worcel. This extract was, however, somewhat frustrating to use on a routine basis, as it requires maintenance of the frogs and exhibits variability over the different seasons of the year. To surmount these problems, Becker and Wu[31] developed an excellent chromatin assembly extract from pre-blastoderm *Drosophila* embryos. We had also developed a larger-scale chromatin assembly extract, termed the S-190, from mostly post-blastoderm *Drosophila* embryos[32]. Both *Drosophila* embryo extracts yield periodic arrays of nucleosomes.

Importantly, in the same time frame, the activity of ATP-dependent nucleosome remodeling factors had been discovered (see, for example, Refs. 33–35), and both of the *Drosophila* chromatin assembly extracts[31,32] were found to contain ATP-driven chromatin remodeling enzymes[33,36]. Thus, with the S-190 chromatin assembly extract, we were able to assemble periodic arrays of nucleosomes that were mobilized by ATP-dependent chromatin remodeling factors. This enabled the combined analysis of transcriptional activation and chromatin dynamics.

With the S-190 assembly system, we initially analyzed the properties of the model transcription factors GAL4(1-147) [the GAL4 DNA-binding domain lacking an activation region] and GAL4-VP16 [GAL4(1-147) fused to the VP16 transcriptional activation region][36]. With chromatin templates, we observed strong transcriptional activation with GAL4-VP16 but not with GAL4(1-147). In contrast, both GAL4(1-147) and GAL4-VP16 were able to activate transcription with naked DNA templates or histone H1-repressed DNA templates. Hence, transcription that is dependent upon the VP16 activation region was observed only with the dynamic nucleosomal template. We also examined the structure of the chromatin template and found that both GAL4(1-147) and GAL4-VP16 were able to direct the ATP-dependent reorganization of nucleosomes in the promoter region. These findings indicated that the DNA-binding domain is sufficient for the initial remodeling of the chromatin structure of the promoter region and that the activation region is required for transcription (Fig. 3).

A notable feature of the S-190-based system is that approximately the same amount of transcriptional activation was observed when the GAL4-VP16 activator was added to preassembled chromatin or to naked DNA prior to chromatin assembly[36]. This is consistent with the dynamic nature of the chromatin. We also observed qualitatively similar results in the absence or presence of histone H1, the major difference being lower levels of transcription in the presence of histone H1. These findings suggested that transcriptional regulation is mediated primarily by dynamic nucleosomes and secondarily by histone H1.

We further tested the S-190 chromatin transcription system with different transcription factors and promoters. In collaboration with the laboratory of Katherine A. Jones on the HIV-1 promoter, we were able to observe transcriptional activation with Sp1 and NF-κB p65, which has an activation region, but not

with Sp1 and NF-κB p50, which lacks an activation region[37]. Moreover, with Sp1 and either NF-κB p65 or NF-κB p50, we observed a chromatin structure that resembles that of the integrated HIV-1 provirus. Thus, by using the S-190 system with a natural promoter and its cognate transcription factors, we were able to recreate the chromatin structure and transcriptional regulation that is seen in cells.

We additionally examined the transcriptional properties of the human estrogen receptor α (ERα) with the S-190 system[38]. With the dynamic chromatin templates, we observed ligand-dependent transcriptional activation by ERα. Specifically, we observed activation with the estrogen 17β-estradiol but not with the anti-estrogens trans-hydroxytamoxifen or ICI 164,384. These studies further revealed that ERα and p300 function cooperatively to increase the efficiency of transcription initiation, whereas ERα can also promote transcription reinitiation.

The assembly of dynamic chromatin with the S-190 extract substantially advanced our ability to study the role of chromatin in the regulation of transcription by RNA polymerase II. These experiments showed how chromatin functions as an integral component of the transcription process. When it is considered that chromatin has existed for perhaps about a billion years, this finding should perhaps not be a surprise! It is also useful to note that chromatin represses basal transcription in the absence of activators but allows the ssTFs to activate transcription. In this manner, the genome is maintained in a transcriptionally repressed state that can be activated at specific loci by the action of the ssTFs.

Stage 4. Dynamic chromatin assembled with purified assembly factors and customized histones. In spite of the success of the S-190 system, there remained many improvements to be made to the chromatin transcription system. For instance, it would be better to assemble the chromatin with purified and defined components than with the crude S-190 extract, which contains many unknown components. With this point in mind, we devoted approximately seven years to the biochemical fractionation of the S-190 and the purification and cloning of the assembly factors. This endeavor resulted in a purified recombinant chromatin assembly system. Today, periodic nucleosome arrays can be assembled with an ATP-driven motor protein (such as ACF, Chd1, or RSF), a core histone chaperone (such as NAP1 or dNLP), core histones, DNA, and ATP (see, for instance, Refs. 39–42). Most commonly, ACF and NAP1

are used to assemble chromatin onto circular plasmid DNA with native or recombinant histones. Because this system uses purified components, the resulting chromatin consists only of the specifically added histones.

Importantly, in the same time frame as the purification and cloning of the assembly factors, there was a large expansion in research on the covalent modifications of the histones. These modifications, which function at least in part as signals[43,44], added another dimension to the study of chromatin. There was also, in this same time period, an emerging appreciation of the histone variants (*i.e.*, S-phase independent histones). Thus, with the innumerable possible functions of the multifarious histone modifications and histone variants, the analysis of chromatin and transcription had become extremely complicated. In this context, it was fortunate that the purified assembly system could be used for the generation and characterization of chromatin with specific histone modifications and variants.

With the purified assembly factors, we and others embarked on the transcriptional analysis of dynamic chromatin. In some cases, the experiments were carried out with native histones[45,46]. The general trend has been, however, to study the transcriptional properties of chromatin that is assembled with defined recombinant histones (see, for example, Refs. 47–50). In a particularly notable study, chromatin containing an analogue of trimethylated histone H3 lysine 4 (H3K4me3) was used to show that H3K4me3 facilitates the assembly of the transcription preinitiation complex via interaction with the TAF3 subunit of the TFIID complex[51]. Thus, we can now make customized chromatin for the study of transcription and other nuclear processes.

Stage X – 1. The future. Over the 50 years since the discovery of RNA polymerases I, II, and III, a significant fraction of our understanding of eukaryotic transcription has emerged from biochemical experiments. In such studies, the use of chromatin templates has provided new insights into the mechanisms by which transcription factors function with chromatin to regulate gene expression. In the course of this work, new discoveries frequently opened up new and unexplored areas of investigation – that is, "new worlds to conquer", as stated by Sir Humphry Davy. With regard to my personal experiences in transcription, it has been a fun and exciting journey with wonderful people as colleagues.

What lies ahead? When I think about how to design a project, I sometimes ask myself, "How would scientists do these experiments 50 years from now?" When considering that question, one could also look backwards and think about the state of the field 50 years ago. For eukaryotic transcription, that was, of course, when RNA polymerases I, II, and III were first discovered. How much further will we advance in the next 50 years?

Rather than leave the reader unsatisfied without an attempt to answer that question, I suggest one possible future direction – the generation and use of synthetic chromatin. After researchers have gained a moderate understanding of natural chromatin (*i.e.*, become bored with studying normal chromatin), they will explore and develop unnatural molecules that form a "synthetic chromatin" with specialized functions that do not exist in natural chromatin. This synthetic chromatin would be generated at specific desired locations in the genome, and its varied and powerful activities would be easily regulated. For instance, the synthetic chromatin would enable the activity of any particular gene to be increased or decreased as simply as turning a dial. OK, let's get started! It's another world to conquer!

ACKNOWLEDGMENTS

This perspective is dedicated to Robert G. ("Bob") Roeder in recognition of his immeasurable contributions to our understanding of transcription in eukaryotes. I thank E. Peter Geiduschek, Shannon Lauberth, George Kassavetis, Jia Fei, Grisel Cruz, Long Vo ngoc, and Cassidy Huang for reading the manuscript and providing helpful suggestions. I would like to express my gratitude to all of the past and present Kadonaga lab members as well as to colleagues in the chromatin and transcription fields over the years. I apologize for any oversights or omissions. J.T.K. is the Amylin Chair in the Life Sciences. Research in the Kadonaga lab is supported by a grant from the NIH/National Institute of General Medical Sciences (R35 GM118060) to J.T.K.

REFERENCES

1. Roeder, R. G. & Rutter, W. J. Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. *Nature* **224**, 234–237 (1969).
2. Kedinger, C., Gniazdowki, M., Mandel, J. L. Jr., Gissinger, F. & Chambon, P. α-Amanitin: a specific inhibitor of one of two DNA-dependent RNA polymerase activities from calf thymus. *Biochem. Biophys. Res. Commun.* **38**, 165– 171 (1970).
3. Weil, P. A., Luse, D. S., Segall, J. & Roeder, R. G. Selective and accurate initiation of transcription at the Ad2 major late promoter in a soluble system dependent on purified RNA polymerase II and DNA. *Cell* **18**, 469–484 (1979).
4. Manley, J. L., Fire, A., Cano, A., Sharp, P. A. & Gefter, M. L. DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract. *Proc. Natl. Acad. Sci. USA* **77**, 3855-3859 (1980).
5. Matsui, T., Segall, J., Weil, P. A. & Roeder, R. G. Multiple factors required for accurate initiation of transcription by purified RNA polymerase II. *J. Biol. Chem.* **255**, 11992–11996 (1980).
6. Davison, B. L., Egly, J. -M., Mulvihill, E. R. & Chambon, P. Formation of stable preinitiation complexes between eukaryotic class B transcription factors and promoter sequences. *Nature* **301**, 680–686 (1983).
7. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11**, 1475–1489 (1983).
8. Knezetic, J. A. & Luse, D. S. The presence of nucleosomes on a DNA template prevents initiation by RNA polymerase II in vitro. *Cell* **45**, 95–104 (1986).
9. Lorch, Y., LaPointe, J. W. & Kornberg, R. D. Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. *Cell* **49**, 203–210 (1987).
10. Matsui, T. Transcription of adenovirus 2 major late and peptide IX genes under conditions of in vitro nucleosome assembly. *Mol. Cell. Biol.* **7**, 1401–1408 (1987).

11. Workman, J. L. & Roeder, R. G. Binding of transcription factor TFIID to the major late promoter during in vitro nucleosome assembly potentiates subsequent initiation by RNA polymerase II. *Cell* **51**, 613–622 (1987).

12. Knezetic, J. A., Jacob, G. A. & Luse, D. S. Assembly of RNA polymerase II preinitiation complexes before assembly of nucleosomes allows efficient initiation of transcription on nucleosomal templates. *Mol. Cell. Biol.* **8**, 3114–3121 (1988).

13. Workman, J. L., Abmayr, S. M., Cromlish, W. A. & Roeder, R. G. Transcriptional regulation by the immediate early protein of pseudorabies virus during in vitro nucleosome assembly. *Cell* **55**, 211–219 (1988).

14. Workman, J. L., Roeder, R. G. & Kingston, R. E. An upstream transcription factor, USF (MLTF), facilitates the formation of preinitiation complexes during in vitro chromatin assembly. *EMBO J.* **9**, 1299–1308 (1990).

15. Meisterernst, M., Horikoshi, M. & Roeder, R. G. Recombinant yeast TFIID, a general transcription factor, mediates activation by the gene-specific factor USF in a chromatin assembly assay. *Proc. Natl. Acad. Sci. USA* **87**, 9153–9157 (1990).

16. Corthésy, B., Léonnard, P. & Wahli, W. Transcriptional potentiation of the vitellogenin B1 promoter by a combination of both nucleosome assembly and transcription factors: an in vitro dissection. *Mol. Cell. Biol.* **10**, 3926–3933 (1990).

17. Workman, J. L., Taylor, I. C. A. & Kingston, R. E. Activation domains of stably bound GAL4 derivatives alleviate repression of promoters by nucleosomes. *Cell* **64**, 533–544 (1991).

18. Becker, P. B., Rabindran, S. K. & Wu, C. Heat shock-regulated transcription in vitro from a reconstituted chromatin template. *Proc. Natl. Acad. Sci. USA* **88**, 4109–4113 (1991).

19. Han, M. & Grunstein, M. Nucleosome loss activates yeast downstream promoters in vivo. *Cell* **55**, 1137–1145 (1988).

20. Han, M., Kim, U.-J., Kayne, P. & Grunstein, M. Depletion of histone H4 and nucleosomes activates the *PHO5* gene in *Saccharomyces cerevisiae*. *EMBO J.* **7**, 2221–2228 (1988).

21. Kayne, P. S., Kim, U.-J., Han, M., Mullen, J. R., Yoshizaki, F. & Grunstein, M. Extremely conserved histone H4 N terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. *Cell* **55**, 27–39 (1988).

22. Kadonaga, J. T. Regulation of RNA polymerase II transcription by sequence-specific DNA-binding factors. *Cell* **116**, 247–257 (2004).

23. Kerrigan, L. A., Croston, G. E., Lira, L. M. & Kadonaga, J. T. Sequence-specific transcriptional antirepression of the *Drosophila Krüppel* gene by the GAGA factor. *J. Biol. Chem.* **266**, 574–582 (1991).

24. Croston, G. E., Kerrigan, L. A., Lira, L., Marshak, D. R. & Kadonaga, J. T. Sequence-specific antirepression of histone H1-mediated inhibition of basal RNA polymerase II transcription. *Science* **251**, 643–649 (1991).

25. Sadowski, I., Ma, J., Triezenberg, S. & Ptashne, M. GAL4-VP16 is an unusually potent transcriptional activator. *Nature* **335**, 563–564 (1988).

26. Stein, A. Reconstitution of chromatin from purified components. *Methods Enzymol.* **170**: 585–603 (1989).

27. Laybourn, P. J. & Kadonaga, J. T. Role of nucleosomal cores and histone H1 in regulation of transcription by RNA polymerase II. *Science* **254**, 238-245 (1991).

28. Laybourn, P. J. & Kadonaga, J. T. Threshold phenomena and long-distance activation of transcription by RNA polymerase II. *Science* **257**, 1682–1685 (1992).

29. Paranjape, S. M., Kamakaka, R. T. & Kadonaga, J. T. Role of chromatin structure in the regulation of transcription by RNA polymerase II. *Annu. Rev. Biochem.* **63**, 265–297 (1994).

30. Glikin, G. C., Ruberti, I. & Worcel, A. Chromatin assembly in *Xenopus* oocytes: in vitro studies. *Cell* **37**, 33–41 (1984).

31. Becker, P. B. & Wu, C. Cell-free system for assembly of transcriptionally repressed chromatin from *Drosophila* embryos. *Mol. Cell. Biol.* **12**, 2241–2249 (1992).

32. Kamakaka, R. T., Bulger, M. & Kadonaga, J. T. Potentiation of RNA polymerase II transcription by Gal4-VP16 during but not after DNA replication and chromatin assembly. *Genes Dev.* **7**, 1779–1795 (1993).

33. Tsukiyama, T., Becker, P. B. & Wu, C. ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature* **367**, 525–532 (1994).

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

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

36. Pazin, M. J., Kamakaka, R. T. & Kadonaga, J. T. ATP-dependent nucleosome reconfiguration and transcriptional activation from preassembled chromatin templates. *Science* **266**, 2007–2011 (1994).

37. Pazin, M. J., Sheridan, P. L., Cannon, K., Cao, Z., Keck, J. G., Kadonaga, J. T. & Jones, K. A. NF-κB-mediated chromatin reconfiguration and transcriptional activation of the HIV-1 enhancer in vitro. *Genes Dev.* **10**, 37–49 (1996).

38. Kraus, W. L. & Kadonaga, J. T. p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation. *Genes Dev.* **12**, 331–342 (1998).

39. Ito, T., Bulger, M., Pazin, M. J., Kobayashi, R. & Kadonaga, J. T. ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* **90**, 145–155 (1997).

40. Ito, T., Levenstein, M. E., Fyodorov, D. V., Kutach, A. K., Kobayashi, R. & Kadonaga, J. T. ACF consists of two subunits, Acf1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. *Genes Dev.* **13**, 1529–1539 (1999).

41. Fyodorov, D. V. & Kadonaga, J. T. Chromatin assembly in vitro with purified recombinant ACF and NAP-1. *Methods Enzymol.* **371**, 499–515 (2003).

42. Khuong, M. T., Fei, J., Cruz-Becerra, G., and Kadonaga, J. T. A simple and versatile system for the ATP-dependent assembly of chromatin. *J. Biol. Chem.* **292**, 19478–19490 (2017).

43. Pazin, M. J. & Kadonaga, J. T. What's up and down with histone deacetylation and transcription? *Cell* **89**, 325–328 (1997).

44. Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* **403**, 41–45 (2000).

45. Jiang, W., Nordeen, S. K. & Kadonaga, J. T. Transcriptional analysis of chromatin assembled with purified ACF and dNAP1 reveals that acetyl CoA is required for preinitiation complex assembly. *J. Biol. Chem.* **275**, 39819–39822 (2000).

46. Santoso, B. & Kadonaga, J. T. Reconstitution of chromatin transcription with purified components reveals a chromatin-specific repressive activity of p300. *Nature Struct. Mol. Biol.* **13**, 131–139 (2006).

47. Levenstein, M. E. & Kadonaga, J. T. Biochemical analysis of chromatin containing recombinant *Drosophila* core histones. *J. Biol. Chem.* **277**, 8749–8754 (2002).

48. An, W., Palhan, V. B., Karymov, M. A., Leuba, S. H. & Roeder, R. G. Selective requirements for histone H3 and H4 N termini in p300-dependent transcriptional activation from chromatin. *Mol. Cell* **9**, 811–821 (2002).

49. An, W. & Roeder, R. G. Reconstitution and transcriptional analysis of chromatin in vitro. *Methods Enzymol.* **377**, 460–474 (2004).

50. Guermah, M., Kim, J. & Roeder, R. G. Transcription of in vitro assembled chromatin templates in a highly purified RNA polymerase II system. *Methods* **48**, 353–360 (2009).

51. Lauberth, S. M., Nakayama, T., Wu, X., Ferris, A. L., Tang, Z., Hughes, S. H. & Roeder, R. G. H3K4me3 interactions with TAF3 regulate preinitiation complex assembly and selective gene activation. *Cell* **152**, 1021–1036 (2013).

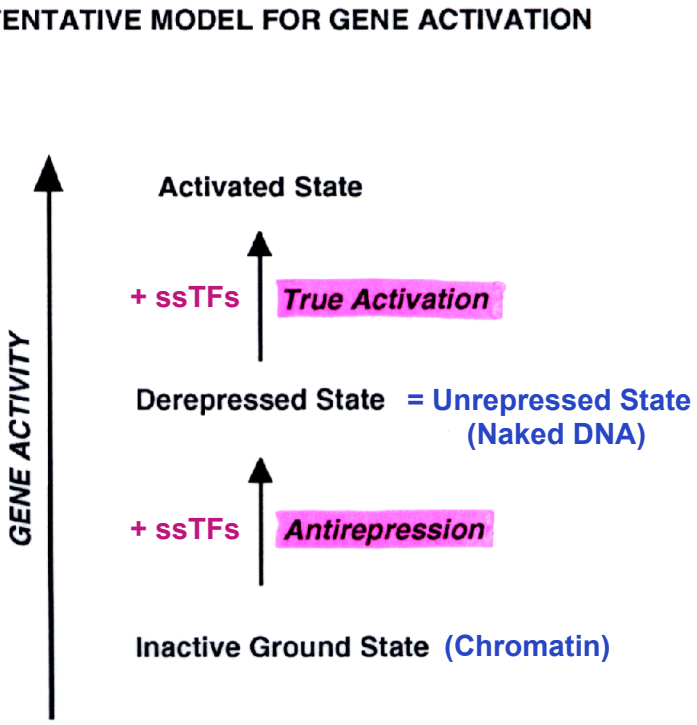
FIGURE LEGENDS

Figure 1. A model for transcriptional activation in which sequence-specific DNA-binding transcription factors (ssTFs) function to counteract chromatin-mediated repression (antirepression). In biochemical experiments, the unrepressed (derepressed) state corresponds to transcription with naked DNA (histone free) templates, whereas the repressed (inactive) state corresponds to chromatin- or histone H1-repressed templates. In "True Activation", the ssTFs increase the rate and/or efficiency of the intrinsic transcription process. In "Antirepression", the ssTFs function to relieve chromatin- or histone H1-mediated repression. The roles of ssTFs in Antirepression and, in some cases, True Activation are discussed in the text. This figure is adapted from Paranjape et al.[29].

Figure 2. Perspectives on the perceived importance of chromatin in the study of transcriptional regulation. These are lecture slides that were used by the author in the early 1990s. The y-axes show differing degrees of love or hatred of chromatin. The horizontal lines represent the truth. (**a**) Is chromatin important for the regulation of transcription? This slide optimistically imagines that the sentiment toward chromatin would converge at the truth. The position of the Early 1990s was added to the original slide. (**b**) Another scenario? This slide considers the possibility that the love or hatred of chromatin could become amplified in the future.

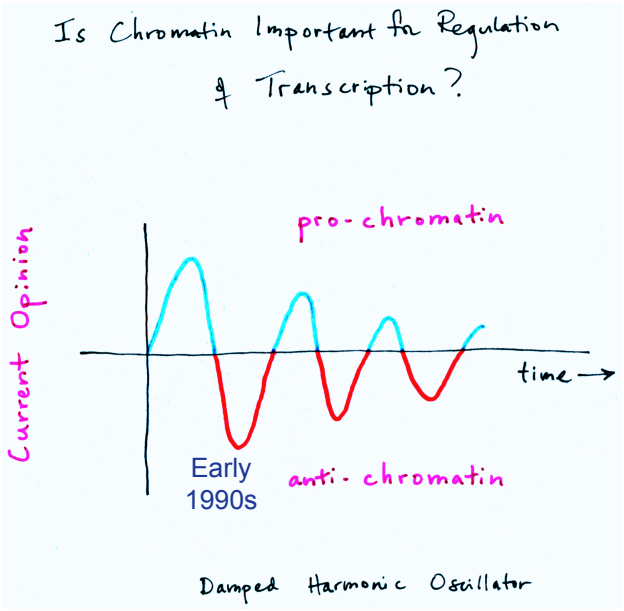
Figure 3. The 1994 version of the steps leading to the activation of transcription. First, sequence-specific DNA-binding factors bind to chromatin in a manner that does not require a transcriptional activation region. Next, a "preactivated" or "competent" promoter state is achieved by the reconfiguration of chromatin structure by ATP-dependent nucleosome remodeling factors. Then, transcription is activated in a process that requires a transcriptional activation region in the sequence-specific DNA-binding factor. This figure is from Pazin et al.[36].

Note: this figure will be redrawn by Nature artists before publication

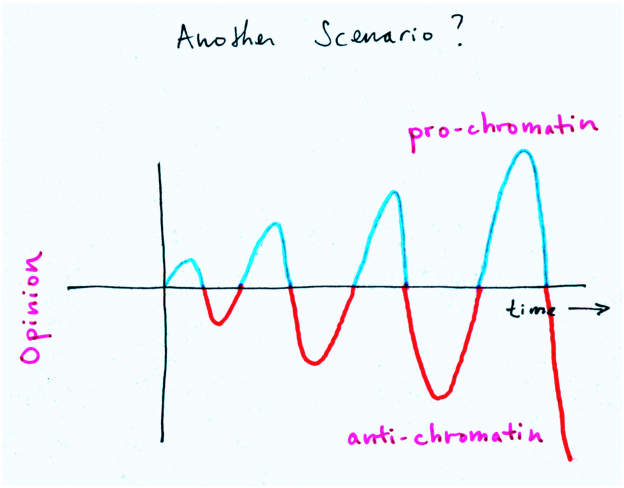


Kadonaga, Fig. 1

a

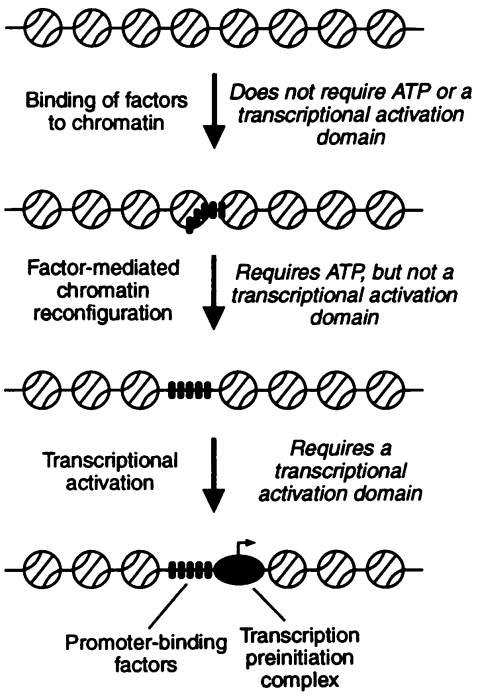


b



Kadonaga, Fig. 2

Note: this figure will be redrawn by Nature artists before publication



Kadonaga, Fig. 3