Identity of the GAM3 Gene with ADR6, Each Required for Transcription of the STA1 or ADH2 Gene in Saccharomyces cerevisiae

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Among a number of Saccharomyces species, S. cerevisiae var. diastaticus is notable for its ability to secrete glucoamylase extracellularly and to ferment starch. The enzyme is encoded by one of three polymorphic genes, STA1–STA3.2–4 The regulation of STA1 expression appears to be complex. Transcription of STA1 has been reported to be negatively regulated at three levels: glucose repression,5,6 heterozygosity (a/a) at the mating-type locus,6,7,8 and an inhibitory gene, INHI.9 Genetic analysis showed that most strains of S. cerevisiae contain INHI but S. diastaticus does not.9 In addition to negative regulation, transcription of STA1 requires three positive regulatory genes, GAM1–GAM3.10–12 GAM1 is allelic to SNF2,11 which is required for transcription of many genes subject to different regulatory systems and for healthy growth and sporulation. GAM1 protein was predicted to be a 194-kDa highly charged protein with a glutamine-rich tract and localized in the nucleus,6,13 consistent with its role as a general transcriptional activator.

To elucidate the role of GAM3 protein and its interaction with GAM1/SNF2 protein in transcription, we have cloned the GAM3 gene by complementation of the defect in glucoamylase production caused by a gam3-1 mutation. The restriction map of the yeast insert cloned in the originally isolated plasmid, pMO101, is shown in Fig. 1. Genomic Southern analysis confirmed that the cloned insert is intact and unique within the genome (data not shown). Deletion analysis (Fig. 1) of the insert showed that the unique SacI site is essential for GAM3 function. A mutation (gam3-1:URA3) (Fig. 1), created by deleting the 3.1-kilobase-pair (kb) Hpal-BglII fragment encompassing the SacI site, was recessive and failed to complement the gam3-1 mutation for glucoamylase production (data not shown), indicating that the cloned locus is GAM3.

The 6.3-kb BamHI–PstI fragment was sequenced and appeared to be identical to the ADR6 locus12,13 which is required for transcription of the ADH2 encoding a glucose-repressible alcohol dehydrogenase.14 The sequence encodes the ADR6 protein of 1314 amino acids with a molecular mass of 148 kDa. We found a silent substitution at the codon of Leu877: CTG is replaced with TGT. The deletion analysis (Fig. 1) indicates that the ADR6 protein is required for GAM3 function. These results indicate that the GAM3 gene is the same as ADR6. Taguchi and Young13 reported that mutations of ADR6 created by inserting a HIS3 DNA fragment into either the BglII or BamHI site, both located in the ADR6-protein coding region, cause pleiotropic phenotypes such as slow growth on either glucose or glycerol and sporulation deficiency. We also found that cells carrying the gam3-1:URA3 mutation, which deletes the carboxy-terminal 2/3 of the ADR6-protein coding region, showed the above phenotypes (data not shown). In addition, we found that gam3-1:URA3 strains were incapable of growing on acetate, and this defect was complemented by either low- or high-copy GAM3 (Fig. 2, panels A and B). Furthermore, gam3-1:URA3 homoygotes failed to arrest as unbudded cells or undergo meiotic nuclear division in 1% potassium acetate (sporulation) medium (panel C). Thus, GAM3/ADR6 is not an essential gene, but it is important for normal growth and meiosis.

RNA blot analysis was done to identify GAM3 RNA and to find transcriptional controls of GAM3, GAM1, and STA1 (Fig. 3). The wild-type strain produced a 4.3-kilobase GAM3 RNA and a minor transcript of smaller size (lane 1). The GAM3 RNA was expressed at an

**Fig. 1.** Restriction Map and the Deletion Analysis of GAM3.

The gam3-1 haploid strain (YUT305, a leu2 STA1 mth) was transformed with a yeast DNA library in a multi-copy number plasmid vector, pY11, which carries LEU2, URA3, and 2μm origin of replication. Transformants carrying a putative GAM3 were selected as described previously.13 Plasmid pMO101 is an original one. Plasmids pHY120 and 115 are centromere-based low-copy plasmids which were constructed by inserting the yeast fragments into plasmid pHY20 carrying LEU2 and URA3. The restriction map of GAM3 is shown at the top, with GAM3 open reading frame indicated by the closed bar. The restriction sites for BamHI (B), BglII (BII), BglII (B), EcoRI (E), HindIII (H), Hpal (Hp), PstI (P), PvuII (Pv), Sdx (S), SacI (S), SacI (S), SacI (S), SacI (S), and XbaI (X) are indicated. The gam3-1:URA3 mutation was constructed as follows. A 5.3-kb Sdx–XbaI GAM3 fragment was filled-in and cloned into EcoRV site of the bacterial plasmid pBR322,21 yielding plasmid pHY11. The plasmid pHY11 was digested with Hpal and BglII to release a 3.1-kb fragment. The 1.2-kb HindIII URA3 fragment of plasmid YEP24 was ligated between the Hpal and BglII sites after the fill-in reaction, yielding pHY112. A 3.6-kb BamHI–HindIII fragment of the plasmid pHY112 was isolated and used to transform a wild-type haploid strain (YHY416, a leu2 ura3 STA1 mth) to Ura+. The substitution events were confirmed by Southern blot analysis. The following strains were cultured at 28°C in YPG (yeast extract-peptone-glycerol-lactate) medium for 3 days: the wild-type strain (YHY416), its isogenic gam3-1:URA3 strain (YHY416, a leu2 STA1 mth) was used as a control. Culture fluids were obtained by centrifugation, and assayed for glucoamylase activity.20 Total activity is presented in units per 5-ml culture. The gam3-1 strain (YUT309) transformed with the vector plasmid pH11 produced 2.6–7.9 units of the activity.
Fig. 2. Pleiotropic Phenotypes of gam3::URA3 Strains.

Effects of the gam3::URA3 mutation on cell growth and sporulation were examined. The wild-type strain (YI416; column 1), the isogenic gam3::URA3 strain (d416-3-9; column 2), and the latter strain transformed with either pY120 (low-copy GAM3; column 3) or pMO101 (multi-copy GAM3; column 4) were cultured at 28°C either on YEPD (extract-extract-peptone-glucose) plates for 2 days (panel A) or on YPA (extract-extract-peptone-acetate) plates for 3 days (panel B). Panel C, isogenic diploid strains (a/a ura3/ura3 leu2:: his2:: STA1/STA1 ind/ind) heterozygous (strain YI416·YI130) or homozygous (strain d416-3-9·YI130) for the gam3::URA3 were incubated with shaking in 1% potassium acetate at 28°C for 2 days, then stained for DNA with DAPI as described previously; micrographs (upper, phase-contrast; lower, DAPI-stained) were taken in the same field.

equivalent level under both derepressive (lane 1) and glucose-repressive (lane 2) conditions, while STA1 expression was inhibited by glucose repression (lanes 1 and 2) as described previously. The gam3::URA3 strain also produced the GAM3 RNA (lane 4), suggesting that functional GAM3 protein is not required for transcription of GAM3. These results are in accordance with the results of Taguchi and Young. The STA1 RNA was absent in either a gam3::URA3 (lane 4) or gam3::URA3 (lane 5) strain, and these defects were complemented by low- or multi-copy GAM3 (lane 6 or 7, respectively). Accumulation of GAM1 RNA was not regulated by GAM3 (lanes 3–7); likewise, GAM3 expression was not regulated by GAM1 (lanes 8–13).

We can conclude that GAM3/ADR6 protein is required for transcription of both STA1 and ADR2 and also for normal growth and the initiation of meiosis. The protein is located in the nucleus and contains a potential zinc-finger (DNA-binding) domain near its carboxy-terminus, consistent with its role as a positive regulator for transcription. These lines of evidence suggest a general role of GAM3/ADR6 protein in transcription. Recently, Laurent et al. reported that transcriptional activation by SNF2/GAM1 protein is dependent on SNF5 and SNF6 proteins and proposed that these three proteins form a heteromeric complex. This complex may either include or functionally interact with GAM3/ADR6 protein. Alternatively, GAM1 and GAM3 may function as part of a regulatory cascade.

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References