Glutamine-rich Domains Activate Transcription in Yeast Saccharomyces cerevisiae*

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Activation domains of eukaryotic transcription factors can be classified into at least three distinct types based on their amino acid composition: acidic, proline-rich, and glutamine-rich. Acidic activators, such as yeast GAL4 and GCN4 and herpes simplex virus VP16, have been shown to stimulate transcription in various higher and lower eukaryotic cells. Similarly, proline-rich activators also function in both mammalian and yeast cells. These activators are regarded to possess “universal” activating potentials. By contrast, several studies have suggested that glutamine-rich activators such as human Sp1 are active in higher (mammalian) but not lower (yeast) eukaryotic cells. One interpretation is that lower eukaryotic cells lack a critical co-factor necessary for a glutamine-rich domain. This reasoning is counter-intuitive because many native yeast activator proteins contain glutamine-rich domains. Here, we have investigated the activity of a glutamine-rich GAL4-Sp1 domain A (Sp1A) hybrid protein in yeast Saccharomyces cerevisiae. We show that GAL4-Sp1A activated a GAL1-lacZ reporter by more than 200-fold over basal when the reporter was carried on a 2μ vector. The generality of the Sp1A results is supported by our finding that yeast glutamine-rich domains from HAP2 and MCM1 are also transcriptionally active in S. cerevisiae. Interestingly, we found that glutamine-rich domains are considerably less potent when responsive promoters (i.e. GAL1-lacZ) are integrated into yeast chromosome. Thus our results segregate the inherent transcriptional activity of a glutamine-rich domain in yeast S. cerevisiae from its apparent lack of activity when assayed on chromosomally embedded promoters.

Activation of transcription by RNA polymerase II is dictated by the interaction of upstream activator proteins with factors at the core promoter (1). Activators usually contain two modular domains: one for binding to DNA and another for functional contact with the transcriptional machinery (1, 2). At least three types of activation domains have been identified and characterized (1). These include the acidic (e.g. GCN4, GAL4, and VP16), the proline-rich (e.g. CTF/NF1), and the glutamine-rich domains (e.g. Sp1). Acidic activators have been shown to stimulate transcription in a range of eukaryotic cells from yeast to human (3–5). Thus, the acidic GAL4 activator of Saccharomyces cerevisiae can activate transcription when introduced into Drosophila (6), plants (7), and mammalian cells (8). Conversely, the “mammalian” acidic activator, VP16, functions efficiently when assayed in yeast.

Similarly, proline-rich domains (e.g. CTF/NF1) also stimulate transcription in higher (mammalian) and lower (yeast) eukaryotic cells (9–11), although in both settings the potency of proline-rich activators is lower than that of acidic activators. In assessing the ubiquitous ability to activate transcription in higher and lower eukaryotic cells, an apparent exception emerges for glutamine-rich activators, prototypically represented by human transcription factor Sp1. Thus, several investigators have reported that glutamine-rich domains are active in higher eukaryotes but are apparently inert for transcription in yeast (10–14). This had led to the reasoning that a necessary co-factor(s) for glutamine-rich domains might be absent in yeast. Such an idea appears to be counter-intuitive because glutamine-rich domains exist in many yeast transcriptional factors such as HAP1 (15), HAP2 (16), MCM1 (17), PHO2 (18), and GAL11 (19). Formally, the prevalence of glutamine-rich domains in yeast activator proteins does not prove a functional significance. Indeed, as yet, the glutamine-rich sequences native to yeast proteins have not been explicitly tested for transcriptional activity. In re-examining the question as to why glutamine-rich domains might be selectively inactive in yeast, we unexpectedly found that a GAL4-human Sp1A fusion protein activated transcription from a GAL1-lacZ reporter in yeast S. cerevisiae by more than 200-fold over basal. This activation by the glutamine-rich domain of Sp1A was found to be dependent on the GAL1-lacZ reporter being on an extrachromosomal 2μ-based vector; GAL4-Sp1A showed little or no activity when GAL1-lacZ was integrated into yeast chromosome. These results suggest that glutamine-rich activators are not transcriptionally inactive in yeast (e.g. missing a necessary co-factor), but rather they may be subjected to limitations exerted by position effects (e.g. access to chromosomally embedded promoters) in lower eukaryotic cells.

EXPERIMENTAL PROCEDURES

Media and Strains—Standard media were prepared (20). Strain GGY1:171, which harbors a GAL1-lacZ reporter integrated at URA3 locus (21), was from Mark Ptashne. Strain HXY1 is the same as GGY1:171, except that it lacks the GAL1-lacZ reporter (9).

Site-directed Mutagenesis and Plasmid Constructions—All DNA manipulations were carried out using standard methods (22). GAL4 derivatives were constructed in yeast vector, pHGXI (9). pGAL4-VP16 contains the C-terminal 79 amino acids of VP16 (23), pGAL4-CTF1 contains amino acids 399–499 of CTF/NF1 (24), and GAL4-Sp1A contains the glutamine-rich domain A of human Sp1 (25). GAL4-Sp1A(Qm) contains alanines substituted for the 14 glutamines contained in the stretch of amino acids from 83 to 124. To construct GAL4-HAP2 and GAL4-MCM1, DNA fragments containing the respective ORFs were polymerase chain reaction-amplified from yeast genomic DNA and fused to the GAL4 DNA-binding domain in pHGXI. GAL4-HAP2 contains the entire HAP2 sequence (16), and GAL4-HAP2(Qm) is the same as GAL4-HAP2 except for an internal deletion of the glutamine-rich segment that contains 29 glutamines. GAL4-MCM1N contains the N-terminal portion of amino acids 1–121 of MCM1 (17), and GAL4-MCM1Q contains the C-terminal glutamine-rich portion (amino acids 78–286) of

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1 The abbreviations used are: ORF, open reading frame; TBP, TATA-binding protein; UAS, upstream activating sequence.
MCM1. GAL1-lacZ plasmid was as described previously (26). GAL-CYC1-lacZ was constructed by replacing the native CYC1 upstream regulatory sequences with a synthetic 17-mer GAL4-binding site inserted at the XhoI site (position -178) of a CYC1-lacZ reporter (27). HSE-CYC1-lacZ is the same as GAL-CYC1-lacZ except that the reporter is under a synthetic 15-base pair HSE (28). HIS3-lacZ was constructed by placing the Escherichia coli lacZ coding sequence under the yeast his3 promoter region from position -2161 to -140 of the yeast his3 gene that contain all the regulatory sites (29). GAL-HIS3-lacZ was constructed by replacing the GCN4-binding site with a synthetic 17-mer GAL4-binding site. PHO5-lacZ was constructed by placing the E. coli lacZ coding sequence under the control of the yeast Pho5 promoter (30, 31).

**RESULTS AND DISCUSSION**

Of the three types of activators based on amino acid composition, published literature suggests that acidic and proline-rich activators (e.g. VP16 and CTF1, respectively) are active, whereas glutamine-rich activators (e.g. Sp1) are inactive for transcription in yeast, S. cerevisiae (for example, see Ref. 10). We have previously studied Sp1-viral activator interactions in mammalian cells (34, 35). Because we wished to construct an Sp1-responsive promoter system in yeast, we sought to re-examine the reason(s) as to why glutamine-rich domains are seemingly selectively inactive in lower eukaryotes. To this end, we constructed chimeric proteins that contained each of the three types of activation domains fused to the DNA-binding portion of GAL4 (Fig. 1A). As controls for the human glutamine-rich Sp1, GAL4-Sp1A, and black bars indicates activities from lacZ reporters activated by GAL4-Sp1A, and white bars indicates activities from lacZ reporters activated by the corresponding endogenous yeast factors. Yeast transformation and β-galactosidase assays were performed as described in the legend to Fig. 2. All lacZ reporters are on a 2μ vector.

Yeast Transformation and β-Galactosidase Assays—Plasmids were introduced into yeast as described previously (32). β-Galactosidase activity was determined using chlorophenol red-β-D-galactopyranoside (Boehringer Mannheim) as substrate (9).

Western Blot Analysis of GAL4 Derivatives—Yeast whole cell extracts were prepared as described (33) and analyzed by immunoblotting using antibody directed to the GAL4 DNA-binding domain (Santa Cruz Biotechnology, Inc.) followed by chemiluminescence detection (Tropix).

**FIG. 1.** Schematic representations of GAL4 fusion proteins and reporter plasmids. All DNA manipulations were carried out using standard methods (22). A, all GAL4 derivatives contain the GAL4 DNA-binding domain (amino acids 1-147) under the yeast heat shock factor gene promoter in pHGX1 (9). B, lacZ reporters were cloned into a yeast 2μ plasmid vector. See “Experimental Procedures” for details.

**FIG. 2.** Glutamine-rich Sp1A stimulates transcription in yeast. GAL4 fusions and lacZ reporters (see Fig. 1 for details of the constructs) were transformed into XHY1, and transcriptional activities were assessed by β-galactosidase assays as described under “Experimental Procedures.” Relative β-galactosidase activities represent the averages from three to five independent assays, each varying less than 10% (as indicated by the error bars).

**FIG. 3.** Comparisons of transcriptional activities of GAL4-Sp1A and native yeast activator proteins. Grey bars indicate activities from lacZ reporter activated by GAL4-Sp1A, and black bars indicates activities from lacZ reporters activated by the corresponding endogenous yeast factors. Yeast transformation and β-galactosidase assays were performed as described in the legend to Fig. 2. All lacZ reporters are on a 2μ vector.
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GAL-CYC1-lacZ, or GAL-HIS3-lacZ, respectively, it activated each of these reporters in the range of 300–8000-fold over basal, as expected (Fig. 2, A, B, and C). Similar co-introductions using GAL4-CTF1 in place of GAL4-VP16 also activated the same promoter-reporters by 10–100-fold over basal (Fig. 2, A, B, and C). Next, we tested GAL4-Sp1A. Unexpectedly, we found that glutamine-rich GAL4-Sp1A activated GAL1-lacZ by more than 200-fold over basal (Fig. 2A). To verify this finding, we assessed the function of GAL4-Sp1A on two additional promoters. Results in Fig. 2 (B and C) show that GAL4-Sp1A activated both GAL-HIS3-lacZ and GAL-CYC1-lacZ by more than 20-fold over basal (Fig. 1B). That targeting of the glutamine-rich domain to the promoter is responsible for activity was assessed by assaying GAL4-Sp1A on reporter-plasmids lacking GAL4-binding sites. Failure of CYC1-lacZ and HIS3-lacZ (both plasmids lack GAL4-binding sites) to be activated by GAL4-Sp1A (Fig. 2D) confirmed this supposition.

Activation by GAL4-Sp1A in yeast measures about 2% of that observed for GAL4-VP16. However, this magnitude for GAL4-Sp1A remains approximately 2-fold higher than that observed for the proline-rich activator, GAL4-CTF1 (Fig. 2, A, B, and C). Interestingly, the latter (but not the former) is commonly regarded as a “universal” activator in both higher and lower eukaryotic cells (9–11). Two additional points suggest that the Sp1A activity in yeast is biologically significant. First, one notes that the relative potencies of GAL4-Sp1A and GAL4-VP16 are variable activities, we reassessed GAL4-Sp1A function in the yeast strain, GGY1:171 (used in previous studies), which harbors GAL1-lacZ integrated at the URA3 locus. C, levels of activation of GAL1-lacZ by Gal4-MCM1. Yeast transformation and β-galactosidase assays were performed as described in the legend to Fig. 2.

The above finding that the Sp1A glutamine-rich domain is inert in yeast (10–14). In comparing the conflicting findings, we noted that a major difference exists in the format of the reporters used to assay for responsiveness to glutamine-rich domains. The responsive GAL1-lacZ reporter used in our study was on a 2μm vector, whereas in previous studies the GAL1-lacZ reporter was integrated into yeast chromosome (for example, see Ref. 10). To check whether this difference accounts for the variable activities, we reassessed GAL4-Sp1A function in the yeast strain, GGY1:171 (used in previous studies), which harbors GAL1-lacZ integrated at the URA3 chromosomal locus (21). In this setting, integrated GAL1-lacZ was indeed not activated by GAL4-Sp1A but was activated by GAL4-VP16 and GAL4-CTF1 (Fig. 4, compare A with B). Hence, unlike the acidic VP16 and the proline-rich CTF1, activity of the glutamine-rich Sp1A is affected by the physical context of the promoter. This finding represents another example consistent with the concept of position effects on gene expression in yeast (38).

The above finding that the Sp1A glutamine-rich domain is active in yeast, although initially unexpected, is consistent with our current understanding of transcriptional activation in yeast. The supposition that Sp1 was not active for transcription in yeast was based on the assumption that yeast lacked an Sp1-specific TBP-associated factor, which bridges Sp1 and the basal transcription machinery (14). However, recent evidence shows that these TBP-associated factor factors are not generally required for transcriptional activation in yeast (39, 40). Moreover, phylogenetic analysis of yeast proteins also suggests that activity from the Sp1 glutamine-rich domain should be
expected. A cursory examination of the yeast genome reveals the abundant presence of Sp1-like glutamine-rich motifs in many proteins. Indeed, based on known experimental results, several glutamine-rich yeast proteins (for example, HAP1 (15), HAP2 (16), MCM1 (17), PHO2 (18), and GAL11 (19)) are clearly indicated to be transcriptional activators. However, because, as yet, no yeast glutamine-rich motif has been tested explicitly for transcriptional activity, it remains formally possible that each of these proteins uses other discrete domains for function.

To confirm the generality of activity from glutamine-rich domains in yeast, we next constructed fusions of two glutamine-rich yeast factors, HAP2 and MCM1, to GAL4 DNA-binding domains (Fig. 5, compare A and B). Both mutants lost the respective transcriptional activities expected. A cursory examination of the yeast genome reveals the prevalence of glutamine-rich factors in yeast and because, as yet, no yeast glutamine-rich motif has been tested explicitly for transcriptional activity, it remains formally possible that each of these proteins uses other discrete domains for function.

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To rule out that domains other than glutamine-rich sequences accounted for the observed transcriptional activities, we next generated several mutant proteins to better define activities. First, we mutated Sp1A by substituting alanines for glutamine residues (as opposed to qualitative) issue. Overall, our results support a general conservation that includes the important class of glutamine-rich activators of transcriptional mechanisms in lower and higher eukaryotes.

For some glutamine-rich domains, access to integrated promoters seems to be a limiting step. However, the activity from HAP2 indicates that this might be a quantitative rather than a qualitative barrier. Overall, our results support a general conservation that includes the important class of glutamine-rich activators of transcriptional mechanisms in lower and higher eukaryotes.

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