Mol Gen Genet (1997) 253:766–769

SHORT COMMUNICATION

N. Tavernarakis · G. Thireos

The DNA target sequence influences the dependence of the yeast transcriptional activator Gcn4 on co-factors

Received: 12 July 1996 / Accepted: 10 September 1996

Abstract The yeast transcriptional activator Gcn4 requires the Ada2/Gcn5/Ada3 co-activator complex to exert part of its activation potential. Here we show that the sequence of the DNA target modulates the function of Gcn4 by modifying this requirement. Promoter configurations were generated that rendered Gcn4-induced transcription either completely dependent or completely independent of the Ada2/Gcn5/Ada3 complex. The topological constraints imposed by these configurations suggest that Gcn4 makes multiple contacts with the basic transcription machinery that are subject to modification by the incident DNA target. We propose that these modifications further determine the direction on the chromosome in which an otherwise symmetric, dimeric transcription factor will activate.

Key words Ada2 · Gcn5 · Ap1 site · Upstream Activating Sequences · Transcriptional regulation

Introduction

Regulation of gene expression at the transcriptional level plays a pivotal role in the process of unveiling genetic information (Tjian and Maniatis 1994). Departure from a precise pattern of transcriptional activation may dramatically affect the fate of a living cell. Establishment and maintenance of such a pattern is mostly achieved by DNA-binding transcription factors that, through a network of interactions, function to increase the rate of formation of productive transcription preinitiation complexes (Berger et al. 1992; Choy and Green 1993; Klein and Struhl 1994).

Typical transcriptional activators often have a modular structure comprised of two independently operating domains, the DNA-binding domain and the transcriptional activation domain (Ptashne 1988). The topology of the DNA-protein complex can influence the overall function of a transcriptional activator. For example, binding of the yeast transcriptional activator PRTF/Mcm1 to an imperfect DNA site masks the function of its activation domain (Tan and Richmond 1990). The NF-κB p50 subunit adopts an active conformation induced by certain κB DNA motifs (Fujita et al. 1992), while the VP16 activation domain becomes non-functional when tethered to a repressor DNA element (Lipkin et al. 1992). Similarly, binding of CREB to a sub-optimal CRE variant, attenuates its activity (Benbrook and Jones 1994). Finally, it has been proposed that DNA acts as an allosteric effector, directing the glucocorticoid receptor to contact components of the transcription machinery that interact with the activation domains of activators (Lefstin et al. 1994).

Both direct interactions with basic transcription factors such as TBP or TFIIB (Choy and Green 1993; Klein and Struhl 1994) and indirect interactions via proteins that serve as co-activators (Berger et al. 1990; Kelleher et al. 1990; Flanagan et al. 1991; Chiang et al. 1993, Kim et al. 1994, Lefstin et al. 1994) mediate ordered assembly of a productive transcription preinitiation complex and determine the strength of transcriptional activation. In *Saccharomyces cerevisiae*, Ada2, Gcn5 and Ada3 constitute a co-activator complex that potentiates the activity of a number of transcriptional regulators (Berger et al. 1992; Georgakopoulos and Thireos 1992; Pina et al. 1993; Horiuchi et al. 1995).

Communicated by C. P. Hollenberg

N. Tavernarakis · G. Thireos
Institute of Molecular Biology and Biotechnology,
Foundation for Research and Technology, PO Box 1527,
Heraklion 711 10, Crete, Greece

N. Tavernarakis (✉)
Department of Molecular Biology and Biochemistry,
Center for Advanced Biotechnology and Medicine,
Room 314, Rutgers, The State University of New Jersey,
l679 Hoes Lane, Piscataway, NJ 08855, USA

One such potentiated transcriptional activator is Gcn4, which binds the symmetric AP1 site as a dimer (Hope and Struhl 1987; O'Neil et al. 1990; Ellenberger et al. 1992). Interestingly, when Gcn4 activates transcription through an AP1 site, half of its activation potential depends on the Ada2/Gcn5/Ada3 co-activator complex (Georgakopoulos and Thireos 1992; Tavernarakis and Thireos 1995). However, transcription of Gcn4-regulated genes is differentially affected when this co-activator is absent (Georgakopoulos and Thireos 1992). Such genes are transcribed to variable maximal levels from promoters with asymmetric Gcn4 DNA targets, whose sequences depart from that of the optimal AP1 site (Hill et al. 1986; reviewed in Hinnebusch 1990). To investigate the influence of the DNA target sequence on Gcn4 activation function, we quantified the effects of an *ADA2* deletion on transcription driven by minimal, well-defined promoters bearing variant AP1 sites.

Materials and methods

Plasmids

Reporter genes were constructed by inserting appropriate double-stranded oligonucleotides between the *Bam*HI and *Eco*RI sites of a *HIS3*-based *lacZ* fusion, driven by a promoter bearing only the "regulated" TATA of *HIS3*, 18 bp downstream of the *Eco*RI site (Kim et al. 1993). The same double-stranded oligonucleotides were used for in vitro binding assays. The complete sequences of the upper strands of these oligonucleotides are as follows. 5′-gatccatggATGACTCATttttttg-3′ (GCRE); 5′-gatccatggAAGACTCTTttttttg-3′ (− 3A, + 3T); 5′-gatccatggATTACTAATttttttg-3′ (−2T, +2A); 5′-gatccatggATGACTAATttttttg-3′ (+2A), 5′-gatccatggATTAGTCATttttttg-3′ (−2T); 5′-gatccatggAAGACTAATttttttg-3′ (−3A, +2A) and 5′-gatccatggATTACTCTTttttttg-3′ (−2T, +3T). The Gcn4 binding site is capitalized; (+2A), (−2T), (−3A, +2A) and (−2T, +3T) bear asymmetric Gcn4 DNA targets, while (+2A) comprises of an optimum left half-site and (− 2T) of an optimum right half-site. The imperfect half-site in these two targets was derived from a target specific for the C242/Q246 Gcn4 mutant to which *wild-type* Gcn4 does not bind in vitro or transactivate in vivo (Kim et al. 1993). For (−3A, +2A) the target was assembled from a left half-site specific for the W235 Gcn4 mutant and a C242/Q246-specific right half-site. (−2T, +3T) comprised a left half-site specific for the C242/Q246 mutant, whereas the right half site was specific for the W235 Gcn4 mutant derivative.

Strains and other methods

Yeast strains were all S288C derivatives (*MAT*α, *mal*, *gal2*). To avoid interference from endogenous Gcn4, all experiments described here were performed with a *gcn4Δ* strain, constructed by removing a 0.8 kb *Bam*HI-*Pvu*II DNA fragment of *GCN4* gene and replacing it with a 1 kb *Hin*dIII DNA fragment containing the *URA3* gene. *GCN4* was then expressed in this genetic background under the control of the *DED1* promoter (Hope and Struhl 1986). The *ADA2* gene was disrupted by inserting the same *URA3* fragment at a unique *Afl*II site within the *ADA2* coding region. *LacZ* expression assays, in vitro synthesis of Gcn4 and mobility shift assays were performed essentially as previously described (Struhl 1991).

Results

Gcn4-induced activation through asymmetric DNA targets

To exacerbate the natural situation of imperfect Gcn4 binding sites, two asymmetric DNA targets were designed. In target +2A the left half-site and in target −2T the right half-site, with respect to the TATA element, were optimal (Fig. 1A). The other half-site was derived from a target that Gcn4 cannot bind in vitro or activate transcription in vivo (Kim et al. 1993; N. Tavernarakis and G. Thireos, unpublished observations). Despite the engineered asymmetry Gcn4 was found to transactivate through these asymmetric targets in vivo (Fig. 1B) and to interact in vitro, with affinities comparable to those obtained with the optimum AP1 target (Fig. 1C). The two engineered DNA targets (+2A, −2T) were inverted with respect to each other to facilitate optimal Gcn4 binding either to the left or to the right of the target. Therefore, target-2T carries a G at position 0 which is complementary to C0 of target +2A (G0; Fig. 1A). Similar results were obtained when a −2T variant target carrying a C at position 0 was employed (not shown).

Interestingly, disruption of the Ada2/Gcn5/Ada3 co-activator complex caused by introduction of an *ADA2* deletion, had a different impact on transcription through each target. The strength of the promoter harboring the optimal AP1 site was attenuated to approximately 50% the normal levels (GCRE, Fig. 1B). In contrast, transcription was completely dependent upon Ada2 when the left half-site was optimal. In the opposite configuration, with an optimal right half-site, transcription was insensitive to *ADA2* deletion (+2A vs. − 2T, Fig. 1B).

Transcriptional activation by Gcn4 mutant derivatives with altered binding specificity

To extend the above observations we tested two Gcn4 mutants with altered binding specificities. Each one has been shown to recognize a specific symmetrically mutated Gcn4 DNA target (Kim et al. 1993). The W235 Gcn4 mutant derivative binds the sequence AAGACTCTT and the C242/Q246 mutant recognizes the sequence ATTACTAAT (Fig. 2A). Both mutant proteins bind to the optimal AP1 with equal affinity but fail to cross-recognize their specific targets; i.e., the W235 Gcn4 mutant derivative does not detectably bind the sequence ATTACTAAT that corresponds to the target of the C242/Q246 Gcn4 mutant derivative, and the C242/Q246 Gcn4 mutant derivative does not recognize the W235 specific site (Kim et al. 1993; our unpublished observations). The *wild type* Gcn4 could not bind in vitro or transactivate in vivo through these mutant DNA sites.

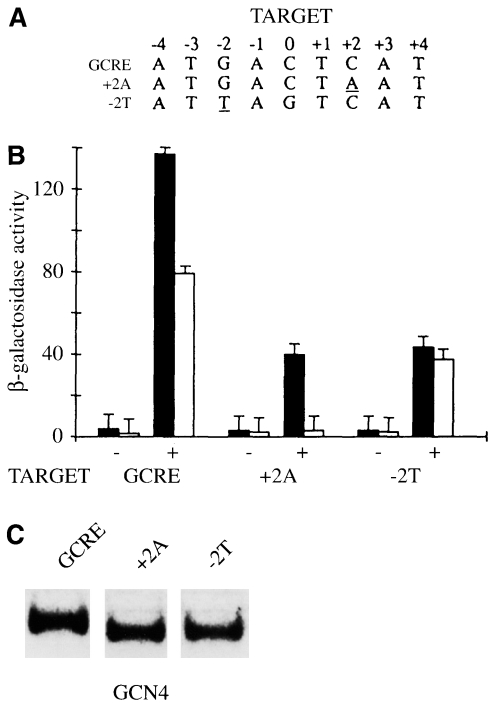

Fig. 1A–C Requirement for the Ada2 protein for transcriptional activation by Gcn4 at symmetric and asymmetric targets. **A** Sequence of DNA targets. Numbering of DNA target bases is according to Kim et al. (1993). Altered bases are underlined. +2A consists of a perfect left half-site, whereas in −2T the right half-site is optimal. In both DNA targets the other half-site derived from the C242/Q246 Gcn4–specific target, to which *wild-type* Gcn4 cannot bind in vitro or transactivate in vivo (Kim et al. 1993). **B** Transcriptional activation of the above reporter genes by Gcn4 in vivo as a function of target site and presence (*shaded bars*) or absence of Ada2 (*open bars*). The promoter activities in the presence (+) or absence (−) of Gcn4, are shown (values are in Miller units and represent the average of three independent experiments with less than 10% deviation). **C** In vitro binding of Gcn4 to the optimum target (GCRE) as well as to the asymmetric targets + 2A and − 2T

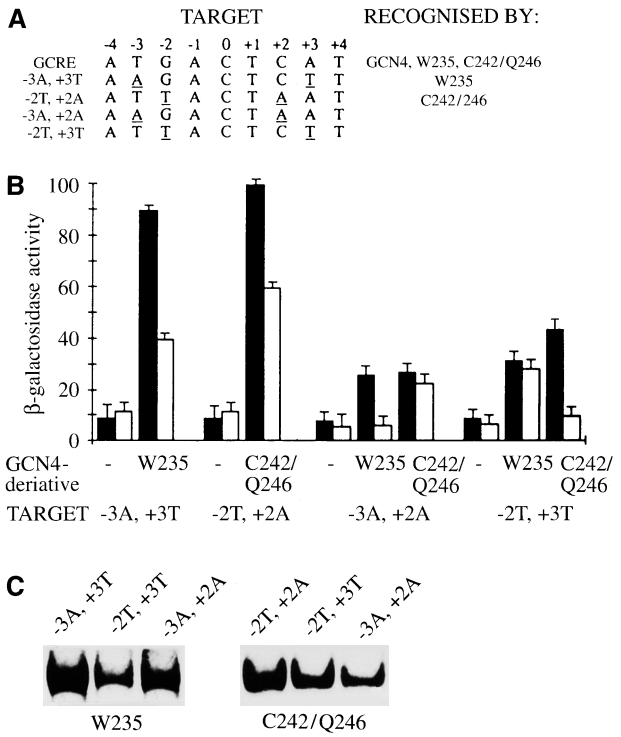

Fig. 2A–C Requirement for Ada2 for transcriptional activation by Gcn4 mutant derivatives through their corresponding symmetric and asymmetric DNA targets. **A** Sequence of targets and their known specificities for binding Gcn4 and its mutant derivatives. Numbering of DNA target bases is according to Kim et al. (1993). Altered bases are *underlined*. **B** Transcriptional activation of a *lacZ* reporter gene in vivo, as a function of DNA target, Gcn4 mutant derivative and the presence (*shaded bars*) or absence of Ada2 (*open bars*). The *DED1* promoter directed the expression of mutant *GCN4* alleles. Genetic backgrounds and assay conditions were as in Fig. 1B. **C** In vitro binding of W235 and C242/Q246 Gcn4 mutant derivatives to their optimum targets (−3A, +3T and −2T, +2A, respectively) and to the asymmetric targets −3A, +2A and −2T, +3T

Hybrid DNA targets were constructed consisting of two asymmetric half-sites, each one specific for only one mutant (Fig. 2A). W235 and C242/Q246 Gcn4 mutant derivatives could activate trascription through such targets in vivo (Fig. 2B) and interact with them in vitro (Fig. 2C). Activation by each mutant derivative through its respective target was only partly dependent on Ada2 (Fig. 2B). Again, when binding to the right half-site was compromised, Ada2 was essential for transcription. Ada2 was dispensable when right half-site was optimal (Fig. 2B). Identical results were obtained by similarly analyzing Gcn4-mediated activation in *gcn5* deletion strains (not shown).

Discussion

Gcn4 binds the symmetric AP1 site as a dimer (Hope and Struhl 1987; O'Neil et al. 1990; Ellenberger et al. 1992) and, when activating transcription through this site, half of its activation potential depends on the Ada2/Gcn5/Ada3 co-activator complex (Georgakopoulos and Thireos 1992; Tavernarakis and Thireos 1995). However, the absence of this co-activator affects the transcription of Gcn4-regulated genes differentially (Georgakopoulos and Thireos 1992). These genes are transcribed from promoters with asymmetric Gcn4 DNA targets (Hill et al. 1986; Hinnebusch 1990). Here, we demonstrate that the absence of a co-activator has a different impact on transcription from non-palindromic sites, depending on the sequence of the DNA target.

Taken together, our evidence suggests that binding of the Gcn4 activator to asymmetric DNA targets imposes topological constraints on the protein-DNA complex that perturb interactions with co-activators or parts of the transcriptional apparatus. The polar nature of these interactions suggests that functionally different surfaces of a dimeric activator are exposed towards the TATA element. Forced binding to a non-cognate left

half-site rendered Gcn4-induced activation insensitive to *ADA2* deletion, indicating that interaction with the Ada2/Gcn5/Ada3 complex was abolished. When binding to the right half-site was compromised, any contribution of other interactions was quenched, resulting in a strict requirement for this complex to reveal the residual Gcn4 activity. Therefore, Gcn4 function can be mediated by at least two pathways in a manner that is dependent on contact with the DNA target. We propose that modification of interactions between the activator and other proteins by DNA sequence-mediated constraints underlies the phenomena observed where the binding site dictates the final transcriptional outcome (Tan and Richmond 1990; Fujita et al. 1992; Lipkin et al. 1992; Benbrook and Jones 1994; Lefstin et al. 1994).

The biological relevance of the observations reported here derives from their implication of DNA binding sites in the establishment of polarity in the action of the activator. This polarity could account for unidirectional function of otherwise symmetric, dimeric activators (Hope and Struhl 1987; O'Neil et al. 1990; Ellenberger et al. 1992), in genomes tightly packed with genes such as that of yeast (Oliver et al. 1992). Thus, in the case of the divergently transcribed genes *HIS3* and *PET56*, a Gcn4 binding site located in the middle of a short intragenic region, consisting of an optimum left half-site with respect to *HIS3* TATA, regulates only *HIS3* (Struhl 1985).

Acknowledgments We thank Dimitris Tzamarias for providing strains and plasmids and Niki Gounalaki for the *ada2Δ* strain. We also thank Monica Driscoll, Abram Gabriel, Robyn Lints and Harbinder Singh for critical reading of the manuscript. This work was supported by funds for regional development, provided by the European Union.

References

Benbrook DM, Jones NC (1994) Different binding specificities and transactivation of variant CREs by CREB complexes. Nucleic Acid Res 22:1463–1469

Berger SL, Cress WD, Cress A, Triezenberg SJ, Guarente L (1990) Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell 61:1199–1208

Berger SL, Pina B. Silverman N, Marcus GA. Agapite J, Regier JL, Triezenberg SJ, Guarente L (1992) Genetic isolation of *ADA2*: a potential transcription adaptor required for function of certain acidic activation domains. Cell 70:251–265

Chiang CM, Ge Wang Z, Hoffmann A, Roeder RG (1993) Unique TATA-binding protein-containing complexes and cofactors involved in transcription by RNA polymerases II and III. EMBO J 12:2749–2762

Choy B, Green MR (1993) Eukaryotic activators function during multiple steps of preinitiation complex assembly. Nature 366:531–536

Ellenberger TE, Brandl CJ, Struhl K, Harrison SC (1992) The GCN4 basic region leucine zipper binds DNA as a dimer of uninterrupted alpha helices: crystal structure of the protein-DNA. Cell 71:1223–1237

Flanagan PM, Kelleher III RJ, Sayre MH, Tschochner H, Kornberg RD (1991) A mediator required for activation of RNA polymerase II transcription in vitro. Nature 350:436–438

Fujita T, Nolan GP, Ghosh S, Baltimore D (1992) Independent modes of transcriptional activation by the p50 and p65 subunits of NF-κB. Genes Dev 6:775–787

Georgakopoulos T, Thireos G (1992) Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. EMBO J 11:4145–4152

Hill DE, Hope IA, Macke JP, Struhl K (1986) Saturation mutagenesis of the yeast *his3* regulatory site: requirements for transcriptional induction and for binding by GCN4 activator protein. Science 234:451–457

Hinnebusch AG (1990) Transcriptional and translational regulation of gene expression in the general control of amino acid biosynthesis in *Sacharomyces cerevisiae*. Prog Nucleic Acid Res Mol Biol 38:195–240

Hope IA, Struhl K (1986) Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell 46:885–894

Hope IA, Struhl K (1987) GCN4, a eukaryotic transcriptional activator protein, binds as a dimer to target DNA. EMBO J 6:2781–2784

Horiuchi J, Silverman N, Marcus GA, Guarente L (1995) ADA3, a putative transcriptional adaptor, consists of two separable domains and interacts with ADA2 and GCN5 in a trimeric complex. Mol Cell Biol 15:1203–1209

Kelleher RJ III, Flanagan PM, Kornberg RD (1990) A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell 61:1209–1215

Kim J, Tzamarias D, Ellenberger T, Harrison SC, Struhl K (1993) Adaptability at the protein-DNA interface is an important aspect of sequence recognition by bZIP proteins. Proc Natl Acad Sci USA 90:4513–4517

Kim YJ, Björklund S, Li Y, Sayre MH, Kornberg RD (1994) A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell 77:599–608

Klein C, Struhl K (1994) Increased recruitment of TATA-binding protein to the promoter by transcriptional activation domains in vivo. Science 266:280–282

Lefstin JA, Thomas JR, Yamamoto KR (1994) Influence of a steroid receptor DNA-binding domain on transcriptional regulator functions. Genes Dev 8:2842–2846

Lipkin SM, Nelson CA, Glass CK, Rosenfeld MG (1992) A negative retinoic acid response element in the rat oxytocin promoter restricts transcriptional stimulation by heterologous transactivation domains. Proc Natl Acad Sci USA 89:1209–1213

O'Neil KT, Hoess RH, DeGrado WF (1990) Design of DNA-binding peptides based on the leucine zipper motif. Science 249:774–778

Oliver SG et al. (1992) The complete DNA sequence of yeast chromosome III. Nature 7:38–46

Pina B, Berger S, Marcus GA, Silverman N, Agapite J, Guarente L (1993) *ADA3*: a gene, identified by resistance to GAL4-VP16, with properties similar to and different from those of *ADA2*. Mol Cell Biol 13:5981–5989

Ptashne M (1988) How eukaryotic transcriptional activators work. Nature 335:683–689

Struhl K (1985) Nucleotide sequence and transcriptional mapping of the yeast *pet56-his3-ded1* gene region. Nucleic Acids Res 36:8587–8601

Struhl K (1991) Reverse biochemistry: methods and applications for synthesizing yeast proteins in vitro. Methods Enzymol 194:520–535

Tan S, Richmond TJ (1990) DNA binding-induced conformational change of the yeast transcriptional activator PRTF. Cell 62:367–377

Tavernarakis N, Thireos G (1995) Transcriptional interference by *GCN4* overexpression reveals multiple interactions mediating transcriptional activation. Mol Gen Genet 247:571–578

Tjian R, Maniatis T (1994) Transcriptional activation: a complex puzzle with few easy pieces. Cell 77:5–8