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The *Saccharomyces cerevisiae* GAM2/SIN3 protein plays a role in both activation and repression of transcription

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Summary. We have cloned *GAM2*, which is required for transcription of *STA1*, a gene encoding an extracellular glucoamylase in *Saccharomyces cerevisiae* var. *diastaticus*. DNA sequence analysis revealed that *GAM2* is the same gene as *SIN3*, known to be a general negative regulator of yeast genes. RNA blot analysis indicated that *GAM2/SIN3* also acts as a positive regulator of *GAM3/ADR6*, which in turn is required for transcription of *STA1* and *ADH2*. These results suggest that *GAM2* regulates *STA1* expression through transcriptional activation of *GAM3* and indicate that GAM2/SIN3 protein is a transcriptional regulator that can play a role in both activation and repression of transcription.

Key words: *GAM2* – *SIN3* – Transcriptional regulator – Glucoamylase – Yeast

Saccharomyces cerevisiae var. *diastaticus* secretes glucoamylase (Yamashita et al. 1986) and ferments starch. The enzyme is encoded by one of three polymorphic genes, *STA1–STA3* (Yamashita et al. 1985a, 1985b; Lambrechts et al. 1991). Genetic analysis suggests that three genes (*GAM1–GAM3*) are required in trans to activate transcription of *STA1* (Okimoto et al. 1989). *GAM1* (Yoshimoto and Yamashita 1991) is the same gene as *SNF2* (Laurent et al. 1991) which is required for transcription of many genes subject to control by various regulatory systems. GAM1/SNF2 protein was predicted to be a 194 kDa, highly charged protein with a glutamine-rich tract, which is located in the nucleus (Yoshimoto and Yamashita 1991; Laurent et al. 1991). Laurent et al. (1991) also reported that transcriptional activation by *SNF2* is dependent on *SNF5* and *SNF6* functions. Recently, we have cloned *GAM3* (Yoshimoto et al. 1992) and found that *GAM3* is allelic to *ADR6* (Taguchi and Young 1987b; O'Hara et al. 1988), which is required for transcription of *ADH2*, the gene that encodes the glucose-repressible alcohol dehydrogenase II (Taguchi and Young 1987a). GAM3/ADR6 protein was predicted to be a 148 kDa nuclear protein with homopolymeric stretches of asparagine, threonine, or glutamine and a potential zinc finger (DNA-binding) domain (O'Hara et al. 1988). *GAM1–GAM3* are also required for normal growth on nonfermentable carbon sources and for sporulation (Okimoto et al. 1989; Yoshimoto et al. 1992).

To elucidate the role of *GAM2* and its interaction with *GAM1/SNF2* and *GAM3/ADR6* in transcription, we have cloned *GAM2* by complementation of the defect in glucoamylase production caused by the mutation *gam2–1*. A restriction map of the complementing yeast insert cloned in plasmid pMO1 is shown in Fig. 1. Genomic Southern analysis confirmed that the cloned sequence is intact and unique within the genome (data not shown). The functional *GAM2* sequence was localized on the insert by deletion analysis (Fig. 1). A null mutation (*gam2::URA3*; Fig. 1), created by deleting the 3.1 kb *Hin*dIII fragment located in the functional unit, was recessive and failed to complement *gam2–1* for glucoamylase production, growth on nonfermentable carbon sources, and sporulation (data not shown), indicating that the cloned locus is *GAM2*.

The functional *GAM2* region (a 5.8 kb *Hin*dIII fragment) was sequenced and appeared to be identical to the *SIN3* locus (Wang et al. 1990). The sequence encodes the SIN3 protein comprising 1538 amino acids, with a molecular mass of 175 kDa. The deletion analysis (Fig. 1) indicates that SIN3 is required for *GAM2* function. These results indicate that *GAM2* is the same gene as *SIN3*, also known as *SDI1*, *UME4*, and *RPD1* (Wang et al. 1990). Interestingly, these genes were all identified as negative regulatory genes, in contrast to the positive role of *GAM2* in *STA1* expression. The *SIN3/SDI1* function (Nasmyth et al. 1987; Sternberg et al. 1987) was identified by a mutation that suppresses loss-of-function mutations in *SWI5*, a gene required for transcription of *HO*. SIN3 protein is required to repress *HO* expression in daughter cells. The *UME4* function was identified by

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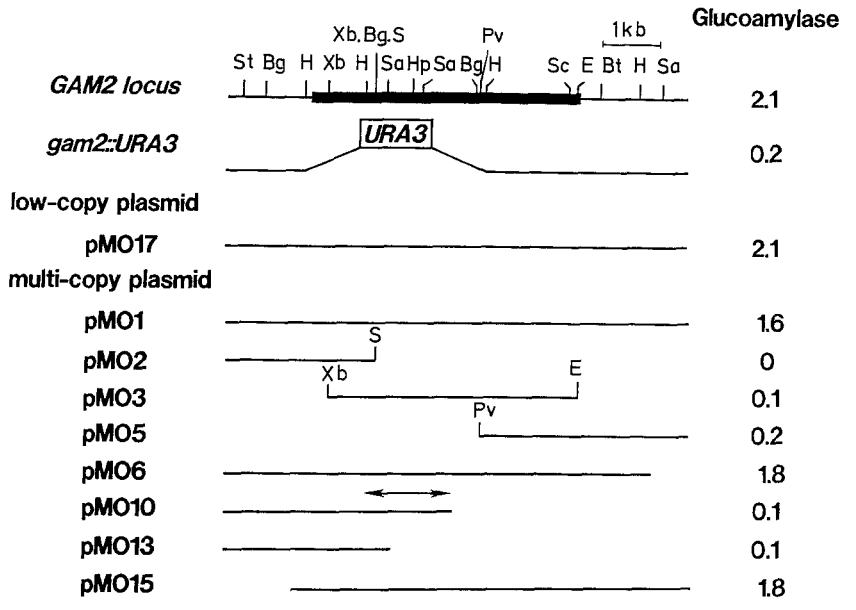

Fig. 1. Restriction map and deletion analysis of *GAM2*. The *gam2–1* haploid strain (YUT216; α *leu2 his2 STA1 inh*°) was transformed with a yeast DNA library (Yamashita et al. 1987) in the multicopy plasmid vector pYI1 (Suzuki et al. 1983), which carries *LEU2* and *URA3* and the 2-μm DNA origin of replication. Transformants carrying a putative *GAM2* were selected as described previously (Yoshimoto and Yamashita 1991). Plasmid pMO1 is the *GAM2* clone originally isolated. Plasmid pMO17 is a centromere-based low copy plasmid that was constructed by inserting the yeast fragment indicated into plasmid pHY20 (Yoshimoto and Yamashita 1991) carrying *LEU2* and *URA3*. Other plasmids are deletion derivatives of plasmid pMO1. The restriction map of *GAM2* is shown at the top, with the *GAM2* open reading frame indicated by the *closed bar*. The fragment indicated by *inverted arrows* was used as a probe to detect *GAM2* RNA. Restriction sites for *Stu*I (St), *Bgl*II (Bg), *Hin*dIII (H), *Xba* I (Xb), *Sal*I (S), *Sac*I (Sa), *Hpa*I (Hp), *Pvu*I (Pv), *Sca*I (Sc), *Eco*RI (E), and *Bst*EII (Bt) are indicated. The *gam2::URA3* was constructed as follows. A 6.2 kb *Stu*I-*Bst*EII *GAM2* fragment was cloned into the *Hin*dIII site of the bacterial plasmid pBR322 (Bolivar et al. 1977) after the fill-in reaction, yielding plasmid pMO202. The plasmid pMO202 was digested with *Hin*dIII to release a 3.1 kb fragment. The 1.2 kb *Hin*dIII *URA3* fragment of plasmid YEp24 (Botstein et al. 1979) was ligated between these *Hin*dIII sites, yielding pMO205. A 4.6 kb *Bam*HI-*Ban*III fragment of the plasmid pMO205 was isolated and used to transform a wild-type strain (YIY416; a *leu2 ura3 STA1 inh*°) to Ura$^+$. The substitution events were confirmed by Southern blot analysis. The wild-type and its isogenic *gam2::URA3* (YMO6) strains and *gam2–1* strain (YUT2l2; *a leu2 lys7 STA1 inh*°) transformed with each plasmid were cultured at 28° C in 5 ml of YPGL (yeast extract-peptone-glycerol-lactate) (Yoshida et al. 1990) for 3 days. Culture fluids were obtained by centrifugation, and assayed for glucoamylase activity (Yamashita et al. 1984). Total activity is presented in units per 10^7 cells

a mutation that permitted unscheduled expression of meiotic genes (*SPO11, 13, 16*) in mitotic cells (Strich et al. 1989). *RPD1* was identified as a mutation that increased expression of *TRK2* (Vidal et al. 1990).

RNA blot analysis was carried out to identify *GAM2* RNA and to examine the transcriptional regulation of *STA1* and *GAM1–GAM3* (Fig. 2). The wild-type strain, when probed for *GAM2* RNA, revealed a 5.5 kb RNA (Fig. 2, lane 1), which was absent in the isogenic *gam2::URA3* strain (Fig. 2, lane 2). The *gam2::URA3* strain transformed with the low copy *GAM2* plasmid (pMO17) produced the same RNA (Fig. 2, lane 3). The *gam2::URA3* strain transformed with the multicopy *GAM2* plasmid (pMO1) produced greatly elevated levels of this RNA (Fig. 2, lane 4). These results indicate that the 5.5 kb RNA detected is the *GAM2* transcript. The level of *STA1* RNA was reduced in the *gam2::URA3* strain (Fig. 2, lane 2), compared to that in the wild-type strain (Fig. 2, lane 1) or the *gam2::URA3* strain carrying *GAM2* on either a low-copy (Fig. 2, lane 3) or multi-copy (Fig. 2, lane 4) vector, confirming that *GAM2* is a positive regulator of *STA1* expression. The *GAM3* RNA level was also reduced in the *gam2::URA3* strain (Fig. 2, lane 2). Transformation with *GAM2* cloned on low copy number (Fig. 2, lane 3) or multicopy (Fig. 2, lane 4) vector restored RNA accumulation to the wild-type level (Fig. 2, lane 1). These results indicate that *GAM2* also acts as a positive regulator of *GAM3* expression. Since *GAM3* is required for transcription of *STA1*, *GAM2* may regulate *STA1* expression through transcriptional activation of *GAM3*. However, we cannot exclude the possibility that *GAM2* also plays a direct role in *STA1* expression. The *GAM1* RNA level was not regulated by *GAM2* (Fig. 2, lanes 1–4). Likewise, the *GAM2* RNA level was not regulated by either *GAM1* (Fig. 2, lanes 5–8) or *GAM3* (Fig. 2, lanes 9–12). The wild-type strain produced the same level of *GAM2* RNA under both derepression (Fig. 2, lane 13) and glucose-repression conditions (Fig. 2, lane 14), while *STA1* expression was inhibited by glucose repression (Fig. 2, lanes 13 and 14) as described previously (Dranginis 1989; Yoshimoto and Yamashita 1991), indicating that glucose repression of *STA1* expression is not mediated through transcriptional control of *GAM2*. Expression of the *GAM2* RNA was not dependent on *GAM2* function; equal amounts of *GAM2* RNA were detected in *gam2–1* and wild-type cells (data not shown).

In conclusion, our data indicate that *GAM2* is a pos-

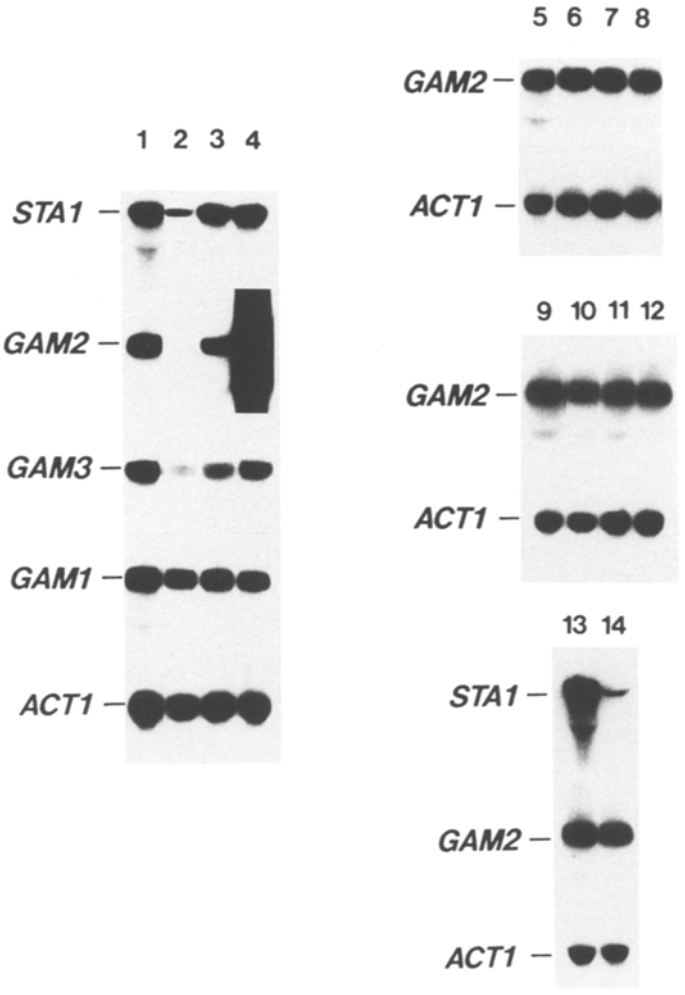

Fig. 2. RNA blot analysis. *STA1, GAM1–GAM3* and *ACT1* (yeast actin) transcripts were detected by RNA blot analysis of total RNA extracted from cells that had been cultured at 28° C to an optical density at 660 nm of 1.0 in YPGL, unless otherwise stated. The probe for *GAM2* RNA was a 1.5 kb *GAM2* fragment (indicated by *inverted arrows* in Fig. 1) located in the *GAM2* open reading frame. The probes for *STA1, GAM1* (Yoshimoto and Yamashita 1991), *ACT1* (Achstetter 1989), and *GAM3* (Yoshimoto et al. 1992) RNAs were described previously. Lanes: 1, 5, 9, 13, and 14 (the wild-type strain YIY416; lane 14, cells were cultured in YPGL plus 5% glucose); 2 (the *gam2::URA3* strain YMO6 transformed with the control plasmid pHY20; Yoshimoto and Yamashita 1991); 3 (the *gam2::URA3* strain with the low-copy *GAM2* plasmid pMO17); 4 (the *gam2::URA3* strain with the multicopy *GAM2* plasmid pMO1); 6 (an isogenic *gam1::URA3* strain d416–1–4; (Yoshimoto and Yamashita 1991) with the control plasmid); 7 (the *gam1::URA3* strain with a low-copy *GAM1* plasmid pHY9; Yoshimoto and Yamashita 1991); 8 (the *gam1::URA3* strain with a multi-copy *GAM1* plasmid pHY10, created by inserting into the plasmid pYI1 the same fragment as that cloned on the plasmid pHY9); 10 (an isogenic *gam3::URA3* strain d416–3–9; (Yoshimoto et al. 1992) with the control plasmid); 11 (the *gam3::URA3* strain with a low-copy *GAM3* plasmid pHY120; Yoshimoto et al. 1992); 12 (the *gam3::URA3* strain with a multi-copy *GAM3* plasmid pMO101; Yoshimoto et al. 1992)

itive transcriptional regulator of *GAM3/ADR6*, which is required for transcription of *STA1* and *ADH2*, and is the same gene as *SIN3* (also known as *SDI1, UME4*, and *RPD1*), a negative transcriptional regulator of several other genes (*HO, SPO11, SPO13, SPO16*, and *TRK2*). These results suggest that GAM2/SIN3 protein plays a role in both positive and negative regulation of transcription. Wang et al. (1990) proposed that SIN3 protein regulates, at the level of protein-protein interaction, the binding of a repressor protein to the *HO* promoter. It remains to be seen whether *GAM2* regulates DNA-protein interaction on a *GAM3* promoter.

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