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Yeast GCN4 transcriptional activator protein interacts with RNA polymerase II *in vitro*

(gene regulation/promoters/affinity chromatography/mRNA initiation/eukaryotic transcription)

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ABSTRACT **Regulated transcription by eukaryotic RNA polymerase II (Pol II) requires the functional interaction of multiple protein factors, some of which presumably interact directly with the polymerase. One such factor, the yeast GCN4 activator protein, binds to the upstream promoter elements of many amino acid biosynthetic genes and induces their transcription. Through the use of affinity chromatography involving GCN4- or Pol II-Sepharose columns, we show that GCN4 interacts specifically with Pol II *in vitro*. Purified Pol II is retained on the GCN4-Sepharose column under conditions in which the vast majority of proteins flow through. Moreover, Pol II can be selectively isolated from more complex mixtures of proteins. Conversely, GCN4 protein, synthesized *in vitro* or in *Escherichia coli*, specifically binds to the Pol II-Sepharose column under equivalent conditions. Using deletion mutants, we also show that the DNA-binding domain of GCN4 is both necessary and sufficient for this interaction. We suggest the possibility that this GCN4–Pol II interaction may be important for transcription *in vivo*.**

Although *Escherichia coli* RNA polymerase holoenzyme binds to promoters and initiates mRNA synthesis, activator proteins binding adjacent to the polymerase can increase the level of transcription, presumably by direct protein–protein contact (1–3). The regions of activator proteins involved in this RNA polymerase interaction have been implicated by mutations that increase or decrease the level of transcriptional stimulation without affecting DNA binding. These mutations are generally located within the DNA-binding domain, specifically in the α-helix of the helix-turn-helix motif that is not involved in direct contacts to DNA.

In eukaryotic organisms, regulated transcription by RNA polymerase II (Pol II) requires multiple proteins that become spatially associated as a result of their specific binding to upstream (enhancer) or TATA promoter elements (for reviews, see refs. 4–6). Moreover, auxiliary factors distinct from these specific promoter DNA-binding proteins and from the ≈10 Pol II subunits are also required for transcription *in vivo*. However, it is unknown which, if any, of these transcription factors directly contact Pol II.

Amino acid starvation of yeast cells results in the synthesis of GCN4 (7, 8), a protein that binds upstream of many amino acid biosynthetic genes and induces their transcription (9, 10). GCN4 contains 281 amino acids and binds as a dimer (11) to a 9-base-pair (bp) dyad whose consensus sequence, ATGA$\overset{C}{G}$TCAT, is optimal for DNA binding (12). The 60 C-terminal residues of GCN4 are sufficient for specific DNA binding and for dimerization (11, 13). In addition to the DNA-binding domain, transcriptional activation *in vivo* by GCN4 requires a short acidic region centrally located within the protein (13, 14). GCN4 is structurally and functionally related to the jun oncoprotein (15–17), an oncogenic version of the vertebrate AP-1 transcription factor (18, 19).

It has been proposed that GCN4, like other yeast activator proteins, stimulates transcription by directly contacting other components of the transcriptional machinery (5, 13, 20, 21). Evidence against the idea that upstream activator proteins function by increasing chromatin accessibility comes from the observation that GAL4 cannot stimulate transcription by bacteriophage T7 RNA polymerase in yeast (22). In contrast, a poly(dA-dT) sequence, which is hypothesized to cause a local disruption in chromatin structure, enhances transcription by T7 RNA polymerase (22). Previously, we suggested that GCN4 might contact proteins bound to the TATA sequence because it activates transcription in combination with only one of the two TATA elements in the *HIS3* promoter (5, 23, 24). In support, it has been shown that upstream activator and TATA-binding proteins can cooperatively interact with DNA (25–27) and that the acidic transcriptional activation region might be involved (27).

The hypothesis that GCN4 interacts with a TATA-binding protein does not exclude the possibility that it might directly contact Pol II. Here, we use GCN4-Sepharose and Pol II-Sepharose affinity columns to show that GCN4 interacts specifically with Pol II *in vitro*. Moreover, as with bacterial activator proteins, the region of GCN4 that contacts Pol II resides within the DNA-binding domain. We suggest the possibility that this GCN4–Pol II interaction may be important for transcriptional regulation by GCN4 *in vivo*.

MATERIALS AND METHODS

Expression and Purification of GCN4 from *E. coli*. The DNA fragment encoding GCN4 from pSP64-Sc4342 (13) was adapted for expression in *E. coli* by replacing sequences upstream of the initiation codon with an oligonucleotide containing an efficient ribosome binding site. The resulting fragment was cloned downstream of the bacteriophage λP_L promoter, and the resulting plasmid was introduced into a strain that expresses the cI^{857} temperature-sensitive λ repressor from a second plasmid. GCN4 was synthesized after temperature induction of the resulting strain, and a crude cell lysate was prepared by conventional methods. GCN4, which represented ≈0.2% of the total protein in the crude extract, was purified by a procedure developed by C. R. Wobbe that involved phosphocellulose chromatography and sequence-specific DNA-affinity chromatography (28).

GCN4-Sepharose Affinity Chromatography. A 50-μl GCN4 affinity column was constructed by coupling GCN4 to CNBr-activated Sepharose (Pharmacia) essentially as described for a Pol II affinity column (29). The final concentration of GCN4 on the column was ≈1 mg/ml, with the coupling efficiency being >95%. An equivalent Sepharose column containing bovine serum albumin (BSA) was also constructed. Protein

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Abbreviations: Pol II, RNA polymerase II; BSA, bovine serum albumin.

preparations containing Pol II were applied to the columns as described in Table 1 and Fig. 1, and Pol II activity was monitored by a standard enzyme assay (30). For the experiment shown in Fig. 1 *Center*, plasmid DNA containing the optimal GCN4 binding site (12) was digested with *Msp* I and *Hin*dIII, labeled with ^{32}P at the 5′ ends, and applied to the GCN4 affinity column in buffer A [20 mM Tris·HCl (pH 7.5) supplemented with 1 mM EDTA, 10% (vol/vol) glycerol, 0.5 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, and 2.5 μg of antipain, 0.2 μg of aprotinin, 0.4 μg of pepstatin, and 0.5 μg of leupeptin per ml] containing 100 mM NaCl. The column was eluted in succession with buffer A containing 0.1, 0.3, 0.5, and 1.0 M NaCl, and the DNA eluted at each step was visualized by autoradiography after polyacrylamide gel electrophoresis. For the experiment shown in Fig. 1 *Right*, yeast proteins were labeled *in vivo* by growth of strain KY114 (23) in the presence of [^{35}S]methionine and prepared by ammonium sulfate precipitation of cell extracts. Approximately 1 μg of protein (≈10^5 cpm) was applied to 50 μl of GCN4-Sepharose and BSA-Sepharose columns in buffer A containing 100 mM NaCl. The columns were washed successively with 150 μl of buffer A containing 100 μg of BSA per ml and 0.1 M or 0.3 M NaCl. The 0.1 M NaCl eluant was included with the flow-through fraction. Protein bands were visualized by fluorography after separation on a denaturing polyacrylamide gel.

Pol II Affinity Chromatography. Pol II was purified from *Saccharomyces cerevisiae* cells by conventional means involving sequential chromatography on phosphocellulose, heparin-agarose, DEAE-cellulose, and DNA-cellulose. Although it is impossible to determine the absolute purity of a multisubunit complex in which the subunit composition is not precisely defined, our Pol II preparation appeared to be similar in quality to the most highly purified preparations that have been published (e.g., ref. 29) by virtue of its specific activity and its analysis on a denaturing polyacrylamide gel. The gel analysis indicated that the largest subunit was essentially unproteolyzed, and it revealed only one visible contaminant, a very low molecular protein of ≈5–10 kDa that flowed through a GCN4 column. Pol II was coupled to Sepharose as described (29). GCN4 and deletion derivatives, either purified from *E. coli* or synthesized *in vitro* (9, 13), were applied and eluted from the Pol II-Sepharose column as described in the legends to Fig. 2 and 3. For the experiment in Fig. 2, GCN4 was assayed by its ability to shift the electrophoretic mobility of a [^{32}P]DNA fragment containing the GCN4 binding site from the *HIS3* upstream region. Fractions were incubated in the presence of 160 μg of poly(dI-dC) per ml with a fragment containing *HIS3* sequences from −135 to +85 that had been end-labeled with T4 polynucleotide kinase and [^{32}P]ATP. The reaction products were subjected to electrophoresis in a 5% native acrylamide gel (31, 32).

RESULTS

Pol II Interacts with GCN4-Sepharose. To identify molecules with which GCN4 interacts, we constructed GCN4 affinity columns. GCN4 was expressed in *E. coli*, purified to apparent homogeneity by a series of chromatographic steps culminated by sequence-specific DNA-affinity chromatography (Fig. 1 *Left*), and covalently coupled to CNBr-activated Sepharose. Coupled GCN4 retained its functional integrity as shown by its ability to selectively bind DNA containing the GCN4 recognition site (Fig. 1 *Center*).

As Pol II was an obvious component of the transcriptional machinery with which GCN4 might interact, a preparation of purified Pol II was applied to the GCN4 column. Pol II activity, verified by its α-amanitin sensitivity, was retained

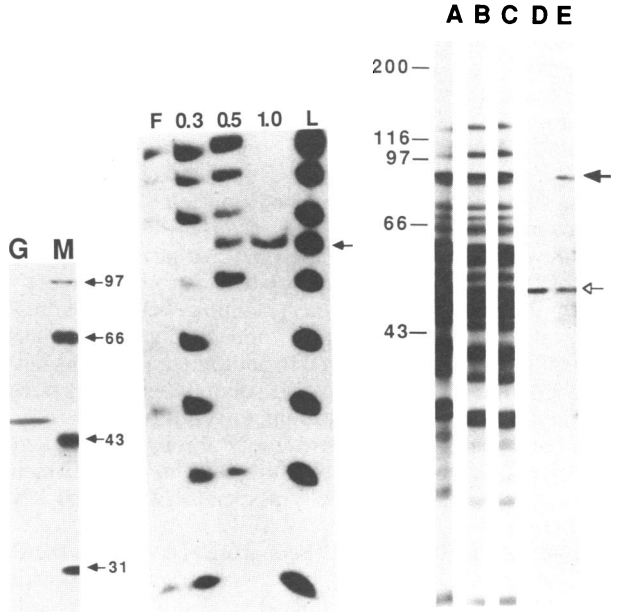

FIG. 1. GCN4 affinity chromatography. (*Left*) Twenty nanograms of purified GCN4 (lane G) and molecular weight markers (lane M) subjected to electrophoresis in a sodium dodecyl sulfate/10% polyacrylamide gel and visualized by silver-staining. (*Center*) 5′-End-labeled restriction fragments that were loaded on (lane L), flowed through (lane F), or were eluted from the GCN4-Sepharose column at the indicated molar concentrations of NaCl. The arrow indicates the fragment representing the GCN4 binding site. (*Right*) Analysis of ^{35}S-labeled total yeast protein applied to GCN4-Sepharose and BSA-Sepharose: yeast protein applied to columns (lane A), flow-through fractions from BSA-Sepharose (lane B) and GCN4-Sepharose (lane C), and bound proteins from BSA-Sepharose (lane D) and GCN4-Sepharose (lane E) that were eluted in 0.3 M NaCl. The large solid arrow indicates the protein that binds specifically to GCN4-Sepharose, and the small open arrow indicates the protein that binds to both the GCN4 and BSA columns. Sizes are shown in kDa.

on the GCN4 affinity column at 100 mM NaCl (Table 1). Examination of the elution profile showed that Pol II activity partitioned 90% and 10% in 200 mM NaCl and 300 mM NaCl step fractions, respectively. Pol II could be selectively isolated from more complex mixtures because a preparation of lower purity by a factor of 20 was retained equally well on the column. As controls, Pol II did not bind to an equivalent

Table 1. Binding of RNA Pol II to a GCN4 affinity column

	GCN4		BSA		E		ΔGCN4
Sepharose	Pol II*	^{35}S†	Pol II*	^{35}S†	Pol II*	^{35}S†	Pol II*
Loaded	86	100	64	100	106	100	220
Flow-through	10	87	78	92	56	44	4
NaCl							
0.3 M	94	8	2	4	3	2	194
1.0 M	ND	5	ND	4	ND	1	5

Approximately 1 μg (10^5 cpm) of ^{35}S-labeled total yeast protein and 5 μg of the Pol II fraction were applied in buffer A containing 0.1 M NaCl to 50-μl Sepharose columns containing bound GCN4, BSA, an N-terminally deleted version of GCN4 containing amino acids 180–281 (ΔGCN4), or a control column that was blocked by ethanolamine (E) (for methodological details, see the legends to Figs. 1 and 2). The columns were washed successively with 150 μl of buffer A containing 100 μg of BSA per ml and 0.1, 0.3, and 1 M NaCl; the 0.1 M NaCl eluant was included with the flow-through fraction.

*Pol II activity represents the number of pmol of [^3H]UTP incorporated into acid-insoluble material in 30 min with a denatured salmon sperm DNA template.

†^{35}S represents the percentage of ^{35}S-labeled yeast protein precipitated by trichloroacetic acid.

column containing BSA nor to an ethanolamine-blocked Sepharose column (Table 1).

To examine selectivity of binding in more detail, ^{35}S-labeled total yeast protein was combined with Pol II, and the resulting mixture was loaded on the GCN4 column. Under conditions in which 90% of Pol II bound to the GCN4 column, only 8% of total yeast protein was retained (Table 1). Some of the ^{35}S-labeled protein retained on the column probably reflects nonspecific interactions because 4% of the labeled protein was also retained on the BSA-Sepharose column. Of the approximately 25 major ^{35}S-labeled proteins visible by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, only one (≈90 kDa) was retained specifically on the column at 100 mM NaCl (Fig. 1 *Right*). In addition, all proteins in the Pol II fractions besides the Pol II subunits visible by silver-staining flowed through the column even though these proteins copurified with Pol II activity through several ion-exchange columns (data not shown). These experiments indicate that GCN4 specifically associates with yeast RNA polymerase II.

GCN4 Interacts with Pol II-Sepharose. To confirm the Pol II–GCN4 interaction, affinity chromatography was carried out in a reciprocal manner. Specifically, Pol II was coupled to Sepharose, and *E. coli*-produced GCN4 was applied to the column as described above. GCN4, assayed by its DNA-binding activity, was retained on the Pol II-Sepharose column at 100 mM NaCl and was eluted in the 300 mM NaCl step fraction (Fig. 2). Under equivalent conditions, GCN4 did not bind to either BSA-Sepharose or to ethanolamine-Sepharose. As previous results have shown that Pol II affinity columns are selective for binding proteins (29), these experiments provide independent evidence for the specificity of the GCN4–Pol II interaction. Moreover, the conditions used for binding to and eluting from both the GCN4 and Pol II columns are comparable to those used for other protein–protein interactions (29, 33).

DNA Binding Domain of GCN4 Is Required for Interaction with Pol II. To determine if the acidic activation region of GCN4 is required for the Pol II interaction, a protein containing only the 100 carboxyl-terminal amino acids of GCN4 was purified by sequence-specific DNA-affinity chromatography and coupled to Sepharose. As with the intact protein, an affinity column containing this short GCN4 derivative retained Pol II (Table 1). Therefore, contacts for Pol II must exist outside of the activation domain.

To confirm this observation and to localize further the GCN4 region important for the Pol II interaction, ^{35}S-labeled GCN4 derivatives were synthesized *in vitro* (9, 13) and applied to the Pol II column. In accord with the reciprocal experiments described above, the full-length protein as well as derivatives containing only the 83 or 60 C-terminal residues (gcn4-C83 and gcn4-C60, respectively) bound to the affinity matrix (Fig. 3). All C-terminal deletions tested failed to bind to the Pol II column. In particular, the gcn4-N270 derivative, which lacks only the 11 C-terminal amino acids, bound extremely poorly under conditions in which gcn4-C83 protein was almost quantitatively retained (Fig. 3). As removal of only 11 residues does not significantly affect the overall amino acid composition, this result provides additional strong evidence in favor of a specific interaction between GCN4 and Pol II. Taken together, these observations indicate that the dimeric DNA-binding domain is both necessary and sufficient for the Pol II interaction. The failure of gcn4-N270 to interact with Pol II could indicate that the 11 C-terminal residues are specifically involved in the association, or more likely that a structurally intact DNA-binding domain is required.

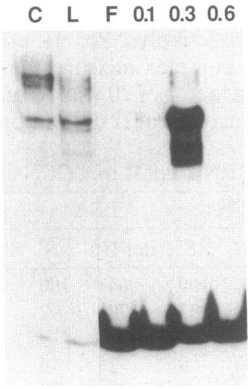

FIG. 2. Binding of GCN4 to a yeast RNA Pol II affinity column. Lanes: L, loaded sample; F, flow-through fraction; 0.1–0.6, eluted fractions at the indicated NaCl concentrations from the Pol II column; C, a GCN4 control sample. GCN4 (450 ng) in buffer A containing 100 μg of BSA and 0.1 mg of gelatin per ml, 0.5 mM dithiothreitol, and 50 mM NaCl was applied to a 65-μl column. The column was washed successively with 200 μl of the same buffer but containing 100, 300, and 600 mM NaCl. Each fraction was assayed for GCN4 by its ability to shift the electrophoretic mobility of a [^{32}P]DNA fragment containing the GCN4 binding site from the *HIS3* upstream region as described. The differences in relative intensities of the bands representing the GCN4–DNA complexes are due primarily to different salt concentrations during the DNA-binding assay.

DISCUSSION

Evidence for a Specific Interaction Between GCN4 and RNA Pol II. Taken together, the results in this paper are internally consistent, and they indicate that GCN4 and Pol II interact selectively *in vitro*. The interaction is observed in reciprocal experiments under conditions used previously to demon-

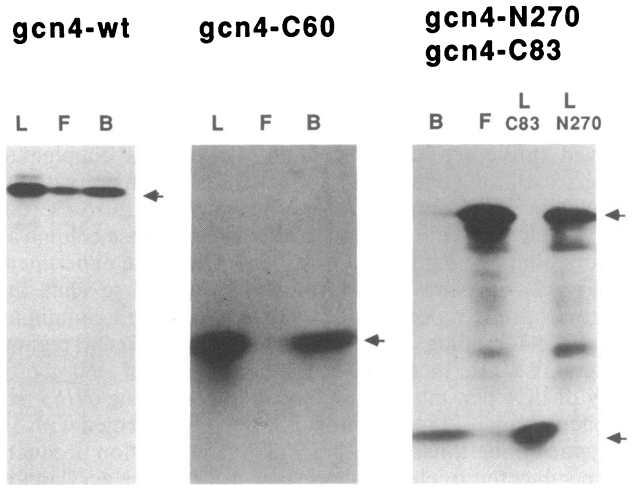

FIG. 3. Binding of GCN4 derivatives to RNA Pol II. Approximately 0.02 pmol of ^{35}S-labeled wild-type (wt) GCN4, gcn4-N270 (lacking the 11 C-terminal amino acids), gcn4-C83, and gcn4-C60 (containing only the 83 and 60 C-terminal residues, respectively) were (*i*) incubated for 30 min at 37°C in 0.5 M NaCl/10 mM $CaCl_2$/50 mM Tris·HCl, pH 8.5/1500 units of micrococcal nuclease per ml, (*ii*) diluted 1:9 in buffer A containing 100 μg of BSA per ml, and (*iii*) applied to the 65-μl Pol II column described in Fig. 2. The column was washed with 150 μl and 200 μl of buffer A containing 0.1 M NaCl and 0.4 M NaCl, respectively. For each protein (arrows), the loaded (lane L), bound (lane B), and unbound (lane F) fractions are indicated. The C83 and N270 derivatives were combined prior to loading on the column, but the proteins are shown individually prior to mixing. In addition, with the exception of the gcn4-C83/gcn4-N270 experiment, the 0.1 M NaCl wash was included with the flow-through fraction. Equivalent percentages of each fraction were analyzed by denaturing polyacrylamide gel electrophoresis followed by fluorography.

strate specific protein–protein interactions, and the same part of GCN4 is necessary and sufficient in both cases. It is unlikely that the interaction is due to an artifactual ionic association because Pol II is retained on the GCN4-Sepharose column, whereas proteins that copurify over three or four different ionic exchange columns flow through. Moreover, Pol II interacts similarly with the highly basic GCN4 DNA-binding domain and with the full-length protein, which has an overall net negative charge. Conversely, deletion of the 11 C-terminal residues of GCN4 has a minor effect on the overall charge of the protein, yet it eliminates the Pol II interaction.

The fact that the GCN4-Pol II association is observed with highly purified protein preparations strongly suggests that the interaction is direct and does not involve an intermediary protein. In this regard, it is very unlikely that the TATA-binding protein TFIID is involved in the observed GCN4–Pol II interaction because it is easily separated from Pol II during the purification procedure (25–27). It is also unlikely that a contaminant in the Pol II preparation is involved in the GCN4–Pol II interaction because immobilization to a solid support should physically separate the contaminant from Pol II (unless the "contaminant" is closely associated with Pol II). However, the experiments cannot distinguish between GCN4 interacting with a "true" subunit(s) of Pol II and a protein very tightly associated with Pol II (a distinction more semantic than real).

The *in vitro* experiments in this paper may be considered nonphysiological in the sense that affinity chromatography permits the components to be present at very high local concentrations. However, it seems reasonable that such high local concentrations of GCN4 and Pol II might occur *in vivo* when both proteins are associated with DNA. Although GCN4 normally activates transcription when bound relatively far upstream and at variable positions of the mRNA initiation sites (12), the GCN4–Pol II interaction could still occur by DNA looping (reviewed in ref. 34). The observed GCN4–Pol II interaction may be weak in comparison to specific protein–DNA interactions, but the selectivity of the interaction suggests the possibility that the association might be important for transcriptional activation of the amino acid biosynthetic genes *in vivo*.

Possible *in Vivo* Significance of the GCN4–Pol II Interaction. Several considerations are consistent with but do not prove a functional role for the GCN4–Pol II interaction. First, GCN4 can activate transcription in the absence of a TATA element when bound very close to the transcriptional start site (35–37). Second, the association between GCN4 and Pol II may be analogous to those between bacterial activator proteins and RNA polymerase in that the positive control mutations of cI, CAP, and cro that presumably identify the polymerase interaction sites are located in one of the crucial α-helices within the DNA-binding domains (1–3). Third, LexA hybrid proteins containing the intact GCN4 DNA-binding domain activate transcription when bound to a *lexA* operator 5-fold more efficiently than related derivatives lacking this domain (13). However, this effect may also be due to increased dimerization and DNA binding of the LexA domain (16, 17). In addition, the observations are in accord with models postulating that the repeated heptapeptide tail at the C terminus of the largest Pol II subunit interacts with transcription factors (38).

The hypothesis that the GCN4–Pol II interaction is functionally important *in vivo* suggests that it might be possible to obtain mutations in the GCN4 DNA-binding domain that do not affect the protein–DNA interaction but reduce the level of transcriptional activation. Although such GCN4 derivatives do not yet exist, the analogous positive control mutants of bacterial activator proteins were isolated only by using very specialized genetic selections that have not yet been used for any eukaryotic activator protein (1–3). However, the I488* derivative of the human glucocorticoid receptor, which deletes sequences in the vicinity of the DNA-binding domain, may be a positive control mutant because it appears to bind DNA normally but fails to activate transcription (39).

The small size and nonstringent sequence requirements of yeast transcriptional activation sequences has led to the view that these regions might act as acidic surfaces for contacting other essential components of the transcriptional apparatus (5, 13, 14, 20–22). Our inability to observe a strong interaction between the acidic region of GCN4 and Pol II suggests that this activation region may contact another component of the transcriptional machinery. In fact, genetic and biochemical evidence suggests that these acidic regions might be used for interactions with TATA-binding factors (5, 23–27). However, the requirement for the acidic activation region does not bear on the question of whether the GCN4–Pol II interaction observed here is also important for transcription *in vivo*. For example, GCN4 might facilitate (but not be absolutely required for) the formation of an active initiation complex by utilizing different regions of the protein for contacting both Pol II and a TATA-binding factor. It remains to be seen if interactions between Pol II and upstream activator proteins are generally used during the transcriptional activation process.

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1. Hochshild, A., Irwin, N. & Ptashne, M. (1983) *Cell* **32,** 319–325.
2. Irwin, N. & Ptashne, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8315–8319.
3. Bushman, F. D. & Ptashne, M. (1988) *Cell* **54,** 191–197.
4. McKnight, S. L. & Tjian, R. (1986) *Cell* **46,** 795–805.
5. Struhl, K. (1987) *Cell* **49,** 295–297.
6. Guarente, L. (1987) *Annu. Rev. Genet.* **21,** 425–452.
7. Thireos, G., Penn, M. D. & Greer, H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5096–5100.
8. Hinnebusch, A. G. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6442–6446.
9. Hope, L. A. & Struhl, K. (1985) *Cell* **43,** 177–188.
10. Arndt, K. & Fink, G. R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8516–8520.
11. Hope, I. A. & Struhl, K. (1987) *EMBO J.* **6,** 2781–2784.
12. Hill, D. E., Hope, I. A., Macke, J. & Struhl, K. (1986) *Science* **234,** 451–457.
13. Hope, I. A. & Struhl, K. (1986) *Cell* **46,** 885–894.
14. Hope, I. A., Mahadevan, S. & Struhl, K. (1988) *Nature (London)* **333,** 635–640.
15. Vogt, P. K., Bos, T. J. & Doolittle, R. F. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3316–3319.
16. Struhl, K. (1987) *Cell* **50,** 841–846.
17. Struhl, K. (1988) *Nature (London)* **332,** 649–650.
18. Bohmann, D., Bos, T. J., Admon, A., Nishimura, T., Vogt, P. K. & Tjian, R. (1987) *Science* **238,** 1386–1392.
19. Angel, P., Allegretto, E. A., Okino, S. T., Hattori, K., Boyle, W. J., Hunter, T. & Karin, M. (1988) *Nature (London)* **332,** 166–171.
20. Brent, R. & Ptashne, M. (1985) *Cell* **43,** 729–736.
21. Ma, J. & Ptashne, M. (1987) *Cell* **51,** 113–119.
22. Chen, W., Tabor, S. & Struhl, K. (1987) *Cell* **50,** 1047–1055.
23. Struhl, K. (1986) *Mol. Cell. Biol.* **6,** 3847–3853.
24. Struhl, K., Brandl, C. J., Chen, W., Harbury, P. A. B., Hope, I. A. & Mahadevan, S. (1988) *Cold Spring Harbor Symp. Quant. Biol.* **53,** 701–709.
25. Sawadogo, M. & Roeder, R. G. (1985) *Cell* **43,** 165–175.
26. Horikoshi, M., Hai, T., Lin, Y.-S., Green, M. & Roeder, R. G. (1988) *Cell* **54,** 1033–1042.
27. Horikoshi, M., Carey, M. F., Kakidani, H. & Roeder, R. G. (1988) *Cell* **54,** 665–669.

28. Kadonaga, J. T. & Tjian, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5889–5893.
29. Sopta, M., Carthew, R. W. & Greenblatt, J. (1985) *J. Biol. Chem.* **260,** 10353–10360.
30. Dahmus, M. E. & Kedinger, C. (1983) *J. Biol. Chem.* **258,** 2303–2307.
31. Garner, M. & Revsin, A. (1981) *Nucleic Acids Res.* **9,** 3047–3060.
32. Fried, M. & Crothers, D. (1981) *Nucleic Acids Res.* **9,** 6505–6525.
33. Formosa, T., Burke, R. L. & Alberts, B. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2442–2446.
34. Ptashne, M. (1986) *Nature (London)* **322,** 697–701.
35. Chen, W. & Struhl, K. (1989) *EMBO J.* **8,** 261–268.
36. Aebi, M., Furter, R., Prantl, F., Niederberger, P. & Hutter, R. (1984) *Curr. Genet.* **8,** 165–172.
37. Zalkin, H., Paluh, J. L., vanCleemput, M., Moye, W. S. & Yanofsky, C. (1984) *J. Biol. Chem.* **259,** 3985–3992.
38. Allison, L. A., Wong, J. K., Fitzpatrick, V. D., Moyle, M. & Ingles, C. J. (1988) *Mol. Cell. Biol.* **8,** 321–329.
39. Hollenberg, S. M., Giguere, V., Segui, P. & Evans, R. M. (1987) *Cell* **49,** 39–46.