

Synergistic transcriptional enhancement does not depend on the number of acidic activation domains bound to the promoter

(transcriptional activation/eukaryotic promoters/cooperativity/enhancers/Fos and Jun oncoproteins)

SALVATORE OLIVIERO AND KEVIN STRUHL

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

Communicated by Keith R. Yamamoto, October 1, 1990 (received for review July 31, 1990)

ABSTRACT Many eukaryotic transcriptional activator proteins contain a DNA-binding domain that interacts with specific promoter sequences and an acidic activation region that is required to stimulate transcription. Transcriptional enhancement by such activator proteins is often synergistic and promiscuous; promoters containing multiple binding sites for an individual protein or even for unrelated proteins can be 10–100 times more active than promoters with single sites. It has been suggested that such synergy reflects a nonlinear response of the basic transcription machinery to the number and/or quality of acidic activation regions. Here, we determine the transcriptional activity of Jun–Fos heterodimers containing one or two GCN4 acidic activation regions on promoters containing one or two Ap-1 target sites. Surprisingly, heterodimers with one or two acidic regions activate transcription with similar efficiency and are equally synergistic (10- to 15-fold) on promoters containing two target sites. Thus, transcriptional synergy does not depend on the number of acidic activation regions but rather on the number of proteins bound to the promoter. This suggests that synergy is mediated either by cooperative DNA binding or by alternative mechanisms in which the DNA-binding domain plays a more direct role in transcription (e.g., changes in DNA structure, nucleosome displacement, or direct interactions with the transcriptional machinery).

Eukaryotic transcription factors contain distinct DNA-binding and transcriptional activation functions that are generally located in separate regions of the protein (1–3). Transcriptional activation domains are often defined by short acidic regions that function autonomously when fused to heterologous DNA-binding domains (3–5). Although many acidic sequences can serve as transcriptional activation regions and negative charge is clearly important (3–8), the level of transcriptional stimulation is influenced by other structural features such as the length of the region and possibly the α -helical character (8, 9). It has been hypothesized that the DNA-binding domain serves merely to bring the protein to the DNA target, whereupon the acidic activation region can interact with a component(s) of the basic transcription machinery. Since acidic regions are necessary for yeast activator proteins to function in mammalian cells (10, 11) and for mammalian activator proteins to function in yeast cells (5, 12), it is likely that they contact some part of the basic transcription machinery that is conserved functionally throughout the eukaryotic kingdom.

Transcriptional enhancement by activator proteins is synergistic in that promoters containing multiple binding sites upstream of a “TATA” element are often 10–100 times more active than analogous promoters containing single binding sites (for review, see ref. 13). Moreover, transcriptional

synergy is frequently observed when the multiple binding sites are recognized by distinct, and even evolutionarily distant, proteins. For example, the combination of the mammalian glucocorticoid receptor and the yeast GAL4 protein stimulates transcription much more effectively than either protein alone. Such promiscuous synergy between activator proteins is a fundamental aspect of eukaryotic transcription and constitutes an important basis for the extraordinary diverse patterns of gene expression mediated by enhancers.

Cooperative DNA binding of transcription factors to adjacent promoter sites represents a simple mechanism that is likely to account for at least some instances of transcriptional synergy. Such cooperative binding has been observed *in vitro* for some proteins (14–16) and generally reflects highly specific protein–protein interactions that contribute to the overall stability of the protein–DNA complexes (17, 18). However, the apparent requirement for highly specific protein–protein interactions makes it difficult to invoke cooperative DNA binding for explaining the promiscuous nature of transcriptional synergy. Moreover, synergistic activation has been observed *in vitro* under conditions where the binding sites for a given activator protein are fully occupied (19, 20). For all of these reasons, it seems very likely that there must be alternative mechanisms of synergy beyond cooperative DNA binding.

One such alternative mechanism is that acidic activation regions associated with DNA-binding proteins that are bound to adjacent promoter sequences interact synergistically with a common target of the basic transcription machinery (for review, see ref. 13). In such a model, the common target would respond functionally in a nonlinear fashion to the number and/or quality of acidic activation regions. Here, we test this proposal by determining whether transcriptional synergy depends on the number of acidic activation regions. To vary the number of acidic regions without changing the number of DNA-bound proteins, we compare the level of transcriptional activation mediated by DNA-binding heterodimers carrying either one or two acidic regions. In contrast to the predictions of the above model, the results indicate that synergistic enhancement does not depend on the number of acidic activation domains but rather on the number of proteins bound to the promoter.

MATERIALS AND METHODS

DNA Manipulations and Construction of Yeast Strains. DNAs encoding the various chicken Jun derivatives were derived from YCp88, a *ura3* vector that utilizes the *ded1* promoter for expression of the proteins (3). JunGa was constructed by inserting the *Xba*I–*Eco*RI fragment encoding the Jun DNA-binding domain in place of the yeast *GCN4* DNA-binding domain of LexA-gcn4- Δ 20 (8) and then replacing the *Escherichia coli* LexA region with a *Bss*HII–*Sal*I fragment containing the *ded1* promoter fused to AGC-TACGCGTACAAAGAAAATGAGTATTTCTTCCAGGG-TAAAAGCAAAGAATT that provides the AUG initia-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

tion codon and 12 additional amino acids in-frame with the GCN4 coding sequence. Jun Δ 4 and Jun Δ 9 were obtained from the LexA derivatives (5) by substitution of the LexA region as described for JunGa. The mouse Fos molecules (cloned in a derivative of YCp88 that contains *trp1* as the selectable marker) were constructed by combining the *Bss*HII-*Eco*RI/filled-in fragment carrying the *ded1* promoter and the above oligonucleotide sequence, the *Fsp* I-*Xho* II fragment (to which a *Sal* I linker was added at the *Xho* II site) from *v-fos* (21), and the *Sal* I-*Eco*RI fragments of *GCN4* deletions that either do (N125) or do not (N71) contain the *GCN4* activation domain (3).

To construct yeast strains KY371 and KY372, the relevant DNAs were introduced into the *his3* locus of KY329 (22) by gene replacement. The plasmid carrying the two optimal *GCN4* binding sites (*his3*-282) was obtained by cloning the oligonucleotide shown in the bottom line of Fig. 1 as an *Eco*RI-*Sac* I fragment into YIp55-Sc4099 (23), a derivative with a deletion between positions -447 and -83 of the wild-type *his3* promoter. The plasmid containing one optimal *GCN4* binding site (*his3*-281) was obtained by deletion of the above DNA between the *Eco*RI and *Sal* I sites. Plasmid DNAs containing the various Jun and Fos derivatives were introduced into KY371 and KY372 by selecting for the appropriate markers.

RNA Analysis. RNAs from KY371 and KY372 derivatives were hybridized to completion with an excess of ³²P-end-labeled oligonucleotides for *his3* and for *ded1* (the internal control) and treated with S1 nuclease as described (24). His3 RNA levels, normalized to *ded1* RNA levels in the same lane, were quantitated by densitometry; the level of transcription activated by *GCN4* in strain KY372 was defined as 100.

DNA-Binding Experiments. The *Eco*RI-*Sac* I fragment from the *his3*-282 promoter, which contains two *GCN4* optimal binding sites, was end-labeled and incubated with various amounts of *GCN4* protein produced in *E. coli* cells and purified to near homogeneity as described (25). The resulting protein-DNA complexes were separated from unbound DNA by electrophoresis in a 5% native polyacrylamide gel. The percentage of input DNA found in complex I (contains one bound *GCN4* dimer) or complex II (contains two bound *GCN4* dimers) was quantitated by densitometry. The protein composition of the two electrophoretically distinct complexes was inferred from the observation that only complex I is formed on the analogous fragment containing one

optimal binding site (data not shown). Independent binding of *GCN4* to the adjacent sites can be seen most easily under conditions of moderate occupancy (50 and 100 ng) where the fraction of DNA bound in complex II equals the square of the fraction bound in complex I.

RESULTS

Experimental Design. The crucial issue to be addressed in this paper is whether synergy depends on the number of acidic activation domains or on the number of proteins bound upstream of the TATA element. Normally, increasing the number of protein binding sites upstream of the TATA element results in the concomitant increase of both the number of acidic regions and DNA-binding proteins at the promoter. To alter the number of acidic domains independently of the number of DNA-binding proteins, we devised an experimental situation in which transcription was activated by DNA-binding heterodimers. In this way, acidic activation regions could be fused to one or both partners of the heteromeric complex.

To obtain such heterodimers, we fused the *GCN4* acidic activation region (3, 8) to the DNA-binding domains of the Jun and/or Fos oncoproteins (JunGa and FosGa; Fig. 1). As controls, we utilized Jun deletions that remove part (Jun Δ 4) or all (Jun Δ 9) of the Jun activation region (5) as well as a deletion of Fos (Fos Δ 1). The Jun and Fos DNA-binding domains preferentially form a heterodimer that binds specifically to a transcription factor AP-1 site (26-29), a dyad-symmetric sequence that is essentially identical to the *GCN4* recognition site (22, 23). Fos is unable to form homodimers and can only bind DNA as a heterodimer with Jun. Jun homodimers can be formed, but in yeast cells they are very inefficient in activating transcription from promoters containing AP-1/*GCN4* sites, presumably due to relatively weak dimerization and DNA-binding activity (5, 22). Thus, the transcriptional activity of Jun-Fos heterodimers containing one or two *GCN4* acidic regions can be measured directly on promoters that contain a required AP-1 site(s) without significant interference from the contributions of Jun homodimers and Fos homodimers.

Transcriptional Activity of Fos-Jun Heterodimers on Promoters Containing One AP-1 Site. Plasmid DNAs capable of expressing the Jun and Fos derivatives were introduced into yeast strain KY371, which is deleted for the *GCN4* gene and contains *his3*-281, an allele with one optimal AP-1 binding site

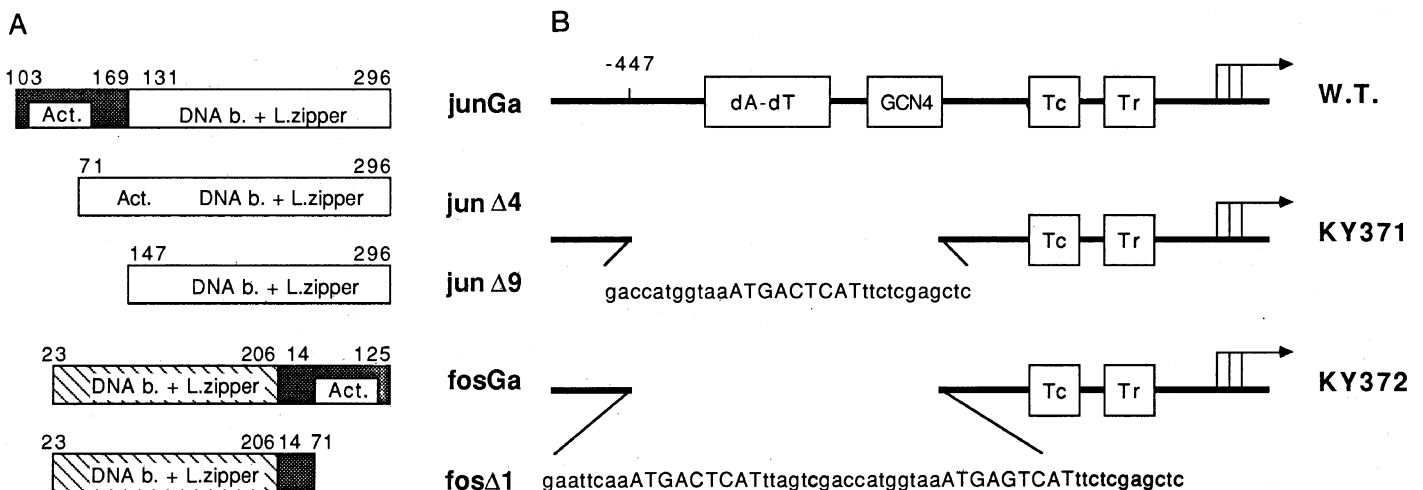


FIG. 1. Structure of proteins and promoters. (A) Proteins composed of the indicated regions (numbers correspond to amino acid residues) of Jun (open box), Fos (hatched box), and *GCN4* (shaded box); the DNA-binding and dimerization domains [basic (b) region and leucine (L) zipper] and acidic activation (Act.) regions are indicated. (B) *his3* promoters in wild-type (W.T.) strains [contains the poly(dA-dT) element, *GCN4* binding site, T_C and T_R TATA elements, and +1, +13, and +22 initiation sites indicated by arrows] and in KY371 and KY372, strains where the region between positions -447 and -83 has been replaced by the indicated sequence that contains one or two optimal *GCN4* binding sites (crucial residues are capitalized).

upstream of the TATA element and mRNA coding region. The resulting strains were assayed for their levels of *his3* RNA (Fig. 2A). In the absence of any Jun or Fos derivative, *his3* transcription was extremely low and in fact the cells were unable to grow in the absence of histidine; this reflects the absence of GCN4 protein as well as the upstream pro-

motor elements necessary for constitutive *his3* transcription (30, 31). When tested alone, Jun Δ 4 was barely effective, and Jun Δ 9 or either of the Fos derivatives was inactive. Thus, with the possible exception of JunGa (see below), *his3* transcription cannot be significantly induced by any of the Jun or Fos homodimers. In addition, the results rule out the possibility of endogenous yeast proteins that can heterodimerize with the Jun or Fos derivatives to produce a transcriptionally active complex with the AP-1 binding site.

The fact that heterodimers containing a single GCN4 acidic region can activate *his3* transcription was most clearly illustrated by the combination of FosGa and Jun Δ 9 (Fig. 2A; compare lanes 5 and 6 with lane 12). Neither protein can activate transcription alone because FosGa is not able to bind DNA and Jun Δ 9 lacks an activation region. Activation by the FosGa-Jun Δ 9 heterodimer depends primarily on the single GCN4 acidic region fused to FosGa because only 20–30% as much *his3* transcription was observed in cells containing Jun Δ 9 and Fos Δ 1. Thus, dimerization of the GCN4 acidic region is not necessary for transcriptional activation.

Interestingly, all the Fos-Jun combinations that contain either one or two activation regions stimulated *his3* transcription to comparable levels (Fig. 2A, lanes 8–12). This result also confirmed the expectation that Jun homodimers do not significantly contribute to transcription in cells containing both Jun and Fos derivatives. Specifically, similar RNA levels were obtained when either JunGa or Jun Δ 9 was combined with FosGa even though JunGa homodimers could contribute to *his3* expression, whereas Jun Δ 9 homodimers could not contribute and might possibly interfere with *his3* expression by binding to the AP-1 site. These observations indicate that the activation domain is a monomeric structure and that the number of acidic regions on a DNA-bound protein does not significantly affect the level of transcription.

Transcriptional Activity of Fos-Jun Heterodimers on Promoters Containing Two AP-1 Sites. When the Jun-Fos combinations were introduced into KY372, a strain containing the *his3*-282 promoter, which has two adjacent AP-1 sites, *his3* RNA levels were approximately 10-fold higher than observed in the corresponding KY371 derivatives that contain one AP-1 site (Fig. 2B, lanes 8–12). Again, heterodimers containing one acidic region activated transcription almost as well (60–90%) as heterodimers containing two acidic regions (Fig. 2C). Thus, there was a dramatic difference in transcriptional activation when two acidic domains were located on two DNA-binding proteins as opposed to the situation when the same two acidic regions were located on a single DNA-bound molecule. This demonstrates that transcriptional synergy does not depend on the number of acidic activation regions but instead depends on the number of proteins bound to the promoter. Although the length, quality, and probably number of acidic domains clearly contribute to the level of transcriptional activation (3, 6–9), they do not appear to be responsible for the synergistic effects.

Several other observations should be noted. (i) Some synergistic activation was observed with JunGa and Jun Δ 4 homodimers as well as with Jun Δ 9-Fos Δ 1 heterodimers (the residual activity was probably provided by the Fos Δ 1 moiety), even though the absolute levels of transcription were low. (ii) Activation by Jun-Fos heterodimers from a single AP-1 site resulted in *his3* transcription initiated equally from the +1 and +13 sites, a pattern typically associated with constitutive *his3* transcription (32). In contrast, Jun-Fos activation through two AP-1 sites was initiated with a strong preference for the +13 site, the pattern observed during activation by GCN4 (Fig. 2A; ref. 32) or by GAL4 (33).

Synergistic Activation by GCN4. Parallel experiments carried out in strains KY371 and KY372 indicated that wild-type GCN4 protein also synergistically activated transcription;

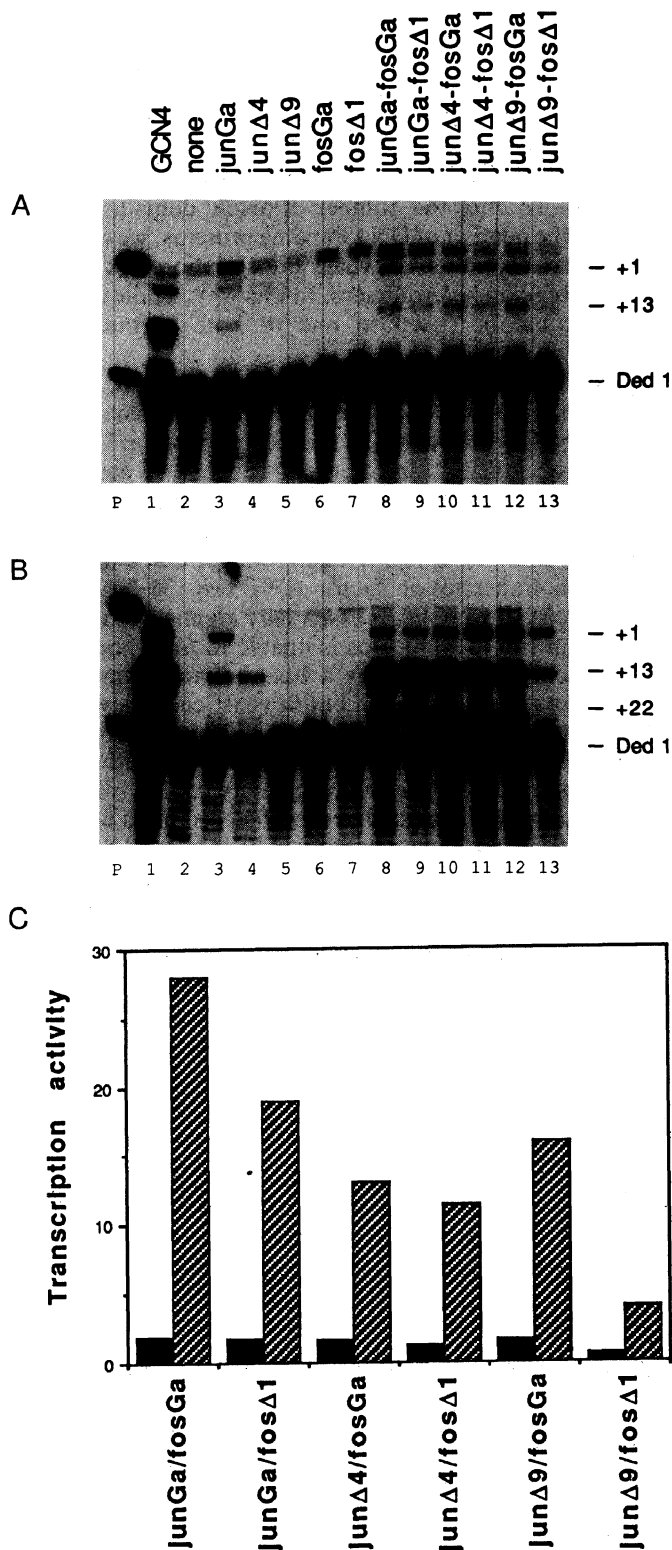


FIG. 2. Analysis of *his3* transcription. RNAs from KY371 derivatives, which contain a *his3* promoter with one optimal binding site (A), or KY372 derivatives, which contain a *his3* promoter with two optimal binding sites (B) were hybridized to 32 P-labeled *his3* and *ded1* probes; positions corresponding to the *ded1* and *his3* +1 and +13 transcripts are indicated. *His3* RNA levels, normalized to *ded1* RNA levels in the same lane, are shown in C (100 is defined as the level achieved by GCN4 in strain KY372).

his3 RNA levels were 15-fold higher on the promoter containing two AP-1 sites (Fig. 2). Transcription activated by GCN4 was 4–7 times more efficient than transcription activated by the Jun–Fos heterodimers. Although this could be due to differences in DNA-binding affinity, it more likely reflects the fact that only a portion of the GCN4 activation region was fused to the Jun and Fos DNA-binding domains. Given the homodimeric nature of GCN4, it is not possible to explicitly separate the contributions of the DNA-binding domain and the transcriptional activation region. However, since the number of GCN4 activation regions present on the Jun–Fos heterodimers does not account for synergistic enhancement, it seems unlikely that the number of GCN4 acidic domains would explain the synergy mediated by GCN4 itself.

GCN4 Binds Noncooperatively to Adjacent Sites. As mentioned in the introduction, cooperative DNA-binding is a simple mechanism to account for transcriptional synergy. To examine this possibility for the case of synergy by GCN4, we carried out standard DNA-binding titrations using *E. coli*-produced GCN4 protein and an oligonucleotide derived from the his3-281 promoter that contains two adjacent binding sites. Complexes containing one or two bound GCN4 molecules were distinguished by their electrophoretic mobility in native acrylamide gels. As shown in Fig. 3, the data are entirely in accord with noncooperative binding. Independent binding of GCN4 to the adjacent sites could be seen most easily under conditions of moderate occupancy (50 and 100 ng) where the fraction of DNA bound in complex II equaled the square of the fraction bound in complex I. Similar results using DNase I footprinting on other promoters containing multiple GCN4 binding sites have been obtained in this laboratory by C. R. Wobbe (unpublished data).

DISCUSSION

The GCN4 Acidic Activation Domain Is Monomeric. Detailed analyses of the GCN4 activation domain indicate that the level of transcriptional stimulation is moderately corre-

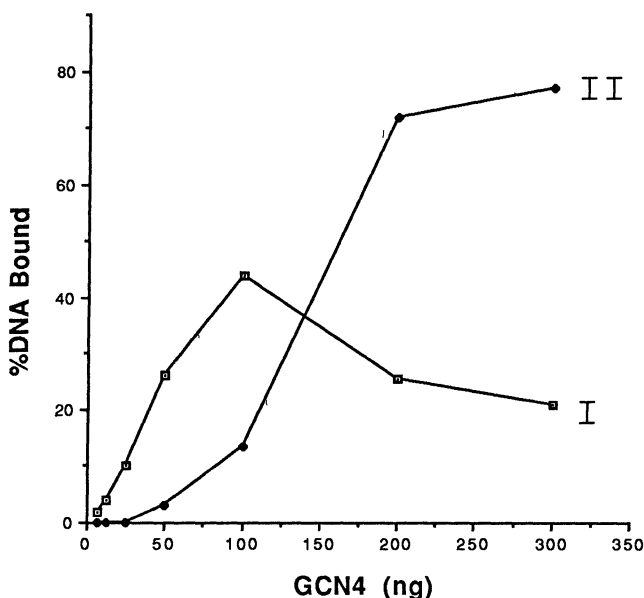


FIG. 3. Noncooperative binding of GCN4. DNA containing two GCN4 binding sites was incubated with the indicated amounts of *E. coli*-produced GCN4 protein, and the percentage of input DNA found in complex I (contains one bound GCN4 dimer) or complex II (contains two bound GCN4 dimers) was determined. Independent binding to the adjacent sites is best seen under conditions of moderate occupancy (50 and 100 ng) where the fraction of DNA bound in complex II equals the square of the fraction bound in complex I.

lated with the number of acidic residues but is influenced by other structural features such as the length of the region and possibly α -helical character (3, 8). For several reasons including the fact that GCN4 binds to DNA as a dimer (34), it was suggested that activation might require dimerization of the acidic region (8). However, the basic observation that Fos–Jun heterodimers containing a single GCN4 acidic region are efficient transcription factors indicates that the GCN4 activation domain is a monomeric structure. Furthermore, the results indicate that proteins lacking an activation region can stimulate transcription if they can associate into heteromeric DNA-binding complexes with partners that contain an activation domain. Conversely, the ability of a gene product to stimulate transcription does not necessarily indicate that the protein itself contains an activation function. In fact, conventional mapping of the transcriptional activation function on such a protein would instead uncover a motif necessary for oligomerization and/or DNA binding. Most importantly, however, the monomeric nature of acidic activation domains increases the complexity of regulation that can be mediated by protein families that contain common dimerization motifs such as the leucine zipper (35) and the helix–loop–helix (36).

Mechanism of Synergistic Transcriptional Activation. The results here argue against the prevailing view that synergy reflects a nonlinear response of a “common target” to the number and/or quality of acidic activation regions (13). We cannot exclude, but consider unlikely, the formal possibility that one activation region can inhibit the activity of a second activation region on the same DNA-bound molecule but not on an adjacent bound molecule. The GCN4 activation region is unstructured and physically separate from the DNA-binding domain (8), and it can function as a monomer; hence, it is likely to have considerable flexibility in finding the putative target in the transcription machinery. In this regard, the two activation regions in the JunGa–FosGa heterodimer lie in opposite configurations with respect to the DNA-binding domains. Finally, heterodimers with two GCN4 acidic regions activate somewhat better (30–60%) than heterodimers with only a single acidic region (Fig. 2B).

One explanation for synergistic activation invokes cooperative DNA binding on adjacent target sites. Although this model is probably valid for specific situations (14, 16), it does not easily account for the many examples of synergistic activation by different (and in some cases evolutionarily distant) proteins and hence is unlikely to be generally correct. Cooperative DNA-binding involves specific protein–protein interactions that are generally mediated by regions of the protein that are distinct from the DNA-binding domain (14, 17, 18). In contrast, the Fos and Jun moieties here are extensively deleted such that the potential contacts between adjacent heterodimers would have to involve the DNA-binding domains themselves or immediately adjacent regions of the protein. In this case, we suspect, but cannot demonstrate, that cooperative binding does not account for the observed synergy.

For synergistic activation by GCN4, three observations argue against a cooperative binding mechanism. (i) Such cooperativity has not been observed in DNA-binding experiments carried out *in vitro* using *E. coli*-synthesized GCN4 protein and the promoter DNAs described here (Fig. 3). (ii) Expression of the GCN4 DNA-binding domain lacking the acidic activation region results in repression of many GCN4-regulated yeast genes (3). (iii) At equivalent *in vivo* protein concentrations as employed here, GCN4 can repress transcription when bound at a single site located immediately downstream of the required TATA element in a *gal-his3* promoter (37). These two examples of repression strongly suggest that *in vivo* a single binding site is frequently occupied by GCN4 and hence that cooperative binding cannot increase

promoter occupancy by a factor of 10. Since the number of GCN4 acidic regions does not account for synergistic activation by the Fos-Jun heterodimers, it would seem unlikely to explain the synergism mediated by GCN4 itself.

In situations where transcriptional synergy is not due to cooperative DNA binding, we suggest an alternative explanation in which the DNA-binding domain plays a more direct role in transcription than simply targeting the protein to the promoter. This idea, though contrary to some suggestions (13), is supported by the existence of glucocorticoid receptor or HAP1 protein derivatives that bind DNA normally but fail to activate transcription (38, 39). In addition, the GCN4 DNA-binding domain can interact selectively with RNA polymerase II *in vitro* (40). Given the constraints imposed by the results in this paper, several noncooperative binding models for synergistic enhancement could be imagined. The DNA-binding domain might alter DNA structure, affect nucleosome distribution on the chromatin template, or directly interact with the basic RNA polymerase II transcriptional machinery. By any of these models, the acidic activation region presumably would carry out a different function than the DNA-binding domain in the overall process of transcriptional enhancement.

We acknowledge Pehr A. B. Harbury for valuable contributions during the initial phase of this work and for providing DNAs and Alfred S. Ponticelli for purified GCN4 protein. This work was supported by postdoctoral fellowships to S.O. from the European Molecular Biology Organization and from the Damon Runyon-Walter Winchill Cancer Fund and by grants to K.S. from the National Institutes of Health (GM 30186) and the Lucille Markey Trust.

1. Brent, R. & Ptashne, M. (1985) *Cell* **43**, 729-736.
2. Keegan, L., Gill, G. & Ptashne, M. (1986) *Science* **231**, 699-704.
3. Hope, I. A. & Struhl, K. (1986) *Cell* **46**, 885-894.
4. Ma, J. & Ptashne, M. (1987) *Cell* **48**, 847-853.
5. Struhl, K. (1988) *Nature (London)* **332**, 649-650.
6. Ma, J. & Ptashne, M. (1987) *Cell* **51**, 113-119.
7. Gill, G. & Ptashne, M. (1987) *Cell* **51**, 121-126.
8. Hope, I. A., Mahadevan, S. & Struhl, K. (1988) *Nature (London)* **333**, 635-640.
9. Giniger, E. & Ptashne, M. (1987) *Nature (London)* **330**, 670-672.
10. Kakidani, H. & Ptashne, M. (1988) *Cell* **52**, 161-167.
11. Webster, N., Jin, J. R., Green, S., Hollis, M. & Chambon, M. (1988) *Cell* **52**, 169-178.
12. Schena, M. & Yamamoto, K. R. (1988) *Science* **241**, 965-967.
13. Ptashne, M. (1988) *Nature (London)* **335**, 683-689.
14. Keleher, C. A., Goutte, C. & Johnson, A. D. (1988) *Cell* **53**, 927-936.
15. Schmid, W., Strahle, U., Schutz, G., Schmitt, J. & Stunnenberg, H. (1989) *EMBO J.* **8**, 2257-2263.
16. Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1989) *Cell* **57**, 443-448.
17. Johnson, A. D., Meyer, B. J. & Ptashne, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5061-5065.
18. Hochschild, A. & Ptashne, M. (1986) *Cell* **44**, 681-687.
19. Carey, M., Lin, Y.-S., Green, M. R. & Ptashne, M. (1990) *Nature (London)* **345**, 361-364.
20. Lin, Y.-S., Carey, M., Ptashne, M. & Green, M. R. (1990) *Nature (London)* **345**, 359-360.
21. Lech, K., Anderson, K. & Brent, R. (1988) *Cell* **52**, 179-184.
22. Struhl, K. (1987) *Cell* **50**, 841-846.
23. Hill, D. E., Hope, I. A., Macke, J. P. & Struhl, K. (1986) *Science* **234**, 451-457.
24. Chen, W., Tabor, S. & Struhl, K. (1987) *Cell* **50**, 1047-1055.
25. Oliphant, A. R., Brandl, C. J. & Struhl, K. (1989) *Mol. Cell. Biol.* **9**, 2944-2949.
26. Nakabeppu, Y., Ryder, K. & Nathans, D. (1988) *Cell* **55**, 907-915.
27. Halazonetis, T. D., Georgopoulos, K., Greenberg, M. E. & Leder, P. (1988) *Cell* **55**, 917-924.
28. Kouzarides, T. & Ziff, E. (1988) *Nature (London)* **336**, 646-651.
29. O'Shea, E. K., Rutkowski, R., Stafford, W. F. I. & Kim, P. S. (1989) *Science* **245**, 646-648.
30. Struhl, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8419-8423.
31. Struhl, K. & Hill, D. E. (1987) *Mol. Cell. Biol.* **7**, 104-110.
32. Struhl, K. (1986) *Mol. Cell. Biol.* **6**, 3847-3853.
33. Struhl, K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7865-7869.
34. Hope, I. A. & Struhl, K. (1987) *EMBO J.* **6**, 2781-2784.
35. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. (1988) *Science* **240**, 1759-1764.
36. Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buskin, J. N., Hauschka, S. D., Lassar, A. B., Weintraub, H. & Baltimore, D. (1989) *Cell* **58**, 537-544.
37. Brandl, C. J. & Struhl, K. (1990) *Mol. Cell. Biol.* **10**, 4256-4265.
38. Schena, M., Freedman, L. P. & Yamamoto, K. R. (1989) *Genes Dev.* **3**, 1590-1601.
39. Kim, K. S. & Guarente, L. (1989) *Nature (London)* **342**, 200-203.
40. Brandl, C. J. & Struhl, L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2652-2656.