ATP-Dependent Nucleosome Reconfiguration and Transcriptional Activation from Preassembled Chromatin Templates

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GAL4-VP16–mediated nucleosome reconfiguration and transcriptional activation were observed with preassembled chromatin templates that contained regular and physiological nucleosome spacing. Both processes were dependent on adenosine triphosphate (ATP), although binding of GAL4-VP16 to the chromatin was ATP-independent. Factor-mediated nucleosome reconfiguration was not, however, sufficient for transcriptional activation. These experiments recreate in vitro the active participation of nucleosomal cores in the regulation of transcription that occurs in vivo, and they suggest a multistep pathway for transcriptional activation in which factor- and ATP-dependent nucleosome reconfiguration is followed by facilitation by the DNA-bound activator of transcription from the repressed chromatin template.

The proper control of gene transcription is essential for an organism's growth, development, and response to the environment. Alterations in chromatin structure that correlate with changes in transcriptional activity have been observed in budding yeast, fruit flies, and mammalian cells (*1*). Evidence indicates that regularly spaced nucleosomal templates are transcriptionally repressed relative to histone-free DNA and that promoter- and enhancer-binding factors function, in part, to counteract the chromatin-mediated repression. It is therefore essential to study the mechanisms of transcriptional activation in the context of the native chromatin template.

To investigate the relation between chromatin structure and transcriptional activity in a biochemical system, we have studied chromatin assembly in a *Drosophila* embryo extract, termed S-190, that contains chromatin assembly factors that efficiently reconstitute chromatin from exogenously added histones and plasmid DNA in an ATP-dependent manner (*2*, *3*). The chromatin that is assembled with S-190 closely resembles native chromatin, as judged by several criteria, including nuclease digestion products, protein composition, template topology, sedimentation properties, and appearance when examined by electron microscopy (*2*, *3*). This reconstituted chromatin consists of periodic arrays of fully assembled nucleosomes with properly incorporated histone H1 and physiological nucleosome spacing and is therefore ideal for the study of chromatin structure and transcription.

We analyzed the transcriptional properties of these chromatin templates with derivatives of the yeast transcriptional activator GAL4. The GAL4 derivatives have been widely used in the study of transcriptional activation both in vivo and in vitro and therefore serve as a useful reference in these experiments. We used three GAL4 derivatives: (i) GAL4(1–94), the transcriptionally inactive DNA-binding segment of the GAL4 protein (*4*); (ii) GAL4(1–147), which is either inactive (*5*) or weakly active (~5% of the activity of full-length GAL4) (*6*) in vivo, but contains a cryptic activation region that functions in vitro (*7*–*9*); and (iii) GAL4-VP16 [a hybrid protein that consists of GAL4(1–147) fused to the transcriptional activation region of the herpes virus protein VP16], which is a potent activator both in vivo and in vitro (*5*, *7*, *8*).

We initially tested the ability of the GAL4 derivatives to activate transcription from the reconstituted chromatin templates (Fig. 1A). These experiments revealed that the relative magnitude of activation by the GAL4 derivatives was similar to that observed in vivo. With the chromatin templates, GAL4-VP16 was a strong activator, GAL4(1–147) was a weak activator (with typically 3 to 10% of the activity of GAL4-VP16), and GAL4(1–94) was inactive. Moreover, the relative ability of the GAL4 derivatives to activate transcription was not altered by the presence or absence of histone H1, which suggests that the differential effects were predominantly the result of nucleosome-mediated repression rather than H1-mediated repression (*10*). As a control, primer extension deoxyribonuclease I (DNase I) footprinting (*11*) was done with portions of the identical samples of chromatin used in the transcription analysis, and the results demonstrated that each of the GAL4 derivatives was bound to the chromatin templates (Fig. 1B). Thus, the VP16

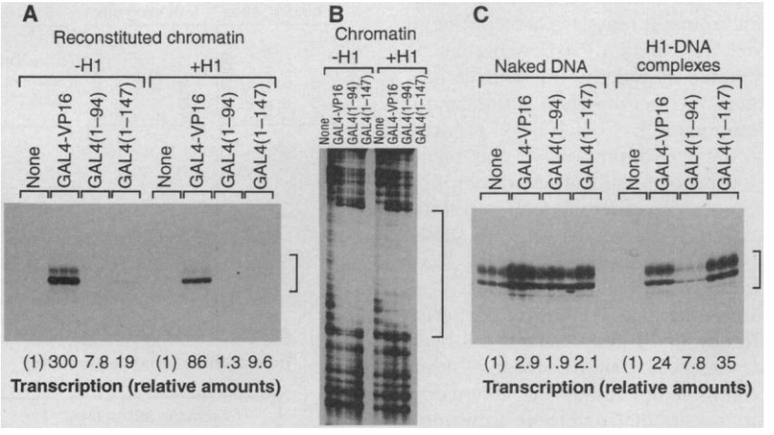

Fig. 1. Transcriptional activation by GAL4 derivatives with chromatin versus nonchromatin templates. (**A**) Activation of transcription from chromatin templates by the VP16 domain in GAL4-VP16. Chromatin was reconstituted with pGIE-0 plasmid DNA, which contains five GAL4 binding sites immediately upstream of the adenovirus E4 minimal promoter (TATA box and RNA start site), in either the presence or absence of exogenously added histone H1 (200 nM). The resulting samples (containing 75 ng of DNA) were subjected to in vitro transcription analysis with the soluble nuclear fraction as the source of basal transcription factors (*25*). Where indicated, GAL4 derivatives were included during chromatin assembly such that the concentrations of the factors in the transcription reactions were as follows: GAL4-VP16, 120 nM; GAL4(1–94), 420 nM; and GAL4(1–147), 140 nM. (**B**) Binding of the GAL4 derivatives to the chromatin templates. Portions (containing 250 ng of DNA) of the identical samples of chromatin used in the transcription reactions shown in (A) were also subjected to DNase I digestion and primer extension analysis (*11*). (**C**) Lack of requirement for the VP16 domain in activation of transcription from nonchromatin templates. Transcription reactions were done with pGIE-0 as either naked DNA (75 ng) or transcriptionally repressed H1-DNA complexes [containing template DNA (75 ng) and *Drosophila* histone H1 (150 nM)]. Where noted, GAL4-VP16 (63 nM), GAL4(1–94) (420 nM), and GAL4(1–147) (140 nM) were included in the reactions. The reverse transcription products (A and C) or DNase I footprints (B) are indicated by brackets.

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domain can function to counteract the nucleosome-mediated repression, whereas the cryptic in vitro activation region in GAL4(1–147) has little transcriptional effect with the nucleosomal templates (*12*).

With naked DNA templates, the VP16 domain in GAL4-VP16 did not increase the magnitude of transcriptional activation beyond that observed with GAL4(1–147) (Fig. 1C). In addition, the inclusion of histone H1, which nonspecifically binds to DNA, in the transcription reactions (*13, 14*) decreased the amount of basal transcription by a factor of 20 but did not alter the magnitude of transcription with GAL4-VP16 relative to that with GAL4(1–147) (Fig. 1C). These results reveal that the mechanism by which transcription factors counteract nucleosome-mediated repression is distinct from transcription factor–induced derepression of nonspecific inhibition of transcription by histone H1 (*15*), and they suggest that transcription from the chromatin templates resembles that which occurs in vivo more closely than transcription from the nonchromatin templates.

One possible explanation for the increased dependence on the VP16 activation domain with the nucleosomal templates is that there may be a more stringent requirement for high-affinity interactions between the activation domain and components of the transcriptional machinery with the highly repressed chromatin templates than with nonchromatin templates. According to this model, the potent VP16 domain may be more effective at establishing critical protein-protein interactions than is the cryptic activation domain in GAL4(1–147). Alternatively, the activation domain may mediate alterations or modifications of chromatin composition or structure, such as histone phosphorylation or acetylation, that subsequently facilitate transcription by the basal factors from the chromatin templates.

In the biochemical analysis of transcription with chromatin templates, it has been generally observed that the reconstitution of chromatin templates in the absence of activators results in irreversible inhibition of transcription, whereas the binding of factors to the DNA templates either before or during chromatin assembly allows transcription to occur from the subsequently reconstituted chromatin (*1*). Under the latter conditions, the transcription factors are presumably bound to the DNA templates before or during the assembly of chromatin, as might occur during DNA replication. However, studies of gene activation in vivo also indicate that chromatin reconfiguration and transcriptional activation can occur in the absence of DNA replication (*16*). We therefore examined replication-independent mechanisms of gene activation by the addition of transcription factors to preassembled chromatin templates.

We had previously shown that the addition of GAL4-VP16 to preassembled H1-containing chromatin purified by sucrose gradient centrifugation results in low levels of transcriptional activation (a 10-fold increase), whereas the inclusion of GAL4-VP16 during chromatin assembly with the S-190 extract yields high levels of transcriptional activity (a 50- to 80-fold increase), relative to chromatin to which GAL4-VP16 had not been added (*2*). On the basis of these observations, we postulated that an auxiliary activity may be present in the S-190 chromatin assembly extract that is required for full activation of transcription from preassembled chromatin templates, and that this activity is lost on purification of the chromatin. To test this hypothesis, we did studies in which newly assembled H1-containing chromatin was transcribed in the presence of the S-190 extract, instead of being subjected to purification by sucrose gradient sedimentation before transcription. In these experiments, GAL4-VP16 was able to activate transcription to the same extent whether the factor was added to the templates before or after chromatin assembly was complete (Fig. 2A).

This effect was further characterized. First, the activation of transcription by GAL4-VP16 was dependent on the presence of GAL4 binding sites in the promoter (Fig. 2A), and therefore required the specific binding of GAL4-VP16 to the template. Moreover, the presence of GAL4-VP16 did not affect the efficiency of bulk chromatin assembly, as determined by micrococcal nuclease digestion analysis of the identical samples of chromatin used in the transcription reactions (Fig. 2B). In addition, DNase I footprinting experiments demonstrated that GAL4-VP16 was bound to the chromatin with similar affinity when added to the templates either before or after chromatin assembly was complete (*17*).

Thus, by the inclusion of the S-190 extract in the reaction medium, we have been able to reconstitute DNA replication–independent potentiation of transcription from preassembled, H1-containing chromatin templates. These results, together with data from previous studies with purified chromatin templates (*2*), also support the hypothesis that there is an auxiliary activity in the S-190 extract that mediates transcriptional activation by GAL4-VP16 from preassembled chromatin templates.

To investigate further the mechanism of transcriptional activation from chromatin templates, we examined the effects of factor binding on chromatin structure. To map

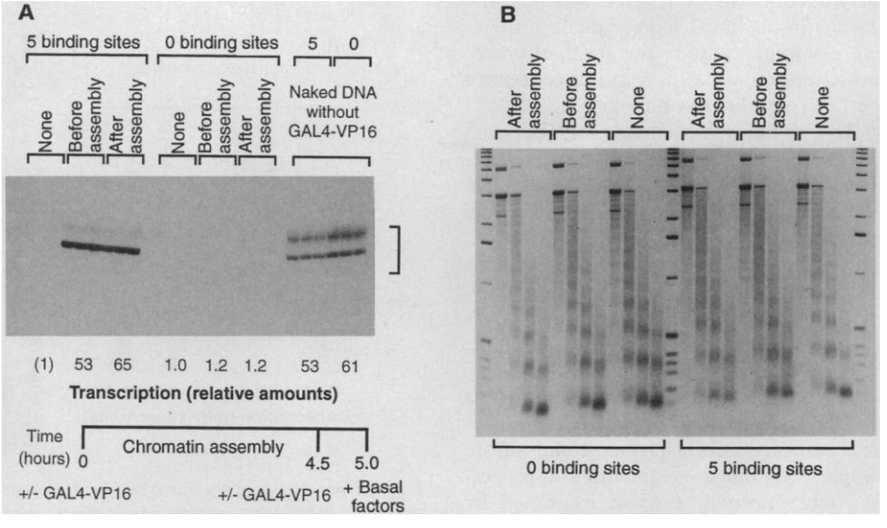

Fig. 2. Activation of transcription from H1-containing chromatin templates by GAL4-VP16 when added either before or after completion of chromatin assembly in the presence of S-190 extract. (**A**) In vitro transcription analysis. H1-containing chromatin was reconstituted with either pGIE-0 or pIE-0, which contain five or zero GAL4 binding sites, respectively, upstream of the minimal adenovirus E4 promoter. Where indicated, GAL4-VP16 (674 nM) was added to the reconstitution reactions either before (0 hours) or after (4.5 hours) chromatin assembly. The resulting samples (containing 75 ng of DNA) were subjected to in vitro transcription analysis on addition of basal factors (5 hours). In addition, in a demonstration that pGIE-0 and pIE-0 contained basal promoters of comparable activity, reactions were also done in parallel with naked DNA templates (75 ng) in the absence of GAL4-VP16. The slight difference in start site selection with the chromatin versus the naked DNA templates is due to a higher concentration of ATP in the reactions with the chromatin templates. The reverse transcription products are indicated by a bracket. (**B**) Micrococcal nuclease digestion analysis. Portions (containing 250 ng of DNA) of the identical samples used in the transcription reaction shown in (A) were also subjected to micrococcal nuclease digestion and agarose gel electrophoresis. The DNA was visualized by staining with ethidium bromide. Molecular markers: 1-kb DNA ladder (Life Technologies).

the positioning of nucleosomes in the promoter region, we subjected the reconstituted chromatin samples to partial digestion with micrococcal nuclease and subsequent indirect end-labeling analysis (*18*). These experiments were done in the absence of basal transcription factors and of detectable transcriptional activity.

When either H1-containing or H1-deficient chromatin was reconstituted in the presence of GAL4-VP16, positioned nucleosomes were observed flanking the GAL4 binding sites, as deduced from the regions of the promoter that were protected from nuclease digestion (Fig. 3A) (*19*). The TATA box and transcription start site were in a nuclease-sensitive region, and protection of the GAL4 binding sites was apparent. The factor-induced nucleosome positioning was dependent on the presence of GAL4 binding sites, which indicates that the effects were not the result of nonspecific interactions of the activator with template or proteins (*17*). Moreover, the factor-mediated positioning of nucleosomes was identical whether GAL4-VP16 was added to the templates before or after chromatin assembly, or in the presence or absence of H1 (*17*). Thus, GAL4-VP16 is able to reconfigure the chromatin structure in the promoter region, because it appears to induce the positioning of nucleosomes to locations that flank its binding sites (*20*). These effects were observed before the addition of exogenous basal transcription factors and in the absence of detectable transcription, and therefore demonstrate that the GAL4-VP16–mediated chromatin reconfiguration was not the consequence of transcription.

To examine whether an activation domain is required for factor-mediated chromatin reconfiguration, we tested the ability of truncated GAL4 derivatives to alter the chromatin structure. Both the weakly active GAL4(1–147) and the inactive GAL4(1–94) induced reconfiguration of chromatin similar to that observed with GAL4-VP16 (Fig. 3B). Furthermore, GAL4(1–94) was able to reconfigure chromatin structure when it was added either before or after assembly, and in the presence or absence of H1 (*17*). Thus, the transcriptional activation domain is not required for the factor-induced positioning of nucleosomes in vitro, and this factor-mediated reconfiguration of chromatin structure is therefore not sufficient for transcriptional activation.

The reconfiguration of chromatin structure by GAL4-VP16 was further studied by analysis of the nucleosomal arrays in the promoter region. Southern (DNA) blot analyses were done with DNA ladders that were obtained by partial digestion of chromatin with micrococcal nuclease, such as those shown in Fig. 2B. With a labeled

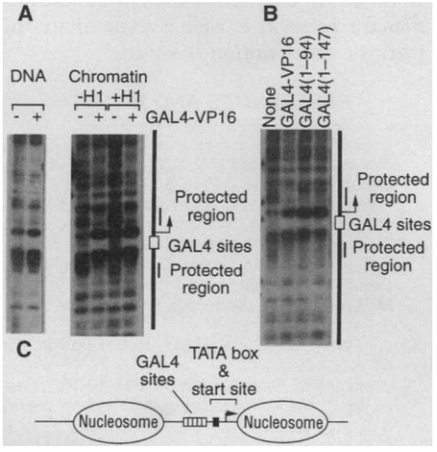

Fig. 3. Reconfiguration of chromatin structure by GAL4 derivatives. (**A**) GAL4-VP16–induced nucleosome positioning in vitro. Chromatin was reconstituted with pGIE-0 plasmid DNA, and, where indicated, GAL4-VP16 (100 nM) or histone H1 (200 nM) or both were included in the assembly reactions. Reconstituted chromatin preparations, together with naked pGIE-0 DNA as a control, were subjected to partial digestion with micrococcal nuclease and subsequent indirect end-labeling analysis. (**B**) Lack of requirement for the transcriptional activation domain in reconfiguration of nucleosomes by GAL4 derivatives. H1-containing chromatin was reconstituted with pGIE-0 DNA in the presence of the indicated GAL4 derivatives at the following concentrations: GAL4-VP16, 400 nM; GAL4(1–94), 1.4 μM; and GAL4(1–147), 450 nM. The resulting chromatin was subjected to indirect end-labeling analysis. (**C**) Schematic interpretation of nucleosome reconfiguration that is induced by binding of GAL4 derivatives.

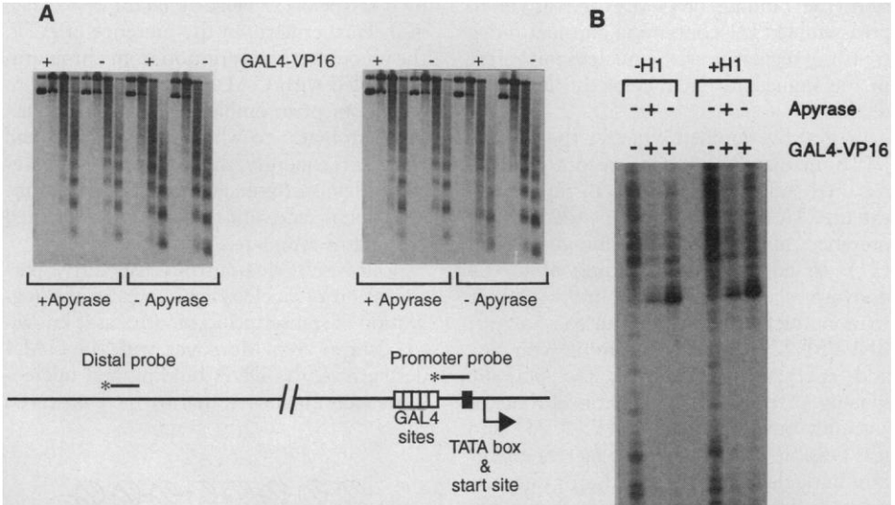

Fig. 4. ATP dependence of GAL4-VP16–mediated nucleosome reconfiguration and transcriptional activation. (**A**) Inhibition of GAL4-VP16–mediated disruption of the nucleosomal array at the promoter by apyrase treatment of chromatin. Newly reconstituted H1-containing chromatin was incubated in the presence or absence of apyrase (2 units per milliliter; 15 min at 27°C) (Sigma). GAL4-VP16 (300 nM) was added, where noted, to the samples, which were then incubated for an additional 30 min at 27°C. Southern (DNA) blot analysis of the nucleosomal arrays was then done as follows. The chromatin preparations were digested with micrococcal nuclease, and the resulting DNA fragments were subjected to agarose gel electrophoresis, transferred to nitrocellulose, and sequentially hybridized to oligonucleotide probes that correspond to sequences either between the GAL4 sites and the RNA start site (Promoter) or ~900 bp upstream of the RNA start site (Distal). (**B**) Effect of apyrase treatment of chromatin on GAL4-VP16 binding to GAL4 sites in preassembled chromatin. Newly reconstituted chromatin was incubated in the presence or absence of apyrase. GAL4-VP16 (200 nM) was added to the samples, where noted, which were then incubated for an additional 30 min at 27°C. The chromatin preparations were then subjected to DNase I digestion and primer extension analysis (*11*). (**C**) ATP dependence of GAL4-VP16–mediated activation of H1-containing chromatin. H1-containing chromatin was assembled with pGIE-0 DNA, and the crude preparation was partially purified by chromatography with Sephadex G-25 (*26*). Where noted, GAL4-VP16 (240 nM in the transcription reaction) was added to the chromatin either before or after assembly and gel filtration. Subsequent to the addition of GAL4-VP16 after gel filtration, each of the samples was incubated for 30 min at 22°C in either the presence or absence of ATP (Mg^{2+} salt) (0.5 mM) before addition of the basal transcription factors.

probe (Distal) that corresponds to sequences ~900 base pairs (bp) upstream of the transcription start site, periodic nucleosomal arrays were observed in either the presence or the absence of GAL4-VP16. In contrast, when the same blot was rehybridized with a probe (Promoter) that corresponds to sequences between the GAL4 binding sites and the RNA start site, disrupted nucleosomal arrays were observed with chromatin samples to which GAL4-VP16 was added (Fig. 4A). This effect was observed when GAL4-VP16 was added to the templates either before or after chromatin assembly; was dependent on the presence of GAL4 binding sites in the template; and was also observed with GAL4(1–94), which lacks the transcriptional activation domain (*17*). Thus, in the absence of basal transcription factors and detectable levels of transcription, the binding of GAL4 derivatives to preassembled H1-containing chromatin disrupts the regularity of the nucleosomal array in the immediate vicinity of the promoter region.

We also examined whether the GAL4-VP16–mediated chromatin reconfiguration required ATP. Nucleosome disruption by the GAGA factor is ATP dependent in the presence of a *Drosophila* embryo extract (*21*). In addition, the binding of GAL4 derivatives and TATA box binding protein to mononucleosomes is facilitated by a yeast SNF2/SWI2 protein–containing complex and ATP (*22*). Moreover, the periodic spacing of nucleosomes during chromatin assembly in vitro requires ATP (*2*, *23*), and it is possible that some of the factors important in nucleosomal spacing during chromatin assembly also participate in factor-mediated nucleosome reconfiguration.

Therefore, to investigate the ATP dependence of chromatin reconfiguration by GAL4-VP16 in the presence of the S-190 extract, we treated samples of fully reconstituted chromatin with apyrase, which catalyzes the hydrolysis of the pyrophosphate bonds in ATP, before the addition of GAL4-VP16. Analysis of the chromatin structure revealed that depletion of ATP with apyrase inhibited the disruption by GAL4-VP16 of the periodic nucleosomal arrays at the promoter (Fig. 4A), but did not affect the binding of GAL4-VP16 to the template (Fig. 4B). These results indicate that the binding of GAL4-VP16 is ATP independent and is not sufficient for the ATP-dependent reconfiguration of nucleosome structure. Together with previous observations of ATP-dependent chromatin reconfiguration (*21*, *22*), our data also suggest that factor-mediated alteration of chromatin structure requires ATP for energy, such as for the movement or reconfiguration of nucleosomes, or for the action of a kinase.

To provide a functional link between ATP-dependent chromatin reconfiguration and transcriptional activation, we investigated whether GAL4-VP16–mediated activation with preassembled, H1-containing chromatin was stimulated by ATP. H1-containing chromatin was assembled, subjected to gel filtration with Sephadex G-25 (under conditions that should remove free ATP), incubated in the presence or absence of ATP or GAL4-VP16, and then transcribed (Fig. 4C). Full activation of the preassembled, partially purified chromatin was dependent on ATP (Fig. 4C). When GAL4-VP16 was added before chromatin assembly (which was then done in the presence of ATP), the resulting template showed greater transcriptional activity than preassembled ATP-depleted chromatin to which GAL4-VP16 was added (activation by a factor of 35 versus a factor of 8) (Fig. 4C). Furthermore, in the presence of ATP, the amount of transcription from chromatin assembled with GAL4-VP16 was similar to that from preassembled and partially purified chromatin to which GAL4-VP16 had been subsequently added. Thus, these results demonstrate ATP-dependent transcriptional activation from preassembled chromatin templates.

Our results demonstrate the active participation of nucleosomal cores in the regulation of transcription in vitro, as is known to occur in vivo. Moreover, with the GAL4 derivatives, the DNA binding and nucleosome reconfiguration that we have observed in vitro are consistent with the properties of the factors that have been detected in vivo (*24*). On the basis of these results, we suggest the following pathway for transcriptional activation (Fig. 5). First, binding of factors to the chromatin template occurs, which requires neither ATP nor a transcriptional activation domain. The template-bound factors then mediate an ATP-dependent reconfiguration of nucleosome structure. This nucleosome reconfiguration requires only the DNA-binding domain and is not sufficient for transcriptional activation. For instance, with GAL4(1–94), nucleosome reconfiguration occurs but the template is transcriptionally repressed. Hence, there is at least one subsequent step, which requires a transcriptional activation domain and at which the template-bound activator facilitates the transcription process, perhaps during initiation or elongation. The basal transcription machinery, by itself, is incapable of productive transcription with the repressed chromatin template in the absence of the activator. Thus, some transcriptional activators possess the ability to bind to chromatin and then to mediate transcription together with the basal factors. In this manner, an activator may function both to counteract the chromatin-mediated repression as well as to facilitate the intrinsic transcription reaction.

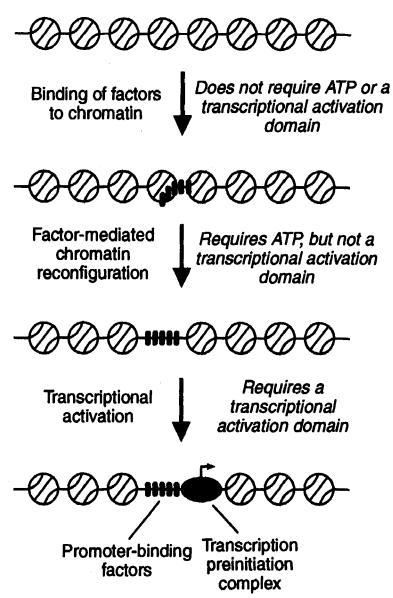

Fig. 5. Model for the steps leading to the activation of transcription. A description of this working model for transcriptional activation in the context of the native chromatin template is given in the text. In the first step (binding of factors prior to nucleosome reconfiguration), a precise location of the GAL4 factors relative to the nucleosome cores is not intended to be implied.

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10. In our studies with chromatin reconstituted with the S-190 extract, the incorporation of histone H1 into the chromatin resulted in a greater repression of transcription, but the biochemical properties of H1-containing chromatin were otherwise similar to those of H1-deficient chromatin. In previous studies with chromatin reconstituted with polyglutamic acid (*14*) [P. J. Laybourn and J. T. Kadonaga, *Science* **254**, 238 (1991); *ibid.* **257**, 1682 (1992)], a more pronounced effect of H1 was observed. We attribute this difference in the magnitude of repression by histone H1 to the active participation of the nucleosomal cores in transcription with S-190–assembled chromatin, which was not observed with chromatin

assembled with polyglutamic acid. Thus, our current data indicate that histone H1 has an important function in transcriptional repression, but with the S-190–assembled chromatin the participation of the nucleosomal cores relative to that of H1 has increased when compared to results of previous studies in which the cores were static rather than active.
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12. Previous studies of transcription from either chromatin templates or H1-DNA complexes showed that GAL4-VP16 activates transcription more effectively than GAL4(1–94) (*4, 14*), but transcriptional activation by GAL4(1–147) was not examined.
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15. These observations are similar to those of previous studies in which potent activation of transcription by GAL4(1–147) was apparent in vitro (*7, 8*), although higher levels of transcription in vitro by GAL4-VP16 relative to GAL4(1–147) have also been observed with different experimental systems (*7, 9*). Other treatments that reduced basal transcription, such as the inclusion of the S-190 extract (in the absence of exogenously added histones, in which case chromatin assembly does not occur), reduction of the template concentration (by a factor of 5 or 25), or addition of nonspecific competitor DNA, had little effect on the relative potency of GAL4-VP16 versus GAL4(1–147) (*17*). It therefore appears that the effects were not the result of a nonspecific decrease in transcriptional efficiency. In addition, transcriptional inhibition by excess GAL4-VP16, commonly known as squelching [G. Gill and M. Ptashne, *Nature* **334**, 721 (1988)], is not observed with the *Drosophila* basal transcription factors (R. T. Kamakaka and J. T. Kadonaga, unpublished data).
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19. The conclusion that nucleosomal cores flank the GAL4 binding sites is based on the data shown in Fig. 3 together with the following results: First, by indirect end-labeling analysis with low-resolution agarose gels, we have observed a region of protection flanking the GAL4 binding sites, interpreted to be due to positioned nucleosomes, when probes from either side of the GAL4 binding sites were used and when the GAL4 sites were moved 1000 bp upstream of the transcription start site (*17*). Second, micrococcal nuclease digestion and primer extension analyses (at nucleotide resolution) of the chromatin in the presence or absence of GAL4 derivatives further supported the conclusion that nucleosomal cores flank the GAL4 binding sites (*17*).
20. We use the term "nucleosome reconfiguration" to denote changes in chromatin structure, but we do not intend to suggest that this alteration is necessarily a disruption, dissolution, or unfolding of the nucleosomes, or a displacement of the core histones. For example, some of the changes in chromatin structure may involve sliding or translational repositioning of the nucleosomes to another location on the DNA template. In addition, in the studies of factor-mediated nucleosome positioning, we cannot discern whether the GAL4 derivatives induce the adjacent positioning of nucleosomes by exclusion of the nucleosomes from the DNA segment that is occupied by the factors or by directed placement of the nucleosomes next to the factors.
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25. Chromatin assembly, in vitro transcription, and primer extension analysis were performed as described previously, except that the chromatin templates were not subjected to purification by sucrose gradient sedimentation before transcription with the soluble nuclear fraction as a histone-deficient source of basal transcription factors (*2, 3, 8*). The S-190 extract alone is not competent for basal transcription (*17*). Moreover, in control reactions containing S-190 and the soluble nuclear fraction, from which the ribonucleoside 5′-triphosphates were omitted, transcription was not observed (*17*). Hence, these extracts are not contaminated with endogenous ribonucleoside 5′-triphosphates. Quantitation of the reverse transcription products was done with a Phosphorimager (Fuji or Molecular Dynamics). All experiments were done a minimum of two times (but more commonly about four times) to ensure reproducibility of the data. In the course of these experiments, we used three S-190 extracts, two preparations of soluble nuclear fraction, three preparations of GAL4-VP16, two preparations of core histones, two preparations of histone H1, and several plasmid preparations. Thus, the results were consistently reproducible with different preparations of reagents. The plasmid pGIE-0 (M. Bulger and J. T. Kadonaga, unpublished data) contains five tandem consensus GAL4 binding sites immediately upstream of the adenovirus E4 minimal promoter (TATA box and RNA start site; from −38 to +250 relative to the RNA start site) and closely resembles pG_5E4T (*7*). The plasmid pIE-0 is identical to pGIE-0 except that it does not contain GAL4 binding sites.
26. Sephadex G-25 SF (Pharmacia) was equilibrated with buffer [12.5 mM Hepes-KOH (pH 7.5), 5% (v/v) glycerol, 0.05 mM EDTA, and 50 mM KCl]. Chromatin (300 μl, containing 3 μg of DNA) was applied to 3 ml of resin and subjected to chromatography in the same buffer. The chromatin eluted in the void volume.
27. We are grateful to H. Weintraub for inspiration and enlightenment regarding the role of chromatin structure in the regulation of gene expression. We thank B. Zimm, B. Emerson, E. Blackwood, J. Tyler, C. George, M. Bulger, S. Paranjape, and A. Wurster for critical reading of the manuscript. J.T.K. is a Presidential Faculty Fellow. Supported by grants to J.T.K. from NIH, NSF, and the Council for Tobacco Research.

9 August 1994; accepted 25 October 1994

Specific Association of Human Telomerase Activity with Immortal Cells and Cancer

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Synthesis of DNA at chromosome ends by telomerase may be necessary for indefinite proliferation of human cells. A highly sensitive assay for measuring telomerase activity was developed. In cultured cells representing 18 different human tissues, 98 of 100 immortal and none of 22 mortal populations were positive for telomerase. Similarly, 90 of 101 biopsies representing 12 human tumor types and none of 50 normal somatic tissues were positive. Normal ovaries and testes were positive, but benign tumors such as fibroids were negative. Thus, telomerase appears to be stringently repressed in normal human somatic tissues but reactivated in cancer, where immortal cells are likely required to maintain tumor growth.

Telomeres are specialized structures at the ends of eukaryotic chromosomes that appear to function in chromosome protection, positioning, and replication (*1, 2*). In vertebrates, telomeres consist of hundreds to thousands of tandem repeats of the sequence TTAGGG and associated proteins (*2, 3*). Analysis of chromosome terminal restriction fragments (TRFs) provides the composite lengths of all telomeres in a cell population (*4–6*). In all normal somatic cells examined to date, TRF analysis has shown that the chromosomes lose about 50 to 200 nucleotides of telomeric sequence per cell division (*4–6*), consistent with the inability of DNA polymerase to replicate the ends of linear DNA (*7*). This shortening of telomeres has been proposed to be the mitotic clock by which cells count their divisions (*8*), and a sufficiently short telomere may be the signal for replicative senescence in normal cells (*5, 6, 9*). In contrast, all immortal cells examined to date show no net loss of telomere length or sequence with cell division, suggesting that maintenance of telomeres is required for cells to escape from replicative senescence and proliferate indefinitely (*10–12*).

Telomerase is a ribonucleoprotein that synthesizes telomeric DNA onto chromo-

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