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Yeast GAL11 protein stimulates basal transcription in a gene-specific manner by a mechanism distinct from that by DNA-bound activators

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Abstract The *GAL11* gene encodes an auxiliary transcription factor required for full expression of many, if not all, genes of the yeast *Saccharomyces cerevisiae*. We have recently shown that *GAL11*-encoded protein (Gal11p) enhances basal transcription from the *CYC1* promoter in a cell-free transcription system [(1993) Proc. Natl. Acad. Sci. USA 90, 8382–8386]. Here we indicate that Gal11p stimulates basal transcription in a gene-specific manner in vitro. We further suggest that the mechanism underlying the transcriptional stimulation by Gal11p is distinct from that by DNA-bound activators, since Gal11p stimulated transcription in a reaction system where activators were unable to enhance transcription due to the lack of intermediary factors.

Key words: GAL11 protein; Basal transcription; Gene specificity; Intermediary factor; In vitro transcription; *Saccharomyces cerevisiae*

1. Introduction

Recent biochemical studies have revealed that regulated synthesis of eukaryotic mRNA involves numerous proteins in addition to RNA polymerase II. These proteins can be classified into at least three distinctive groups. The first group is a set of proteins called basal transcription factors which form a preinitiation complex together with RNA polymerase II on the so-called core promoter comprising the TATA box and the initiation site [1,2]. The second class, referred to as activators in this report, consists of proteins that bind DNA in a most gene-specific fashion by recognizing specific DNA sequences, the enhancer or upstream activation sequence (UAS) [3]. The third class can be categorized as intermediary factors which mediate the activation signal of an activator(s) to the preinitiation complex [1,4]. In the yeast *Saccharomyces cerevisiae*, cell-free transcription has been reconstituted with basal transcription factors designated a, b, d, e, and g by the studies of Kornberg and his associates [5–7]. They further demonstrated that the activator-induced transcription requires two additional intermediary factors including a mediator [8,9] and a protein(s) contained in so-called fraction c [5,10].

The regulatory gene *GAL11* [11], also named *SPT13* [12], of *S. cerevisiae* was first identified in a weak fermentor of galactose, and later found to encode a global transcription factor exerting its function on the expression of many genes [13]. Molecular genetic experiments had once suggested that Gal11p was one of the intermediary factors for various activators, such as Gal4p, Grf1p, or Ppr1p [14,15]. Later, we showed that *GAL11* is required for efficient basal transcription from the core promoter of *CYC1* in yeast [16]. We also revealed that Gal11p does not bind to a specific DNA sequence but stimulates in vitro transcription from the *CYC1* core promoter in the presence or absence of activators which include GAL4-VP16, GAL4-AH, and Grf1p. We further suggested that the apparent potentiation of the activators by Gal11p was accounted for by the stimulation of basal transcription, and that the stimulation occurs at the step of preinitiation complex formation [16]. In this paper, we addressed the question why normal functioning of *GAL11* is required for full expression of many but not all the yeast genes in the cell. Here we demonstrate that the gene-specific transcriptional stimulation by Gal11p depends on core promoters but not on UASs/activators. We also suggest that the mechanism of transcriptional enhancement by Gal11p is distinct from that by DNA-bound activators. In the light of these results we suggest that Gal11p is a novel class transcription factor which regulates the efficiency of transcription initiation through interaction with basal transcription factors in a gene-specific manner.

2. Materials and methods

2.1. Template DNAs for in vitro transcription

pSK119 [16] contained a single copy of a Gal4p-binding sequence (UAS_G) upstream of the *CYC1* promoter/G-free cassette fusion, such that the distance between UAS_G and the TATA box became 29-base pairs. The UAS-less template pSK115 was described previously [16]. pSK145 was constructed as follows: Plasmid pMT24-271 containing the *GAL7* gene [17] was digested with *Mbo*II which cleaves *GAL7* DNA at nucleotide position +43 with respect to the transcription initiation site at +1 [18]. After the ends were blunt-ended by T4 DNA polymerase, the *Bam*HI–*Mbo*II fragment (position from −271 to +43) was cloned into the *Bam*HI and *Sma*I sites of pSK140, a derivative of pUC118 with modification at the *Sac*I site which had been converted to *Bgl*II site. pSK164 containing the core promoter of *GAL7* was constructed as follows: pSK145 was digested with *Nhe*I (position −73), blunt-ended by mung bean nuclease, and digested with *Bgl*II. The liberated fragment containing the core promoter of *GAL7* was cloned into the *Sma*I and *Bgl*II sites of pSK140. pSK161 containing the *GAL80* gene was constructed by subcloning of the *Dra*I–*Bgl*II fragment (position from −218 to +83 [19]) into the *Sma*I and *Bgl*II sites of pSK142, a derivative of pUC119 with the same modification as pSK140.

2.2. Preparation of transcription factors and extracts

Yeast Gal11p and recombinant GAL4-VP16 were purified as described [16]. Recombinant yeast TATA-binding protein (TBP) produced in *Escherichia coli* was a kind gift from H. Handa. Yeast nuclear extract was prepared from *gal11* null strain HS301 as described [16]. Yeast fractionated whole cell extract was prepared from HS301 as

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described [10], and the final sample was dialyzed against buffer A containing 50 mM potassium acetate [5]. Fraction c′ was prepared from nuclear extract of strain BJ2168, a *GAL11* wild-type derivative of HS301, exactly as fraction c [5], except that the heat treatment was omitted (the heat treatment abolished the activity in our preparation possibly because of the difference in strain).

2.3. In vitro transcription

The transcription assay using the yeast nuclear extract was carried out as described previously [16] except that the reaction was performed at 25°C for 90 min. The whole cell extract transcription system contained the fractionated whole cell extract (9 μg of protein), TBP (50 ng of protein) and 80 ng of template DNA in the same buffer as in the nuclear extract transcription system. The reactions were carried out in the presence or absence of 6 ng of Gal11p. When transcripts were analyzed by primer extension or S1 nuclease mapping, the reaction mixture contained 0.4 mM of each of the four ribonucleoside triphosphates. In the case of primer extension analysis, the reaction was terminated by the addition of 0.2 ml of a stop solution (0.3 M sodium acetate pH 5.2, 0.5% SDS, 5 mM EDTA and 20 μg of proteinase K) and incubated at 30°C for 20 min. Nucleic acids were extracted through two cycles of phenol/chloroform (1:1) treatment and precipitated by the addition of 2.5 volumes of ethanol. The pellet was dissolved in 12 μl of an annealing buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA and 175 mM KCl) containing 60 fmol of 5′-end-labeled RV primer (Takara Shuzo, Kyoto, Japan). After incubation at 37°C for 1 h, the reaction mixture was diluted with 18 μl of a reverse transcription buffer (83 mM Tris-HCl pH 8.0, 16.8 mM dithiothreitol, 16.8 mM $MgCl_2$ and 833 μM of each of the four deoxyribonucleoside triphosphates) containing 3 U of Rous associated virus 2 reverse transcriptase. After incubation at 37°C for 40 min, primer extension products were recovered by ethanol precipitation and analyzed on a 8% polyacrylamide/7 M urea gel. *GAL80* transcripts were analyzed by S1 nuclease mapping. The transcription reaction was terminated by the addition of 5 μg of DNase I and 2 μg of tRNA. After incubation at 25°C for 10 min, samples were treated with the stop solution, and nucleic acids were recovered as above. S1 nuclease mapping was performed as described [20]. Probe DNA was the *Bgl*II–*Pst*I fragment of pSK161 with 5′-end label at the *Bgl*II site (position +88). Fragments protected from S1 nuclease digestion were separated on a 8% polyacrylamide/7 M urea gel.

3. Results

3.1. Gene-specific enhancement of transcription by Gal11p

Previously we showed that normal functioning of *GAL11* is required for the efficient transcription of *GAL1*, *GAL2*, *GAL7* or *GAL10* but not of *GAL80* in yeast cells [11,13], despite the fact that all these genes are under the control of Gal4p/UAS_G [21]. To investigate what determines the gene-specificity of *GAL11* function, we analyzed the effect of Gal11p on transcription of the *GAL7* and *GAL80* genes in a cell-free system (Fig. 1A). Fragments from −271 to +43 of *GAL7* or from −218 to +83 of *GAL80* were subcloned into plasmids and used as templates. When the in vitro transcripts of *GAL7* were analyzed by primer extension, transcription was initiated at +1 and +28 (lane 1). Addition of GAL4-VP16 resulted in a 10-fold activation of transcription (lane 3), since this template has two UAS_G [17]. Gal11p enhanced both basal and GAL4-VP16-activated transcription by a factor 4. The apparent potentiation of GAL4-VP16 by Gal11p is attributable to the stimulation of basal transcription as described previously [16]. In case of *GAL80*, the transcripts were analyzed by the S1 nuclease mapping technique. As shown in lane 1 of Fig. 1B, transcription of *GAL80* was initiated at +5 in vitro. Transcription from +5 was also detected in vivo transcripts (lane M). GAL4-VP16 activated transcription from +1, +5 and +37 (lane 3) by binding to the UAS_G lying at −95 [22]. In contrast to the case of *GAL7*, addition of Gal11p to the reaction did not cause a significant

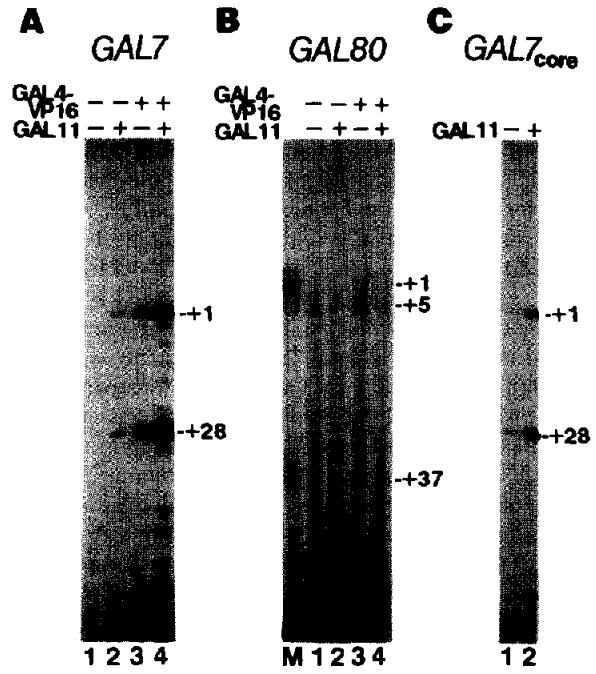

Fig. 1. Gene-specific stimulation by Gal11p. (A) Transcription of *GAL7*. The template DNA pSK145 was transcribed in vitro, and the transcripts were analyzed by primer extension. The reaction mixture contained Gal11p (lanes 2 and 4) and/ or 0.5 pmol of GAL4-VP16 (lanes 3 and 4). Positions of the initiation sites are indicated to the right of the panel with respect to the transcription initiation site at +1 [18]. (B) Transcription of *GAL80*. The template DNA pSK161 was transcribed, and the transcripts were analyzed by S1 nuclease mapping. The reaction mixture contained Gal11p (lanes 2 and 4) and/or 0.5 pmol of GAL4-VP16 (lanes 3 and 4). The total RNA (10 μg) prepared from the wild-type yeasts (HSY5–3C [16]) grown in YPGal [13] was subjected by S1 mapping, and the protected fragments were co-electrophoresed to verify the initiation site of *GAL80* (lane M). Positions of the initiation sites are indicated to the right of the panel with respect to the transrip-tion initiation site at +1 [19]. (C) Transcription of core promoter of *GAL7*. The template DNA pSK164 was transcribed in the absence (lane 1) or presence (lane 2) of Gal11p, and the transcripts were analyzed by primer extension. The transcription initiation sites are indicated to the right as A.

stimulatory effect on transcription of *GAL80* (lanes 2 and 4). We concluded therefore that transcriptional stimulation by Gal11p observed in vitro faithfully reflects the *GAL11* function in vivo. Similarly to the case of the entire *GAL7* promoter (regulatory) region, transcription from a template containing the core promoter of *GAL7* spanning from −69 to +43 (the TATA box is located at −63 [17]) was also stimulated by Gal11p (Fig. 1C). This result suggests that Gal11p exerted its effect on the basal transcription in a gene-specific fashion presumably depending on the core promoter context.

3.2. Non-involvement of intermediary factors in Gal11p function

Previously, Himmelfarb et al. [15] demonstrated that Gal11p, when tethered to upstream region of a reporter gene through the DNA-binding domain of LexAp, functions as a potent DNA-bound activator in yeast. This result might be interpreted to imply that Gal11p stimulates transcription through the same pathway as DNA-bound activators. It has been known that the activators like GAL4-VP16 and Gcn4p require at least two

intermediary factors for their activation function in the yeast cell-free transcription system. The one termed mediator is a protein that relieves squelching interference caused by excess amounts of activator proteins [4,8,9]. The other protein(s) is contained in fraction c, which was separated from the yeast nuclear extract by DEAE and phosphocellulose chromatography [5,10]. Thus we investigated whether these factors were required for transcriptional stimulation by Gal11p. As shown in Fig. 2, transcription of a template containing UAS_G was activated by the addition of 1 pmol of GAL4-VP16 (lane 5). However, addition of an excess amount of GAL4-VP16 (40 pmol) resulted in no activation. Instead, the amount of transcripts decreased to the basal level, presumably due to the squelching [4] of mediator by GAL4-VP16 (compare lanes 3 and 6). It was reported that addition of an excess amount of GAL4-VP16 in the reaction mixture inhibits basal transcription, which is a phenomenon called *cis*-inhibition [23]. In the present experiment, however, basal transcription was not affected as shown in lane 2, possibly because the concentration of template DNA was relatively low [23]. In the presence of excess GAL4-VP16, Gal11p still enhanced transcription by a factor 4 (lane 7). It was unlikely that this stimulation was due to release from the squelching inhibition, since the fold enhancement by Gal11p was the same irrespective of the absence or presence of excess GAL4-VP16 (compare lanes 4 and 7). Therefore these results suggested that the mediator for GAL4-VP16 was not involved in transcriptional stimulation by Gal11p.

We further studied the requirement of fraction c for stimulation by Gal11p using the transcription system derived from yeast whole cell extract [10]. In this system, basal transcription was reconstituted by the addition of TATA-binding protein (TBP). When activators like GAL4-VP16 or Gcn4p were included in the reaction, an additional factor(s) in fraction c was required for their activation function [10]. As shown in Fig. 3A, basal transcription was reconstituted by the addition of TBP to the fractionated extract prepared from the *gal11* null yeasts (lane 2). The Gal11p fraction could not substitute for the TBP activity (lane 3). In the presence of TBP, Gal11p induced transcription by a factor 6 over the basal level (lane 4) under conditions in which GAL4-VP16 could not activate transcription

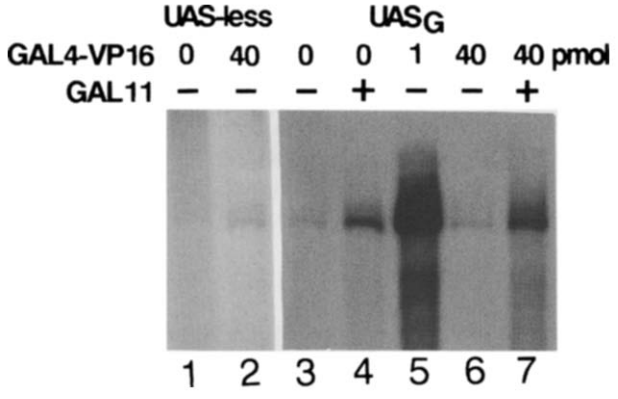

Fig. 2. Transcriptional stimulation by Gal11p under squelching condition. The UAS-less template pSK115 (lanes 1 and 2) or UAS_G-inserted template pSK119 (lanes 3–7) was transcribed in the absence (lanes 1, 3 and 4) or presence of 1 pmol (lane 5) or 40 pmol (lanes 2, 6 and 7) of GAL4-VP16. Gal11p was also added to the reaction (lane 4 and 7).

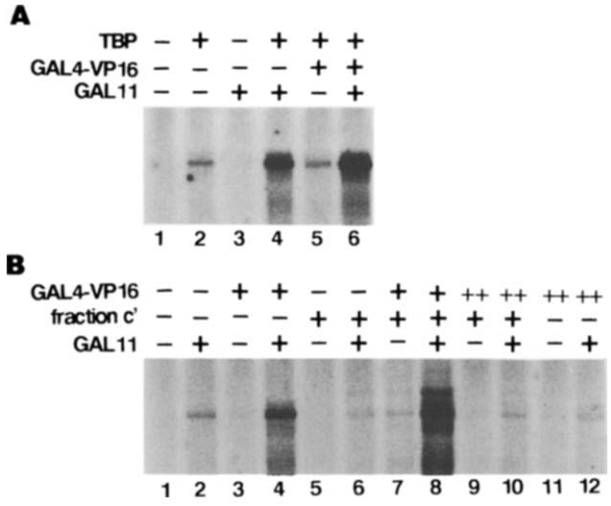

Fig. 3. Gal11p stimulates basal transcription in transcription system using fractionated whole cell extract. (A) Template DNA pSK119 was transcribed in the fractionated whole cell extract. Recombinant yeast TBP was added to the reactions (lanes 2, 4, 5 and 6). The reactions also contained Gal11p (lanes 3, 4 and 6) and/or 0.5 pmol of GAL4-VP16 (lanes 5 and 6). (B) Transcription reactions were carried out in the presence of TBP. Gal11p was added to the reactions (even numbered lanes). The reactions included fraction c′ (10 μg of protein, lanes 5–10) and 0.5 pmol (lanes 3, 4, 7 and 8) or 15 pmol (lanes 9–12) of GAL4-VP16.

(lane 5). In other words, Gal11p but not GAL4-VP16 stimulated transcription in a system composed of the fractionated whole cell extract and TBP. The Gal11p-induced transcription was further enhanced by GAL4-VP16 by 2- to 3-fold (compare lanes 4 and 6 of Fig. 3A or lanes 2 and 4 of Fig. 3B). The meaning of this enhancement remains unknown at this moment. To reconstitute the transcription system which responds to GAL4-VP16, fraction c′ was prepared from yeast nuclear extract (Fig. 3B). Fraction c′ was not strictly the same as the original fraction c [5], since the heat treatment was omitted for the present preparation (see section 2). In agreement with the published result [10], GAL4-VP16 activated transcription by approximately 5-fold in the presence of fraction c′ (compare lanes 3 and 7). As in the experiments with nuclear extract [16], Gal11p further enhanced the GAL4-VP16-activated transcription (lane 8). The transcriptional activation by GAL4-VP16 was not observed when excess amount of GAL4-VP16 was added, presumably due to the squelching of mediator as above. Under such conditions, Gal11p enhanced transcription (lane 10). Furthermore, in the absence of fraction c′ and in the presence of excess GAL4-VP16, Gal11p stimulated transcription by approximately 5-fold, which is the same fold enhancement as by Gal11p alone (compare lanes 2 and 12). These results indicated that Gal11p required neither mediator nor fraction c′ for its function, suggesting that the mechanism of transcriptional stimulation by Gal11p was inherently different from that by activators such as GAL4-VP16 or Gcn4p.

4. Discussion

We have revealed a couple of new features of Gal11p as a distinctive type transcription factor. First, the stimulatory ef-

fect of Gal11p on the basal transcription appears to be specific for the core promoter. Thus Gal11p enhances basal as well as activated transcription from the core promoters of *CYC1* [16] or *GAL7* but not from *GAL80*. Importantly, the in vitro specificity parallels with the *GAL11*-dependency of a gene in vivo. Second, Gal11p enhances basal transcription in the absence of mediator or a protein(s) in fraction c (strictly c'), both of which are required for activated transcription by DNA-bound activators, such as GAL4-VP16 or Gcn4p [8–10]. These results indicate that Gal11p does not require such intermediary factors for its function and stimulates transcription through a pathway different from that of activators. Although the exact mechanism of Gal11p function has remained to be elucidated, it is tempting to imagine that Gal11p interacts with a basal transcription factor(s) and that the formation of the preinitiation complex is regulated at multiple steps by activators and auxiliary factors like Gal11p. In support of this view, we have previously demonstrated that Gal11p was involved in the formation of the preinitiation complex [16]. In in vitro transcription systems of mammalian origin, the requirement for the basal transcription factors has been found to vary in each promoter, suggesting that compositions of the basal factors in the preinitiation complex are variable with promoter [24,25]. We may speculate therefore that Gal11p can interact with the preinitiation complex formed on the *CYC1* or *GAL7* promoters through a basal transcription factor but not with the complex formed on the *GAL80* promoter lacking that factor.

Besides *GAL11*, a number of regulatory genes have been identified, in which mutations cause pleiotropic defects, which likewise appear to affect the expression of many genes. They include *SWI*s and *SIN*s identified as regulatory genes for the *HO* gene, *SNF*s and *SSN*s for *SUC2*, and *SPT*s for transposable elements, Ty or delta (reviewed in [26]). Interestingly, some of the mutants isolated through the different screening systems were identical, which were therefore given different names. In fact, *gal11* mutants were isolated as one of the *spt*s (*spt13* [12]), and also as one of the *snf*s [27]. Successive studies have suggested that those genes encode either transcription factors or proteins involved in chromatin structures. Thus *SPT15* encodes for TBP, *SPT11* and *SPT12* for histones H2A and H2B, respectively, and *SPT2/SIN1* for an HMG-like protein (reviewed in [26]). Here we describe the protein encoded by *GAL11/SPT13* as another example of transcription factor required for efficient basal transcription of some genes.

Another group of global regulatory factors encoded by a set of genes designated *SRB* have been characterized genetically as well as biochemically. Those genes were identified as suppressors of partial truncation mutations of carboxyl terminal domain (CTD) of the largest subunit of RNA polymerase II [28]. Among those genes, *SRB2* and *SRB5*, when disrupted, cause pleiotropic defects but not lethality in yeast as in the case of *GAL11* [28–30]. In vitro transcription experiments have revealed the requirement of Srb2p and Srb5p for efficient transcription initiation [29,30]. They further indicated that the SRB proteins form a complex with RNA polymerase II and a set of basal transcription factors and the complex responses to activators [31]. Most recently, Kim et al. [32] also isolated a complex form of RNA polymerase II, termed 'holoenzyme' of RNA polymerase II, from the fractionated whole cell extract. This complex comprised some 20 polypeptides, including TFIIF, Gal11p, the SRB proteins [28–30], and Sug1p [33,34]. The holoenzyme enhances basal as well as activated transcription in the reconstituted system, in which 'core' RNA polymerase II is unable to support activated transcription [7]. The presence of Gal11p in the holoenzyme suggested that Gal11p contributes the activity to stimulate basal transcription in the holoenzyme. However, it remains to be clarified whether or not Gal11p is also involved in the activity to mediate transcriptional enhancement by activators.

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